EFFECTS OF TEMPERATURE ON SEASONAL CHANGES IN GROWTH AND CARBOHYDRATE PHYSIOLOGY OF ASPARAGUS

(*Asparagus officinalis* L.)

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ABSTRACT

In a temperate climate, most of the visible, seasonal changes in asparagus growth are induced by or dependent on changing temperature regimes. Senescence of ferns in autumn occurred below 13C, but was prevented by 20C. Crowns required chilling at temperatures below 12.5C to release the internal dormancy which occurred during winter. Although budbreak was never completely suppressed, the minimum temperature at which budbreak could occur changed during winter dormancy. Budbreak did not occur at 12.5C in some cultivars at maximum dormancy. The optimum temperature for the growth of young plants was between 25C and 30C.

A model was developed which simulated seasonal changes in carbohydrate accumulation and utilisation, and the changing source-sink relationships within male and female plants. The model used temperature, indirectly, to determine the times at which seasonal changes in plant growth occurred.

The basic unit for carbohydrate production and allocation in cultivars with well defined rhizomes, e.g.'Rutger's Beacon', was a rhizome and it's attached developing axillaries. An axillary rhizome became independent very soon after it had developed fern. The basic unit may differ in cultivars such as 'UC157' which have less well defined rhizomes. The strength of correlative inhibition within a cultivar appears to affect both rhizome morphology and budbreak patterns during spear harvest.

In summer, young fern had a higher mobilising ability for assimilate than older fern or roots in male plants. In late summer-early autumn, roots became a stronger sink than the fern. On female plants, reproductive sinks (i.e, berries) had the highest competitive and mobilising ability.

Crown carbohydrate concentration appeared to reach a physiological maximum of 65% in late summer. Most of the carbohydrate pool was long chain fructans, i.e, with degree of polymerisation above eight. The size of the crown carbohydrate pool increased during autumn and senescence as crown dry weight increased. The concentration of disaccharide increased during senescence indicating that it may have a role in cold tolerance. There was little change in crown dry weight or carbohydrate concentration of chilled plants until after the plants had been chilled for five weeks and the minimum temperature for budbreak had decreased. Respiration then increased as internal dormancy was further released.

Changes in the composition of carbohydrate reserves are associated with the chilling process, and may affect the release of internal dormancy. Dormant plants required exposure to temperatures below 12.5C to increase the monosaccharide concentration above 4.5% dry weight and to depolymerise long chain fructans. Both these factors would decrease the substrate for some energy requiring process which must occur before budbreak can occur.

'Rutger's Beacon' required approximately 500 chilling units (calculated using the Utah model) to release 50% of the basal buds from internal dormancy and permit growth at 12.5C. The chilling response curve for asparagus appears to be flatter than the Utah model.

This thesis confirmed earlier work which indicated that improved agronomic performance may be related to increased partitioning into carbohydrate storage tissue i.e, the crown. Genotypic differences in depth of internal dormancy and spear growth rate will also affect yield.

Differences in carbohydrate metabolism are not the reason for agronomic differences between male and female plants. The strong sink effect of berries on female plants reduces crown dry weight and thus the crown carbohydrate pool.

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Asparagus officinalis L. is a perennial plant which is grown commercially in a wide range of temperature regimes, from the humid tropics of Malaysia and Thailand where fern is present all year, to cool temperate conditions in Canada and the Netherlands where snow falls and the plants are dormant for part of each year.

In temperate climates such as New Zealand, the fern senesces in autumn and the plant appears to be dormant during winter. In spring, buds on the underground rhizomes grow to produce new shoots or spears. The spears are usually harvested until early summer, after which the fern is allowed to develop. Photosynthesis by the fern replaces the carbohydrate reserves used in the production of spears and new fern. Thus plant vigour and survival are strongly influenced by the plant's carbohydrate reserves.

The objectives of the research described in this thesis were to examine some effects of temperature and daylength on the growth of asparagus and relate seasonal changes in the accumulation and utilisation of storage carbohydrates to growth and partitioning.

1.2 BOTANY

1.2.1 BOTANICAL BACKGROUND

The asparagus plant from which edible shoots are normally harvested commercially is *Asparagus officinalis* L. subspecies *officinalis*, a monocotyledonous plant belonging to the family Liliaceae (Tutin et al. 1980). Cultivated asparagus is diploid (n = 10) (Ammal et al. 1966).

Asparagus is normally a dioecious plant, with plant sex usually considered to be inherited in a simple Mendelian factor manner (Larzarte and Palser 1979; Loptien 1979; Benson 1982) with homozygous female plants (XX), heterozygous male plants (XY) (Rick and Hanna 1943) and a few andromonoecious plants (XY and YY) (Sneep 1953a,

b). However, other research indicates that sex inheritance may be more complex (Pierce and Currence 1962; Marks and Cornish 1979).

1.2.2 MORPHOLOGY

Asparagus is a herbaceous perennial. The underground portion of the plant is known as the 'crown', and the foliage is known as the 'fern' (Fig.1.01).

The crown consists of many unbranched fleshy storage roots up to 6 mm thick which are attached to underground stem tissue. Fibrous feeding roots are attached to the storage roots. Each underground stem, and it's attached roots, is commonly called a 'rhizome', although the 'stem' differs from a true rhizome in that it consists of the unelongated basal internodes of many stems (Blasberg 1932; Mullendore 1935). The term 'rhizome' will be used in this thesis. Buds form 'clusters' at the growing tip of the rhizome (Fig. 4.01). Plants normally have several rhizomes, and thus several bud clusters. Rhizome development is examined in Chapters 3 and 4.

The fern consists of many shoots, each of which has developed from a separate bud on the rhizome. Each shoot consists of a central stem which branches many times. The leaves form very small scales at nodes on the stem (Mullendore 1935). Whorls of needle-like branches (cladophylls or phyllodes) grow in the axils of the scale leaves (Arber 1925; Blasberg 1932; Mullendore 1935). The spears harvested from the plant are rapidly growing, immature shoot tips which have not yet branched.

1.3 PHYSIOLOGY

1.3.1 ROOT GROWTH

The development of new storage roots associated with a particular bud occurs when that bud develops into a shoot (Tiedjens 1924, Fisher 1982). Storage roots can grow up to 1 m in length in a growing season (Blasberg 1932). Roots 2 m long have been found on mature plants (Yaeger and Scott 1938). The economic life of the crop increases as depth of soil able to be penetrated by feeding roots increases (Reijmerink 1973) which implies that longevity may be linked to root mass. The life span of storage roots is unknown and probably depends on the growing conditions as healthy storage roots three (Tiedjens 1924) and six (Scott 1954) years old have been reported.

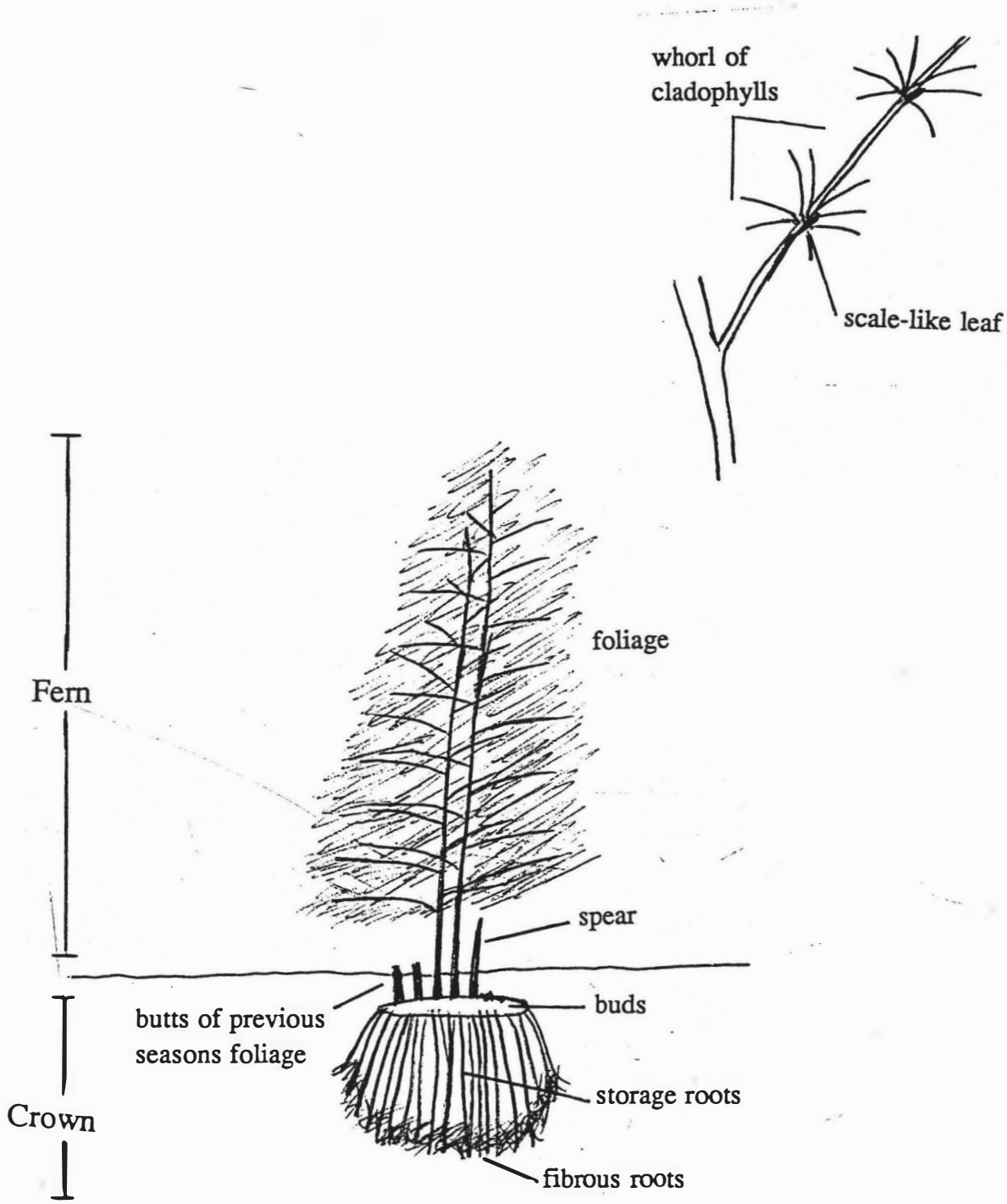


Figure 1.01: Stylised diagram showing the parts of a mature asparagus plant and the terminology used to describe the plant parts.

In 1 and 2 year old plants, root dry weight is nearly constant over winter and decreases in early spring as stored carbohydrates are mobilised to support the development of the new seasons fern (Shelton and Lacy 1980, Fisher 1982, Haynes 1987). Root dry weights in plants harvested for spears have not been reported previously and are examined in Chapters 3 and 5.

1.3.2 SHOOT GROWTH

Shoot growth utilises storage carbohydrate until the cladodes have expanded (Downton and Torokfalvy 1975, Lin and Hung 1978). Thus the production of spears and establishment of the first fern is a major drain on the carbohydrate reserves in the storage roots.

The production of spears and ferns appears to be controlled by correlative inhibition; it is not clear whether correlative inhibition (probably apical dominance) extends to all of a plant or is confined to a bud cluster (Nichols and Woolley 1985; Kretschmer and Hartmann 1985). This issue is examined in Chapter 4.

The cladophylls of asparagus are the main site of assimilation although photosynthesis occurs in all green tissue (Sawada et al. 1962; Downton and Torokfalvy 1975, Inagaki et al. 1989). Asparagus has a C-3 photosynthetic pathway (Downton and Torokfalvy 1975, Lin and Hung 1978) with an optimum temperature for net photosynthesis near 20C (Sawada et al. 1962, Lin 1983, Inagaki et al. 1989). There are no differences in photosynthetic activity of male and female plants (Sawada et al. 1962, Lin and Hung 1978). Differences between varieties in yield and in optimum harvest length may result from differences in their photosynthetic efficiency and/or the distribution of assimilates (Benson and Takatori 1980; Benson 1982; Dirks et al. 1982), but conclusive proof is lacking.

1.3.3 RHIZOME GROWTH

In seedling asparagus, the rhizome extends along a longitudinal axis as new buds progressively develop in the axil of the first scale leaf of the previous stem, following a sympodial branching pattern (Fig.1.02). As the plant ages, lateral buds at the base of

older stems develop and rhizome growth in other directions occurs (Fig.1.03) (Blasberg 1932, Mullendore 1935). The factor(s) which stimulate the growth of lateral buds into axillary rhizomes are not known. Eventually the older parts of the rhizome decay and the growing tips (bud clusters) become physically separated.

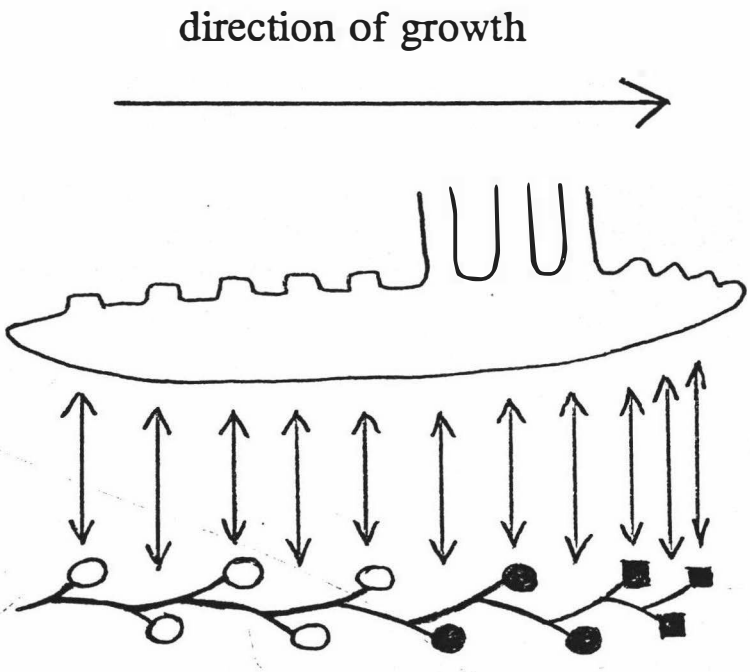
The main period of bud formation is during fern growth following harvest, with few buds being formed during the harvesting season (Tiedjens 1924, 1926). Buds first appear on the growing region of a rhizome as scales, which gradually fill out and assume an individual identity (Tiedjens 1924). From a study of 1170 plants over 4 years, Tiedjens (1924) reported 'a decided uniformity in the number of buds produced from year to year'. He concluded that bud formation was genetically controlled, and that carbohydrate reserves influenced the number of buds which developed into spears and fern, but not the number of buds formed. However, the size of buds is dependent on the amount of fern growth (Tiedjens 1926) and hence the amount of carbohydrate available.

1.3.4 DORMANCY

The traditional areas for growing asparagus are temperate climates where the young shoots are harvested as they emerge in spring. The absence of visible growth during winter may be imposed on a plant by external factors, e.g, low temperatures, or it may be due internal factors, e.g. plant growth hormones, which prevent growth in environmental conditions which would normally permit growth. The effect of temperature on the dormancy of asparagus in a temperate winter is examined in Chapter 4.

1.3.5 VEGETATIVE GROWTH

Differences in the agronomic performance of asparagus plants has been attributed to differences in the rate of growth (Fisher 1982) or in the partitioning of carbohydrates (Benson and Takatori 1980; Dufault and Grieg 1983; Wilcox-Lee and Drost 1990). The effect of temperature on growth and on carbohydrate partitioning during vegetative growth is examined in Chapter 2.



Note: Production of buds follows a sympodial branching pattern, i.e,

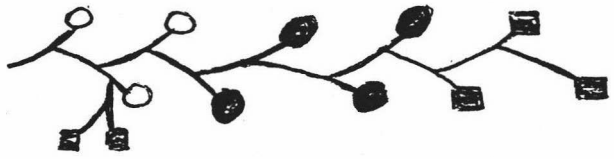
- the apical bud develops into a shoot
- longitudinal growth of the rhizome occurs through the development of successive basal (lateral) buds
- the basal internode of each stem swells to form the rhizome

Key:

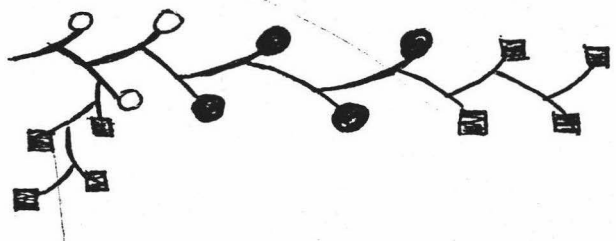
- ○ site of previous seasons fern
- ● site of current seasons fern
- ■ buds yet to develop into spears or fern

Figure 1.02: Stylised diagram to show the relationship between rhizome and sympodial branching pattern.

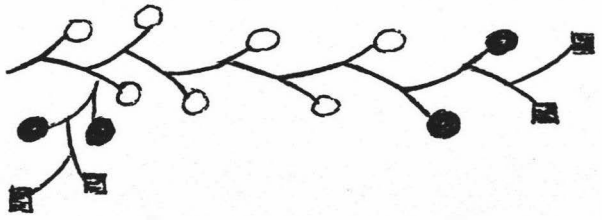
Rhizome development initially occurs along a single axis



Buds beginning to develop at the base of an old shoot



At the end of the growing season, 2 dormant bud clusters exist



In the following spring, shoots (spears or fern) develop from both bud clusters

Key
- dormant bud
- spear or fern
- remains of dead fern

Figure 1.03: Stylised diagram to show the development of axillary rhizomes as a result of sympodial branching.

1.3.6 SEASONAL CHANGES IN CARBOHYDRATES

Seasonal changes in the carbohydrate concentration in storage roots of harvested asparagus is well documented (Scott et al. 1939; Taga et al. 1980; Shelton and Lacy 1980). However changes in the size of the storage carbohydrate pool are unknown as there is no information on seasonal changes in the root mass of harvested plants. This is examined in Chapter 5.

The length of the harvesting season varies with the age of the plants and the climate in which they are grown. Harvesting of most New Zealand asparagus begins in late September. New Zealand growers have determined from experience that they must cease harvesting after 9-10 weeks to maintain crop quality from year to year (Findlay and Ryan 1974). The harvest season for young beds is shorter to allow the plants to develop large crowns.

The storage carbohydrates in the roots of *A. officinalis* are fructans (fructo-oligosaccharides) containing a maximum of 21 hexose sugars (Shelton and Lacy 1980). Fructans are the major carbohydrate reserves present in the vegetative tissues of 12% of angiosperm plants (Hendry 1987).

1.3.7 MALE-FEMALE DIFFERENCES

In a commercial situation, male plants consistently produce a higher marketable yield and have a higher longevity than female plants in the same population (Tiedjens 1924; Robbins and Jones 1925, Yaeger and Scott 1938, Ellison et al. 1960, Moon 1976). Male plants tend to produce spears earlier in spring than the female plants (Tiedjens 1924; Robbins and Jones 1925; Ellison and Schermerhorn 1958; Ellison et al. 1960). The effect of plant sex on spear growth is examined in Chapters 4 and 5.

The lower yield and higher mortality of female plants may be due to competition between berries and roots for carbohydrates. Berries can comprise 32% of the weight of female plants (Robbins and Jones 1925). In an isolated planting of only female plants, the weight of spears produced in the first 2 years was similar to that produced by a male only planting (Lodejwiks 1958). The effect of plant sex on sink-source relationships and carbohydrate metabolism is examined in Chapters 3 and 5.

CHAPTER TWO

THE EFFECTS OF TEMPERATURE AND GENOTYPE ON THE DEVELOPMENT OF YOUNG ASPARAGUS

2.1 INTRODUCTION

There are large differences in the agronomic performance of asparagus genotypes. The reasons for this are unknown. One possible reason is differences in the temperature responses of cultivars. This chapter examines the effect of temperature on growth and carbohydrate partitioning during vegetative growth of four asparagus genotypes.

The growth of plants can be compared using the 'Relative Growth Rate' (RGR) which measures the increase in dry weight per unit of dry weight within a specified time period (Hunt 1978). The RGR of young asparagus plants appeared to decrease as plant size increased regardless of whether the plants are grown in the field (Fisher 1982) or glasshouse (Benson and Takatori 1980; Fisher and Benson 1984) where environmental conditions vary, or in a controlled environment with constant temperature and daylength (Brown et al. 1980; Nichols and Woolley 1985). Decreasing RGR was accompanied by a decrease in partitioning into the shoots (Brown et al. 1980, Nichols and Woolley 1985).

High and low yielding genotypes appeared to differ in partitioning between roots and shoots even though there were no differences in the size or growth rate of the entire plant. For example, a high yielding genotype had a higher root number to shoot number ratio than lower yielding genotypes (Benson and Takatori 1980; Dufault and Grieg 1983) or a higher proportion of dry weight in the roots than in the fern (Wilcox-Lee and Drost 1990). In one study, a rapid increase in root and bud number occurred earlier in the growing season on the high yielding genotype than in the low yielding genotype although there was no difference in crown weight at the end of the growing season (Dufault and Grieg 1983). However, in two other studies, higher yielding genotypes had a higher crown weight and were thus considered more efficient at partitioning assimilate into the crown than low yielding genotypes (Benson and Takatori 1980; Wilcox-Lee and Drost 1990). A higher crown dry weight has been associated with a higher spear yield (Tiedjens 1926).

There are seasonal changes in partitioning between roots, shoots and buds in field grown seedling asparagus. In the first and second year of growth, the number of shoots increases steadily during spring and may then plateau (Dufault and Grieg 1983, Haynes 1987) or decrease (Fisher 1982). The production of new roots tends to lag behind the production of new fern (Dufault and Grieg 1983, Haynes 1987) and is followed by bud formation (Fisher 1982, Haynes 1987). Rhizome dry weight increases steadily throughout the growing season and comprises about 8% of crown dry weight by autumn (Fisher 1982, Haynes 1987). The partitioning of assimilate (i.e, dry matter) between roots and shoots is initially about equal (Fisher 1982, Dufault and Grieg 1983, Haynes 1987), and is followed by an increase in partitioning to the roots (Fisher 1982, Haynes 1987). This change in partitioning occurs earlier in the growing season on larger plants (Fisher 1982, Haynes 1987), and thus it may be a function of plant size. It is unlikely to be due to flowering as young asparagus plants normally do not flower until their second year of growth. None of the studies quoted report the presence or absence of flowers. However, the change in partitioning may be induced by environmental factors.

Any factor which limits growth induces a change in resource allocation by the plant. This results in an increase in the relative size, number or mass of that part of the plant which draws most upon the growth limiting part of the environment (Hunt and Nicholls 1986). Thus nutrient limited plants produce more roots, and shaded plants produce more shoots. The factors controlling the seasonal changes in partitioning in asparagus have not been defined and may include temperature, daylength or water availability. Partitioning between the roots and shoots of seedling asparagus can be altered by plant nutrition (Fisher and Benson 1983; Adler et al. 1984; Precheur and Maynard 1983). Root:shoot ratio (number and/or dry weight) increases as soil moisture decreases (Wilcox-Lee and Drost 1990). However, increasing container volume did not alter partitioning (Brown et al. 1982; Dufault and Waters 1984) indicating that the growth of both root and shoot had been restricted by smaller pots. However, these studies have all been carried out on seedling asparagus, and the yield of mature plants has not been measured within the same trial.

Asparagus yield is related to other agronomic features of mature plants. The yield (spear number and weight) of individual plants and of populations is consistently correlated with number and/or vigour of fern in the preceding autumn (Currence and Richardson 1937; Young 1937; Ellison and Schermerhorn 1958; Ellison and Scheer 1959; Moon 1976) and with earliness of spear production (Ellison and Schermerhorn 1958; Moon 1976). However these correlations are significant only for data collected 6 to 18 months apart i.e, they do not hold beyond one complete growing season. The yield ranking of

individual plants changes over a five or ten year harvest period (Young 1937; Hanna 1938, 1942, Falloon and Nikoloff 1986). However there does appear to be some links between early growth and yield in later years. Some researchers have proposed using cumulative yield from the first two years of harvest as a predictor of long term yield of a cultivar (Bussell et al. 1987).

The work described in this chapter used young asparagus plants to examine the effects of temperature and genotype on growth and carbohydrate partitioning during vegetative growth.

2.2 MATERIALS AND METHODS

2.2.1 TREATMENTS

Temperature Treatments

The temperature treatments used span the conditions experienced in New Zealand during the growing season. The plants were grown in controlled environment rooms at the DSIR Climate Laboratory in Palmerston North at either constant or alternating (12 hour day/night) temperatures, as shown in Table 2.01. A constant vapour pressure deficit of 0.4 kPa was maintained at each temperature.

Genotypes:

The genotypes used were bred in different environments and were chosen because they were expected to show different responses to temperature and thus provide a key to the factors determining plant growth and partitioning.

The four genotypes used were:

1. 'Rutger's Beacon' - a dioecious polycross hybrid from Rutger's University, New Jersey, USA 43°N.
2. 'Jersey Giant' - all male single cross hybrid from Rutger's University which has produced high yields in several New Zealand trials.
3. 'Limbras 126', also known as 'Franklim' - an all male single cross hybrid from Royal Sluis, Holland 52°N. This hybrid is related to 'Limbras 26' which has produced well in New Zealand trials.

4. 'INRA 61' - an all male single cross hybrid from INRA, Versailles, France 45°N. French cultivars usually produce spears earlier in spring than Dutch or USA genotypes.

2.2.2 EXPERIMENTAL DESIGN

This study was conducted as four separate experiments. Each experiment consisted of two temperature treatments (Table 2.01). The results of each experiment have been pooled. The same pair of controlled environment rooms were used throughout the study.

Each experiment was set up as a randomised complete block (RCB) of four replicates with one plant per plot. Plants were visually graded into blocks with the largest plants being placed into replicate 1 and the smallest into replicate 4 before transfer to the treatment temperatures. Each replicate occupied two trolleys within the growth room. Trolleys were rotated weekly to minimise any positional effects of light and water or nutrient supply.

Table 2.01: Conditions in DSIR Climate Laboratory controlled environment rooms for plants at each temperature regime:

Temperature day/night C	Experiment number
10/10	1
15/15	2
20/20	4
25/25	1
30/30	4
20/10	2
25/15	3
30/20	3

2.2.3 PROPAGATION AND GROWING CONDITIONS

The plants were propagated by pre-germinating seed at 25C for 5 days before planting in 5 cell root trainers and growing the seedlings in a controlled environment room at 20C for five weeks. The seedlings were then transplanted into 3 litre pots (Experiment 1 only, 25C and 10C constant temperature regimes) or into 4 litre black plastic bags (PB6's) and transferred to the treatment temperature.

The plants were grown in sand and watered regularly with a nutrient solution. The plants in Experiment 1 were initially fed a modified half strength Hoaglunds A nutrient solution. This was changed to North Carolina State University (NCSU) nutrient solution after 5 weeks at the treatment temperature as the plants were showing chlorotic symptoms typical of iron deficiency. A decrease in chlorosis was visible within a week.

All treatments had a 12 hour photoperiod temperature, adjusted over 2 hours when there was a day/night temperature difference. The plants were lit by 4 x 1000W Sylvania 'Metalarc' high pressure discharge lamps and 4 x 1000W tungsten halogen lamps to a standard irradiance (mean value of 152 W/m^2) and photosynthetic photon flux density (mean value of 710 $\mu mol/m^2/sec$).

2.2.4 PLANT MEASUREMENTS

One plant from each replicate of each genotype x temperature treatment was destructively harvested at approximately 7 day intervals, commencing on the day plants were transferred to the treatment temperature. Each plant was dissected into roots, shoots and underground stem and the number of roots, shoots and buds, and fresh and dry weight of roots, shoots and rhizomes was recorded. Plant samples were dried in a vacuum oven (40C and 2 mm Hg) for at least 24 hours and allowed to equilibrate at 20C and 55% RH before dry weights were recorded.

2.2.5 ANALYSIS OF DATA

Treatment conditions were established at the end of the five week propagation period. The growth of the asparagus seedlings was measured for up to 63 days following transfer to the treatment temperature. Plants at 25C were measured for 35 days and

plants at 30C were measured for 45 days as plant growth beyond these times was restricted by pot size (25C) or by a suspected mite infestation (30C).

Data from plants harvested at the end of the propagation period was analysed by analysis of variance (ANOVA) for RCB (SAS Version 5, PROC GLM) to determine the effects of experiment, genotype and block. Differences between blocks were significant at the P=0.0001 level for total dry weight and at P=0.078 for root to shoot ratio for dry weight. Thus blocking the plants on the basis of plant size removed some of the variation between treatments.

Growth analysis

Initial analysis of the data by the Hunt and Parsons program (Hunt and Parsons 1974) showed that RGR fitted a linear model. RGR (g/g/day) was subsequently calculated as the slope of the line obtained when $\log_e$ of total dry weight (g) was regressed against time (days) using SAS Version 5, PROC GLM.

The first measurements used for analysis of growth were collected 7-8 days after the plants were transferred to the treatment conditions. Measurements taken on the day that the plants were transplanted and transferred to the treatment conditions were not used as growth in the following week was likely to be reduced by transplanting shock.

Root to shoot ratios - allometric relationships

The allometric relationship between root and shoot dry weight and number was examined to overcome problems caused by confounding the root:shoot ratios with changes in plant size as the plants grew, and with differences in plant size between the temperature treatments used.

The allometric ratio for dry weight, K_{dw}, is the ratio of RGR's for the roots and shoots. It was calculated by regressing $\log_e$ of root dry weight against $\log_e$ of shoot dry weight i.e,

$$\log_e (\text{root dry weight}) = \log_e a + K_{dw} \times \log_e (\text{shoot dry weight}).$$

The allometric ratio for root and shoot number, K_{no}, was calculated by regressing $\log_e$ of root number against $\log_e$ of shoot number i.e,

$$\log_e (\text{root number}) = \log_e a + K_{no} \times \log_e (\text{shoot number})$$

The allometric constant, K (K_{dw} or K_{no}) is normally constant for a species and environment during the vegetative phase of growth (Hunt 1978), and normally close to 1. If K does not equal 1, then the relative proportions of root and shoot change with time i.e, the root:shoot ratio changes with time.

Data from this experiment has been reported in Hughes et al. 1990.

2.3 RESULTS

2.3.1 EFFECT OF TEMPERATURE AND GENOTYPE ON RELATIVE GROWTH RATE

Plants at 10C constant died 3-6 weeks after being transferred from the propagation temperature of 20C. The foliage became chlorotic and then senesced. Chlorosis of the foliage was probably due to chlorophyll bleaching which occurs when irradiance is high relative to temperature and the temperature is too low for light induced breakdown of chlorophyll to be repaired. Data from plants in the 10C treatment have not been used in the following analyses.

The increase in plant weight was exponential in each temperature treatment above 10C as the RGR of the seedlings was constant for the period in which growth was analysed (Fig.2.01 A & B). Differences between genotypes were not significant at P=0.05 when analysed by t-test. The optimum temperature for RGR of the whole plant was between 25 and 30C when plants were grown in a constant temperature (Fig.2.01 C). The RGR at 25C was probably underestimated in this experiment as the plants at this temperature appeared to have inadequate iron, and were grown in small containers (Section 2.2.3).

An optimum under alternating temperatures cannot be determined from the treatments used in this experiment (Fig.2.01 C). The RGR increased as the mean temperature increased in the alternating temperature regimes.

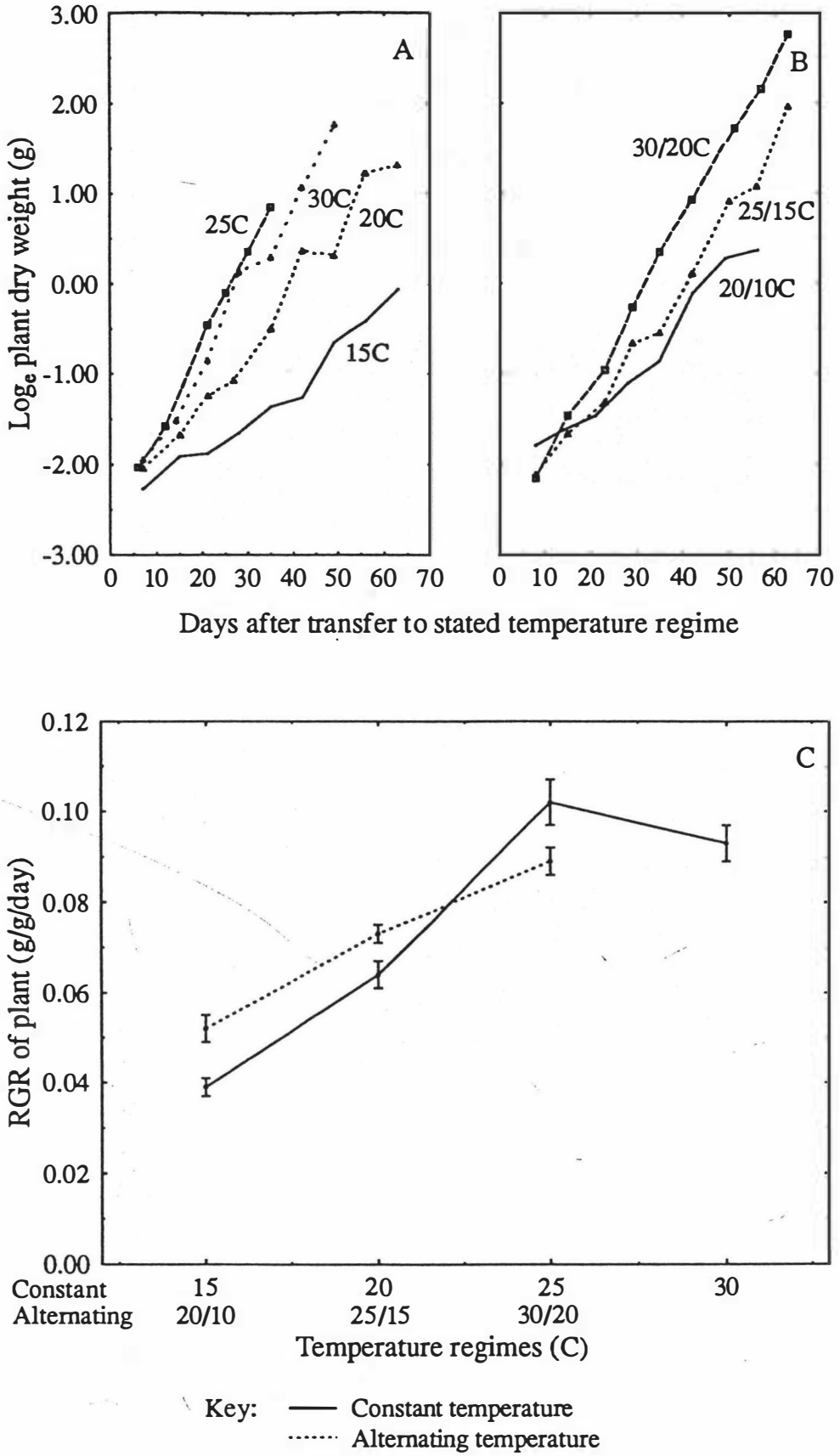


Figure 2.01: Effect of temperature regimes on Relative Growth Rate (RGR) of whole plant. Plant growth is exponential (A, B). Temperatures were constant (A), or alternating (12 hour day/night)(B). Maximum RGR occurred near 25C (C). Bars represent twice standard error of the mean.

2.3.2 EFFECT OF TEMPERATURE AND GENOTYPE ON PARTITIONING

The allometric ratios for dry weight (K_{dw}) changed in the first 1-2 weeks after transfer from 20C to the treatment conditions (Fig.2.02). The K_{dw} at each temperature regime then appeared to be near constant over the remainder of the experiment. Thus, data collected in the first two weeks after transfer to the treatment conditions were omitted from calculation of the allometric ratios (K_{dw} and K_{no}).

Plants with a higher K_{dw} value partitioned more assimilate into their roots than plants with a lower K_{dw}. The pattern for mean K_{dw} was similar to that for RGR of the entire plant i.e, 25C was close to the optimum temperature for partitioning assimilate to the roots (Fig.2.03).

The mean allometric ratios (K_{dw}) for dry weight were below 1 (Fig.2.03) in each of the temperature regimes which indicates that the asparagus seedlings in this experiment tended to partition an increasing proportion of assimilate into shoots as the plants increased in size. Thus the root:shoot ratio for dry weight decreased as the plants increased in size.

The K_{dw} differed between genotypes (P=0.05, t test) in each of the temperature regimes (Fig.2.04). However, there were interactions between genotype and temperature such that consistent trends were difficult to discern. The K_{dw} of each genotype tended to follow the same pattern as the mean value when the plants were grown in constant temperature regimes, but not in alternating temperature regimes. The maximum value for K_{dw} occurred at 25C in 'Rutger's Beacon', 'Jersey Giant' and 'Limbras 126'. However, in 'INRA 61', the maximum occurred at 30C. 'Rutger's Beacon' and 'Jersey Giant' at 25C were the only genotype/temperature combination in which K_{dw} was 1 or higher, i.e, root growth equalled or exceeded shoot growth, and thus root:shoot ratio increased as plant dry weight increased.

The ranking of the genotypes within a temperature regime was the same at 15C and 20/10C where 'Jersey Giant' has a higher K_{dw} than 'Limbras 126' (Fig.2.04). The ranking at 20C is also the same as at 25/15C, but in contrast to 15C and 20/10, the K_{dw} of 'Jersey Giant' was lower than 'Limbras 126' and 'Rutger's Beacon'. However, the ranking of genotypes is not consistently the same at both the constant temperature and the corresponding alternating temperature regime with the same mean temperature: the ranking at 25C is almost the reverse of that at 30/20C.

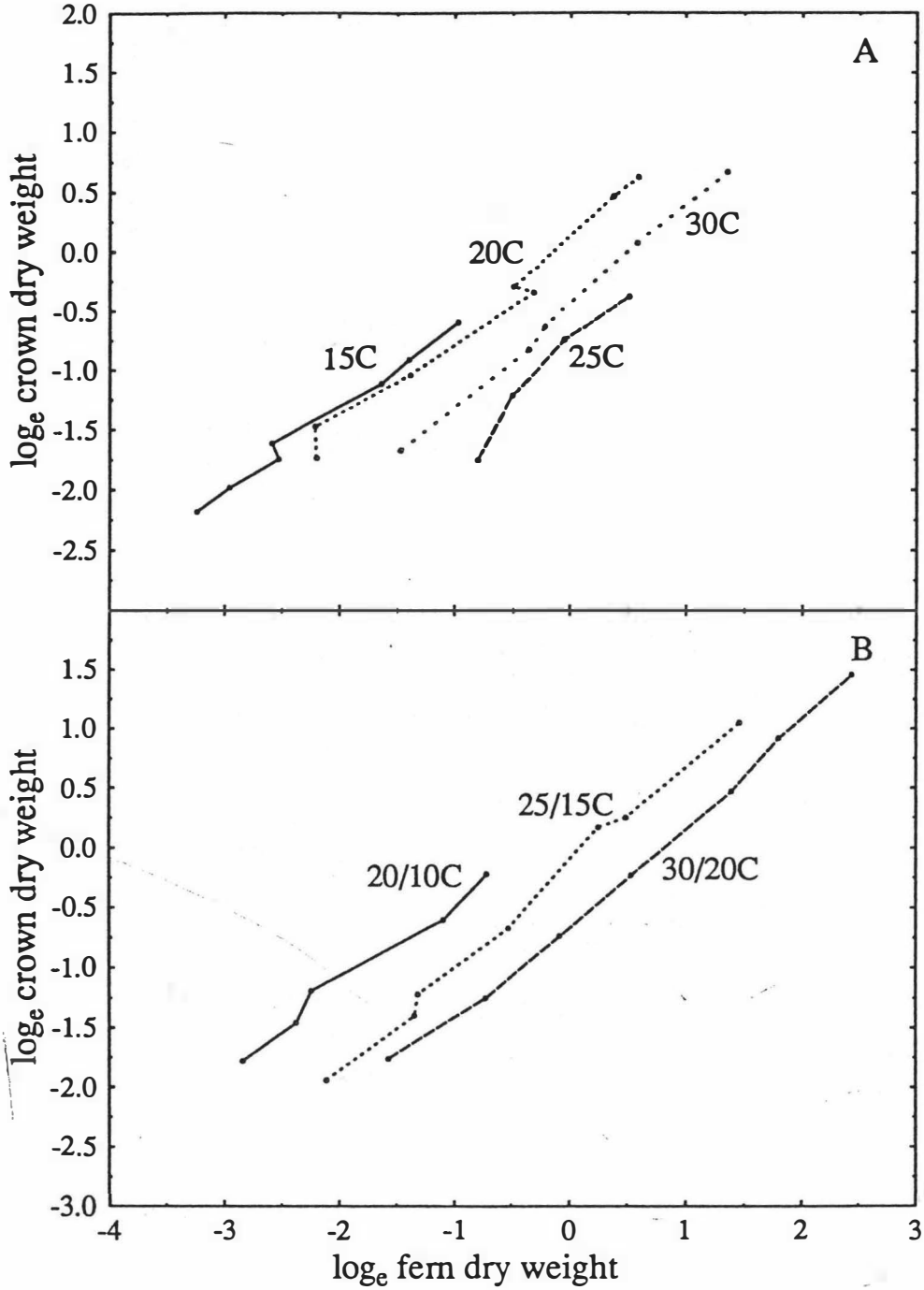
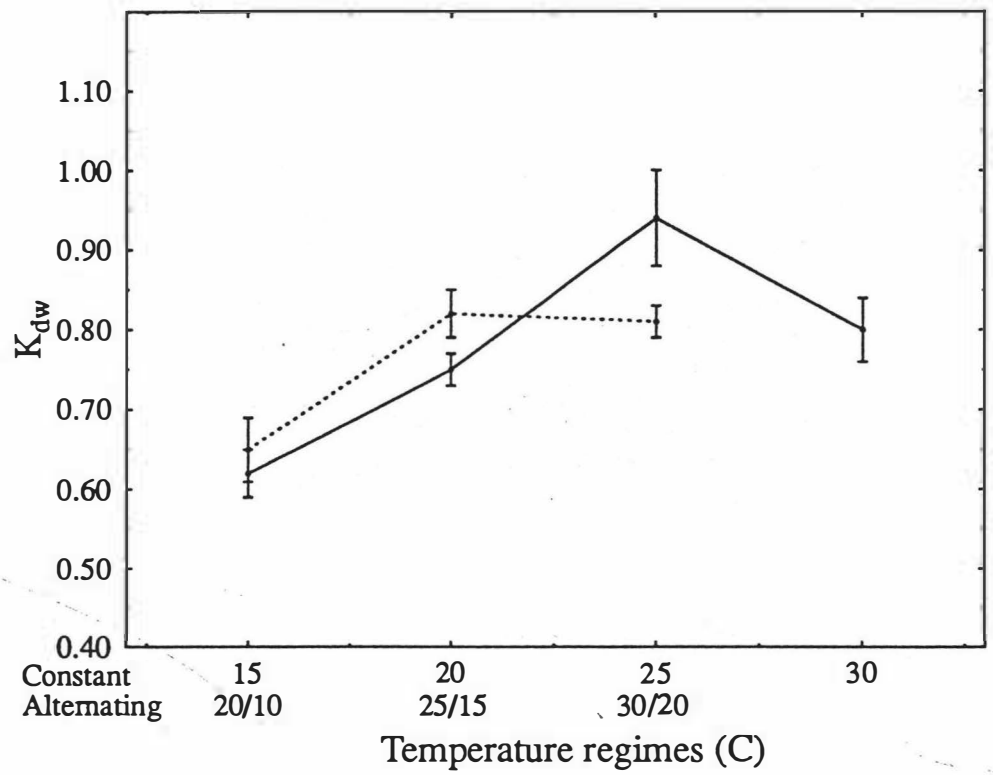


Figure 2.02: Allometric relationship between crown and fern dry weight. Temperatures were constant (A), or alternating (12 hour day/night) (B). Allometric constant, K_{dw}, is slope of line. Data from first 2 weeks after transfer to treatment conditions omitted.

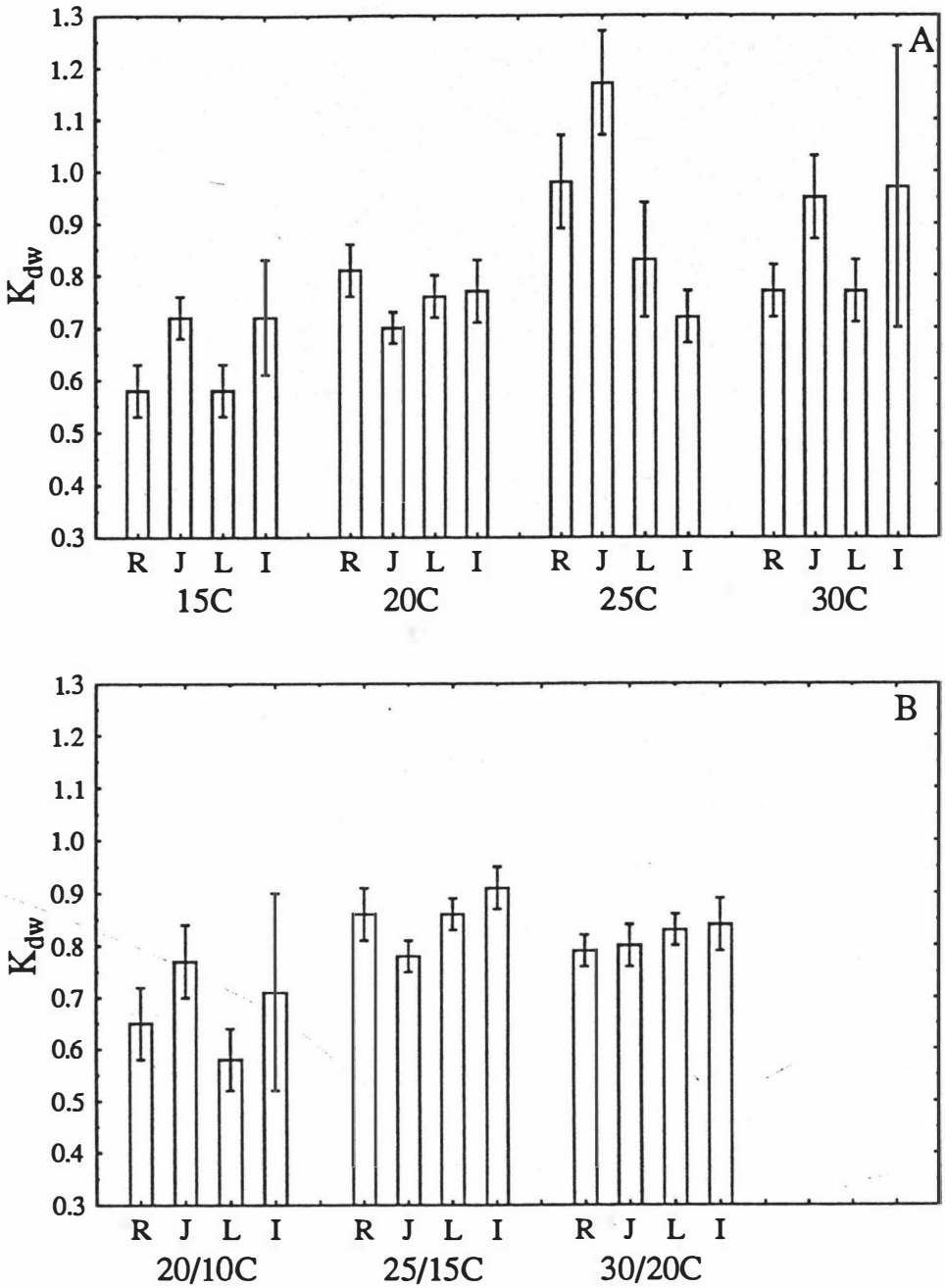


Key: —— Constant temperature
····· Alternating temperature

Figure 2.03: Effect of temperature regimes on K_{dw}, allometric constant for partitioning dry weight. K_{dw} was calculated from the following equation:

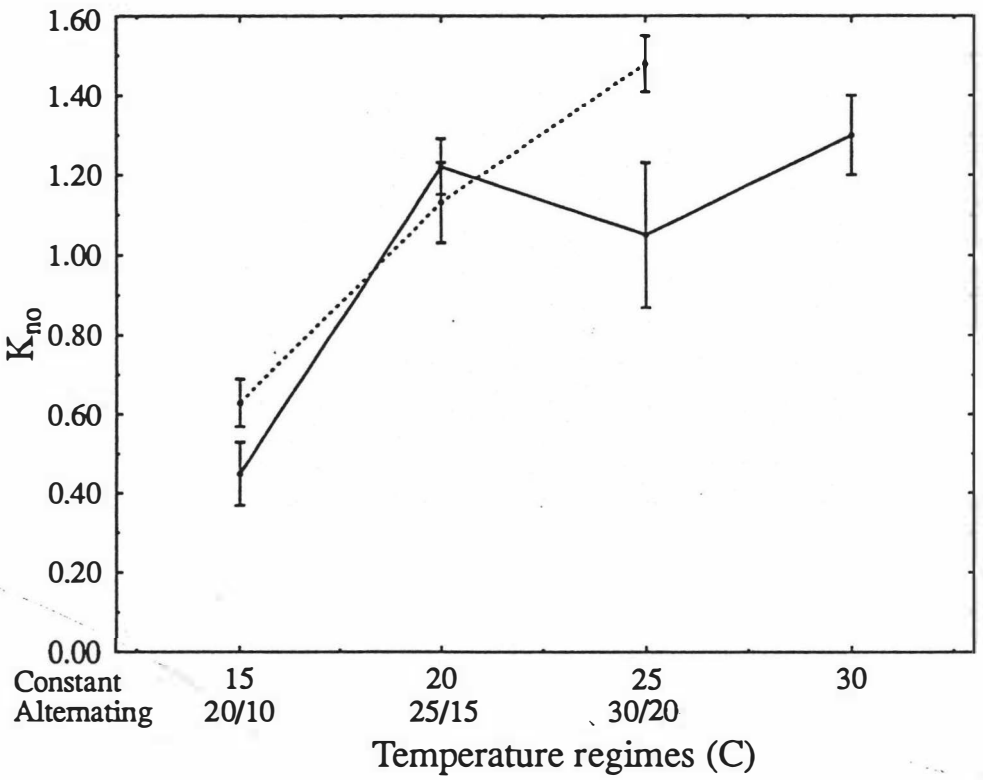
$\log_e$ crown dry weight = $\log_e$ a + (K_{dw} x $\log_e$ fern dry weight)

Temperatures were constant, or alternating (12 hour day/night). Bars represent twice standard error of mean.



Key: R = Rutger's Beacon
J = Jersey Giant
L = Limbras 126
I = INRA 61

Figure 2.04: K_{dw} of four asparagus genotypes in constant (A) and alternating (B) temperature regimes. Bars represent twice standard error of mean.



Key: —— Constant temperature
····· Alternating temperature

Figure 2.05: Effect of temperature regimes on K_{no}, allometric constant for root/shoot number. K_{no} was calculated from the following equation:

$$\log_e(\text{root number}) = \log_e a + (K_{dw} \times \log_e(\text{fern number}))$$

Temperatures were constant, or alternating (12 hour day/night). Bars represent twice standard error of mean.

None of the genotypes consistently had a lower or higher K_{dw} at either lower or higher temperatures (Fig.2.04). The K_{dw} for 'Jersey Giant' was higher than that of 'Rutger's Beacon' at 15C, 25C, 30C and 20/10C, but lower at 20C and 25/15C. When plants were growing in constant temperatures, the K_{dw} of 'Rutger's Beacon' and 'Jersey Giant' was more sensitive to changes in temperature than 'Limbras 126' and 'INRA 61'. However, the same pattern did not occur in alternating temperatures, where there was no difference in the K_{dw} for 'Jersey Giant'.

'INRA 61' tended to have a higher K_{dw} than the other genotypes (Fig.2.04). The K_{dw} for 'INRA 61' was higher than at least one other genotype at 15C, 20C, 30C and each of the alternating temperature regimes. However, it had the lowest K_{dw} at 25C. 'Limbras 126' tended to have a lower K_{dw} than the other genotypes. It's K_{dw} is lower than at least two other genotypes at 15C, 25C, 30C, and 20/10C.

The allometric ratios for root:shoot number, K_{no}, were above 1 when the mean temperature was 20C or higher (Fig.2.05) indicating that the plants were producing roots faster than they were producing shoots. Thus the ratio of root number to shoot number increased as the plants increased in size. However, at the lower temperatures of 15C constant and 20/10C day/night, the plants produced more shoots than roots.

2.4 DISCUSSION

2.4.1 EFFECT OF TEMPERATURE ON RELATIVE GROWTH RATE

The constant RGR as plants increased in size differed from previous studies which reported a decrease in RGR as plants increased in size (Fisher 1982; Benson and Takatori 1980; Fisher and Benson 1984; Brown et. al. 1980; Nichols and Woolley 1985). This result indicated that the phase of exponential growth in seedling asparagus can continue for longer than previously thought as the plants in this experiment were similar in size (up to 10g dry weight) and age (2 to 12 weeks old) to most of those studied previously. However, in Fisher's study (1982), the growth of field grown plants was initially exponential; the decrease in RGR occurred in mid to late summer when the plants were larger than 10g. Thus the decrease in RGR in Fisher (1982) and other studies could be due to restrictions in growth imposed by environmental conditions rather than due to ontogenetic changes in asparagus growth. The effects of the environment on growth appear to be larger than genotypic effects.

Genotype had no effect on the RGR of the young plants at any of the temperatures studied. This was consistent with previous studies which found no differences between high and low yielding genotypes in growth rate or plant size (Benson and Takatori 1980; Dufault and Grieg 1983; Wilcox-Lee and Drost 1990). The results of this experiment suggested that this consistency in growth applied to a wide temperature range, i.e, 15C to 30C. However there was considerable variation in plant size within each genotype which may have obscured genetic differences.

The variation in plant size occurred even though the cultivars used were not open-pollinated. They were either all male single cross hybrids ('Jersey Giant', 'Limbras 126' and 'INRA 61') or a dioecious polycross hybrid ('Rutger's Beacon'). Differences in plant size were visible during propagation at 20C, and may have been due, at least partially, to variation in the date of germination. Blocking plants on the basis of plant size when the plants were transferred to the treatment conditions was not effective as the size ranking was not maintained during the experiment. Thus some of the variation was due to either variability in the growing conditions (e.g. nutrient solution) or to differences in growth responses. Thus four replicates per genotype was too few to effectively determine genotypic differences.

Differences in the RGR between plants grown at a constant temperature and an alternating temperature with the same mean value may have been due to differences in the balance between photosynthesis and respiration. Plants at constant temperatures of 15C and 20C had higher night temperatures than plants at the corresponding mean temperatures of 20/10C and 25/15C and thus would probably have had higher respiratory losses at night. The lower RGR at 30/20C than at 25C may have been due to a lower net photosynthetic rate at the higher day temperature as the optimum temperature for net photosynthesis is near 20C (Sawada et al. 1962; Lin 1983; Inagaki et al. 1989).

2.4.2 EFFECT OF TEMPERATURE AND GENOTYPE ON PARTITIONING

In asparagus, root to shoot ratios represent partitioning between tissue which stores assimilates and tissue which produces assimilate for storage. In a temperate climate, the development of storage tissue is more important than the growth of the entire plant as plant growth (spears or fern) following winter is dependent on assimilate stored in the roots.

Partitioning between roots and shoots of asparagus changed when the temperature at which the plants were growing changed (Fig.2.02), indicating that seasonal changes in partitioning (e.g. Fisher 1982; Haynes 1987) are probably influenced by temperature changes. However, after the plants had adjusted to the change in environment, the K_{dw} of vegetative plants appeared to be constant.

The effect of temperature on K_{dw} was similar to the effect on RGR of the plants, with optimum K_{dw} at 25C for plants growing in a constant temperature regime (Figs.2.03, 2.01). Thus the highest proportion of assimilate was partitioned into the crown at the optimum temperature for growth. These results suggest that either the net photosynthetic rate of plants growing at 25C was higher than that of plants at 20C, and/or that respiratory losses in the root tissue are lower than in the shoot tissue. Both would have increased the assimilate accumulated within the plant.

A higher net photosynthetic rate at 25C than at 20C does not necessarily contradict reports that the optimum temperature for net photosynthesis in asparagus was near to 20C (Sawada et al. 1962; Lin 1983; Inagaki et al. 1989) as plants may not have acclimated to the growing conditions before photosynthesis was measured. Data from this experiment indicate that at least two weeks was required for K_{dw} to stabilise after transfer to the treatment conditions. Nichols and Woolley (1985) suggest that photosynthetic efficiency of asparagus can be increased by the sink effect of a greater root mass. Papers published on photosynthetic rates in asparagus do not provide, in English, any information on the time which elapsed between transfer to various temperatures and measurement of photosynthesis.

The results here indicated that the K_{dw} for asparagus with a constant RGR appeared to be slightly below 1 in each of the temperature regimes, except for 'Rutger's Beacon' and 'Jersey Giant' grown at 25C. Thus, the root:shoot ratio for dry weight tended to decrease as the dry weight of the plants increased. As discussed above, a constant RGR may indicate that conditions were not restricting plant growth whereas a decreasing RGR may indicate that conditions were restricting plant growth. However, these results have been obtained from young plants, prior to flowering, and should not be extrapolated to plants on which spears have been harvested.

In field grown seedling asparagus, root dry weight and shoot dry weight were similar until the RGR began to decrease; root dry weight then increased more rapidly than shoot dry weight (Fisher 1982). A similar increase in root dry weight accompanied a decreasing RGR in controlled environment (at 22.5C)(Nichols and Woolley 1985), and

in glasshouse (Benson and Takatori 1980) experiments. This change in partitioning supports the view that a decrease in RGR is due to restrictions imposed by environmental factors (Hunt and Nicholls 1986). The increase in root growth associated with the decrease in RGR in other studies with young (apparently non-flowering) asparagus plants implies that growth is being limited by a factor related to root function. This could be moisture stress as drought frequently occurs in late summer.

K_{dw} differed between genotypes, but there were few consistent patterns. 'Jersey Giant' tended to have a higher K_{dw} than 'Rutger's Beacon', an earlier release from the same breeding programme. This may indicate that increased yield is associated with partitioning more dry weight to the crown than in a lower yielding genotype. On that basis, 'INRA 61' should be a high yielding genotype in a range of environments as it tended not only to have a higher K_{dw} than the other genotypes, but also appeared to be less sensitive to temperature than 'Rutger's Beacon' and 'Jersey Giant'.

The relative number of roots and shoots appeared to be more sensitive to environmental conditions than partitioning dry weight as the range in K_{no} values was greater than the range in K_{dw}. K_{no} tends to be greater than 1 at temperatures above 15C indicating that the ratio of root:shoot numbers increased as plant size increased. This increase in the number of roots may be a response to the increased water requirement of plants at the warmer temperatures.

2.5 CONCLUSIONS

The optimum temperature for growth of young asparagus plants appears to be between 25C and 30C. In this experiment, maximum RGR occurred at 25C constant. Growth at alternating temperature regimes of 20/10C and 25/15C was faster than in the corresponding mean temperatures, while growth at 30/20C was slower. This is probably due to differences in the balance between photosynthesis and respiration.

The partitioning of dry matter between roots and shoots follows a similar pattern to RGR, with maximum partitioning into roots occurring at maximum RGR. This increased partitioning to the roots may have increased photosynthetic efficiency, and thus induced the increase in RGR.

This experiment appears to confirm earlier work which indicated that agronomic differences between cultivars (e.g. yield, earliness) are not related to the overall growth

of the plant, but may be related to increased partitioning of dry weight into the crown (Benson and Takatori 1980; Dufault and Grieg 1983, Wilcox-Lee and Drost 1990). Differences in partitioning were calculated using the allometric ratio which appears to be constant, after the plants have acclimated to the environmental conditions. K_{dw} differed between genotypes but there were few consistent patterns. Increased yield may be associated with increased partitioning into the crown as the K_{dw} of 'Jersey Giant' tended to be higher than that of 'Rutger's Beacon'. However, the experimental design lacked the robustness required to effectively determine genotypic differences in RGR.

A higher number of replicates should be used in any future assessment of genotypic differences utilising growth analysis techniques. Propagating and growing plants in the treatment conditions would avoid problems associated with acclimation to a changed environment. Propagating a large number of plants and then selecting plants of the same size is not recommended as it would bias the sample used to evaluate genotypic differences.

CHAPTER THREE

SOURCE-SINK RELATIONSHIPS

3.1 INTRODUCTION

3.1.1 DEFINITIONS AND GENERAL PRINCIPLES

The concept of source-sink relationships is widely used to explain assimilate partitioning at whole plant, cellular and biochemical levels of organisation. 'Source' and 'sink' were defined by Warren Wilson (1972) in terms of losses and gains of a substance, and thus can apply to leaf assimilated carbon compounds and root assimilated mineral nutrients. In terms of carbon partitioning, a source exports photoassimilates and a sink imports photoassimilates. The processes of photoassimilate production, transport and storage are known or have well supported hypotheses. However, the regulation of photoassimilate partitioning, particularly the integration of source processes and sink requirements is subject to several hypotheses. Daie (1985) stated that 'although some description of how assimilates are partitioned in plants is emerging, no single aspect of it is fully understood'. This chapter will describe partitioning in asparagus in terms of commonly accepted hypotheses for the regulation of source-sink partitioning at the whole plant level.

Warren Wilson's (1972) descriptive definitions of source and sink were quantified by Wareing and Patrick in 1974. Warren Wilson's definition, source strength equals 'source size x source activity' was considered equivalent to

'rate of assimilation = leaf area x net assimilation rate
per plant per plant per unit area' .

Similarly, Warren Wilson defined sink strength as 'sink size x sink activity'. Quantitative expression of sink strength as

'absolute growth rate = dry weight x relative growth rate'

provides a measure of net sink strength, but does not account for respiratory losses which can be 40-50% of the photoassimilates imported by a sink (Wareing and Patrick 1974). Wareing and Patrick argue that if sink activity is a measure of the rate of assimilate uptake per unit weight of sink, then respiratory losses should be included in the measure of sink activity. Sink activity can also be limited by the supply of photoassimilates and by competition from neighbouring sinks. Wareing and Patrick (1974) propose that the term 'sink strength' be used to refer to the potential capacity to

import and accumulate photoassimilates, and the terms 'mobilising ability' and 'competitive ability' be used to refer to the actual accumulation of dry matter (assimilate) by a sink, and the strength of the sink for the accumulated assimilate respectively.

In the following discussion, I will use the terms 'potential sink strength' to refer to the total assimilate which can be imported by a sink, and 'mobilising ability' to refer to the net assimilate imported when it is necessary to distinguish between the two values; otherwise I will use the term 'sink strength'. Thus

potential sink strength = *sink size* x *gross sink activity*
(weight/time) *(weight)* *(weight/weight/time)*
equals RGR + respiratory losses, assuming
1. assimilate supply not limiting, and
2. no competition from other sinks

and *mobilising ability* = *sink size* x *competitive ability*
(weight/time) *(weight)* *(weight/weight/time)*
equals RGR

3.1.2 FACTORS DETERMINING PARTITIONING

Assimilate is transported between source and sink within the phloem. Assimilate movement along the phloem can occur in either direction as sieve elements form a continuous network throughout a plant. Preferred routes exist in some species, with specific sinks being supplied by specific leaves due to the orthostichy of vascular connections. These preferred routes appear to offer the least resistance to assimilate movement as the translocation pattern is rapidly altered by selective leaf or sink removal, often without any change in flow rate (Gifford et al. 1984).

The most widely accepted model for translocation is a modified 'Munch pressure flow' hypothesis (Gifford et al. 1984; Daie 1988; Patrick 1988) in which assimilates are translocated *en masse* along a turgor pressure gradient which is maintained by the regulation of phloem loading and unloading. Thus flow rates of sucrose from source to sink regions would depend on the sucrose gradient between the regions. Theoretically this gradient can be maintained by either

1. increasing the concentration at the loading site (resulting in feedback inhibition of sucrose production in source leaves if no increase occurs in translocation) or

2. decreasing the sucrose concentration at the sink.

The latter hypothesis is preferred, and supported by continued growth of vegetative and reproductive sinks under drought (Daie 1988). Thus, once loaded, the destination of assimilates is determined by the activity of competing sinks.

The sink strength of various tissues constantly changes during the ontogenetic development of a plant. Young leaves on dicotyledonous plants act as a sink until they are 30 to 50% expanded and net export of photoassimilates commences (Wareing and Patrick 1974). 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 photoassimilates are exported to the plant roots. This sequence can be modified by the development of reproductive or storage organs which usually have a high sink strength.

Biomass gain by a sink also depends on the potential size of the sink. The maximum potential size is mostly determined by cell number which is set at the meristematic phase of development; the actual size is determined by sink activity during expansion and filling. Patrick (1988) believes that the size of the transport pool does not limit delivery to non-storage meristematic sinks as only a small quantity of assimilate is imported. However, CO_2 enrichment will increase the yield of many crops. Thus transport resistance to meristematic sinks must be relatively high compared to larger sinks, probably due to lack of phloem differentiation near the apical dome (Patrick 1988).

The initiation of vegetative storage organs such as bulbs, tubers, and tap roots is probably limited by assimilate supply. Subsequent development may be source or sink limited, depending on the relative activity of secondary meristems in the storage organ. Storage organs such as potato tubers contain no active meristems and their growth is sink limited; sugar beet grows from multiple concentric cambia and appears source limited as growth is strongly dependent on irradiance although the filling of storage cells is not limited by assimilate supply (Patrick 1988).

Partitioning of assimilates within a plant is the end result of many processes and is the integrated result of these processes, spatially and temporally (Patrick 1988). In theory, biomass gain by a sink can be limited by the sink itself and/or by the source.

The sink model for integration of assimilate transport and utilisation has wide empirical support. It proposes that the rate of assimilate 'use' controls assimilate concentration in

sink cytoplasm which then determines the concentration gradient between source and sink and thus the rate of assimilate transport to the sink (Patrick 1988). Sink strength is determined by the ability of the sink to maintain a low symplastic and/or apoplastic sucrose concentration by either metabolism or compartmentation (Daie 1985).

In the source/sink model, assimilate use and transport is integrated by factors other than carbohydrate concentration (e.g. plant growth substances), and thus assimilate gain by the sink is regulated by both source and sink. In contrast to the sink model, the sucrose concentration in the sink cytoplasm may remain constant when assimilate use by the sink varies. There is much evidence that high sink demand increases photosynthetic rates. The contrary evidence is mostly from short term experiments which may reflect lack of adjustment within the plant (Daie 1985). Clarification of the signalling mechanisms operating between source and sink is essential to understanding assimilate partitioning. At present, the nature of the signal(s) between source and sink is subject to much speculation.

The main hypotheses regarding the signalling mechanism involve either a physical signal (e.g. turgor pressure which requires a turgor sensing mechanism) or a chemical signal (e.g. plant growth substances). In his review, Patrick (1988) states no clear preference for a turgor change or a phyto-hormonal signal. Physical and chemical signals may be integrated i.e, turgor levels controlled by sink produced plant growth substances, especially auxin. Patrick (1988) maintained that this form of regulation could extend over short distances only, and thus could operate between source leaves and shoot apices, but would not extend to root apices. More is known about the enzymes regulating assimilate use than about factors regulating translocation. Patrick (1988) has examined enzyme kinetics and established criteria to distinguish between partitioning models.

Patrick (1988) examined both the sink model and the source/sink model using Michealis Menton kinetics with invertase as the enzyme rate-limiting sucrose use. He concluded that partitioning to meristematic sinks is most readily regulated by components of the source/sink system while partitioning to expansion or storage sinks could be confined to sink processes. Thus, source/sink control of partitioning would occur in tissues which produce plant growth substances and which are supplied with assimilate by apoplastic unloading e.g, meristems, developing seeds, and fleshy fruit. Supply of assimilate to storage tissues, such as the fleshy roots of asparagus, would be controlled by the sink and it's ability to maintain a low symplastic concentration of the translocated assimilate.

3.1.3 OBJECTIVES OF EXPERIMENT

The experiment examined the extent of carbohydrate movement between rhizomes, and seasonal changes in source-sink activity within asparagus plants. This included the effects of plant sex which was investigated by examining the effects of berry production by female plants.

The first objective was to determine the extent of carbohydrate translocation between the rhizomes of a plant and thus define the basic physiological unit for carbohydrate production, distribution and utilisation. Without this knowledge, any modelling of the carbohydrate budget for asparagus is of limited value. After this basic unit for carbohydrate production, distribution and utilisation was defined, source-sink relationships of asparagus could be examined.

Source-sink relationships within plants are often examined using growth analysis techniques i.e, examining changes in the dry matter partitioned between various organs by destructively harvesting plants over a period of time. The technique does not allow separate physiological units to be identified. In this experiment, radiolabelled $^{14}CO_2$ was used to indicate assimilate movement within a physiological unit.

The second objective of this experiment was to identify the carbohydrate sinks within each physiological unit, and determine the seasonal changes in their mobilising ability and competitive ability. During the yearly cycle of spear growth, fern growth and dormancy, the roots of asparagus change from being a carbohydrate source to a carbohydrate sink. Production of the asparagus crop and establishment of the fern are dependent on assimilates from the crown when it acts as a source during spring and early summer. Thus the mobilising ability of the crown during summer and autumn when it acts as a carbohydrate sink is important in determining future yield and longevity of the crop.

Mobilising ability was determined using radiotracer and growth analysis techniques. Plants were supplied with a pulse of $^{14}CO_2$ and the fate of the ^{14}C labelled assimilate determined when the plants were sampled several weeks later. Thus the mobilising ability (net sink strength) of a plant part in relation to a particular source was estimated from the total ^{14}C present in the plant part. This technique has been used to determine partitioning and sink strength in several plants (e.g. Pate et al. 1983; Heilmeier and Whale 1986; Danckwerts and Gordon 1987, 1989). Competitive ability was estimated from the concentration of ^{14}C and calculated from mobilising ability and sink dry weight

(sink size). When calculated in this way, mobilising ability and competitive ability are 'snapshot' values which apply only to the time between labelling and sampling the plants.

3.2 MATERIALS AND METHODS

3.2.1 PLANT MATERIAL

All plants used in the source-sink studies were 'Rutger's Beacon', the most widely grown cultivar of edible asparagus in New Zealand. Seed was obtained from Dr Howard Ellison of Rutger's University, New Jersey, USA.

The plants were propagated in December 1985 in 5 cell root trainers in an unheated glasshouse using a peat-sand (1:1 v.v) potting mix. After 4 months they were transferred to 4.8 litre (PB8) black plastic bags and placed in a heated glasshouse for 8 months. The minimum temperature in the glasshouse was 13C with venting at 22C. The plants did not senesce, but continued to grow throughout the winter.

By November 1986, approximately 50% of the plants had flowers. Seventy-five male and 25 female plants were potted into 20 litre buckets. The potting mix consisted of peat-pumice (5:3 v.v) with Dolomite (3.00 mg/l), Micromax (0.94 mg/l), Osmocote 18 (long term)(3.00 mg/l) and Osmocote 14 (short term)(0.63 mg/l). After potting, all plants were placed outdoors on black polythene and staked. The plants were watered by trickle irrigation with 2 whiskers per plant.

By January 1987 the plants were similar in size to field grown plants at the beginning of their third year of growth. Ferns present in late November were removed as the plants were potted up; thus the ferns to be labelled with ^{14}C were similar in age to those on plants whose spears had been harvested until late November. However, unlike 2 year old field grown plants, the females had not produced any berries. Before commencing the experiment, the male and female plants were each separated into five blocks based on fern vigour.

3.2.2 APPLICATION OF $^{14}CO_2$

The $^{14}CO_2$ was generated by reacting ^{14}C sodium bicarbonate ($NaHCO_3$) with an excess of lactic acid in a closed system as indicated in Fig.3.01. The plants were moved to a glasshouse to apply the $^{14}CO_2$ as their outdoor site was not well sheltered and lacked a power supply. The ^{14}C label was applied to the youngest fully expanded fern on the largest rhizome of each plant. The fern to be labelled was enclosed in a mylar bag (350 x 1000 mm) which was firmly sealed by tying elastic over a 75 mm diameter cork at the base of the plant. The cork had been cut into halves and the inside edges lined with a closed cell foam which sealed around the plant stem. One piece of PVC tubing (5 mm internal diameter) was inserted in each half of the cork. The CO_2 was generated in a 25 ml flask with 2 side arms and covered by a rubber septa. A gas pump (0.1 hp) was used to circulate air through the system. CO_2 levels were monitored by a Binos infra-red gas analyser (IRGA) connected to an Epsom HX20 laptop computer.

The IRGA and computer were turned on after all the gas tubing was connected. Three ml of 30% lactic acid was then syringed into the CO_2 generating flask. A mylar bag was placed over the fern to be labelled, cork and gas tubing inserted at the base of the bag and the bag sealed. The gas pump was then turned on and the system checked for leaks.

The $^{14}CO_2$ was fed to actively photosynthesising plants only. The plant was covered with a sheet of black plastic to temporarily stop photosynthesis and 1M $NaHCO_3$ added dropwise to the CO_2 generation flask to increase the CO_2 concentration to about 400 ppm. One-hundred micro-curies of ^{14}C-$NaHCO_3$ was then syringed into the CO_2 generation flask and shaken to mix thoroughly. The black plastic sheet was removed allowing the plant to fix CO_2 until CO_2 concentration stabilised at 60-100ppm. This required 10-20 minutes. The $^{14}CO_2$ fixed was calculated from the maximum and minimum CO_2 concentration and the amount of ^{14}C available. The $^{14}CO_2$ fixed was between 85 and 90 μCuries for most plants.

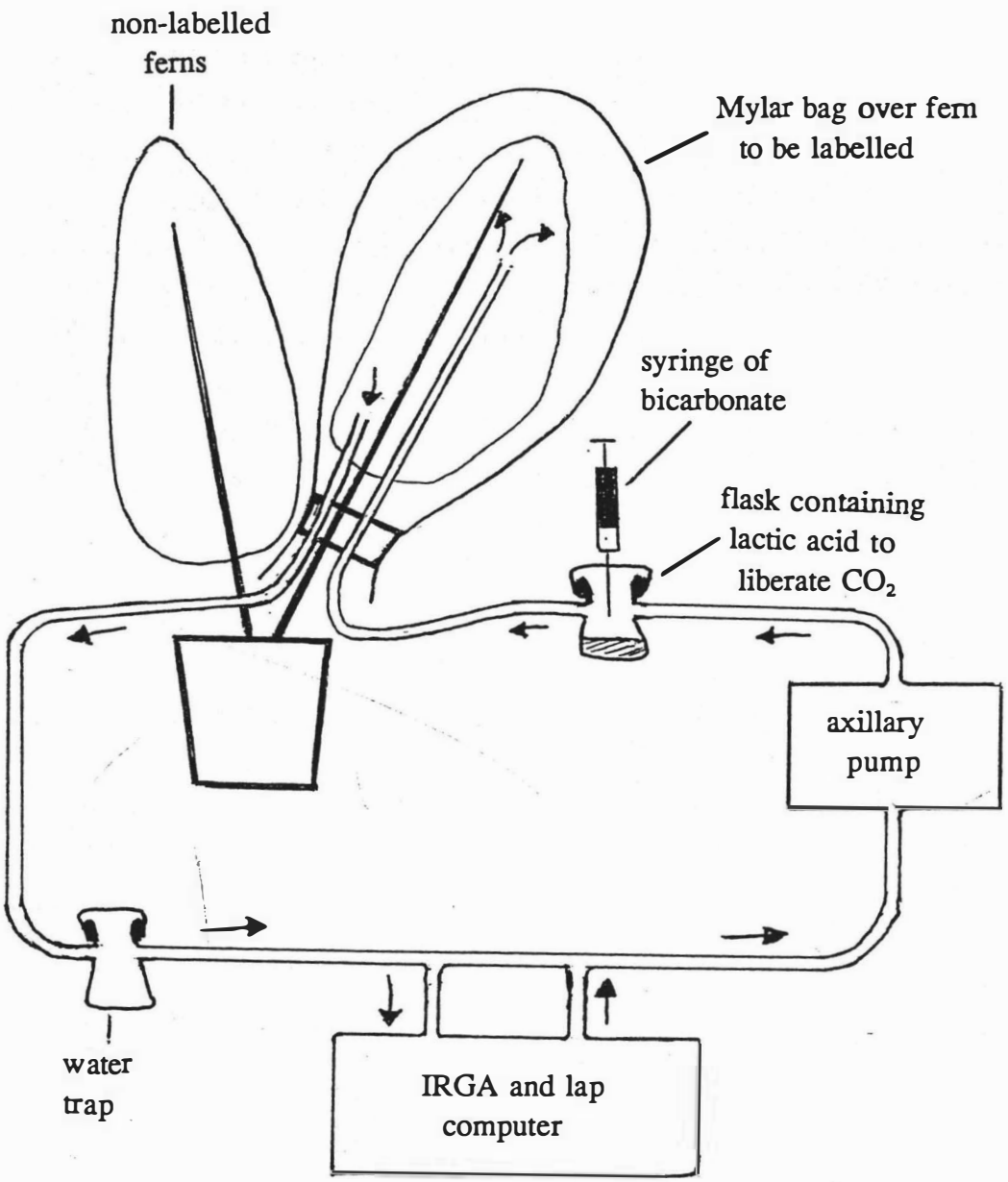


Figure 3.01: Arrangement of $^{14}CO_2$ labelling apparatus and direction of gas flow.

3.2.3 TREATMENTS

Preliminary experiments indicated that the rate of translocation from an asparagus fern is relatively slow: following fixation of $^{14}CO_2$, 10 days was required for 50% of the ^{14}C to move out of the labelled fern. Thus four weeks was the shortest time period over which ^{14}C movement was measured.

The times at which plants were labelled with ^{14}C and subsequently sampled are specified in Table 3.01. The female plants without berries (Treatments 9 & 10) were obtained by removing all flowers and small berries by hand, at weekly intervals. All plants were returned to natural conditions within 24 hours of labelling, and remained there until sampled.

Table 3.01: The labelling and sampling dates for all treatments in the source-sink experiment.

Treatment	Date labelled	Date sampled	Growth stage at sampling	Plant sex	Weeks to sampling
1	Jan 21-23	Feb 18-20	active	male	4
2	Jan 21-23	Mar 18	active	male	8
3	Jan 21-23	Apr 15-18	senescing	male	12
4	Feb 19	Mar 18	active	male	4
5	Feb 19	Apr 16	senescing	male	8
6	Mar 21	Apr 17	senescing	male	4
7	Jan 26-28	Feb 23-25	active	female with berries	4
8	Jan 26-28	Apr 20-21	senescing	female with berries	12
9	Jan 26-28	Feb 23-25	active	female without berries	4
10	Jan 26-28	Apr 20-21	senescing	female without berries	12
11	Jan 29-31	Apr 23	senescing	male	12
12	Jan 29-31	Jun 25	senesced	male	21
13	Jan 29-31	Aug 29	pre spear emergence	male	30
14	Jan 29-31	Oct 13	end of harvest	male	37
15	Jan 29-31	Nov 5	fern established	male	40

3.2.4 SAMPLING

Plants were removed from their containers and potting mix washed from the roots. This removed most of the fine, fibrous feeding roots from the sample. The plants were dissected and fresh weight, fern diameter, rhizome length and number of ferns, buds and storage roots recorded before drying the samples in a vacuum oven (40C, 2 mm Hg) for at least 2 days. If samples could not be placed in the vacuum oven within 4 hours of removal from their container, they were stored in a cool room (2C) to prevent desiccation and biochemical changes. After drying, samples were placed in an equilibration room at 20C and 55% RH for 1-3 hours before dry weights were recorded.

Samples required for ^{14}C analysis were redried in the vacuum oven then held in a forced draft oven at 60C before grinding to pass a 1 mm screen in a hammer mill. Asparagus fructans are hydroscopic and samples stored at 55%RH were too sticky to grind in the hammer mill.

Nomenclature of plant samples

Each rhizome with ferns present was sampled separately. On plants with more than one rhizome, the rhizome which supported the labelled fern was called the 'primary' rhizome, and the other rhizome(s) were called the 'secondary' or 'axillary' rhizomes. Relative positions and sizes of the primary, secondary and axillary rhizomes are shown in Fig.3.05.

There was a clear difference in the size of secondary and axillary rhizomes. Axillary rhizomes were lateral rhizomes which had developed from the primary rhizome, and were less than 30% of the length of the primary rhizome. Many of the axillary rhizomes did not have fern when sampled. Secondary rhizomes were more than 50% of the length of the primary rhizome, and all had ferns.

Each primary and secondary rhizome of plants sampled from February to April was dissected into the following samples for ^{14}C analysis as shown in Fig.3.02 and Fig.3.03:

1. Fern - each fern and spear was sampled separately. The relative ages (age rank) of the ferns and their origin on the rhizome was recorded.
2. Each rhizome was divided into the following segments:
   a. 'bud' or new rhizome with un-expanded buds
   b. 'fern' rhizome supporting the current season's fern
   c. old rhizome which supported the previous season's fern

d. axillary rhizome - sampled separately (if present) when it had a clearly defined shape and supported new roots.
Some plants, especially those harvested in February and March had many small, indistinct axillary bud clusters which were sampled with the parent rhizome.

3. Roots associated with each rhizome segment were sampled separately and divided into:

a. new (i.e. current season's) root tissue which was visually distinguished by it's lighter colour. New roots attached to the rhizome and new tissue on the end of old roots were combined.

b. old (i.e. previous season's) roots, including damaged or dying roots.
One and 2 year old roots could not be distinguished by visual characteristics.

After the data had been analysed, it appeared likely that some of the current season's roots had been sampled with the 'old' root fraction. All root samples were combined in plants sampled after April as it was not possible to distinguish between the rhizome segments after the fern had senesced, or between old and new roots.

3.2.5 ANALYSIS OF TOTAL ^{14}C

Duplicate 100 mg aliquots of dried ground tissue were placed in separate ceramic 'boats' and combusted in a biological oxidiser (Harvey Model OX-600). The samples were combusted at 900C in a stream of oxygen flowing at 350 ml/min; the combustion products passed through a series of catalysts to remove halogens, iron compounds, phosphorous and sulphur before the CO_2 was absorbed as it passed through a trap containing the scintillation cocktail (Fig.3.05). The system was flushed with nitrogen gas following the combustion of each sample.

A non-bubbling scintillation cocktail was used, consisting of toluene: 2-methoxyethanol (ethylene glycol monomethyl ether): ethanolamine (10:7:1) and 5.5 g/l of 2,5 diphenyloxazole (PPO) (Jeffay and Alvarez 1961). The scintillation cocktail was transferred to scintillation vials and counted at least 3 hours later for a maximum of 5 minutes or until a 2% standard error was obtained on a liquid scintillation counter (Beckman Model LS-3801). Counting efficiency was determined by the external standard method using commercially obtained quenched standards.

3.2.6 DATA ANALYSIS

As most of the data obtained from the experiments was unbalanced, an analysis of variance was carried out using the generalised linear model of SAS (Versions 5 and 6) (PROC GLM). A protected LSD was calculated to determine differences between treatments, and between sinks within a treatment, using the harmonic mean of cell size when replication was unequal.

3.3 RESULTS

3.3.1 DISTRIBUTION OF ^{14}C BETWEEN RHIZOMES

The partitioning of ^{14}C between the rhizomes of asparagus plants was examined to test the hypothesis 'that each asparagus plant is a complete unit for carbohydrate production and allocation, and there is no barrier to carbohydrate movement between rhizomes'. Plants labelled from mid January to mid March and sampled four to twelve weeks later from mid February to late April (Treatments 1 to 11, Table 3.01) were used to test this hypothesis. Forty-eight of the 60 plants examined had one or more secondary or axillary rhizomes attached to the primary (i.e, labelled) rhizome (Table 3.02, Fig.3.05). Plants sampled in June onwards (Treatments 12 to 15) were not used as relationships between parts of the plant could not be clearly determined due to fern senescence and decay of old underground stem tissue.

Some labelled carbohydrate was found in both secondary and axillary rhizomes (Table 3.03, Fig.3.06) although most of the ^{14}C recovered was in the primary rhizome. The mobilising ability of the axillary rhizome for the ^{14}C labelled assimilate was correlated with it's size, while the mobilising ability of the secondary rhizome was independent of rhizome size, except for number of roots (Table 3.03). There was no relationship between the size of the primary rhizome and the mobilising ability of the axillary or secondary rhizomes. The mobilising ability of the secondary rhizome increased as the age rank of the labelled fern increased, i.e. as the plants were labelled later in the growing season.

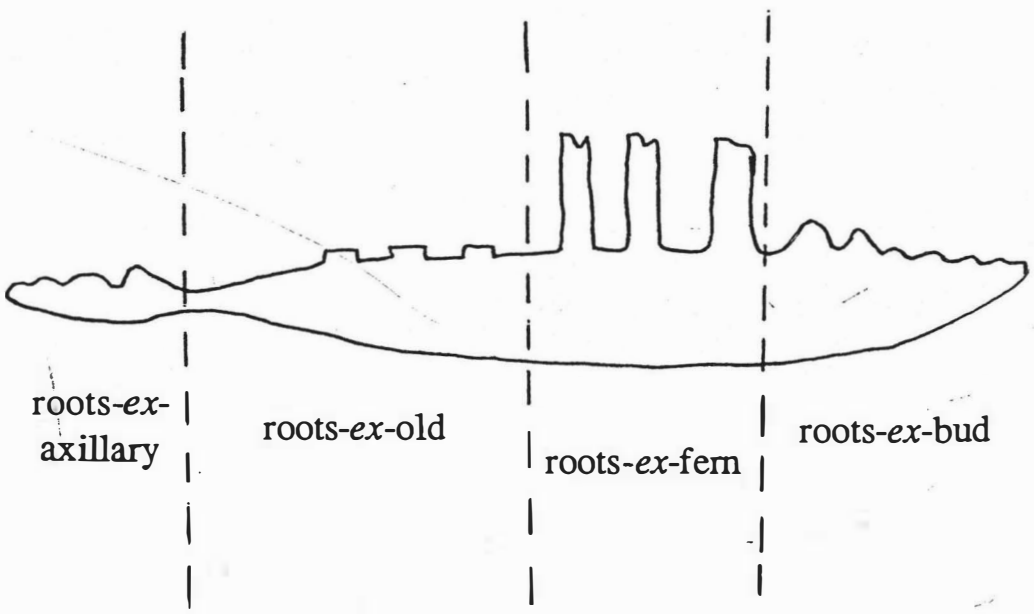
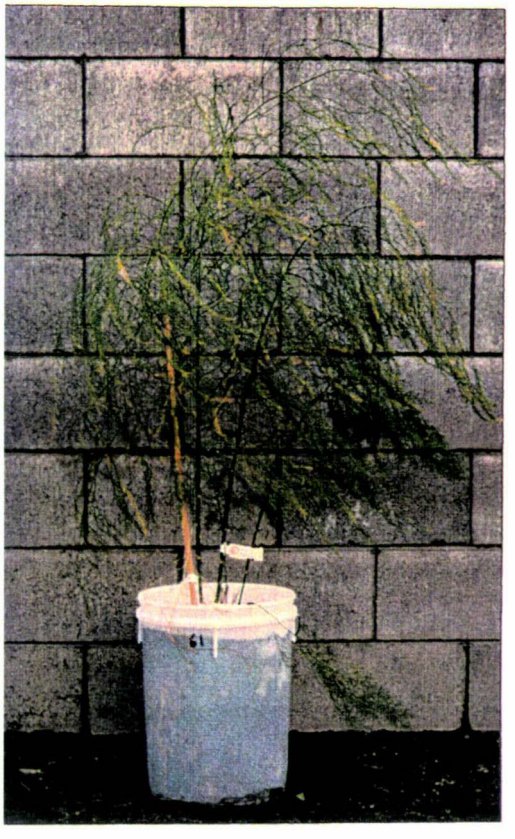


Figure 3.02: Diagrammatic view of an asparagus rhizome, and the nomenclature used to describe the carbohydrate sinks within the crown.



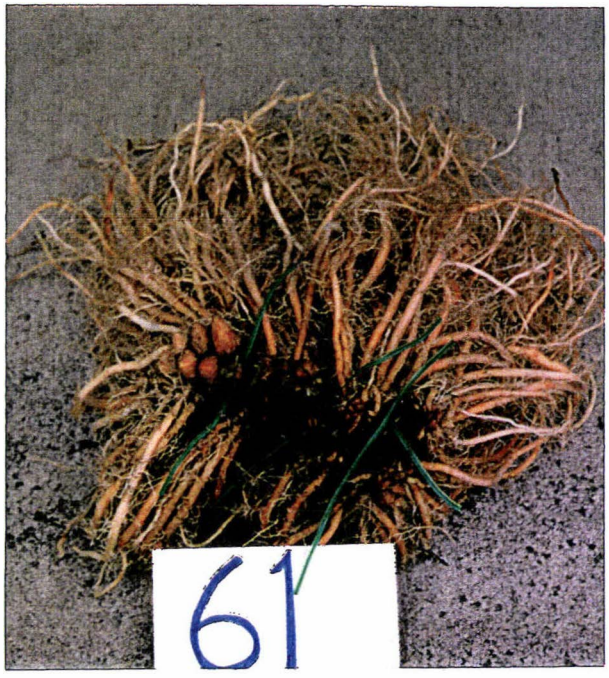
A. Plant in 20 L bucket, before sampling on 15 April. The fern which had been exposed to $^{14}CO_2$ in mid January is tagged.

B.Intact plant after being removed from bucket and partially washed. Ruler on left of fern is 1 m long.

Figure 3.03: Photographs of a dissected asparagus plant, and the nomenclature used to describe the carbohydrate sinks within the crown.

C. Crown of plant after fern removed. The crown was subsequently divided as follows (from left to right):

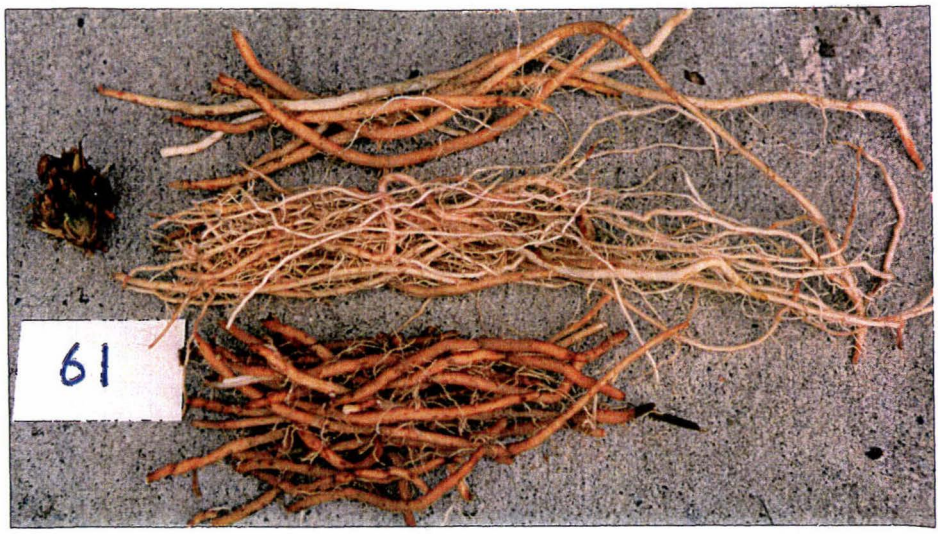
1. developing buds which had not yet produced spears or fern and the attached roots, known as 'roots-*ex*-bud'.
2. buds which had produced fern during the current season and the attached roots, known as 'roots-*ex*-fern'
3. buds which had produced fern during the previous season and the attached roots, known as 'roots-*ex*-old'
4. developing axillary underground stems and attached roots, known as roots-*ex*-axillary



D. Washed rhizome, divided as described above.

Note: 1. small axillary rhizome adjacent to a bud which has not grown on rhizome which supported the current season's fern

2. small axillaries on periphery of old rhizome



E. New (top two sections) and old (lower section) root material derived from rhizome which supported the current season's fern.

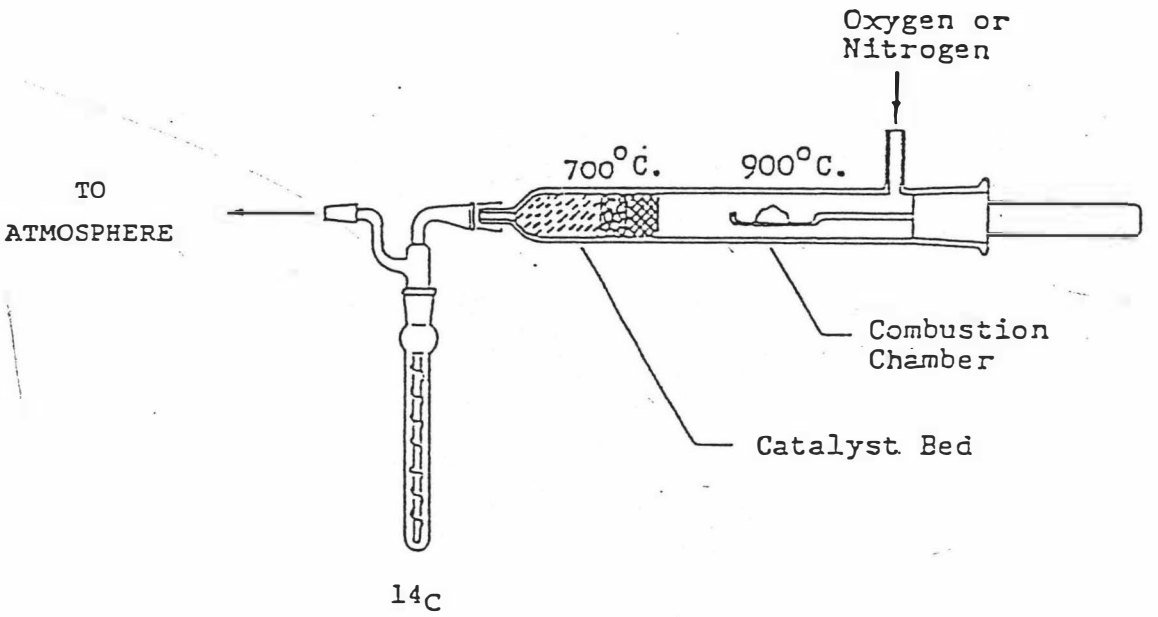


Figure 3.04: Schematic view of biological oxidiser used to combust samples to determine total ^{14}C content.

3.3.2 SOURCE-SINK RELATIONSHIPS: APPROACH USED TO ANALYSE DATA

Data from secondary rhizomes were excluded from analysis of source-sink relationships, and the primary rhizome with it's attached axillaries considered the complete unit when calculating percentage dry weight or ^{14}C in a particular tissue. Secondary rhizomes generally contained less then 5% of the ^{14}C recovered from a plant (Fig.3.06), although they comprised up to 60% of the plant dry weight.

Changes in sink priority for current and stored assimilate were determined by sequential labelling and sampling. The mobilising ability (^{14}C content) and competitive ability (^{14}C concentration) for current assimilates from the labelled source was determined from plants sampled 4 weeks after labelling. Subsequent changes in ^{14}C content and concentration were used to determine mobilising ability and competitive ability for stored assimilate. When data was expressed as a percentage, competitive ability was calculated as the ratio of percentage total ^{14}C recovered to percentage dry weight.

Roots were divided into four 'sinks' based on the section of the rhizome which they subtended (Figs. 3.02, 3.03). 'Roots-*ex*-bud' were attached to the bud cluster at the growing tip of the rhizome; 'roots-*ex*-fern' were attached to the portion of the rhizome which supports the current season's ferns; 'roots-*ex*-old' were attached to the portion of the rhizome which supported the previous seasons ferns and 'roots-*ex*-axillary' were attached to the axillary rhizome(s).

As axillary rhizomes were not present in all plants, data obtained from axillary rhizomes was pooled with roots-*ex*-old. Fifty seven of the sixty axillary rhizomes sampled were attached to the 'old' rhizome segment; the remaining three were on the same plant and were attached to the fern segment of the primary rhizome. Data from this plant was not used. Many other plants had clusters of buds in an axillary position, but the clusters were too small to sample separately. Thus pooling the data from axillary rhizomes and the 'old' rhizome segment ensured that tissue with essentially the same morphological basis was compared.

The old and new roots from within a rhizome segment have been combined in most of the following data analyses as the classification of roots as 'new' and 'old' was more subjective than intended. The dry weight of the 'old' roots increased during the growing season implying that the old fraction contained not only the previous seasons roots but

Figure 3.05: Classification of rhizome arrangements in plants sampled from February to May, 1987. Active rhizomes (those supporting fern) coloured black; dormant rhizomes white.

Rhizome type	Plan view	Number of plants	Description
A		12	1 large active rhizome: axillaries indistinct or too small to sample separately
B		33	1 large active rhizome; 1 or more axillary rhizomes (active or dormant)
C		15	1 large active rhizome with a large secondary rhizome; axillary rhizomes may be present

Table 3.02: Frequency of rhizome types in plants sampled from February to May.

	Rhizome type			Total number of plants sampled
Date sampled	A	B	C	
Male plants:				
Feb 18-20	3	1	1	5
Mar 18	1	2	6	10
Apr 15-18	1	15	4	20
Apr 23	0	4	1	5
Female plants with berries:				
Feb 23-25	2	3	0	5
Apr 20-21	2	1	2	5
Female plants without berries:				
Feb 23-25	2	2	1	5
Apr 20-21	1	4	0	5

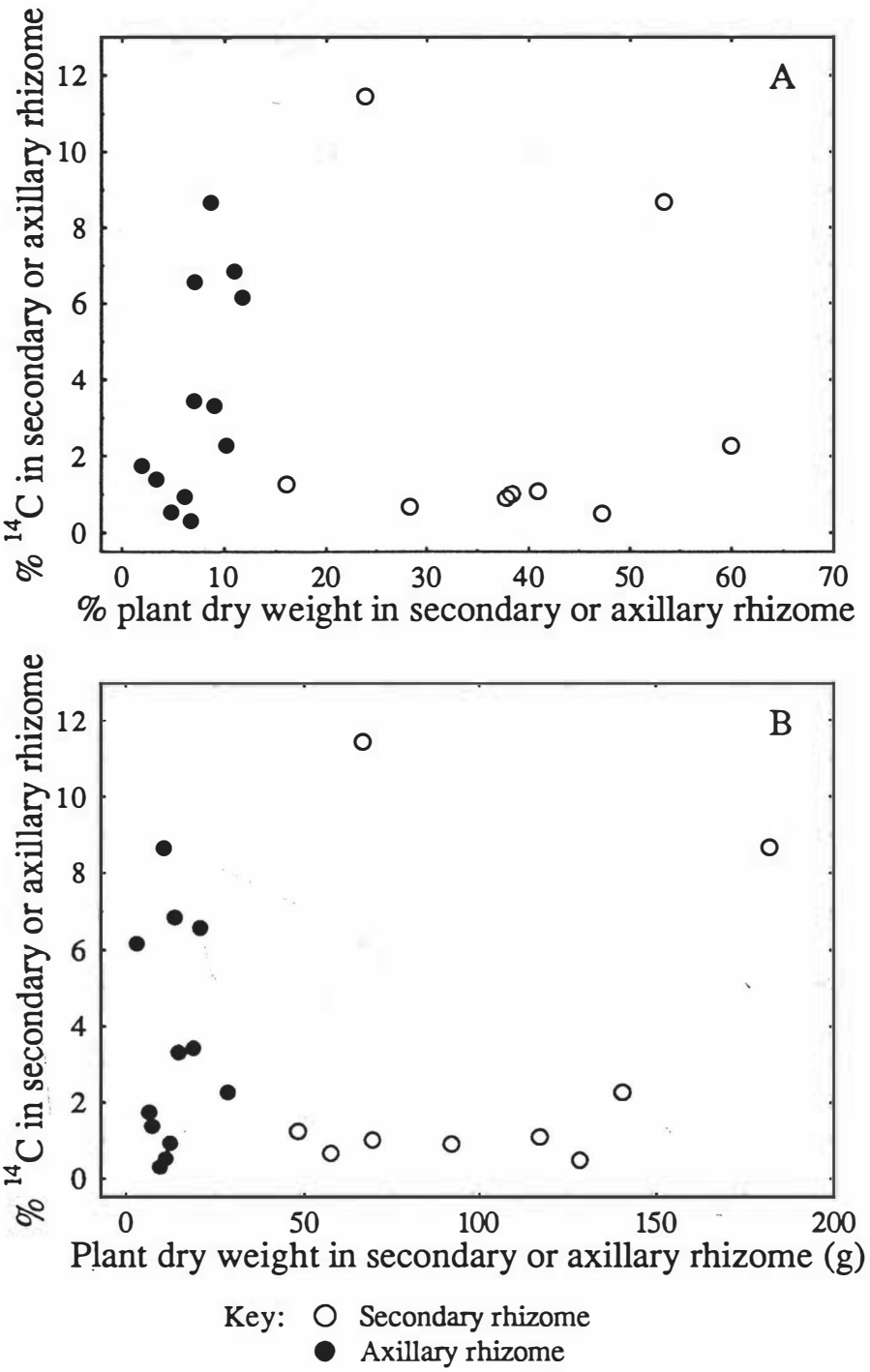


Figure 3.06: Effect of rhizome weight on mobilising ability (% of plant's ^{14}C) of secondary and axillary rhizomes.
A: dry weight as percentage of plant dry weight
B: actual dry weight (g)

Table 3.03: Correlation (r) between mobilising ability (^{14}C content), (with respect to the labelled fern), of axillary or secondary rhizomes and other plant attributes at sampling, and it's significance (P). Plants were labelled by exposing the youngest fern to $^{14}CO_2$ in January, February or March, and sampled 4 to 12 weeks later, before the end of April.

	Correlation with mobilising ability (total ^{14}C) of			
	Axillary rhizome		Secondary rhizome	
Non-labelled rhizome:	r	P	r	P
Dry weight all crown & fern	0.689	0.01	0.605	0.85
Dry weight crown only	0.663	0.02	0.595	0.09
Length of rhizome	0.629	0.03	0.561	0.12
Number of roots	0.637	0.03	0.716	0.03
Labelled rhizome:				
Dry weight all crown & fern	-0.068	0.83	-0.237	0.54
Dry weight crown only	-0.042	0.89	-0.199	0.61
Length	-0.020	0.95	-0.123	0.75
Number of roots	-0.171	0.60	-0.139	0.72
Number of shoots	0.130	0.37	0.392	0.30
Age rank of labelled fern	0.130	0.69	0.631	0.07

also some of the current seasons roots. Thus, the new 'root' fraction contains only the most recently formed roots, and not all of the current season's roots as intended.

Fern older than the labelled fern has been excluded from the following analyses of sink strength and activity as it was never a major sink (the concentration and total ^{14}C in the tissue were both very low) and many of the plants did not have any ferns older than the labelled fern, especially those labelled in mid January.

3.3.3 SOURCE-SINK RELATIONSHIPS IN MALE ASPARAGUS PLANTS DURING SUMMER AND AUTUMN

Current assimilate

Male asparagus plants labelled with 190 x 10^3 dpm of ^{14}C in mid January, mid February and mid March lost approximately 50% of the fixed ^{14}C within four weeks (Fig.3.07, Treatment 1, 4 & 6). The ^{14}C recovered from plants labelled in mid January appeared to be lower than that recovered from plants labelled in mid February but this was not significant at P=0.05.

Based on mobilising ability for the ^{14}C labelled carbohydrate, the sink priority in January-February (Table 3.04a) was younger fern, roots-*ex*-old, then other roots followed by the rhizome. Fern younger than the labelled fern also had the highest competitive ability. Although roots-*ex*-bud had a relatively high competitive ability compared with other root sinks (Table 3.04b, Fig.3.08), they had a lower dry weight (Fig.3.09) and thus a low mobilising ability with respect to the labelled fern (Table 3.04a, Fig.3.07).

In the first four weeks following labelling, plants labelled in mid February tended to retain more ^{14}C in their labelled fern and in the entire plant than plants labelled in mid January (Fig.3.07 Treatment 4, Table 3.04a). The mobilising ability with respect to the labelled fern of the roots-*ex*-fern was now higher than that of the younger fern, although their competitive ability was similar (Fig.3.08, Table 3.04b). Roots-*ex*-fern and roots-*ex*-bud had a higher mobilising ability with respect to the labelled fern than roots-*ex*-old, which was the reverse of the sink priorities within the crown in January-February (Table 3.04). The competitive ability of roots-*ex*-old tended to be higher than in January-February, while the competitive ability of roots-*ex*-bud tended to be lower (Table 3.04b).

The dry weight of ferns older than the labelled fern was higher on plants labelled in mid February than on plants labelled in mid January as more ferns were present when the label was applied to the youngest expanded fern (Fig.3.09). The dry weight of ferns younger than the labelled fern appeared to be less on plants labelled in mid February (Table 3.04c) than on plants labelled in mid January.

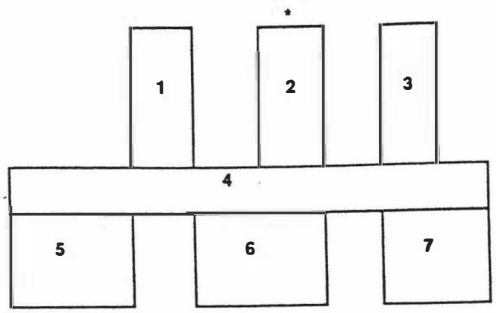
The ^{14}C recovered from the plants in the four weeks following labelling in mid March tends to be less than the total ^{14}C recovered following labelling mid February, and similar to that recovered following labelling in mid January. This was associated with an apparent decrease in the ^{14}C retained by both the labelled and younger fern (Fig.3.07 Treatment 6, Table 3.04a). Within the roots, roots-*ex*-fern and roots-*ex*-bud still appeared to have a higher mobilising ability and competitive ability for the current assimilate than roots-*ex*-old (Fig.3.7 Treatment 6, Table 3.04a & b). The rhizome appeared to have higher competitive ability (Table 3.04b) than the roots-*ex*-bud: however this data was based on only two plants, one of which had a rhizome with a very high sink strength and none of the differences were significant at P=0.05.

Stored assimilate

The ^{14}C recovered from plants labelled in mid January (Fig.3.07 Treatment 2 & 3) continues to decrease between mid March and mid April although the differences were not significant. The ^{14}C content of both labelled and younger fern appeared to decrease between mid March and mid April on the plants labelled in mid January (Fig.3.07, Treatment 2 & 3, Table 3.05a), while the ^{14}C content of the roots-*ex*-old appeared to increase.

The dry weight of each sink tends to increase between mid March and mid April (Table 3.05c) and is associated with an apparent decrease in the concentration of ^{14}C within each sink (Table 3.05b). When sampled in mid April, plants labelled in mid February appeared to retain more of the ^{14}C fixed than plants labelled in mid January or mid March (Table 3.06a).

Key to figures 3.07 to 3.09



Tissues within plants were divided into sinks as follows:

1. Fern older than the labelled fern (if present)
2. Fern labelled with $^{14}CO_2$
3. Fern younger than the labelled fern
4. Rhizome
5. Roots-*ex*-old: storage roots subtending the portion of the rhizome which supported the previous seasons fern
6. Roots-*ex*-fern: storage roots subtending the portion of rhizome which supports the current season's fern
7. Roots-*ex*-bud: storage roots subtending the portion of the rhizome which contains un-expanded buds

All data is presented as treatment means, followed by standard error of the mean (in italics). All plants were male.

Summary of treatments:

Treatment	Labelled in:	Sampled in:
1	mid January	mid February
2	mid January	mid March
3	mid January	mid April
4	mid February	mid March
5	mid February	mid April
6	mid March	mid April

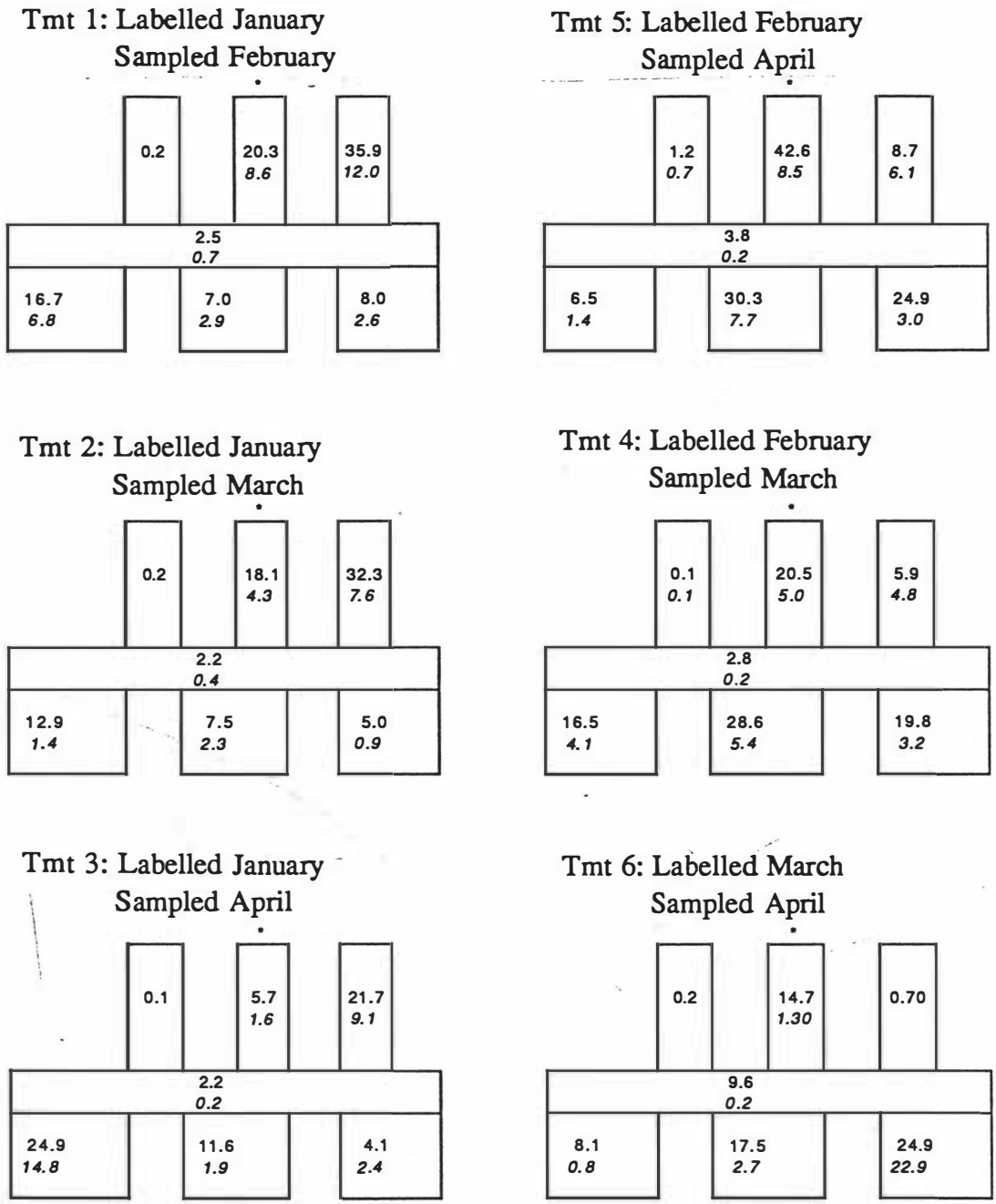


Figure 3.07: Mobilising ability (^{14}C content, dpm x 10^3), with respect to the labelled fern, of sinks within male asparagus plants labelled with a pulse of $^{14}CO_2$ at 3 stages during fern growth and sampled 4 to 12 weeks later. See Table 3.01 for labelling and sampling dates. Standard error of mean is in italics.

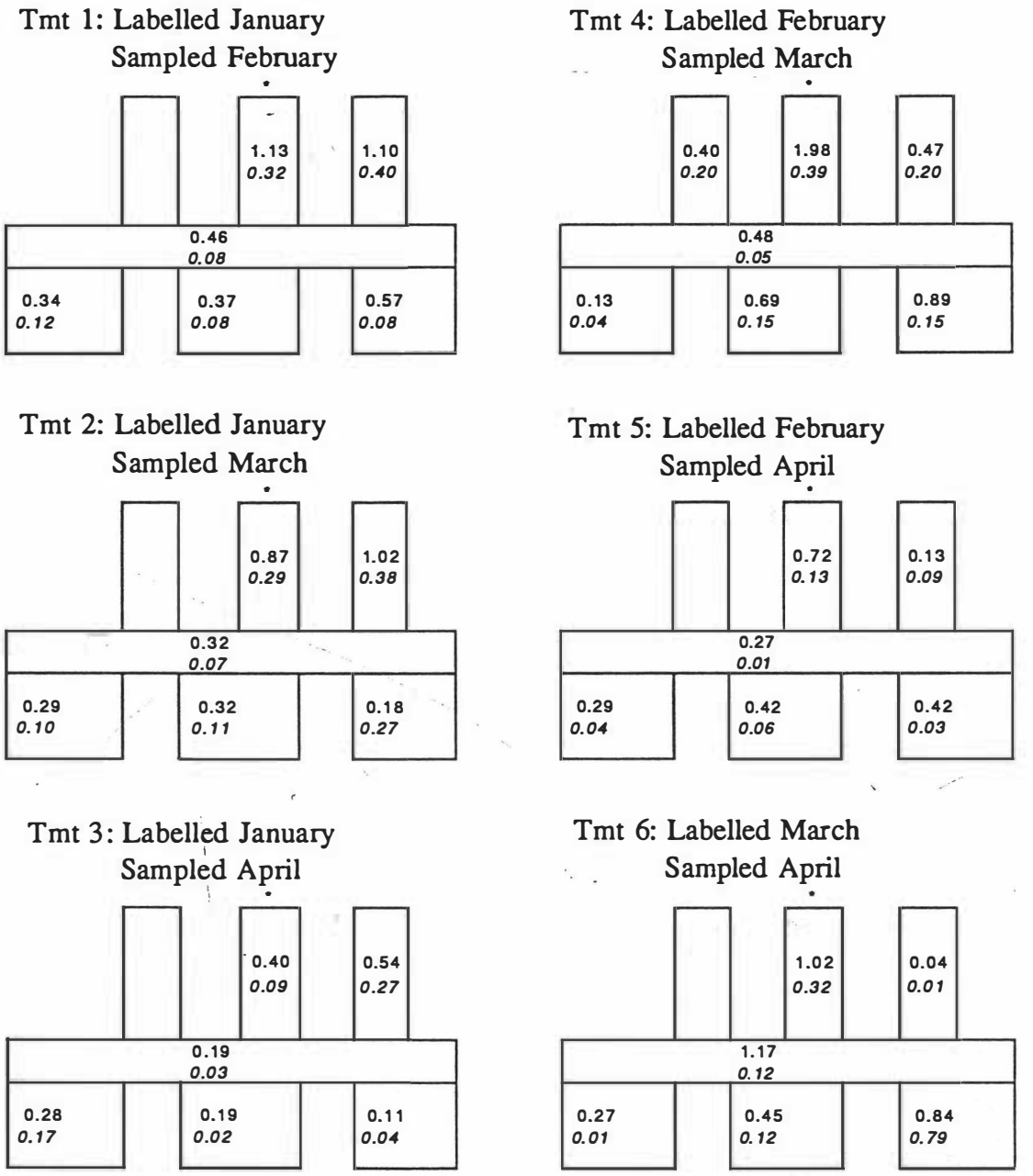


Figure 3.08: Competitive ability (^{14}C concentration, dpm x 10^3 /mg) of sinks, with respect to the labelled fern, within male asparagus plants labelled with a pulse of $^{14}CO_2$ at 3 stages during fern growth and sampled 4 to 12 weeks later. See Table 3.01 for labelling and sampling dates. Standard error of mean is in italics.

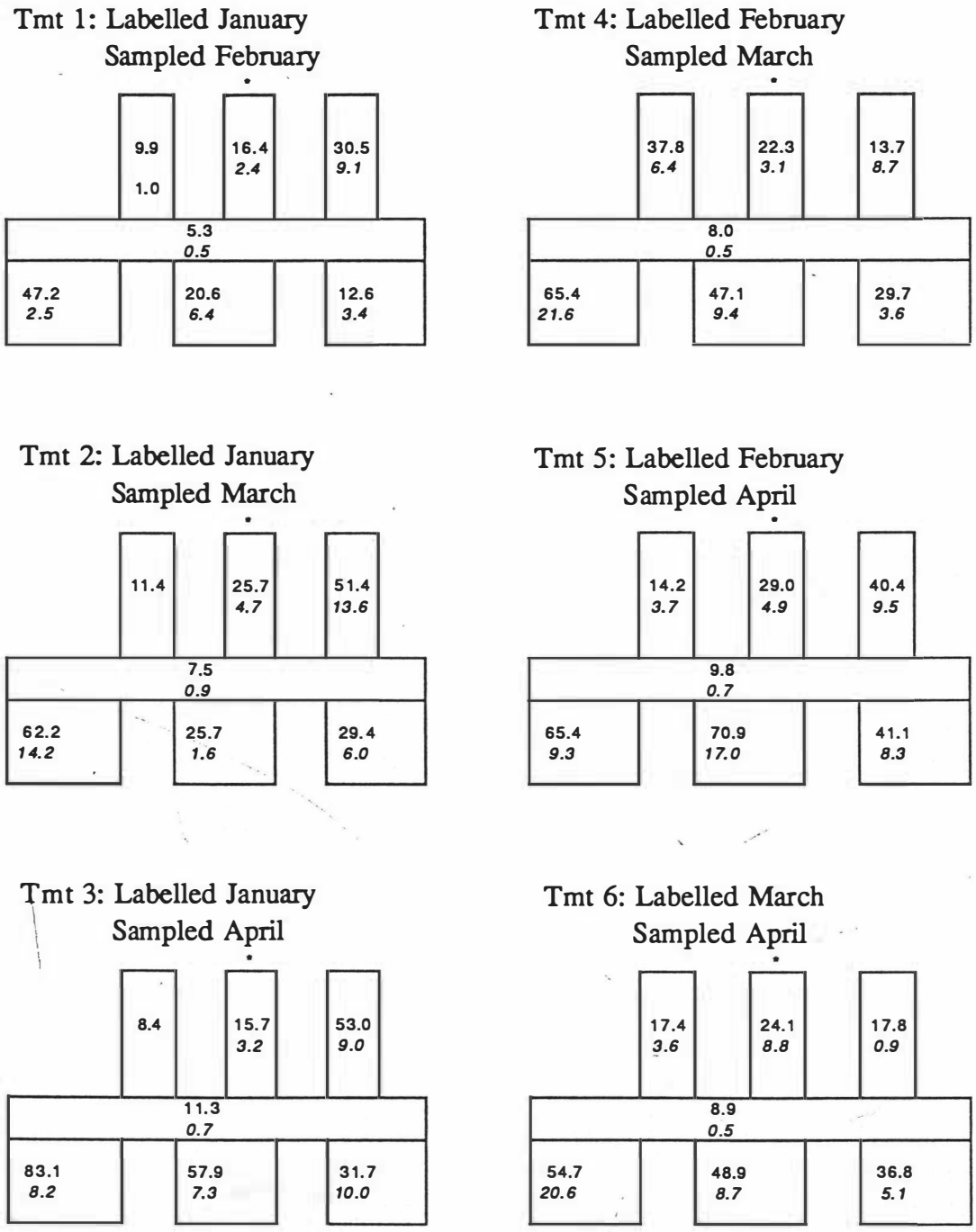


Figure 3.09: Dry weight of sinks within male asparagus plants labelled with a pulse of $^{14}CO_2$ at 3 stages during fern growth and sampled 4 to 12 weeks later. See Table 3.01 for labelling and sampling dates. Standard error of mean is in italics.

Table 3.04a: Total ^{14}C (dpm x 10^3) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: effect of labelling date on mobilising ability, with respect to the labelled fern, for current assimilate, i.e. 4 weeks after labelling. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 17	
Tissue:				
labelled fern	20.3 (8.6)	42.6 (8.5)	14.7 (1.3)	ns
younger fern	35.9 (12.0)	8.7 (6.1)	0.7 -	ns
rhizome	2.5 (0.7)	3.8 (0.2)	9.6 (0.2)	1.4
roots-*ex*-bud	8.0 (2.6)	24.9 (3.0)	24.9 (22.9)	ns
roots-*ex*-fern	7.0 (2.9)	30.3 (7.7)	17.5 (2.7)	ns
roots-*ex*-old	16.7 (6.8)	6.5 (1.4)	8.1 (0.8)	ns
$LSD_{.05}$[a]	20.3	16.3	12.4	
n (b)	4	5 3	4 1	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.04b: Concentration of ^{14}C (dpm x 10^3/g) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: effect of labelling date on competitive ability for current assimilate, i.e. 4 weeks after labelling. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 17	
Tissue:				
labelled fern	1.13 (0.32)	1.98 (0.39)	1.02 (0.32)	ns
younger fern	1.10 (0.40)	0.47 (0.20)	0.04 -	ns
rhizome	0.46 (0.08)	0.48 (0.05)	1.17 (0.12)	0.25
roots-*ex*-bud	0.57 (0.08)	0.89 (0.15)	0.84 (0.78)	ns
roots-*ex*-fern	0.37 (0.09)	0.69 (0.15)	0.45 (0.12)	ns
roots-*ex*-old	0.34 (0.12)	0.13 (0.04)	0.27 (0.01)	ns
$LSD_{.05}$[a]	0.66	0.61	0.21	
n (b)	4	5 3	2 1	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.04c: Dry weight (g) recovered from sinks within male asparagus plants during vegetative growth: sink dry weight 4 weeks after labelling with a pulse of ^{14}C. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 17	
Tissue:				
labelled fern	16.4 (2.4)	22.3 (3.1)	24.1 (8.8)	ns
younger fern	30.5 (9.1)	13.6 (8.7)	17.8 (0.9)	ns
rhizome	5.3 (0.5)	8.0 (0.5)	8.9 (0.5)	1.6
roots-*ex*-bud	12.6 (3.4)	29.7 (3.6)	36.8 (5.1)	12.9
roots-*ex*-fern	20.6 (6.4)	47.1 (9.4)	48.9 (8.7)	ns
roots-*ex*-old	47.2 (2.5)	65.4 (21.6)	54.7 (20.6)	ns
$LSD_{.05}$[a]	14.7	32.2	28.5	
n [b]	4	5 3	4 1	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.05a: Total ^{14}C (dpm x 10^3) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: changes in 'mobilising ability with respect to the labelled fern' over time. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Jan 21-23	Jan 21-23	$LSD_{.05}$[a]	Feb 19	Feb 19	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 15-18		Mar 18	Apr 16	
Tissue:							
labelled fern	20.3 (8.6)	18.1 (4.3)	5.7 (1.6)	ns	42.6 (8.5)	20.5 (5.0)	ns
younger fern	35.9 (12.0)	32.3 (7.6)	21.7 (9.1)	ns	8.7 (6.1)	5.9 (4.8)	ns
rhizome	2.5 (0.7)	2.2 (0.4)	2.2 (0.2)	ns	3.8 (0.2)	2.8 (0.2)	0.8
roots-*ex*-bud	8.0 (2.6)	5.0 (0.9)	4.1 (2.4)	ns	24.9 (3.0)	19.8 (3.2)	ns
roots-*ex*-fern	7.0 (2.9)	7.5 (2.3)	11.6 (1.9)	ns	30.3 (7.7)	28.6 (5.4)	ns
roots-*ex*-old	16.7 (6.8)	12.9 (1.4)	24.9 (14.8)	ns	6.5 (1.4)	16.5 (4.2)	9.4
$LSD_{.05}$[a]	23.3	10.9	ns		16.3	12.4	
n (b)	4	5	4		5 3	4	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, is less than number of plants sampled

Table 3.05b: Concentration of ^{14}C (dpm x 10^3/mg) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: changes in competitive ability over time. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Jan 21-23	Jan 21-23	$LSD_{.05}$[a]	Feb 19	Feb 19	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 15-18		Mar 18	Apr 16	
Tissue:							
labelled fern	1.13 (0.32)	0.87 (0.29)	0.40 (0.09)	ns	1.98 (0.39)	0.72 (0.13)	1.1
younger fern	1.10 (0.40)	1.02 (0.38)	0.54 (0.27)	ns	0.47 (0.20)	0.13 (0.09)	ns
rhizome	0.46 (0.08)	0.32 (0.07)	0.19 (0.03)	0.16	0.48 (0.05)	0.27 (0.01)	0.13
roots-*ex*-bud	0.57 (0.08)	0.18 (0.27)	0.11 (0.04)	ns	0.89 (0.15)	0.42 (0.03)	ns
roots-*ex*-fern	0.37 (0.09)	0.32 (0.11)	0.19 (0.02)	ns	0.69 (0.15)	0.42 (0.06)	ns
roots-*ex*-old	0.34 (0.12)	0.29 (0.10)	0.28 (0.17)	ns	0.13 (0.04)	0.29 (0.04)	ns
$LSD_{.05}$[a]	0.66	0.61	ns		0.61	0.21	
n (b)	4	5	4		5 3	4	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.05c: Dry weight (g) recovered from sinks within male asparagus plants during vegetative growth: changes in sink dry weight over time. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Jan 21-23	Jan 21-23	$LSD_{.05}$[a]	Feb 19	Feb 19	$LSD_{.05}$[a]
Date sampled	Feb 18-20	Mar 18	Apr 15-18		Mar 18	Apr 16	
Tissue:							
labelled fern	16.4 (2.4)	25.7 (4.7)	15.7 (3.2)	ns	22.3 (3.1)	29.0 (4.9)	ns
younger fern	30.5 (9.1)	51.4 (13.6)	53.0 (9.1)	ns	13.6 (8.7)	40.4 (9.5)	ns
rhizome	5.3 (0.5)	7.5 (0.9)	11.3 (0.7)	2.4	8.0 (0.5)	9.8 (0.7)	ns
roots-*ex*-bud	12.6 (3.4)	29.4 (6.0)	31.7 (10.0)	ns	29.7 (3.6)	41.1 (8.3)	ns
roots-*ex*-fern	20.6 (6.4)	25.7 (1.6)	57.9 (7.3)	17.4	47.1 (9.4)	70.9 (17.0)	ns
roots-*ex*-old	47.2 (2.5)	62.2 (14.2)	83.1 (8.2)	ns	65.4 (21.6)	65.4 (9.3)	ns
$LSD_{.05}$[a]	14.7	25.2	21.1		32.2	28.5	
n [b]	4	5	5		5 3	5	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.06a: Total ^{14}C (dpm x 10^3) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: effect of labelling date on plants sampled in April. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Apr 15-18	Apr 16	Apr 17	
Tissue:				
labelled fern	5.7 (1.6)	20.5 (5.0)	14.7 (1.3)	13.3
younger fern	21.7 (9.1)	5.9 (4.8)	0.7 -	ns
rhizome	2.2 (0.2)	2.8 (0.2)	9.6 (0.2)	0.8
roots-*ex*-bud	4.1 (2.4)	19.8 (3.2)	24.9 (22.9)	ns
roots-*ex*-fern	11.6 (1.9)	28.6 (5.4)	17.5 (2.7)	14.7
roots-*ex*-old	24.9 (14.8)	16.5 (4.2)	8.1 (0.8)	ns
$LSD_{.05}$[a]	ns	12.4	ns	
n (b)	4	4	2 1	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled.

Table 3.06b: Concentration of ^{14}C (dpm x 10^3/g) recovered from sinks within male asparagus plants labelled with a pulse of ^{14}C during fern growth: effect of labelling date on plants sampled in April. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Apr 15-18	Apr 16	Apr 17	
Tissue:				
labelled fern	0.40 (0.09)	0.72 (0.13)	1.02 (0.32)	0.40
younger fern	0.54 (0.27)	0.13 (0.09)	0.04 -	ns
rhizome	0.19 (0.03)	0.27 (0.01)	1.17 (0.12)	0.14
roots-*ex*-bud	0.11 (0.04)	0.42 (0.03)	0.84 (0.78)	ns
roots-*ex*-fern	0.19 (0.02)	0.42 (0.06)	0.45 (0.12)	0.19
roots-*ex*-old	0.28 (0.17)	0.29 (0.04)	0.27 (0.01)	ns
$LSD_{.05a}$	ns	0.21	ns	
n (b)	4	4	2 1	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled

Table 3.06c: Dry weight (g) recovered from sinks within male asparagus plants during vegetative growth: effect of labelling date on plants sampled in April. Standard error of mean is in brackets.

Date labelled	Jan 21-23	Feb 19	Mar 21	$LSD_{.05}$[a]
Date sampled	Apr 15-18	Apr 16	Apr 17	
Tissue:				
labelled fern	15.7 (3.2)	29.0 (4.9)	24.1 (8.8)	ns
younger fern	53.0 (9.1)	40.4 (9.5)	17.8 (0.9)	ns
rhizome	11.3 (0.7)	9.8 (0.7)	8.9 (0.5)	ns
roots-*ex*-bud	31.7 (10.0)	41.1 (8.3)	36.8 (5.1)	ns
roots-*ex*-fern	57.9 (7.3)	70.9 (17.0)	48.9 (8.7)	ns
roots-*ex*-old	83.1 (8.2)	65.4 (9.3)	54.7 (20.6)	ns
$LSD_{.05}$[a]	21.1	28.5	ns	
n (b)	5	5	4 3	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - number of plants with younger fern, if less than number of plants sampled.

3.3.4 DETAILED ANALYSIS OF SOURCE SINK RELATIONSHIPS IN MALE PLANTS DURING SUMMER AND AUTUMN.

Individual plants were examined to identify competing sinks and determine some of the causes of variation in mobilising ability and competitive ability for ^{14}C labelled current assimilate within treatments. The effect of axillary rhizomes was also examined. Data on dry weight and ^{14}C content are presented as percentages to remove the variation due to differences in plant size and ^{14}C content.

Mid January to mid February

Younger fern had a higher mobilising ability (^{14}C content), than all of the root sinks combined on three of the four plants examined (Table 3.07a). On these three plants (1A, 1B & 1C), competitive ability (^{14}C concentration) of the younger fern was inversely related to the dry weight and mobilising ability for the ^{14}C labelled assimilate.

The mobilising ability, with respect to the labelled fern, of the sinks within the crown varied in plants 1A, 1B, and 1C. The mobilising ability and competitive ability of new roots was greater than that of the old roots subtending the same section of the rhizome. Old roots-*ex*-old on plant 1A had a relatively high mobilising ability, competitive ability and dry weight.

On the plant with an axillary rhizome (1C), the mobilising and competitive ability of the roots-*ex*-axillary were similar to roots-*ex*-bud, and higher than that of the other root sinks. The presence of an axillary rhizome appeared to have no effect on the competitive ability of the other root fractions as they were similar to that on plants without an axillary rhizome; however the percentage dry weight (Table 3.07a) and mobilising ability of new roots-*ex*-old was lower than on plants 1A and 1B which did not have an axillary rhizome.

The growth of plant 1D appeared to have been restricted as the dry weight of the rhizome was much lower than that of the other plants (Table 3.07c), and the ^{14}C content of new tissues (younger fern and roots-*ex*-bud) was relatively low while the ^{14}C content of old roots-*ex*-old was very high (Table 3.07a).

Table 3.07a: Male plants labelled with a pulse of ^{14}C in mid January and sampled 4 weeks later: mobilising ability with respect to the labelled fern expressed as a percentage of the ^{14}C recovered.

Plant	Labelled fern	Younger fern	Root sinks					
			all *ex*-bud	new *ex*-fern	new *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-fern
1A	13	49	11	14	6.0	-	5.6	1.5
1B	17	55	13	6.8	4.7	-	0.8	2.1
1C	10	57	10	5.1	1.2	14.9	0.5	1.2
1D	52	1.8	1.5	1.2	3.6	-	39	0.7

Table 3.07b: Male plants labelled in mid January with a pulse of ^{14}C and sampled 4 weeks later: competitive ability expressed as percentage ^{14}C recovered/percentage dry weight.

Plant	Labelled fern	Younger fern	Root sinks					
			all *ex*-bud	new *ex*-fern	new *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-fern
1A	1.1	3.0	0.9	0.8	0.6	-	0.2	0.5
1B	2.1	2.0	1.2	0.4	0.5	-	0.1	0.1
1C	0.9	1.7	1.2	0.5	0.5	0.8	0.1	0.1
1D	2.1	0.2	0.4	0.4	0.7	-	0.4	0.8

Table 3.07c: Male plants labelled with a pulse of ^{14}C in mid January and sampled 4 weeks later: total weight, and dry weight as a percentage of total weight.

Plant	Labelled fern	Younger fern	Root sinks						Total weight (g)
			all *ex*-bud	new *ex*-fern	new *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-axillary	
1A	12	17	12	18	11	-	27	3.1	129
1B	7.9	28	12	13	9.5	-	18	6.5	170
1C	11	33	8.6	11	2.4	18	11	4.2	130
1D	25	10	4.1	2.9	5.0	-	51	1.6	94

Mid February to mid March

On plants labelled in mid February and sampled four weeks later, the combined mobilising ability of all root sinks for ^{14}C labelled current assimilate was higher than that of younger fern in all plants (Table 3.08a). The competitive ability of the fern (Table 3.08b) was proportional to both the dry weight (Table 3.09c) and mobilising ability, unlike plants labelled in mid January where an inverse relationship occurred. Compared with plants labelled in mid January, the mobilising ability of roots-*ex*-bud increased while the mobilising ability of new roots-*ex*-old tended to decrease. The mobilising ability and competitive ability of new roots-*ex*-old remained less than new roots-*ex*-fern and roots-*ex*-bud. The mobilising ability and competitive ability of old roots was higher than in mid January. On two plants (4A & 4D), the mobilising ability of the old roots was at least equal to that of new roots, although the competitive ability of old roots tended to be lower than on new roots. The percentage of root tissues classified as old was higher on these two plants than on the other plants (Table 3.08c).

The plant with axillary rhizomes (4C) had relatively high mobilising and competitive ability in both old and new roots-*ex*-fern (Table 3.08a & b). Both these sinks also had a high percentage dry weight (Table 3.08c). The mobilising ability, with respect to the labelled fern, of the axillary rhizome itself was smaller than in January-February, as was it's size (percentage of plant dry weight) and competitive ability.

Table 3.08a: Male plants labelled with a pulse of ^{14}C in mid February and sampled 4 weeks later: mobilising ability with respect to the labelled fern expressed as a percentage of ^{14}C recovered.

Plant	Labelled fern	Younger fern	Root sinks					
			all *ex*-bud	new *ex*-fern	new *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-fern
4A	29	23	20	10	3.4	-	3.7	5.6
4B	28	4.2	27	11	2.4	-	0.6	11
4C	24	0.1	21	35	3.5	5.0	0.4	7.9
4D	30	-	23	15	1.1	-	3.9	18
4E	52	-	17	18	2.0	-	2.5	5.0

Table 3.08b: Male plants labelled with a pulse of ^{14}C in mid February and sampled 4 weeks later: competitive ability expressed as a percentage of the ^{14}C recovered/percentage dry weight.

Plant	Labelled fern	Younger fern	Root sinks					
			all *ex*-bud	new *ex*-fern	new *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-fern
4A	3.5	1.9	1.4	1.7	0.6	-	0.2	0.4
4B	5.5	1.1	2.3	1.1	0.2	-	0.4	0.4
4C	5.5	0.14	1.8	2.2	0.2	0.5	0.03	1.0
4D	1.8	-	1.1	1.5	0.4	-	0.4	0.9
4E	2.3	-	1.1	1.7	0.4	-	0.1	0.8

Table 3.08c: Male plants labelled with a pulse of ^{14}C in mid February and sampled 4 weeks later: total dry weight and dry weight expressed as a percentage of total.

Plant	Labelled fern	Younger fern	Roots						Actual dry weight
			all *ex*-bud	new *ex*-fern	young *ex*-old	all *ex*-axillary	old *ex*-old	old *ex*-fern	
4A	8.4	12	15	5.9	6.0	-	16	13	247
4B	8.6	3.8	12	10	14	-	20	9.6	214
4C	4.4	0.6	12	16	20	11	11	7.7	344
4D	16	-	17	10	2.5	-	10	19.0	143
4E	23	-	15	10	5.1	-	22	6.4	145

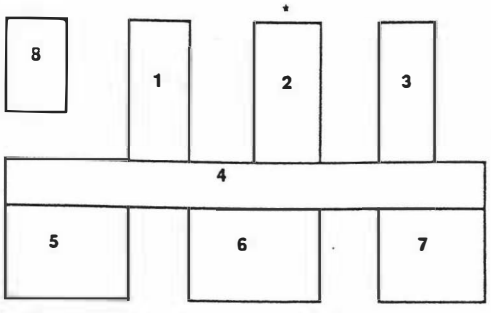
3.3.5 THE EFFECT OF BERRY PRODUCTION ON THE SOURCE-SINK RELATIONSHIPS OF ASPARAGUS PLANTS

Current assimilates

The dry weight of the female plants appeared to be higher than that of male plants near the beginning of berry production (February), while female plants without berries appeared to have a higher dry weight than the other plant types at the end of the first season of berry production (April) (Fig.3.12). The crown dry weight on female plants without berries was almost double that of the female plants with berries. By April, male plants and female plants with berries had a higher percentage of their dry weight in the crown (67%) than female plants with berries (40%). The dry weight of female plants with berries was not significantly lower than that of the male plants, although they tended to have a lower vegetative fern weight, and a lower crown dry weight.

In February, berries were the sink with the highest mobilising ability for current assimilate from the ^{14}C labelled fern in female plants (Fig.3.10. Table 3.09a). The mobilising ability and competitive ability (Table 3.09b) of all other sinks in female plants with berries tended to be low relative to male plants and female plants without berries (Figs. 3.10, 3.11), especially younger fern which was the strongest sink on male plants and females without berries at this time. Within the crown, roots-*ex*-old tended to have the highest mobilising ability with respect to the labelled fern in all plant types and the highest dry weight compared with the other root fractions (Figs. 3.10, 3.11, 3.12; Table 3.09a, b & c). On both male plants and female plants without berries, the roots-*ex*-bud tended to have the highest competitive ability for current assimilates on plants labelled in mid January (Fig.3.11) although their competitive ability was lower than other sinks. However, the dry weight of roots-*ex*-bud was low relative to other root sinks in when sampled in mid February (Fig.3.12), and their mobilising ability, with respect to the labelled fern, appeared to be similar to roots-*ex*-fern on male plants, and lower than roots-*ex*-fern on female plants with and without berries.

Key to figures 3.10 to 3.12



Tissues within plants were divided into sinks as follows:

1. Fern older than the labelled fern (if present)
2. Fern labelled with $^{14}CO_2$
3. Fern younger than the labelled fern
4. Rhizome
5. Roots-*ex*-old: storage roots subtending the portion of the rhizome which supported the previous seasons fern
6. Roots-*ex*-fern: storage roots subtending the portion of rhizome which supports the current season's fern
7. Roots-*ex*-bud: storage roots subtending the portion of the rhizome which contains un-expanded buds
8. Berries - present on female plants only

All data is presented as treatment means, followed by standard error of the mean.
All plants were labelled with $^{14}CO_2$ in mid January.

Summary of treatments:

Treatment	Plant type:	Sampled in:
1	Male	mid February
3	Male	mid April
7	Female with berries	mid February
8	Female with berries	mid April
9	Female without berries	mid February
10	Female without berries	mid April

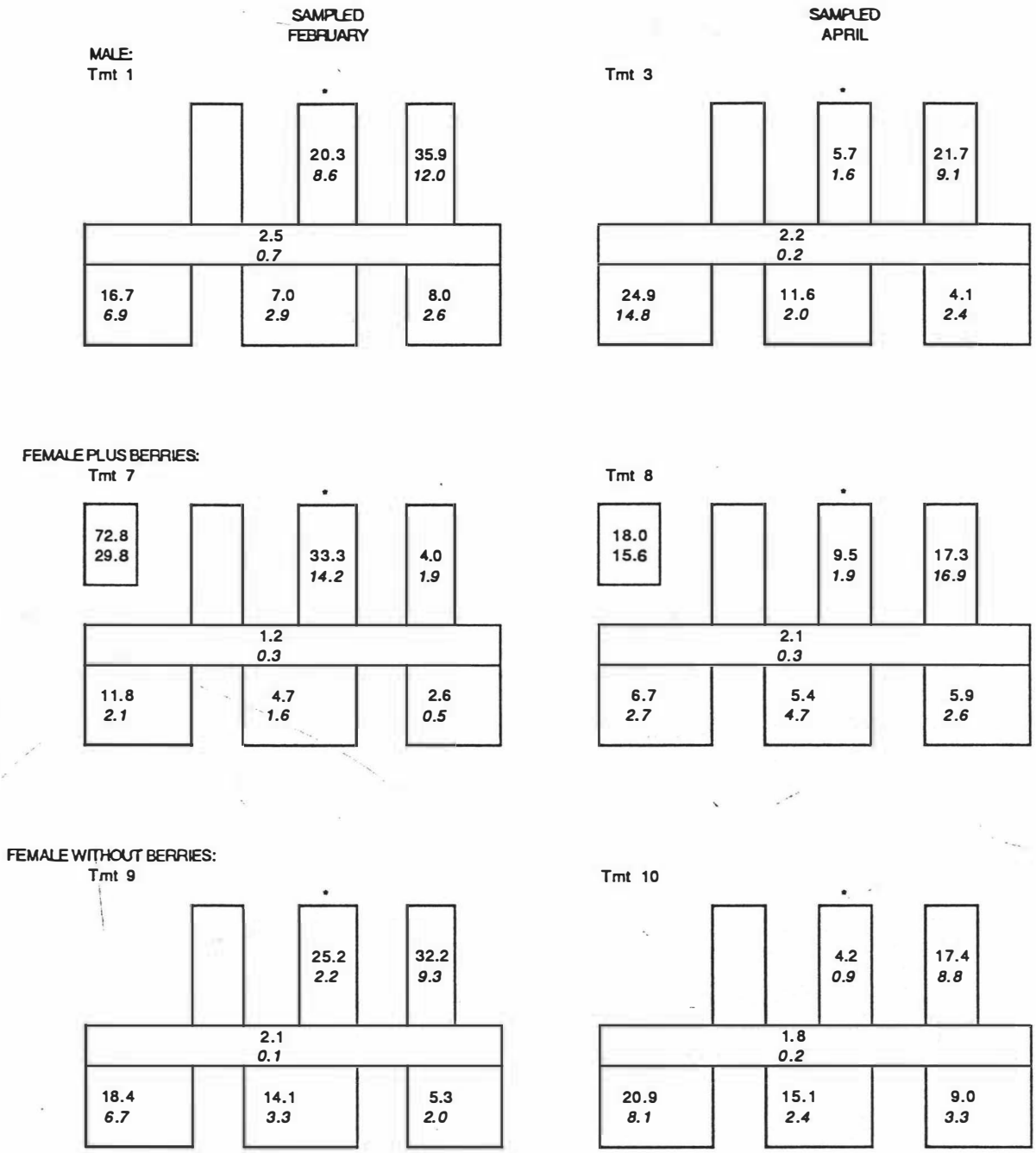


Figure 3.10: Mobilising ability with respect to the labelled fern (^{14}C content, dpm x 10^3) of sinks within male plants, female plants with berries and female plants without berries labelled with a pulse of $^{14}CO_2$ in mid-late January and sampled four and twelve weeks later in mid February and mid April. Standard error of mean is in italics.

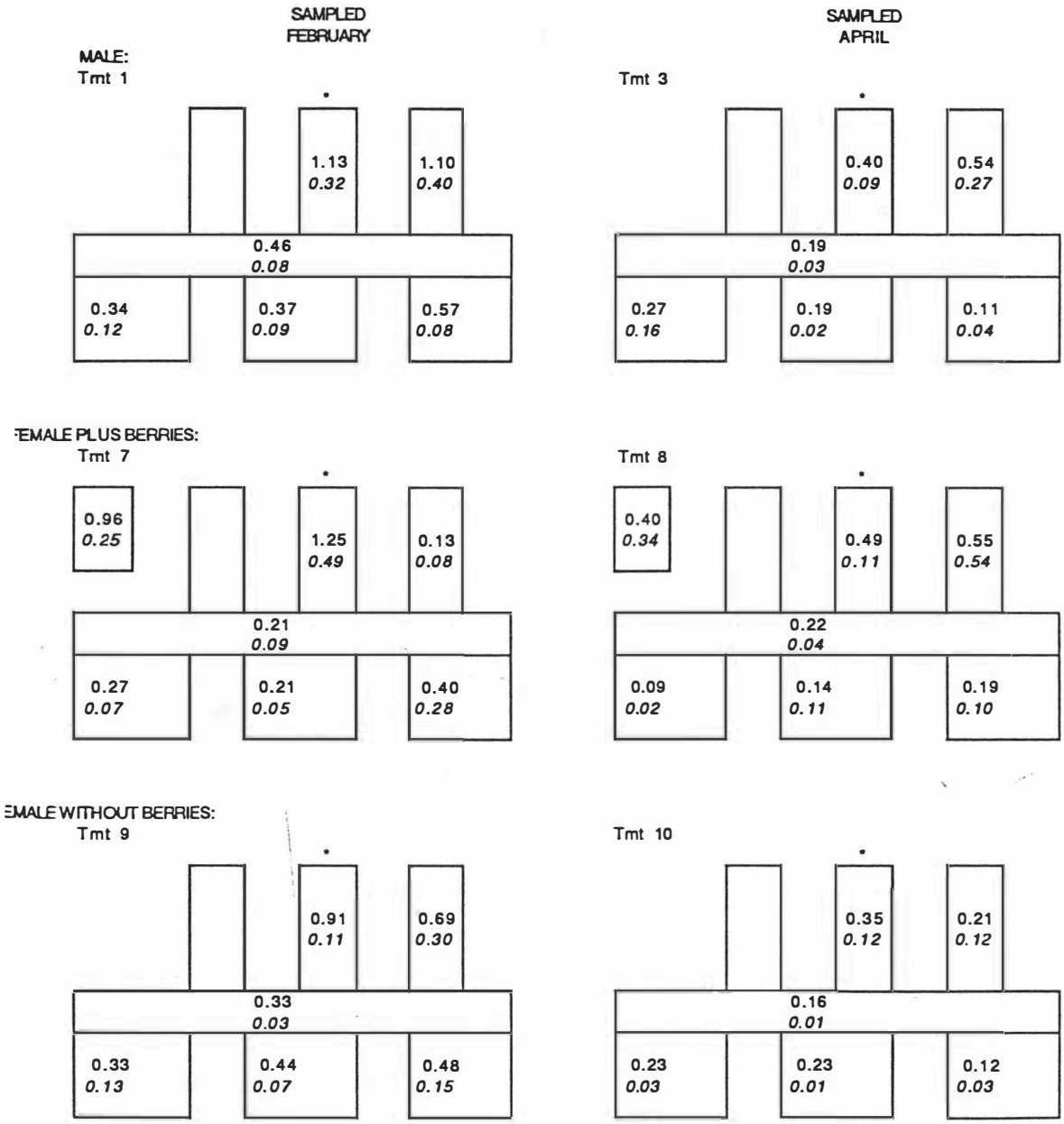


Figure 3.11: Competitive ability (^{14}C concentration, dpm x 10^3/g dry weight) of sinks within male plants, female plants with berries and female plants without berries labelled with a pulse of $^{14}CO_2$ in mid-late January and sampled four and twelve weeks later in mid February and mid April. Standard error of mean is in italics.

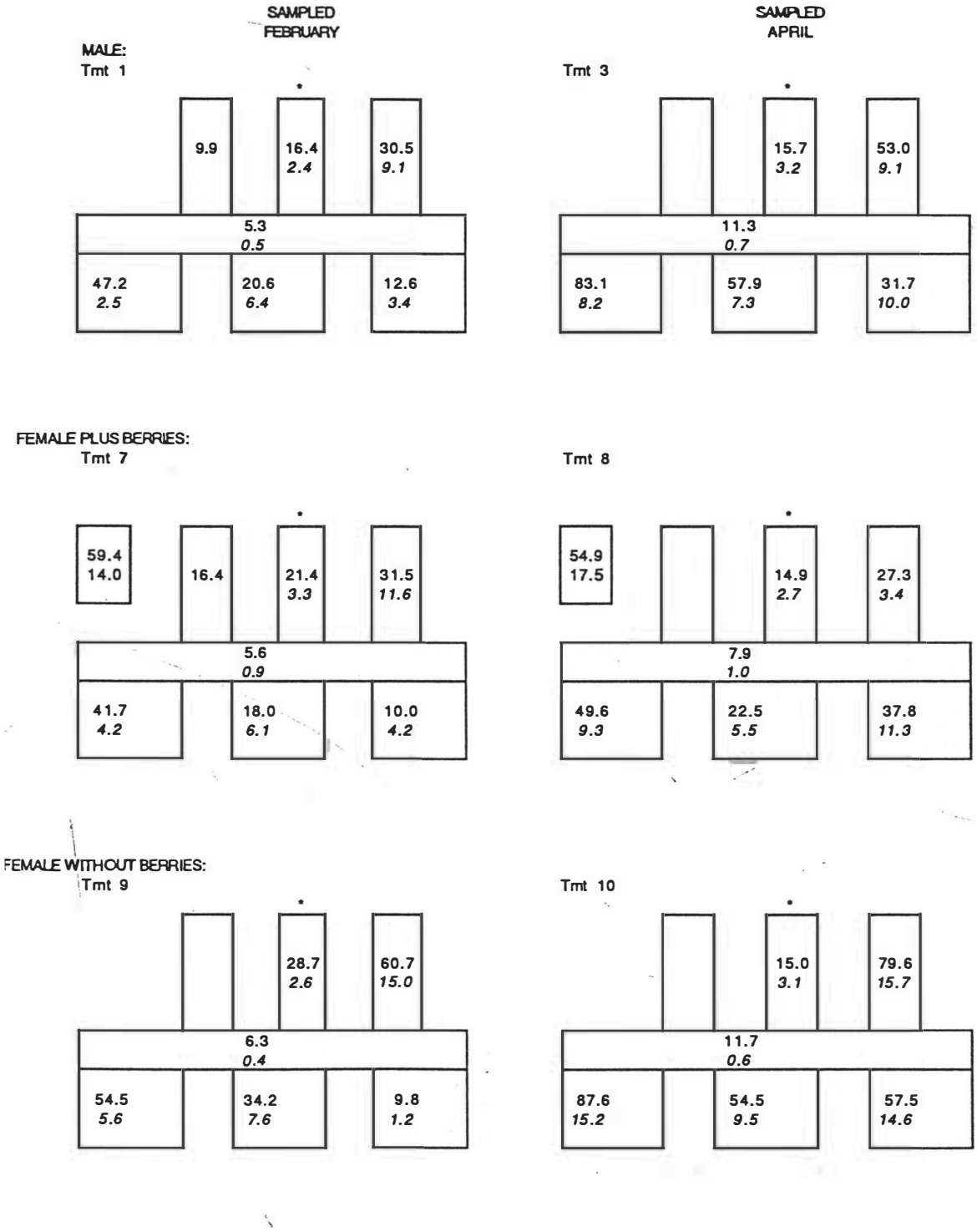


Figure 3.12: Dry weight (g) of sinks within male plants, female plants with berries and female plants without berries labelled with a pulse of $^{14}CO_2$ in mid-late January and sampled four and twelve weeks later in mid February and mid April. Standard error of mean is in italics.

Table 3.09a: Total ^{14}C (dpm x 10^3) recovered from sinks within male asparagus plants, females with berries and females without berries labelled with a pulse of ^{14}C in mid January: differences in mobilising ability with respect to the labelled fern within and between plants. Standard error of mean is in brackets.

Plant type	Male		Female with berries		Female without berries		$LSD_{.05}$ [a] [b]
Date sampled	Feb 18-20	Apr 15-18	Feb 23-25	Apr 20-21	Feb 23-25	Apr 20-21	
Tissue:							
labelled fern	20.3 (8.6)	5.7 (1.6)	33.3 (14.2)	9.5 (1.9)	25.2 (2.2)	4.2 (0.9)	20.5
younger fern	35.9 (12.0)	21.7 (9.1)	4.0 (1.9)	17.3 (16.9)	32.2 (9.3)	17.4 (8.8)	ns
berries [c]			72.2 (29.8)	18.0 (15.6)			
rhizome	2.5 (0.7)	2.2 (0.2)	1.2 (0.3)	2.1 (0.3)	2.0 (0.1)	1.8 (0.2)	ns
roots-*ex*-bud	8.0 (2.6)	4.1 (2.4)	2.6 (0.5)	5.9 (2.6)	5.3 (2.0)	9.0 (3.3)	ns
roots-*ex*-fern	7.0 (2.9)	11.6 (2.0)	4.7 (1.6)	5.4 (4.7)	14.1 (3.3)	15.1 (2.4)	9.3
roots-*ex*-old	16.7 (6.88)	24.9 (14.8)	11.8 (2.1)	6.7 (2.7)	18.4 (6.7)	20.9 (8.1)	ns
$LSD_{.05}$ [a]	20.3	ns	38.0	ns	14.6	ns	
n	4	4	3	2	5	3	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - Main effects significant at P=0.05: Plant type: roots *ex*-fern; sample date: labelled fern

c - Male plants do not form berries; flowers removed from 'females without berries' to prevent berry formation.

Table 3.09b: Concentration of ^{14}C (dpm x 10^3/mg) recovered from sinks within male asparagus plants, females with berries and females without berries labelled with a pulse of ^{14}C in mid January: differences in competitive ability within and between plants. Standard error of mean is in brackets.

Plant type	Male		Female with berries		Female without berries		$LSD_{.05}$ [a] [b]
Date sampled	Feb 18-20	Apr 15-18	Feb 23-25	Apr 20-21	Feb 23-25	Apr 20-21	
Tissue:							
labelled fern	1.13 (0.32)	0.40 (0.09)	1.25 (0.49)	0.49 (0.11)	0.91 (0.11)	0.35 (0.12)	0.76
younger fern	1.10 (0.40)	0.54 (0.27)	0.13 (0.08)	0.55 (0.54)	0.69 (0.30)	0.21 (0.12)	ns
berries [c]			0.96 (0.25)	0.40 (0.34)			
rhizome	0.46 (0.08)	0.19 (0.03)	0.21 (0.09)	0.22 (0.04)	0.33 (0.03)	0.16 (0.01)	0.17
roots-*ex*-bud	0.57 (0.08)	0.11 (0.04)	0.40 (0.28)	0.19 (0.10)	0.48 (0.15)	0.12 (0.03)	0.44
roots-*ex*-fern	0.37 (0.09)	0.19 (0.02)	0.21 (0.05)	0.14 (0.11)	0.44 (0.07)	0.23 (0.01)	0.20
roots-*ex*-old	0.34 (0.12)	0.28 (0.17)	0.27 (0.07)	0.09 (0.02)	0.33 (0.13)	0.23 (0.03)	ns
$LSD_{.05a}$	0.66	ns	0.77	ns	ns	ns	
n	4	4	3	2	5	3	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - Main effects significant at P=0.05:

Plant type: nil

Sample date: labelled fern, rhizome, roots-*ex*-fern, roots-*ex*-bud

c - Male plants do not form berries; flowers removed from 'females without berries' to prevent berry formation.

Table 3.09c: Dry weight (g) recovered from sinks within male asparagus plants, females with berries and females without berries labelled with a pulse of ^{14}C in mid January: differences within and between plant. Standard error of mean is in brackets.

Plant type	Male		Female with berries		Female without berries		$LSD_{.05}$ [a] [b]
Date sampled	Feb 18-20	Apr 15-18	Feb 23-25	Apr 20-21	Feb 23-25	Apr 20-21	
Tissue:							
labelled fern	16.4 (2.4)	15.7 (3.2)	21.4 (3.3)	14.9 (2.7)	28.7 (2.6)	15.0 (3.1)	10.5
younger fern	30.5 (9.1)	53.0 (9.1)	31.5 (11.6)	27.3 (3.4)	60.7 (15.1)	79.6 (15.7)	41.6
berries [c]			59.4 (14.0)	54.9 (17.5)			-
rhizome	5.3 (0.5)	11.3 (0.7)	5.6 (0.9)	7.9 (1.0)	6.3 (0.4)	11.7 (0.6)	2.7
roots-*ex*-bud	12.6 (3.4)	31.7 (10.0)	10.0 (4.2)	37.8 (11.3)	9.8 (1.2)	57.5 (14.6)	33.0
roots-*ex*-fern	20.6 (6.4)	57.9 (7.3)	18.0 (6.1)	22.5 (5.5)	34.2 (7.6)	54.5 (9.5)	28.2
roots-*ex*-old	47.2 (2.5)	83.1 (8.2)	41.7 (4.2)	49.6 (9.3)	54.5 (5.6)	87.4 (15.0)	31.7
$LSD_{.05}$ [a]	14.7	21.1	22.3	26.1	21.6	33.4	
n	4	5	5	5	5	5	

Notes:

a - protected LSD, ns = analysis of variance not significant at P=0.05

b - Main effects significant at P=0.05:

Plant type: rhizome, roots-*ex*-fern, roots-*ex*-old

Sample date: labelled fern, younger fern, rhizome, roots-*ex*-old, roots-*ex*-fern, roots-*ex*-bud

c - Male plants do not form berries; flowers removed from 'females without berries' to prevent berry formation.

Stored assimilates

Between mid February and mid-late April, fern younger than the labelled fern became an active sink for ^{14}C fixed in mid January on female plants with berries as the ^{14}C content of the younger fern appeared to increase between mid February and mid April. The apparent increase in the ^{14}C content of the younger fern was accompanied by an apparent decrease in the ^{14}C content of both roots-*ex*-old and the labelled fern. However, on male plants and females without berries, the ^{14}C content of the younger fern appeared to decrease between mid February and mid April (Table 3.09a).

The dry weight of the rhizome and each of the root sinks increased between mid February and mid April (Table 3.09c), especially on male plants and female plants without berries where the increases were significant at P=0.05. The rhizome was the only sink on female plants with berries which had a significant increase in sink dry weight (P=0.05). The increase in sink dry weight was accompanied by a decrease in competitive ability in all crown sinks except roots-*ex*-old on male plants and female plants without berries. The increase in the dry weight (Fig.3.12) of roots-*ex*-bud on both types of females plants was accompanied by an apparent increase in the ^{14}C content (Fig.3.10). In male plants, the ^{14}C content of roots-*ex*-bud appeared to decrease between February and April.

3.3.6 GROWTH AND SOURCE-SINK RELATIONSHIPS BETWEEN GROWING SEASONS

Male asparagus plants were used to examine the growth of plants and changes in source-sink relationships from one growing season to the next. Plants which were actively growing were labelled with ^{14}C by exposing the youngest fully expanded fern to 190 x 10^3 dpm $^{14}CO_2$ in mid January, as described in Section 3.2.3. Over half of the fixed ^{14}C was lost from the plant, probably via respiration in the first four weeks following labelling (Fig.3.13), and 17% of the ^{14}C which had been fixed was recovered from the crown of the labelled rhizome four weeks after labelling. There was no change (at P=0.05) in the ^{14}C recovered from the crown throughout the experiment, from mid February (four weeks after plants exposed to ^{14}C) to early November (40 weeks after plants exposed to ^{14}C) (Fig.3.13). The ^{14}C recovered from plants labelled late January and sampled late April (Fig.3.13) was extremely variable, as was the rate at which plants senesced.

The dry weight of the crown (of the labelled rhizome) increased from mid February to late April (Fig.3.14). Fern dry weight increased slowly from mid February to mid March, and then decreased. The dry weight of both fern and crown decreased between late April and mid winter (Fig.3.14). Over the same time, the number of roots on the rhizome halved (Fig.3.15). This decrease in crown dry weight and root number was not accompanied by an increase in the loss of ^{14}C from the crown.

No major change in plant dry weight i.e, crown plus spears and new ferns, was recorded from spear emergence until the end of the experiment (Fig.3.14). Crown dry weight and root number (Fig.3.15) decreased very little during harvest, and then decreased rapidly during fern establishment.

3.4 DISCUSSION

3.4.1 DISTRIBUTION OF ^{14}C BETWEEN RHIZOMES

Within asparagus, the basic unit for carbohydrate production and allocation was a rhizome and it's attached, developing axillary rhizomes as over 90% of the ^{14}C recovered was normally present in these tissues (Fig.3.06). The mobilising ability of axillary rhizomes was high relative to secondary rhizomes with a similar dry weight, indicating that they had a much higher competitive ability for the ^{14}C than rhizomes with ferns. The relatively low mobilising and competitive ability of secondary rhizomes for the labelled assimilate may be due to the presence of alternative assimilate sources i.e, the ferns on the secondary rhizome. This suggests that a rhizome becomes 'independent' of it's parent after it has developed fern i.e, after it has changed from a carbohydrate sink to a potential carbohydrate source. Thus developing axillary rhizomes can rapidly become relatively autonomous. This change in source-sink relationships as an axillary rhizome develops is similar to that which occurs in the development of grass tillers (Watson and Casper 1984). In many grass species, a developing tiller changes from almost complete dependence on the parent for assimilate, to a situation where the little translocation which occurs is mostly from the 'daughter' tiller to the parent.

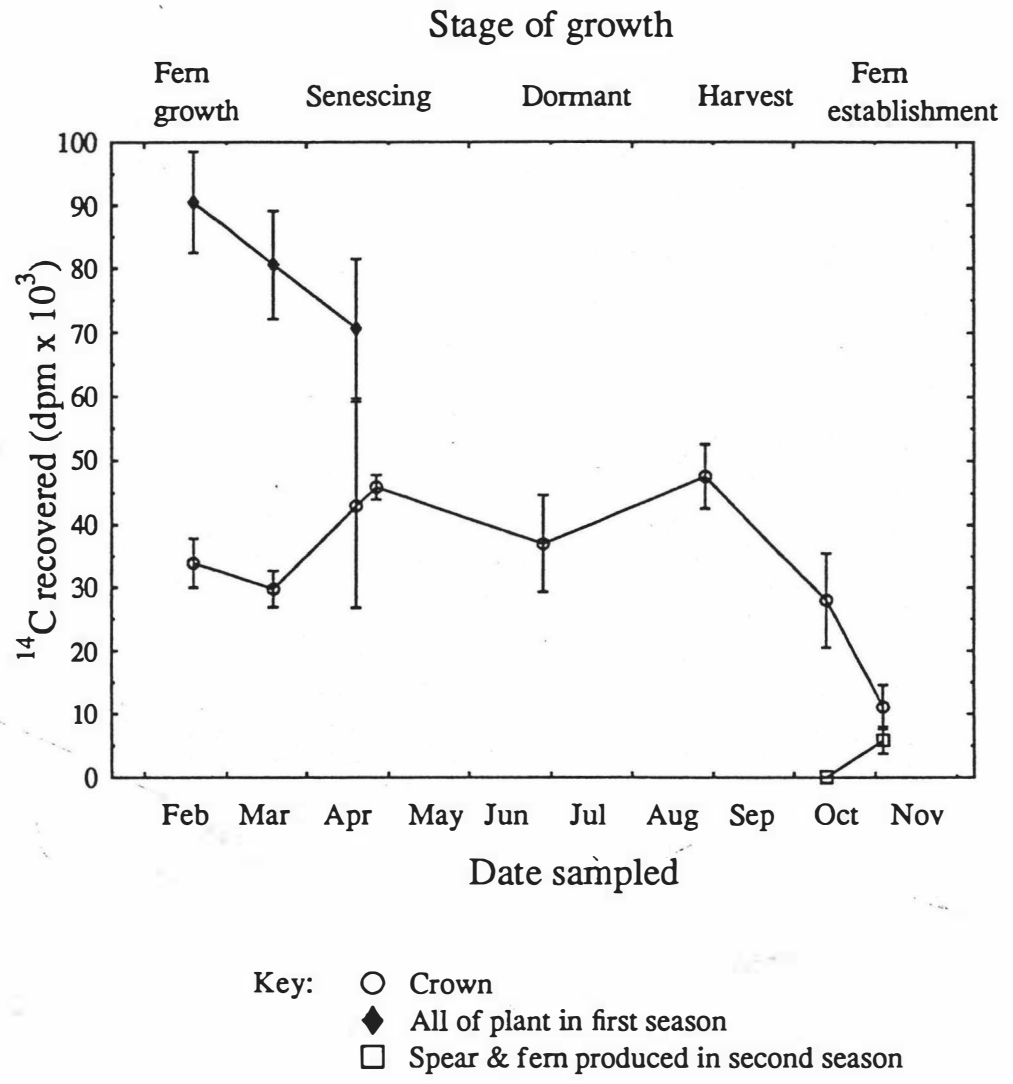


Figure 3.13: Distribution of ^{14}C in male asparagus plants following labelling with 190 x 10^3 dpm in January. Plants in first 3 samplings were labelled 2 weeks earlier than remaining samples. Bars represent twice standard error of mean.

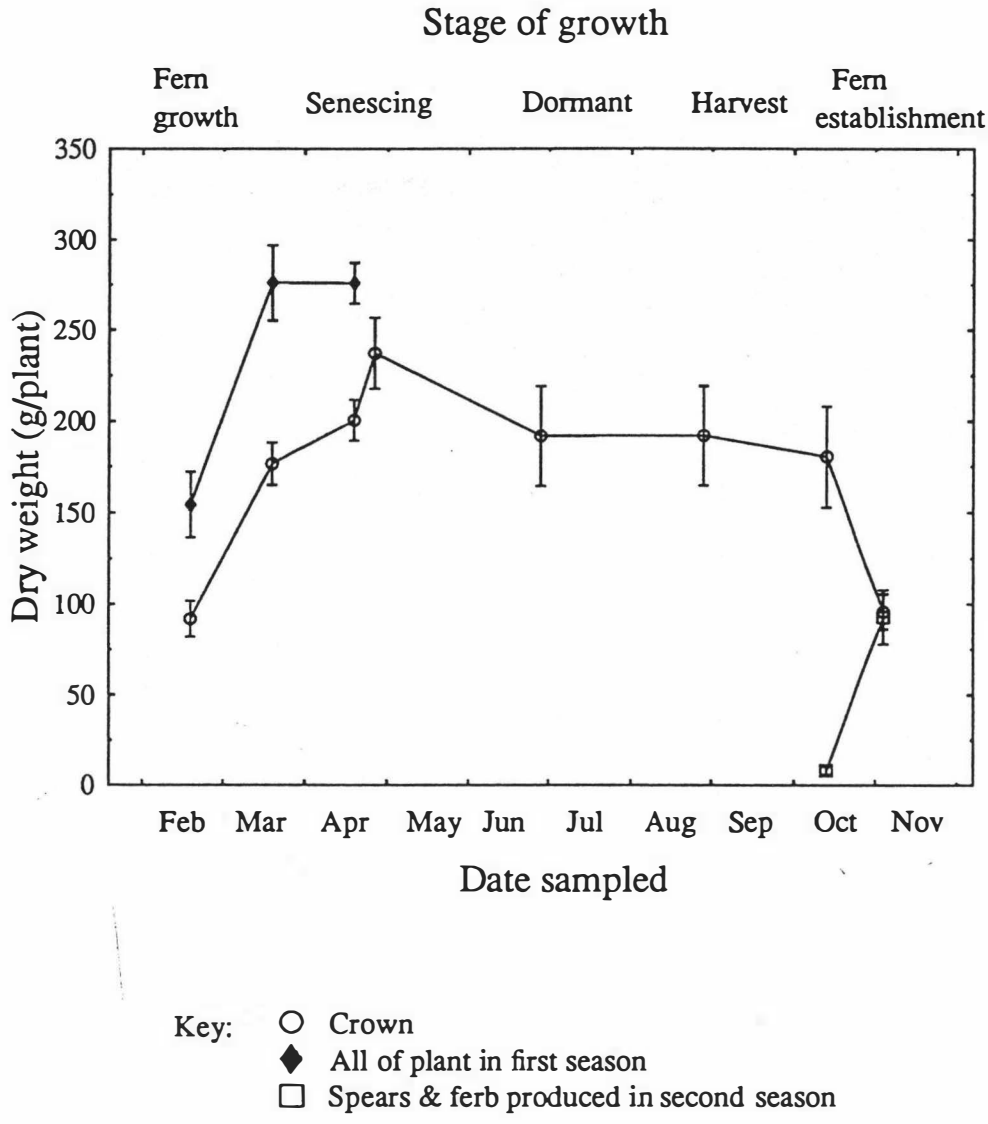


Figure 3.14: Distribution of dry weight in male asparagus plants following labelling with 190 x 10^3 dpm in January. Bars represent twice standard error of mean.

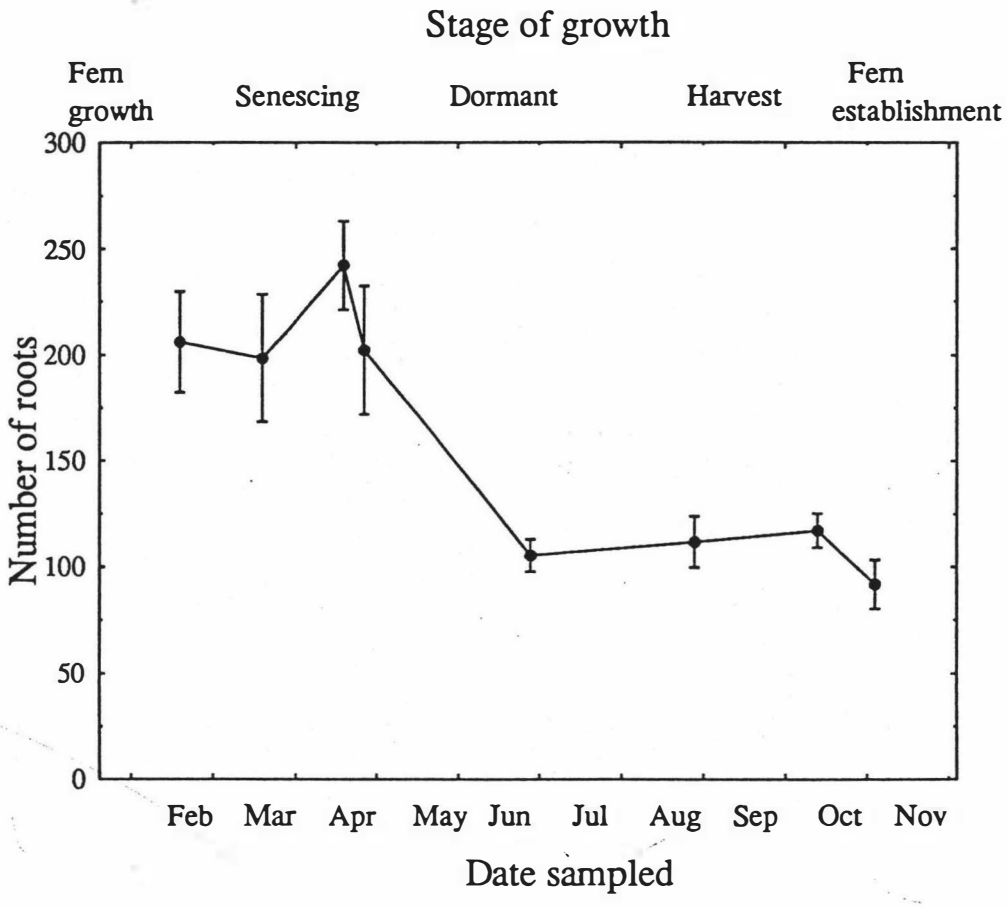


Figure 3.15: Number of roots in main rhizome of male asparagus plants following labelling with 190 x 10^3 dpm in January. Bars represent twice standard error of mean.

However, each rhizome and it's developing axillary rhizomes were not completely autonomous as some $^{14}CO_2$ was translocated to rhizomes with fern i.e, to secondary rhizomes. Unlike the axillary rhizomes, mobilising ability of secondary rhizomes, with respect to the labelled fern, was not related to rhizome dry weight. This indicates that the secondary rhizome obtained most of it's assimilates from it's own fern. Therefore, the amount of assimilate imported from the primary rhizome was probably influenced by the assimilate requirements of the primary rhizome and proximity of the secondary rhizome to the labelled fern.

Asparagus plants are readily able to form new, relatively autonomous rhizomes. Eighty percent of the plants in this experiment had developed at least one axillary or secondary rhizome during their 18 months of growth, while 25% of the plants had developed at least two axillary and/or secondary rhizomes (Table 3.02, Fig.3.05). This amount of development would probably require 30 months in the field as the plants in this experiment were similar in size to plants in their third season of growth. This indicates that the number of independent rhizomes could double in the first three years of growth. Competition for space, water and nutrients would probably prevent this rate of expansion throughout the life of the crop.

Future research in this area should separate rhizomes based on the presence or absence of fern at both the time of labelling and the time of sampling, rather than rhizome length at sampling as in this experiment. Sampling a larger number of plants over a short time frame (i.e. two to four weeks rather than eight or twelve weeks) should clarify the time of transition between a 'meristematic' sink totally dependent on the parent rhizome, and an independent sink producing it's own assimilate.

3.4.2 SEASONAL CHANGES IN RESPIRATORY REQUIREMENTS

Respiration appeared to have the strongest demand on current assimilate because plants lost approximately 50% of the fixed ^{14}C within four weeks (Fig.3.07, Table 3.04a). This level of respiratory loss is similar to that reported for grasses (Danckwerts and Gordon 1987, Gifford et al. 1984, Robson 1980). The respiratory requirements of asparagus appear to change during the year.

Changes in the ^{14}C recovered suggest that respiratory requirements were higher in January-February than in February-March (Table 3.04a, Table 3.06a, Fig.3.13). The high respiratory requirement could have been associated with a period of rapid fern growth.

The mean number of ferns per plant increased from 3.07 (standard error of mean, SE = 0.27) in mid January to 3.70 (SE = 0.21) in mid February, while crown dry weight increased from mid-February to mid-April (Fig 3.14). The increase in fern number was probably associated with an increase in fern dry weight, but unfortunately dry weight was not recorded in January. In other reports on field grown asparagus, an increase in fern dry weight preceded the rapid increase in root dry weight (Fisher 1982, Dufault and Grieg 1983, Haynes 1987). Data from this experiment suggest that respiratory requirements decreased when the plant changed from a phase of predominantly fern growth to a phase of predominantly root growth. Thus the production of a carbohydrate source (fern tissue) appeared to require more current assimilate than the production of carbohydrate storage tissue (roots). However, the apparent difference in respiratory requirement may be due, partially or entirely, to changes in air temperature rather than ontogenetic development.

The plants in this experiment were grown in outdoor conditions where air temperatures during March are cooler than those in January and February (see Table 6.01), and this would have reduced respiratory requirements. Respiratory requirements appeared to change during senescence also.

The low recovery of ^{14}C from plants labelled in mid March (Figs.3.07, 3.09) may have been due to an increase in respiratory requirements associated with senescence. The fern was beginning to senesce when the plants were sampled in late April. Although the plants appeared capable of producing photoassimilates until mid April, the plants appeared to also use stored assimilate for respiration.

Stored assimilate appeared to be respired throughout this experiment (Figs. 3.07, 3.13, Table 3.06a). The rate at which ^{14}C fixed in mid January was lost was greater during the first 4 weeks following fixation, and during fern establishment than at other times (Fig 3.13). The rate at which stored assimilate was utilised did not change from mid February to the end of spear harvest, although an alternative source i.e, actively photosynthesising fern, was present from February to at least the end of March. Unlike current assimilates, there was no increase in utilisation of stored assimilate during senescence.

Plant sex has a major effect on the use of assimilates within asparagus. The ^{14}C translocated to the berries of female plants appeared to be initially 'protected' from respiratory loss. Four weeks after labelling, more ^{14}C was recovered from female plants with berries than from plants without berries. However, the berries contained over 50% of the ^{14}C recovered from the plant (Fig.3.10), thus the ^{14}C recovered from vegetative

tissues of female plants with berries was less than that recovered from male plants or females without berries (Table 3.09a).

3.4.3 CHANGES IN SINK PRIORITIES FOR CURRENT ASSIMILATE: SUMMER-AUTUMN

Two criteria must be considered when assessing sink priority: which sinks have the highest mobilising ability with respect to the labelled fern (total ^{14}C), and which sinks have the highest competitive ability (concentration of ^{14}C). Both mobilising ability and competitive ability of the fern younger than the labelled fern and the various crown tissues changed during this experiment. Interpretation of the results of this experiment must be considered tentative due to the variability of the results and lack of statistically significant differences at P=0.05, which was partly due to the unexpected variability in the morphology of the plants. The results have been interpreted in the context of commonly accepted theories of source-sink relationships outlined in Section 3.1.

Sink priorities for current assimilate within male plants

The competitive ability of young plant tissue was higher than that of older tissue in January-February. At this time, fern younger than the labelled fern tended to be the strongest and most active sink for ^{14}C labelled assimilate within male plants (Figs 3.07 & 3.08 Treatment 1, Table 3.06). This was similar to results obtained with other plants. Shoots of plants can have a high competitive ability against roots when the assimilate supply is low (Ryle 1972). Within the asparagus crown, roots-*ex*-bud had a higher competitive ability than the other two fractions (Fig.3.07, Treatments 1,4,6). However, their mobilising ability was relatively low because of their low dry weight (Fig.3.09). Based on mean mobilising ability for ^{14}C labelled assimilate, the labelled fern, the sink priority (Fig.3.07, Table 3.06a) within plants in January-February was younger fern, roots-*ex*-old, then roots-*ex*-fern and roots-*ex*-bud, followed by the rhizome. However, these conclusions are based on mean values from plants, and sink priorities varied between plants.

If plant growth was restricted by damage to ferns, as in plant 1D, the competitive and mobilising abilities of young fern for the ^{14}C labelled assimilate was very low, while the competitive and mobilising abilities of the old roots-*ex*-old was very high. This plant had lost two current seasons ferns, probably by wind damage, before it was labelled with

$^{14}CO_2$. Thus, the priority of a plant which had suffered fern damage appeared to be replenishment of the carbohydrate used in the production of the fern, rather than the production of new tissues for assimilate production or storage. Sink priorities also changed over time.

The sink priorities of plants labelled with ^{14}C in mid February differed from plants labelled four weeks earlier. Most of the ^{14}C which was translocated out of the labelled fern was recovered from the roots of the plants. This contrasted with plants labelled in mid January when most was recovered from the younger ferns (Table 3.04a). This change in ^{14}C distribution was associated with a change in dry matter partitioning by the plants. Plants labelled in mid February produced less new fern than plants labelled in mid January. The dry weight of the root sinks, especially roots-*ex*-bud increased during February-March (Table 3.04c). The morphological variation of the plants increased: two of the five plants had no younger fern, while only one plant had a similar percentage dry weight in younger fern to plants labelled in mid January (Tables 3.09c, 3.07c). The decrease in the production of young fern and increase in mobilising ability (for ^{14}C labelled assimilate) of the crown was accompanied by an apparent decrease in the respiratory losses of current assimilate.

There was also a change in sink priority within the roots of male plants between January-February and February-March. In February-March, roots-*ex*-fern and roots-*ex*-bud had a higher mobilising ability for ^{14}C labelled assimilate than roots-*ex*-old, which was the reverse of the sink priorities in January-February (Table 3.04a). The competitive ability of roots-*ex*-fern and roots-*ex*-bud tended to increase (Table 3.04b) and was higher than roots-*ex*-old whose competitive ability had decreased since January-February. These sink priorities were retained between mid March and mid-April (Fig.3.07, Table 3.04a). The dry weight of all root sinks appeared to increase between January-February and February-March, while only roots-*ex*-bud increased between February-March and March-April. However, these apparent changes in the competitive and mobilising abilities of sinks within the rhizome may also be due, at least partially, to changes in the age rank of the labelled fern.

In this experiment, $^{14}CO_2$ was applied to the youngest, fully expanded fern in mid January, mid February and mid March. The mean age rank of ferns on male plants labelled in mid January was 1.53 (SE = 0.19), while it increased to 3.16 (SE = 0.40) in mid February and 3.0 (SE = 0.0 i.e, all samples equal) in mid March. Although there was very little change in the physical distance between the base of a fern and each of the root sinks, the presence of fern older than the labelled fern could have altered the

translocation of the ^{14}C labelled assimilate, as these fern would have been active as assimilate sources. There were too few plants of each age rank present to be able to separate the effects of fern position from time of labelling.

Sink priorities based on root age also changed, and were less likely to be affected by fern position than partitioning between the parts of the rhizome. The mobilising ability and competitive ability of new root for ^{14}C labelled assimilate was greater than that of the old root subtending the same portion of the rhizome in January-February (Table 3.07a & b). The apparent increase in the competitive ability of the old root in February-March (Table 3.08a & b) was associated with a decrease in fern growth and a decrease in the rate at which root number was increasing. This suggests that as the meristematic activity associated with the production of new fern and new roots decreased, the competitive ability of new fern and new roots decreased and that of the old root increased. This increase in mobilising and competitive ability of old root may have been partly due to an increase in the percentage of root tissues classified as old, and the inclusion of current seasons tissue in the 'old' fraction. It may also have been due to meristematic activity associated with the elongation of existing roots. Growth rings caused by the cessation and resumption of growth, and changes in the colour of storage root epidermis were observed during sampling, and indicate that existing roots can resume longitudinal growth.

Relatively high mobilising ability is a characteristic of sinks which control partitioning according to the source/sink model, while low mobilising ability is characteristic of sinks which control partitioning according to the sink model. Sinks which utilise the source/sink model tend to have apoplastic unloading from the phloem and includes meristematic and reproductive sinks. Sinks which utilise the sink model tend to have symplastic unloading and includes many storage tissues (Patrick 1988). The relative mobilising ability of sinks within an asparagus rhizome, and their seasonal changes indicate that partitioning into young fern was probably controlled via the source/sink model, while storage roots used both models. The sink model could have been used while the carbohydrate content of existing roots was being restored in early summer, while the source/sink model could have been used firstly, by new roots initiated from the rhizome, and secondly by old roots which had resumed elongation.

In plants labelled in mid February, less ^{14}C appeared to be translocated out of the labelled fern on than in plants labelled in mid January (Fig.3.07, Table 3.04a). This may have been due to the change from translocation to primarily meristematic sinks i.e, young, developing fern and new roots, to primarily expanding storage sinks i.e, increase

in size of existing roots. Alternatively, more ^{14}C may have been incorporated into the structure of the labelled fern.

There was too few plants with axillary rhizomes to clarify their role in source/sink relationships. Roots-*ex*-axillary can be a very strong sink, as in plant 1C, where the mobilising and competitive ability of the roots-*ex*-axillary were similar to roots-*ex*-bud, and considerably higher than that of the other root sinks (Table 3.07a & b). Thus developing axillary rhizomes appeared to control partitioning via the source/sink model.

Effect of berry production on sink priorities for current assimilates

In asparagus, as in many other plants, the mobilising ability and competitive ability of reproductive sinks was much higher than vegetative sinks (Figs 3.10, 3.11, Table 3.09a, b). Berries were the dominant sink in female plants in January-February. The high competitive ability of the berries for the ^{14}C labelled assimilate indicate that partitioning to them was controlled by the source/sink model, which is typical of reproductive sinks. The presence of berries reduced the mobilising ability and competitive ability of all other sinks, especially younger fern and roots-*ex*-bud i.e, especially on the sinks which controlled partitioning via the source/sink model. Based on mobilising ability for the ^{14}C labelled assimilate, the sink priorities for current assimilate in female plants with berries was berries, roots-*ex*-old, young fern and roots-*ex*-fern, followed by roots-*ex*-bud and the rhizome. In female plants without berries, the priority within the crown sinks followed the same pattern; however, like male plants, younger fern has a higher priority than any of the crown sinks.

The relatively high priority of roots-*ex*-old on females with berries was similar to the pattern on a male plant which had lost two of it's three ferns (plant 1D, Table 3.07). This suggests that a similar mechanism was operating in both situations to ensure plant survival i.e, the replenishment of carbohydrate reserve within old storage tissue had a higher priority than the production of new fern or root tissue. This could have been triggered by a low crown carbohydrate content, and may have occurred in other plants, before the ^{14}C label was applied and thus not been detected. However, plant sex may also have had an effect. The priorities within the crown of female plant without berries was the same as in females with berries, although the younger fern had a higher priority than any of the crown sinks. Despite these differences, there were some similarities in partitioning within crowns.

Within the crowns of all plant types, roots-*ex*-bud appeared to have the highest competitive ability for current assimilate (Fig.3.11, Table 3.09b). However, roots-*ex*-old appeared to have the highest mobilising ability, with respect to the labelled fern, in all plant types as their dry weight was higher than the other root fractions (Table 3.09a,c).

3.4.4 TRANSLOCATION OF STORED ASSIMILATE: SUMMER-AUTUMN

Seventeen percent of the ^{14}C which had been fixed by male plants in mid January was recovered from the crown of the labelled rhizome four weeks after labelling. There was no translocation into the crown between mid February and mid March although crown dry weight was increasing (Fig.3.13, Fig.3.14), implying that assimilate imported by the crown between mid January and mid March was recently fixed. Further translocation of ^{14}C into the crown occurred between mid March and mid April when fern dry weight was decreasing. Thus some of the ^{14}C initially translocated to the fern of male plants was remobilised and translocated into the crown during senescence.

The presence of berries had a major effect on the translocation of stored assimilates. In the female plants without berries, as in male plants, some of the ^{14}C initially translocated to the fern in January-February was remobilised and translocated to the crown between mid February and mid April (Table 3.09c, Fig.3.12). However, on females with berries, fern younger than the labelled fern became a very strong and very active sink for ^{14}C fixed in mid January (Table 3.09a, b). Thus the production of berries appeared to delay the development of younger fern as a strong sink. The ^{14}C translocated to the younger fern in female plants with berries appeared to come from the labelled fern, and possibly from roots-*ex*-old (Table 3.09a)

The decrease in ^{14}C recovered from berries probably contributed to the loss in ^{14}C from the female plants with berries rather than to the redistribution of ^{14}C within the plants (Fig.3.10) as it is unlikely the assimilate would be translocated from the berries to the fern. By mid April, the berries were very ripe. Many had fallen from the fern and were rotting. Birds were observed feeding on the berries and would also have contributed to the decrease in ^{14}C recovered from the berries. Future work on source-sink relationships or carbohydrate budgets should enclose the plants in bird-proof netting !

Changes in the ^{14}C content and dry weight of the sinks within the crown between mid February and mid April also appeared to be influenced by the presence of reproductive sinks. The dry weight of all crown sinks tended to increase between mid February and

mid April (Fig.3.12, Table 3.09c). The crown dry weight on male plants and females without berries doubled between February and April, while that of female plants with berries increased by only 56%. In male plants, the increase in dry weight was associated with translocation of both stored and current assimilate from the fern to the crown.

The sink priorities for the stored assimilate differed from that of the current assimilate: roots-*ex*-old and roots-*ex*-fern had the highest mobilising ability for ^{14}C labelled stored assimilate while roots-*ex*-bud had the highest mobilising ability with respect to the labelled fern for current assimilate (Table 3.09a). This may have been due to differences in the proximity of the labelled fern.

In female plants without berries, roots-*ex*-bud appeared to be the major sink for stored assimilate mobilised from the fern. Current assimilate was probably also being translocated to all root sinks on female plants without berries as the dry weight of all sinks, especially roots-*ex*-bud, tended to increase. However, in female plants with berries, there was no net increase in the ^{14}C content of the roots (Table 3.09a, Fig.3.12) which suggests that the increase in crown dry weight between February and April was due to mobilisation of current assimilate with no net contribution from assimilate stored in the fern (Table 3.09c, Fig. 3,12). The only sink which increased in dry weight was roots-*ex*-bud. This was accompanied by an apparent increase in the ^{14}C content of roots-*ex*-bud, and a decrease in ^{14}C content of roots-*ex*-old and labelled fern, i.e, ^{14}C appeared to be remobilised from roots-*ex*-old to roots-*ex*-bud between mid February and mid April.

Unlike both types of female plants, the ^{14}C content of roots-*ex*-bud on male plants appeared to decrease between mid February and mid April (Table 3.09a). Some of this may have been remobilised to other sinks, but it may also have been lost in respiration as the sink dry weight increased (Table 3.09c). This difference in translocation of ^{14}C to or from roots-*ex*-bud was accompanied by differences in the percentage dry weight of roots-*ex*-bud and roots-*ex*-fern.

In mid February, there was no significant difference in the proportion of dry weight in roots-*ex*-bud: it comprised 9 to 15% of crown dry weight. By mid April, roots-*ex*-bud comprised 35% on female plants with berries, 29% on female plants without berries and only 18% on male plants. The percentage of crown dry weight in roots-*ex*-old was similar (41-45%) in all plant types, thus there was a difference in the dry weight of sinks described as roots-*ex*-fern and roots-*ex*-bud in male and female plants, with female plants apparently partitioning more assimilate into roots-*ex*-bud. This may be due to the

length of underground stem tissue associated with each root fraction. Female plants with berries produced less fern than male plants, and thus the length of stem associated with roots-*ex*-fern would be less than on male plants.

3.4.5 GROWTH AND SOURCE-SINK RELATIONSHIPS FROM SENESCENCE TO SPEAR EMERGENCE

The dry weight of both fern and crown of male plants tended to decrease between late April and mid winter (Fig.3.14). The decrease in fern dry weight was due senescence. The decrease in crown dry weight was accompanied by a large decrease in the number of roots on the labelled rhizome (Fig.3.15). Unlike earlier samplings, no new roots were present on the plant in June, implying that the decrease in root weight and number was due to no new roots developing to replace those lost by decay. The decrease in crown dry weight between late April and mid winter was not accompanied by a change in the ^{14}C content of the crown (Fig.3.13). Crown dry weight then remained constant until spear emergence in August (Fig.3.14).

3.4.6 GROWTH AND SOURCE-SINK RELATIONSHIPS FROM SPEAR EMERGENCE TO FERN ESTABLISHMENT

Plant dry weight did not change during spear harvest or fern establishment following spear harvest which implied that carbohydrate losses due to the respiration requirements of growing spears and fern were matched by assimilate production (Fig.3.14). Crown size (dry weight and root number) (Figs. 3.13, 3.15) decreased very little during harvest, and then decreased rapidly during fern establishment. This supports previous work which indicates that the establishment of fern following harvest utilised more storage carbohydrate than harvest itself (Scott et al. 1939, Shelton and Lacy 1980). The data here indicate that this difference was probably due to the transfer of more dry matter from the crown to the fern than to the harvested spears.

In this experiment, a mean decrease of 12 grams in crown dry weight supported a harvest of 8 grams per plant over of six week period. However, during the 3 weeks of fern establishment, an 84 gram decrease in crown dry weight supported a 76 gram increase in fern dry weight. The loss of 4 grams per plant during harvest was close to a 30% respiratory loss; however, the lower respiratory loss during fern establishment (9.5%) suggests that the fern was producing some assimilate during establishment.

3.4.7 EFFECT OF PLANT SEX ON DRY MATTER PRODUCTION

Crown dry weight in female plants without berries was at least as high as that of male plants indicating that the widely reported smaller crowns and reduced vigour of female plants is probably due to the sink activity of berries (Fig.3.12, Table 3.09c). By mid April, berries comprised over 25% of plant dry weight on female plants with berries. This experiment may underestimate berry dry weight as many berries had abscised by mid April; birds were observed feeding on the berries; and the ^{14}C content of the berries had decreased (which is unlikely to be due to translocation).

Female plants appeared to be more efficient at producing dry matter than male plants. In this experiment, female plants which had never produced berries consistently tended to have a higher dry weight than male plants (Fig.3.12). Preventing berry formation could greatly increase the yield and longevity of an asparagus crop as removing berries increased plant dry weight by over 40% and crown dry weight by almost 80% in April. Thus removing berries resulted in an increase in photosynthetic tissue and in the dry matter produced by the plant, not just a redistribution of assimilate. However, the removal of berries appears to decrease the net efficiency of assimilate production: the ratio of sink dry weight (crown and berries) to photosynthetic dry weight (fern) in female plants with berries is 4.1, while in female plants without berries it was 2.2 and in male plants it was 2.7. Thus dry matter production, and presumably photosynthesis in asparagus appeared to be limited by sink demand.

3.4.8 METHODOLOGY USED IN THIS EXPERIMENT

Interpretation of the results of this experiment must be considered tentative due to the variability between plants and the general lack of statistical significance (at P=0.05) between treatment means. This was partly due to the unexpected variability in the morphology of the plants. Blocking plants on the basis of fern vigour at the beginning of the experiment did not allow determination of the number of rhizomes present, or of the relationship between them. In this situation, five replicates was not enough to determine statistically significant treatment effects.

Plant morphology should be treated as a separate variable in future work on source-sink relationships. This would also allow better discrimination between seasonal changes and the effects of plant size and morphology, e.g. the transition between predominantly fern growth in mid summer, to predominantly root growth in late summer/early autumn; and the time at which an axillary rhizome becomes independent of it's 'parent'.

In this experiment, applying $^{14}CO_2$ to the youngest, fully expanded fern at different times of the year confounded the effects of fern position and time of labelling. The labelled fern varied from the oldest to the fourth oldest. Standardising the fern to which the label is applied, and/or systematic removal of selected ferns would improve the confidence of data describing the source-sink relationships between fern and various storage root sinks. The rhizome could also be subdivided into smaller sinks, e.g. the roots physically closest to each fern, without making the samples too small to handle. However, this separation would be fairly arbitrary as the storage roots are closely packed on the rhizome and there is no clear association between a fern and specific roots.

3.5 CONCLUSIONS

Within an asparagus plant, the basic unit for carbohydrate production and allocation was a rhizome, and it's attached, developing axillary rhizomes. A rhizome became independent of its 'parent' very soon after it had developed fern. Data presented here indicate that the number of independent rhizomes could double during the first three years of growth in the field. Respiratory requirements, and source-sink relationships within a rhizome changed in response to the seasonal growth cycle.

Respiration of current assimilate appeared to decrease between January-February and February-March. This coincided with, firstly, a change from a phase of predominantly fern growth to a phase of predominantly root growth, and secondly, to a decrease in air temperatures. The interactions between these three factors could not be separated in this experiment. There was an increase in respiration of current assimilate during March-April i.e, the early stages of senescence.

Sink strength and activity were determined from the mobilising ability and competitive ability for ^{14}C labelled assimilate after the youngest mature fern on plants was pulse labelled. The storage roots on asparagus retained their function as carbohydrate sinks for at least one year after they were produced. Within male plants, the meristematic sinks present in January-February, young fern and roots-*ex*-bud, had a high competitive ability. Young fern were a stronger and more active sink than the entire storage root system. Within the rhizome, roots-*ex*-old had the highest mobilising ability, but a relatively low competitive ability for the ^{14}C labelled assimilate. In contrast, roots-*ex*-bud had a high competitive ability, but a low mobilising ability due to their low dry weight.

The change in source-sink relationships between January-February and February-March appeared to be due to ontogenic development. By February-March, the roots had become a stronger sink than the younger fern. Within the rhizome, roots-*ex*-bud and roots-*ex*-fern had become the strongest and most active sinks for the ^{14}C assimilate, and their dry weight increased. Although the competitive ability of the roots-*ex*-bud and roots-*ex*-fern increased, it was less than that of the younger fern in January-February, which may be why less ^{14}C was translocated from the labelled fern than in January-February.

In asparagus, as in many other plants, the mobilising and competitive ability of a reproductive sink was higher than any vegetative sinks within female plants. The mobilising and competitive ability of younger fern was depressed compared to plants without berries, and was lower than that of the roots within the female plants. Berries appeared to delay ontogenic development within vegetative tissues as the young fern of female plants appeared to become a strong sink later in summer than on plants without berries.

In male plants, both current and stored assimilate were translocated to the crown during senescence. In female plants with berries, no translocation of stored assimilate to the crown was detected between February and April.

There was little change in crown dry weight or ^{14}C content between senescence and the establishment of fern following spear harvest. The establishment of fern following harvest utilised more ^{14}C labelled assimilate than the spear harvest itself, possibly because more dry matter was transferred from the crown the fern than to the harvested spears.

Data from this experiment indicate that the widely reported reduced vigour of female plants compared with males appears to be due to the sink activity of berries. Berries can import at least 50% of the assimilate produced at a given time. At senescence, 25% of plant dry weight was in the berries. This reduced, firstly, the development of photosynthetic tissue i.e, young fern, and secondly, the dry weight of the crown. Female plants which had never produced berries had a higher crown dry weight than male plants. However, the presence of berries may increase photosynthetic efficiency as female plants with berries have a higher ratio of sink dry weight (crown plus berries) to photosynthetic dry weight than plants without berries. Plants with berries also appeared to have a lower respiratory requirement.

CHAPTER FOUR

FACTORS AFFECTING INITIATION OF SPEAR GROWTH IN SPRING AND AUTUMN

4.1 INTRODUCTION

In cool temperate climates, asparagus has a clearly defined cycle of spear harvest, fern growth and dormancy, but in warmer climates spears can be harvested all the year round (Toledo 1990). In the cooler climates, there are no visible signs of growth from late autumn until spears emerge again in late winter-early spring.

The minimum temperatures at which buds break appear to differ between autumn and spring. Spear growth in autumn ceases when air temperatures are higher than those at which spear growth commences in spring (Hughes unpub. data, Nichols pers. comm., Dufault 1990). The minimum temperature for budbreak appears to be higher at the beginning of dormancy than at the end of dormancy.

The initiation of the first shoot in asparagus after a period of dormancy, or after fern has been removed in autumn, appears to be limited primarily by temperature. The experiments reported in this chapter demonstrate that provided the temperature remains above a minimum, this first shoot controls budbreak i.e, commencement of rapid elongation, of other buds on the rhizome. Furthermore, they show that the minimum temperature at which budbreak occurs varies as asparagus progresses through dormancy, and the mechanisms which induce and release this dormancy appear similar to those operating in deciduous fruit trees. Although the effect of daylength on spring spear growth was not studied, daylength had no effect on autumn spear or fern growth.

4.1.1 THE INDUCTION AND RELEASE OF DORMANCY

This section reviews Vegis's model of dormancy (Vegis 1963, 1964) and secondly, chilling unit models for determining the end of dormancy (Richardson et al. 1974, Gilreath and Buchannan 1981, Shaltout and Unrath 1983). Vegis's model is descriptive and underlies the quantitative chilling unit models. Definitions of dormancy vary. Lang's (1987) broad definition of dormancy is used here i.e, 'the temporary suspension of visible growth of any plant structure containing a meristem'. This definition fits

observed behaviour of asparagus plants during winter in cool temperate environments such as New Zealand. But, within this broad definition, Lang (1985, 1987) defines three types of dormancy on the basis of their physiological causes.

Ecodormancy occurs when growth is inhibited or released directly by environmental factors e.g. temperature or drought, without any interaction with other physiological factors. Paradormancy occurs when the stimulus which induces or releases it is detected or produced within the plant but in an organ or site other than the affected one. For example, apical dominance where growth of a shoot is induced by removing the apical bud or adjacent leaves. Endodormancy occurs when growth is regulated by factors within the plant and within the affected organ e.g. meristems which require chilling or a specific photoperiod to release them from dormancy. Both paradormancy and endodormancy involve an interaction between the environment and factors within the plant. Frequently, it is not possible to distinguish between them.

In Vegis's (1963, 1964) model, the depth of internal dormancy (paradormancy and endodormancy) increases to a maximum and then decreases with time. His model is based on results obtained from buds, bulbs, tubers and seeds. On most plants which undergo internal dormancy, meristems lose the ability to grow at both low and high temperatures as internal dormancy deepens. Vegis (1964) stated that the maximum temperature at which growth occurs initially decreases, followed by an increase in the minimum temperature. However, his figures show a symmetrical narrowing of the temperature range i.e, the decrease in maximum temperature occurred at the same time as the increase in minimum. Meristems of some plants retain the ability to grow over a very narrow temperature range at maximum endodormancy, while others will not grow in any environmental conditions. The temperature range at which meristems commence growth then widens as internal dormancy is released (Vegis 1963, 1964). This change in growth response is induced by environmental factors, especially temperature and night length.

Lengthening nights in autumn may induce internal dormancy in temperate fruit crops, although temperatures outside a certain range may counteract it (Vegis 1963, 1964). Buds must then be exposed to 'chilling' to progress through physiological changes necessary before internal dormancy is released. Buds will then grow, if ecodormancy is not operating. If internal dormancy is not released, budbreak can be completely suppressed, or spread over several weeks and vegetative growth can be very weak (Saure 1985, Edwards 1987).

Vegis's model has been used to predict the time at which buds on deciduous plants are released from internal dormancy from 'chilling hours' i.e, the number of hours below a critical temperature. But this assumes that all temperatures below the critical one have equal effectiveness, and that higher temperatures have no effect. Variation in the chilling effect of temperature is accounted for in chill unit models.

4.1.2 CHILL UNIT MODELS

Chill unit models predict the end of internal dormancy from accumulated chill units. Chill units are a weighted accumulation of chilling hours which incorporate variation in the chilling effectiveness of temperatures and the reversal of chilling by high temperatures (Richardson et al. 1974). One chill unit accumulates when the plant is exposed to the optimum chilling temperature for 1 hour (Table 4.01). This is close to 5C for most plants, but ranges from 3.5C to 10C (Fuchigami and Nee 1987). Chill units decrease as the temperature diverges from the optimum. Negative chill units accumulate at warm temperatures which reverse the effect of chilling and prolong internal dormancy (e.g. Erez and Lavee 1971, Erez et al. 1979, 1990).

Chill units are calculated from hourly temperatures. These can be measured, or estimated from daily maximum and minimum temperatures by assuming that temperature changes at a constant rate (Richardson et al. 1974). This assumption holds in locations such as Utah, but not in locations where rapid temperature changes occur during the day, e.g, in maritime climates, at high altitudes or at low latitudes (del Real Laborde 1987, Rojas-Martinez et al.1990). Temperatures are usually measured in standard meterological instrument shelters.

In chill unit models, chilling begins in autumn when the summation of chill units accumulated daily becomes positive. The end of internal dormancy is determined experimentally by transferring buds to warmer temperatures (typical daily mean 20C) and measuring budbreak, as a proportion of the buds which break in a given time, or as the mean time which buds take to break (e.g, Richardson et al. 1974, Gilreath and Buchannan 1981, Shaltout and Unrath 1983). Chill unit curves for three deciduous fruit crops from different locations are given in Table 4.01.

The Utah model, also called the Richardson model, accurately predicted the end of internal dormancy in Utah, Washington and Georgia (Anderson and Richardson 1987). The shape of the three models compared in Table 4.01 is very similar although the

species, and the number of chill units required to release internal dormancy differ. This suggests that there may be substantial similarity between plants in the mechanisms used to release internal dormancy. Perhaps the release of internal dormancy in asparagus is also similar, despite it being a monocotyledonous plant.

Table 4.01: The effect of temperature on chill unit accumulation on fruit trees with a range of chilling requirements (from Saure 1985). The trees were located at the sites the models are named after.

Chill unit values	Corresponding Temperature (C)		
	Utah model	Florida model	N Carolina model
0.0	<1.4	-1.0	-1.1
0.5	1.5-2.4	1.8	1.6
1.0	2.5-9.1	8.0	7.2
0.5	9.2-12.4	10.4	13.0
0.0	12.5-15.9	17.0	16.5
-0.5	16.0-18.0	19.5	19.0
-1.0	>18	21.5	20.7
-1.5			22.1
-2.0			23.3
Fruit	*high chill peaches*	*low chill nectarines*	*high chill apples*
Source	*Richardson et al. 1974*	*Gilreath and Buchannan 1981*	*Shaltout and Unrath 1983*

Chill Unit Models currently provide the most widely used simple means of predicting the end of internal dormancy. They are empirical models, and assume that the efficiency of each temperature in releasing internal dormancy is constant. However the effectiveness of a temperature may vary with the stage of internal dormancy (Kobayashi et al. 1982). Recent modifications of the Utah model take this into account (del Real Laborde et al. 1990). Some physiologically based models have been proposed (e.g. Dennis 1987; Erez et al. 1990) but none are as simple and predictive as the chill unit model. On this basis, the Utah chill unit model, and Vegis's model for internal dormancy will be applied in this chapter to data from experiments on asparagus.

4.1.3 FEATURES OF ASPARAGUS SPEAR GROWTH

Growth cycle in a temperate climate

In temperate climates, there is variation in the time at which asparagus internal dormancy begins and ends at a given location. On spring harvested plants, variations in the timing of fern senescence and of spear emergence of about three weeks have been observed between years, between cultivars, and between juvenile plants i.e, before first flowering, and older plants. On autumn harvested plants, the time at which budbreak ceases varies between cultivars (Hughes unpub. data), and few data are available on variations between years or plant ages.

The reasons for the variations in spring harvested plants are not known. There is some evidence that spring spear growth is limited by temperature. For example, several days of warm temperatures can cause a few spears to emerge up to six weeks before a harvestable growth flush, but subsequent cool temperatures cause spear emergence to cease. Cool spring temperatures can also delay harvest.

Harvesting of a crop normally ends in early December (in New Zealand) and is followed by fern production. Very few new ferns are produced from mid January onwards. The production of new shoots may be inhibited by existing fern, or by summer drought. Frost will induce early senescence, as will summer drought (Nichols pers. comm.).

Large scale commercial harvesting of asparagus in autumn occurs in warm climates such as Taiwan and Mexico where the fern grows all year round (Garza 1970, Lin 1979). Autumn harvesting in temperate climates frequently results in low yields and poor plant survival (Jasmin and Laliberte 1962, Brasher 1956, Farish 1937). Bud break in autumn ceases when the soil and air temperatures are higher than during the spring harvest (Hughes, unpub. data; Nichols, pers. comm.). Factors controlling the cessation of spear growth in autumn are not known. At Palmerston North, growth ceases in mid-late March when the mean daily temperature is higher than in mid-late September (Table 4.02) when spear production commences in spring.

Prerequisites for budbreak

The minimum temperature at which budbreak occurs in the field in spring appears to be close to 5C, although field based trials have not measured budbreak directly. The minimum temperature required for spear growth has been calculated as 5.8C for vigorous 'Martha Washington' (Culpepper and Moon 1939a), and as being below 7.2C for 'Raritan' (Blumenfield et al. 1961). Budbreak on 'UC157' in pots did not occur at 5C constant after one month of natural chilling in mid winter (Nichols and Woolley 1985), but did occur on one year old plants of 'Mary Washington 500W' in a Japanese study (Kim and Sakiyama 1989a).

Table 4.02: Mean air and soil temperatures (30 year normals) during spring and autumn harvest at the Palmerston North DSIR meterological station.

	Temperatures during spring			Temperatures during autumn		
	Sept	Oct	Nov	Feb	Mar	Apr
Air temperature						
Mean	10.6	12.4	14.2	17.6	16.4	13.9
Maximum	14.7	16.6	18.5	22.3	20.9	18.2
Minimum	6.6	8.3	9.8	11.7	9.6	6.8
Soil temperature						
At 10cm	9.9	12.5	15.1	18.1	16.3	13.2

Budbreak pattern

The pattern of budbreak has been studied in detail on 16 relatively young plants of 'UC157' (Nichols and Woolley 1985). Growth always started at one of the two basal buds on a rhizome and proceeded towards the tip of the rhizome. Up to 7 buds commenced growth within a 24 hour period, however only one spear developed from each rhizome. A very strong interaction occurred between spears growing from different rhizomes (Nichols and Woolley 1985). The two extremes were

1. if the initiation of growth by buds was separated by at least 12 hours, the growth of later spears was very slow until the earlier spears were removed.

2. if several spears (3-6) started growing at the same time, they grew at a similar rate, but the growth of other spears on that crown was extremely slow until most of the first group of spears was removed.

Thus the growth of asparagus spears in their study was controlled by correlative inhibition (a type of paradormancy) both within a rhizome and between rhizomes of a plant. This contradicts other results in which the inhibitory effects of a growing spear extended only to that rhizome, implying that the correlative inhibition was restricted to the rhizome bearing the growing spear (Tiedjens 1926; Kretschmer and Hartmann 1979). This difference can probably be explained by the extent of living tissue connections between the rhizomes (see section 1.3.3).

Spear growth rates

The most rapidly growing portion of the spear is just below the spear tip. This zone is more sensitive to temperature than the rest of the spear (Culpepper and Moon 1939a). Thus spear emergence following budbreak tends to be influenced by air temperature more than by soil temperature (Bouwkamp and McCully 1975). Spear growth appears to increase between 7C and 31C (Culpepper and Moon 1939a; Blumenfield et al. 1961, Nichols and Woolley 1985). However, there is one report of reduced spear growth when root temperature was increased from 25C to 30C while air temperature was maintained at 25C (Kim and Sakiyama 1989a). Spear growth above 31C has not been examined.

Spear growth, as determined from measurements of spear length, is exponential with time when spears are 5 to 200mm long (Nichols and Woolley 1985) and when there is plenty of stored carbohydrates (Kim and Sakiyama 1989a). As the carbohydrate available decreases due to plant size or spear harvest, spear growth becomes linear, and then decreases as the stored carbohydrate is exhausted (Kim and Sakiyama 1989a).

When spear growth is exponential, 'relative spear growth rate' (RSGR) can be used to study the effects of temperature and bud position on spear growth (Nichols and Woolley 1985). Nichols and Woolley (1985) define RSGR as the slope of the line obtained when the natural log of spear length is plotted against time. Thus differences in the rate at which spears grow can be represented a single number - the RSGR. The RSGR was considered to be constant from budbreak to harvest, and increased with temperature between 10C and 30C (Nichols and Woolley 1985). Kim and Sakiyama (1989a) report maximum spear growth at root temperatures of 20 to 25C, and an air temperature of 25C.

Effect of plant sex

Although there are no obvious differences in the growth or vegetative morphology of male and female plants, male plants consistently produce a higher marketable yield (spear weight and number) than female plants and live longer (Tiedjens 1924; Robbins and Jones 1925; Yaeger and Scott 1938; Ellison and Schermerhorn 1958; Ellison et al. 1960; Moon 1976). Male plants also tend to produce spears earlier in the spring than female plants (Tiedjens 1924; Robbins and Jones 1925; Ellison and Schermerhorn 1958; Ellison et al. 1960).

4.2 MATERIALS AND METHODS

4.2.1 INTRODUCTION

Factors affecting the induction and release of internal dormancy in asparagus, the growth of spears produced during spring and autumn harvests, and accompanying changes in carbohydrate content and composition were investigated in four related experiments. These experiments used controlled environments to simulate autumn, winter and spring conditions. Data on internal dormancy and spear growth are discussed in this chapter; data on dry weight and carbohydrate changes are discussed in Chapter 5. The aims of each experiment were as follows:

Experiment A: to identify some of the effects of temperature, daylength and plant sex on fern senescence and on spear growth, carbohydrate content and composition in autumn by exposing 'Rutger's Beacon' to natural and controlled conditions in autumn.

Experiment B: to identify some of the effects of 'autumn', 'winter' and 'spring' temperature, length of chilling (simulated winter) and plant sex on budbreak, spear growth, carbohydrate content and composition during a spring harvest by exposing 'Rutger's Beacon' to a range of controlled temperatures.

Experiment C: to identify some of the effects of 'autumn' and 'winter' temperature, and length of chilling on budbreak and spear growth during a spring harvest by exposing 'Rutger's Beacon' to natural conditions during autumn and winter.

Experiment D: to identify some of the effects of 'autumn', 'winter' and 'spring' temperature, length of chilling (simulated winter) and genotype on budbreak and spear

growth during a spring harvest by exposing 'Jersey Giant' and 'UC157' to a range of controlled temperatures.

4.2.2 PLANT MATERIAL

Experiments A, B and C: 'Rutger's Beacon'

Plants of 'Rutger's Beacon' were propagated in 5 cell root trainers in an unheated glasshouse using a peat-sand (1:1 v.v) potting mix on 18 December 1985. They were transferred to 4.8 litre black polythene bags when 4 months old and placed in a heated glasshouse where vegetative growth continued for the following 8 months. Heating within the glasshouse commenced at 15C and venting at 22C. By November 1986, the plants had begun to flower. In November-December 1986 the plants were potted into 10.8 litre black polythene bags (after cutting off the existing fern), placed on polythene laid on the open ground and staked. After propagation, the potting mix used was peat-pumice (5:3 vv) with dolomite (3 mg/l), micromix (0.94 mg/l), long term Osmocote (Osmocote 18) (3 mg/l) and short term Osmocote (Osmocote 14) (0.63 mg/l). The plants were watered by trickle irrigation every 2-3 days as required during summer.

By January 1987, the plants were similar in size to field grown plants at the beginning of their third year of growth. Before commencing the experiment, the male and female plants were identified and each separated into 5 blocks on the basis of fern vigour. Plants required for Experiments A and B were returned to a heated glasshouse on 23 February to prevent exposure to temperatures below 13C. Previous experience indicated that night temperatures below 13C induced fern senescence. Plants required for Experiment C remained outdoors.

Five male and five female plants of 'Rutger's Beacon' were exposed to each treatment in Experiments A and B (Table 4.03, Table 4.04), except where indicated. Insufficient male plants were available for all treatments as the decision to grow plants at both 12.5C and 20C in Experiment B was made after Experiment A had started. Plants exposed to natural chilling in Experiment C (Table 4.05) were not blocked.

Experiment D: 'Jersey Giant' and 'UC157'

Six month old 'Jersey Giant' and 'UC157' were used for Experiment D. The plants had been propagated in cells, transplanted in January 1987 into 3 litre black polythene bags

using a 50:50 peat:sand UC type potting mix and grown in an unheated glasshouse. The plants were separated into 6 blocks on the basis of plant size before the experiment commenced on 23 February. The plants were exposed to the same temperatures as in Experiment B.

Six plants of each cultivar were exposed to each treatment in controlled environment conditions. It was not possible to identify the male and female plants in 'UC157' before the experiment as the plants had not flowered. 'Jersey Giant' is an all male cultivar.

4.2.3 ENVIRONMENTAL REGIMES

The plants for Experiments A, B and D were transferred from outdoors to a heated glasshouse (minimum temperature 13C) on 23 February. Experiment A commenced on 22 March, and Experiments B and D on 26 May. Experiment C consisted of plants which remained in natural conditions until required.

In Experiment A, plants of 'Rutger's Beacon' were exposed to temperature and daylength regimes as summarised in Table 4.03.

Table 4.03: Temperature regimes applied to plants in Experiment A: fern senescence and spear growth in autumn [a]

Total daylength (hours) [b]	8	16	8	16	natural
Photoperiod lights[c] (hours)		8		8	
Temperature (C)	12.5	12.5	20.0	20.0	natural

Note:

a Experiment commenced on 22 March

b Photosynthetically active light of similar quality in each growth room

c Photoperiod was extended 4 hours before and 4 hours after the 8 hours of day 'lighting'

d Mean daily minimum 7.5 +- 1.1C, daily maximum 18.7 +- 0.6C in the two weeks following transfer to natural conditions

The temperature regimes used to simulate winter and spring in Experiments B and D are given in Table 4.04. Table 4.05 contains the temperature regimes used in Experiment C.

Table 4.04: Temperature regimes applied to plants in Experiments B and D: Effect of simulated winter temperatures on subsequent spear growth.

'Winter conditions'		'Spring' Temperature C	Chilling Units [c] received before transfer to 'spring' temperature	Date and season transferred to 'spring' temperature
Length of chilling (weeks)	Chilling temperature C [b]			
0		12.5	0	May 26 (day 0) Early winter
0		20		
5	10	12.5	420	July 2 (day 36) Mid winter
5	10	20		
5	5	12.5	840	
5	5	20		
5	2	12.5	420	
5	2	20		
10	10	12.5	840	August 10 (day 75) Early spring
10	10	20 [a]		
10	5	12.5	1680	
10	5	20		
10	2	12.5	840	
10	2	20 [a]		

Note: a - female plants only

b - Plants chilled at 2C were held at 0C for the first week, and transferred to 2C after a breakdown of the equipment maintaining the cool room at 0C.

c - Chilling Units calculated using the Utah model (Richardson et al. 1974)

Table 4.05: Temperature regimes applied to plants in Experiment C: Plants exposed to natural conditions before transfer to 12.5C

'Winter' conditions		'Spring' temperature C	Chilling units[a] received before transfer	Date transferred to 'spring' temperature
Length of chilling (weeks)	Chilling temperature			
0	natural	12.5	267	May 26 (day 0)
5	natural	12.5	773	July 2 (day 36)
10	natural	12.5	1356	August 10 (day 75)

Note a: Chilling Units calculated using Utah model (Richardson et al. 1974)

The controlled environment facilities used were at several locations as follows:
Experiment A:

12.5 ± 0.5C Growth room at Climate Laboratory, DSIR Fruit and Trees
20.0 ± 0.5C Growth room at Climate Laboratory, DSIR Fruit and Trees

Experiments B, C, and D:

10 ± 1C Cool room at Plant Growth Unit, Massey University
5 ± 1C Cool room at Plant Growth Unit, Massey University
2 ± 2C Cool store at Fruit Unit, Massey University
20 ± 3C Growth cabinets at Ag/Hort Faculty, Massey University
12.5 ± 0.5C Growth room at Climate Laboratory, DSIR Fruit and Trees

Within the growth rooms and growth cabinets, plants on which spears were harvested were watered by hand, while plants with fern (Experiment A only) were trickle irrigated. A vapour pressure deficit of 0.4 kPa was maintained at 12.5C and at 20C in Experiment A and at 12.5C in Experiment B, C and D.

Photosynthetic photon flux density at the Climate Laboratory controlled growth rooms was measured with a Licor LI 185 Meter containing a LI 190S Quantum Sensor; photosynthetic irradiance was measured with a Licor LI 185 Meter containing a LI 19SE Flat response photosynthetic irradiance sensor. The mean photosynthetic photon flux density was 697 µmol/m^2/sec in Experiment A and 690 µmol/m^2/sec at 12.5C in Experiments B, C and D. The mean photosynthetic irradiance was 149 W/m^2 in Experiment A and 147 W/m^2 for plants at 12.5C in Experiments B and C. The light

levels at 20C in Experiment B and D were lower than at 12.5C. Photoperiod lights in Experiment A were used for 4 hours before and 4 hours after the main lights.

4.2.4 DATA COLLECTION

Measurement of fern senescence

The plants on which senescence was evaluated were placed on 2 trolleys and their positions changed twice a week to reduce any variation in plant growth due to position within the growth room (Experiment A).

The chlorophyll content of the fern was measured following extraction in dimethylformamide (DMF) (Inskeep and Bloom 1985, Moran and Porath 1980, Moran 1982).

Measurement of Chlorophyll content

Cladodes and their supporting branches were removed from the main structural branches of the fern and cut into 2-3 cm lengths. Duplicate 2 gm sub-samples were extracted in 40 ml of DMF in the dark at 5C for 7 days. The DMF was decanted and the tissue extracted with a further 40 ml of DMF for 7 days. The DMF was then decanted off, the combined extracts filtered and made up to 100 ml. Absorbance was measured at 664.5 and 647 nm, after dilution if required.

Preliminary experiments demonstrated that neither Waring blender or pestle and mortar were suitable for breaking up the tissue. The tissue partially disintegrated in the DMF and all colour was removed by the procedure above.

Chlorophyll content of the cladodes was calculated using the following equations (Inskeep and Bloom 1985):

Total chlorophyll = $17.90\ A_{647} + 8.08\ A_{664.5}$

Chlorophyll a = $12.90\ A_{664.5} - 2.79\ A_{647}$

Chlorophyll b = $20.70\ A_{647} - 4.62\ A_{664.5}$

where A = absorbance in 1.0 cm cuvettes, and chlorophyll content is in mg per litre.

Calculation of Spear Growth Rate

On plants required for spear growth measurements, the fern was cut off before the plants were transferred to either the growing temperatures or chilling temperatures.

The buds on the rhizome were exposed by removing potting mix immediately after the plants were transferred to the 'growing' temperatures (12.5C or 20C). Exposing the buds had several advantages:

- it ensured that spear diameter was influenced primarily by physiological factors such as size of bud and carbohydrate availability, and not by the depth of the potting mix as spear diameter increases as soil depth increases (Williams and Kendall 1976)
- it allowed spear growth to be measured from the time of budbreak rather than from the time of emergence through the potting mix
- it ensured that budbreak and subsequent spear growth occurred at the same temperature, as potting mix is likely to have a higher temperature than the air.

Preliminary studies indicated that permanently exposing the buds did not damage any plant tissues, and did not affect spear growth rates.

The height of spears on 'Rutger's Beacon' plants was recorded for at least 5 weeks, the actual time depending on when (or if) the plants produced spears. The height of spears on the 'UC157' and 'Jersey Giant' plants was recorded for 3 weeks at 20C and for 6 weeks at 12.5C.

Spear growth on plants at 20C was measured twice daily (8 to 12 hours apart); spear growth on plants at 12.5C was measured once every 2-3 days. Length was measured from the base of the spear with a flexible metal ruler whose origin was placed on the apical side of the spear. Spears were harvested when at least 200mm high by snapping them off at the rhizome. This produced a clean break occurred along what appeared to be an abscission layer.

When the temperature is constant, the elongation of asparagus spears is exponential and can be described by a log-linear equation (Nichols and Woolley 1985). In this experiment, exponential growth was preceded by a period in which the bud slowly swelled but remained covered by purply-white scales. Exponential stem elongation commenced when the bud height was 1.5-2 times greater than bud diameter and was accompanied by the development of green colour below the tip of the developing spear.

The relative spear growth rate (RSGR) of the spears was calculated by regressing $\log_e$ (spear height) (mm) against time (days)

i.e. $\log_e(\text{spear height}) = \log_e(a) + (\text{RSGR} \times \text{time})$ *(Eq.4.1)*

All the data obtained from selected plants are shown in Figs.4.08 and 4.14. Spear heights less than twice the spear diameter were excluded from the regression as these measurements preceded the beginning of exponential growth. Time was measured as the number of days after transfer to the 'growing' temperature. The regression coefficient (r^2) of most equations was between 98.0% and 99.7%.

In this experiment, 'time of budbreak' was calculated as the time at which the spear height equalled twice the diameter of the harvested spear as observations suggested that this was the height at which exponential growth commenced. Actual spear diameter was used for 'Rutger's Beacon'; a spear diameter of 5 mm was used for 'Jersey Giant' and 'UC157'.

i.e. $t_0 = \log_e(2 \times \text{spear diameter}) - \log_e(a) / \text{RSGR}$ *(Eq.4.2)*

Spear diameter was measured 5-10 cm from the basal end of the spear using digital callipers. If the spear cross-section was oval, the average of the maximum and minimum diameter was recorded.

All spears can be related to a specific rhizome whose location is known. Sketch maps of each crown were drawn to show the location of the rhizomes and the location of buds within each rhizome (see Figs. 4.08 and 4.14).

Position of the first bud to grow

The basal bud, i.e, the bud closest to the previous season's fern is the largest bud on a dormant rhizome. The buds become smaller and younger towards the growing tip of the rhizome (Fig.4.01a&b). Dormant buds were numbered, starting from the basal position, and ending with the smallest visible bud near the tip. Thus the basal bud occupies 'Position 1' , the adjacent bud 'Position 2' etc. as shown in Fig.4.01b.

Calculation of chilling units

The chilling units accumulated by 'Rutger's Beacon' in Experiments B and C were calculated using the Utah model (Richardson et al. 1974). The hourly temperature response for chilled plants is given in Table 4.01. In this model, the chilling response at 5C is twice that at 2C or 10C. This model assumes that chilling temperatures 'push' the plants into internal dormancy, and subsequently 'pull' the plants out of dormancy. It has no allowance for a change in the optimum temperature required for firstly, the deepening of internal dormancy, and secondly, the release of internal dormancy.

For plants exposed to natural chilling (Experiment C), the hourly temperature was estimated by a linear interpolation of the daily maximum and minimum temperatures recorded at the Palmerston North DSIR meterological station which was approximately one kilometre from the experimental site. It was assumed that the minimum temperature occurred at 5.00 hours and the maximum at 14.00 hours. Effective chilling units commenced accumulating on 23 April as any chilling which had accumulated prior to this date was negated by subsequent warm temperatures.

The chilling accumulated by plants stored at constant temperatures was calculated using the same chilling responses as for the plants in natural conditions. The accumulation of chilling units commenced when the plants were transferred from the heated glasshouse to the storage conditions as heating at 15C and venting at 22C would have maintained the mean temperature above 15.95C, the temperature at which chilling commences in this model.

The chilling units which had accumulated when plants were transferred to 'spring' temperatures are given in Tables 4.04 and 4.05.

Morphological data and carbohydrate sampling:

At the end of each part of the experiment, all plants of 'Rutger's Beacon' were destructively sampled to determine crown morphological data and obtain samples for carbohydrate analysis. This part of the experiment is covered in Chapter 5.

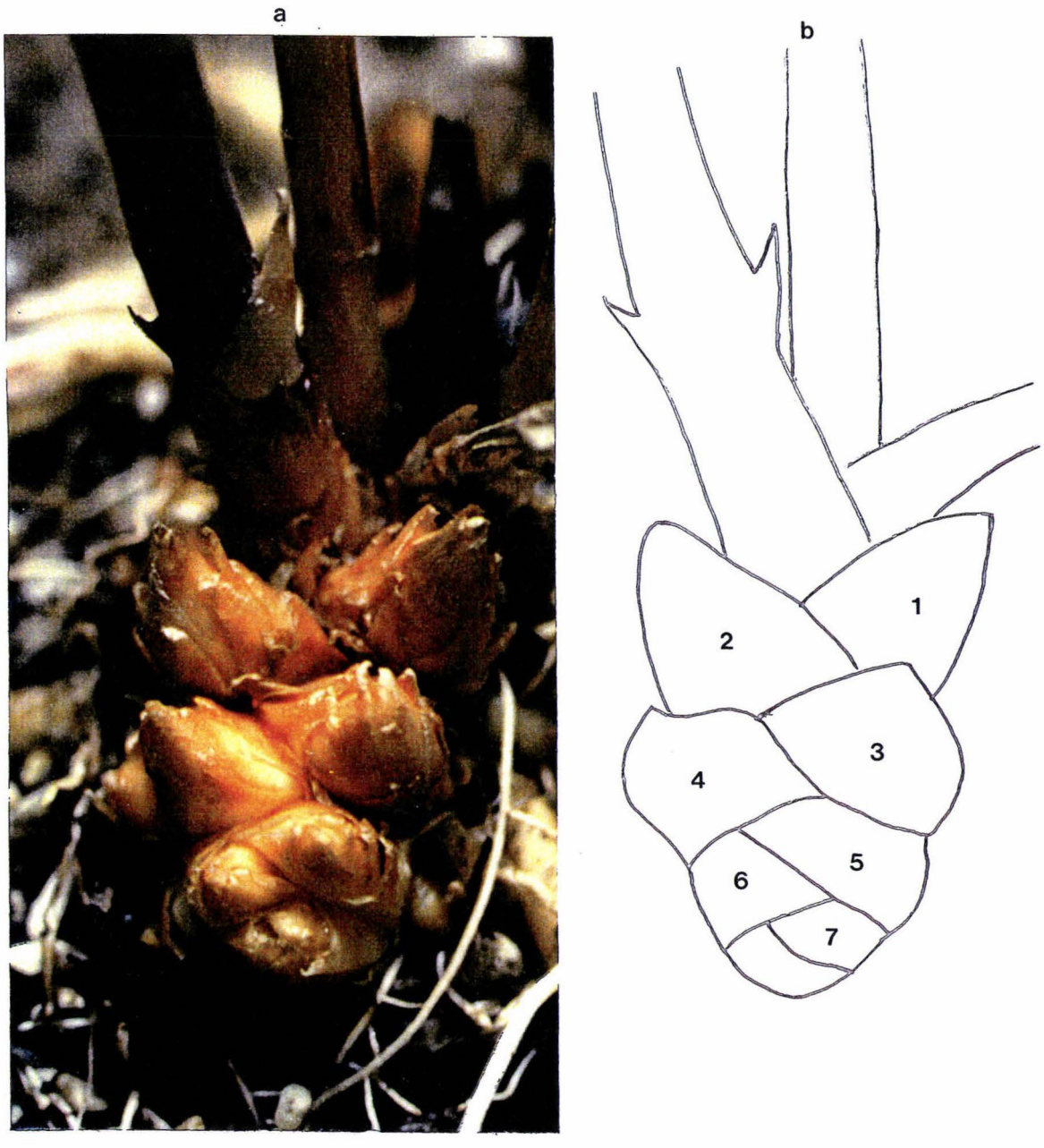


Figure 4.01: Position of buds at the apex of a dormant rhizome.

a. Photograph of dormant rhizome.

b. Schematic drawing - numbers denote position of bud. Position 1 is the oldest bud i.e, the basal bud.

4.2.5 DATA ANALYSIS

All analyses were carried out using the SAS package, Version 6.03 (SAS Institute Inc, 1988).

The experimental designs were set up as follows as it was expected to be able to treat each plant as a replicate:
Experiment A: randomised block with plants separated into 5 blocks on the basis of plant size
Experiment B: unbalanced randomised block with plants separated into 5 blocks on the basis of plant size. Plants from same population as Experiment A.
Experiment C: five replicates (not blocked). Plants from same population as Experiment A.
Experiment D: six replicates (blocked)

However, much of the data was subsequently analysed on a per rhizome basis which destroyed the blocking effect. Most of the data presented on spear growth in 'Rutger's Beacon' was obtained from the first three spears of rhizomes which produced three or more spears. This restricted the data to the main rhizome(s) on each plant. Rhizomes which produced less than three spears during the experiment were excluded from all analyses except number of days to budbreak to ensure that there was the same number of spears of each birth rank.

Analysis of variance

As most of the data obtained from the experiments was unbalanced, the analysis of variance was carried out using the generalised linear model of SAS (PROC GLM).

Differences in number of days to budbreak and spear relative growth rate (RSGR) were evaluated by conducting an analysis of variance with the main effects only in the model. This tested the effects of each factor independently and eliminated the problem of confounding due to the unbalanced experimental design i.e,
(i) in some conditions no plants grew,
(ii) plants chilled for zero time could not be assigned to any chilling temperature,
(iii) no male plants chilled for the 10 week, 2 and 10C treatments.

Interactions between main effects were determined using the same protocol on subsets of the data which formed balanced factorial designs. The main effects and all possible interactions of the relevant factors were entered in the model, and the non-significant effects removed until all effects remaining in the model were significant at P=0.05.

Chi-squared analysis

Chi-squared analyses were carried out using the procedure PROC FREQ within SAS.

4.3 RESULTS

4.3.1 SENESCENCE OF FERN: EXPERIMENT A

Fern on plants transferred from the heated glasshouse to 12.5C or to outdoor conditions on 22 March senesced over the following seven weeks while plants transferred to 20C remained green and produced several new ferns (Fig.4.02). Chlorophyll content was measured in older fern, but not in new fern on plants at 20C i.e, only in fern which was present at the beginning of the experiment. After 19 days, non-senescing ferns on plants at 20C were visibly greener and contained more chlorophyll (Fig.4.03) than the senescing ferns of plants outdoors or growing at 12.5C. Plants in the 8 hour photoperiod were greener and had more chlorophyll than plants in the 16 hour photoperiod.

4.3.2 SPEAR GROWTH BY RUTGER'S BEACON: EXPERIMENTS A and B

Effect of internal dormancy on spear growth

The following changes in budbreak and spear growth characteristics were expected as asparagus buds were released from internal dormancy:

- a decrease in the number of days to budbreak
- a decrease in the spread of budbreak
- a change in the position of the first spear to grow
- an increase in the RSGR of the first spear to grow.

These features are characteristic of buds and seeds which require chilling to break their dormancy and induce growth.

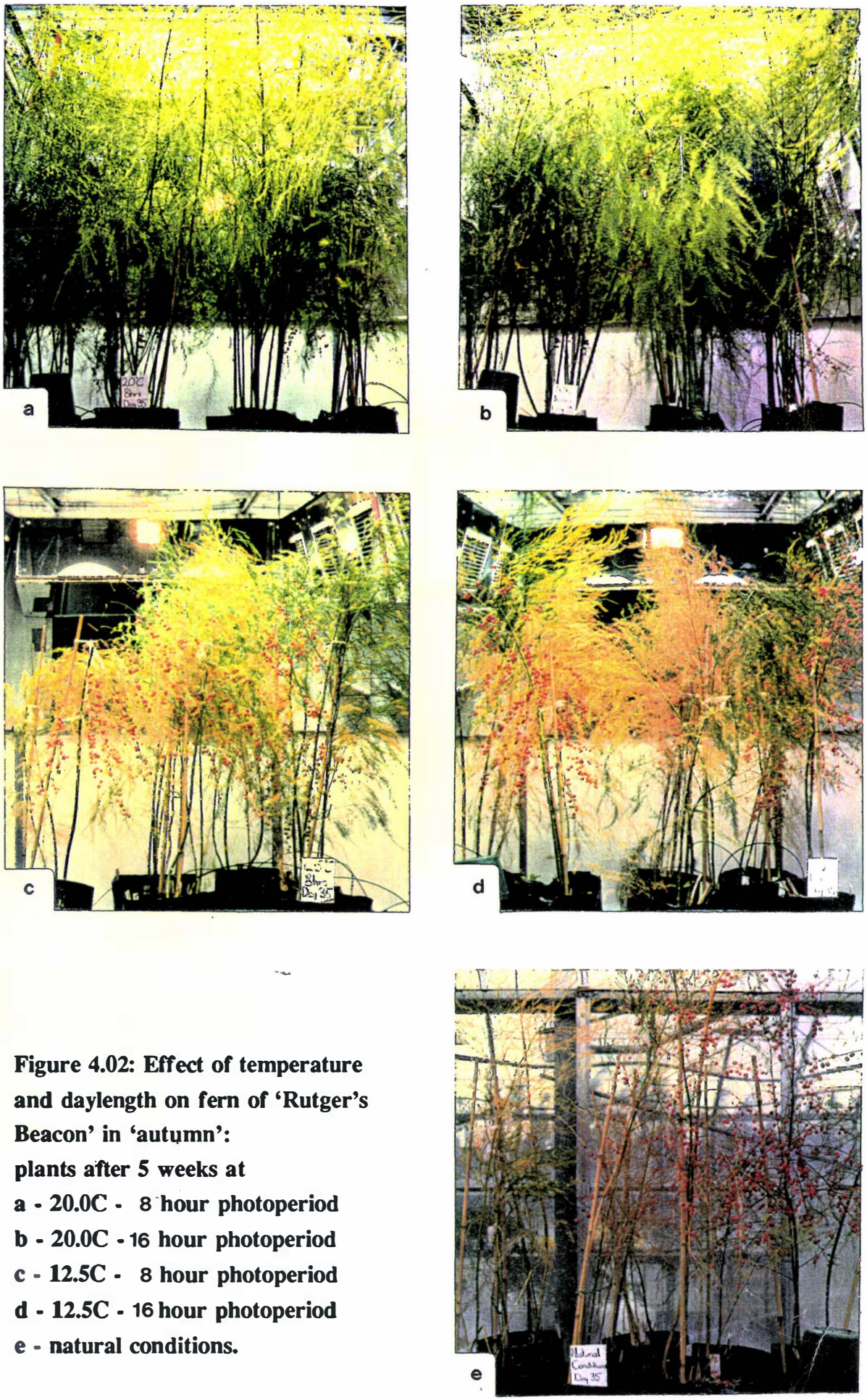
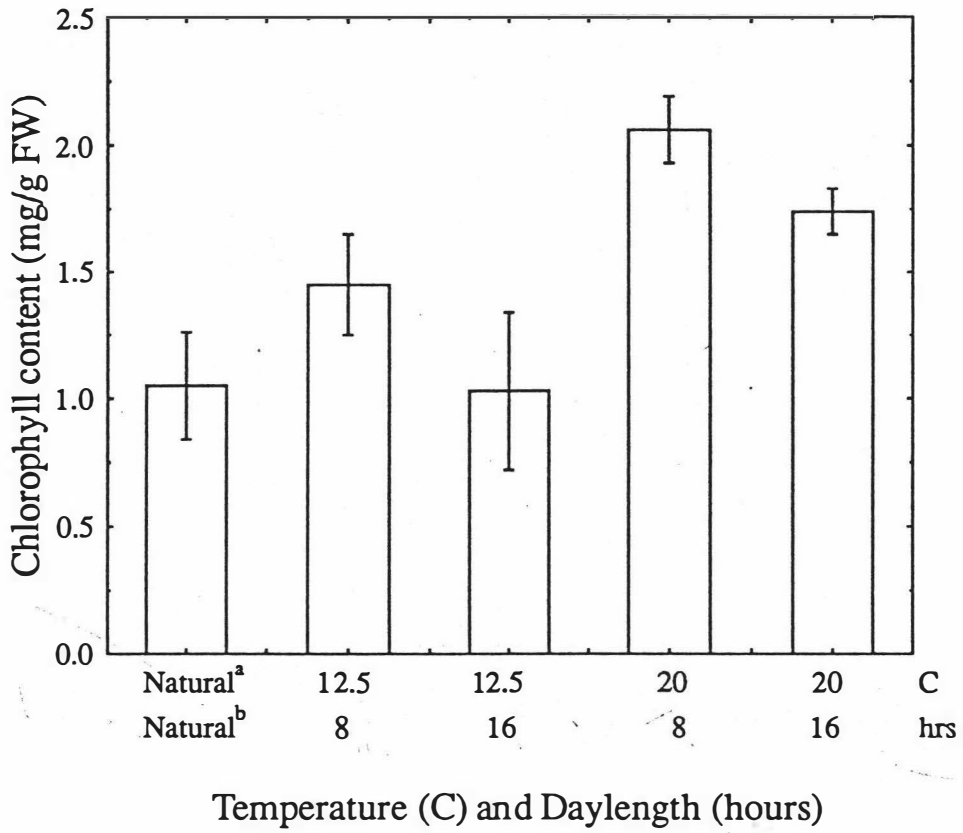


Figure 4.02: Effect of temperature and daylength on fern of 'Rutger's Beacon' in 'autumn':
plants after 5 weeks at
a - 20.0C - 8 hour photoperiod
b - 20.0C - 16 hour photoperiod
c - 12.5C - 8 hour photoperiod
d - 12.5C - 16 hour photoperiod
e - natural conditions.



Note: a - Mean maximum 14.4C; mean minimum 9.7C
(30 year normals for April)
b - Daylength range: 12.6 to 11.5 hours

Figure 4.03: Effect of temperature and daylength on chlorophyll content of 'Rutger's Beacon' in simulated autumn: chlorophyll content (mg/g/FW) 19 days after the experiment began on 22 March. Plants transferred to natural conditions or to 12.5C senesced; plants transferred to 20C produced a growth flush. Bars represent twice standard error of mean.

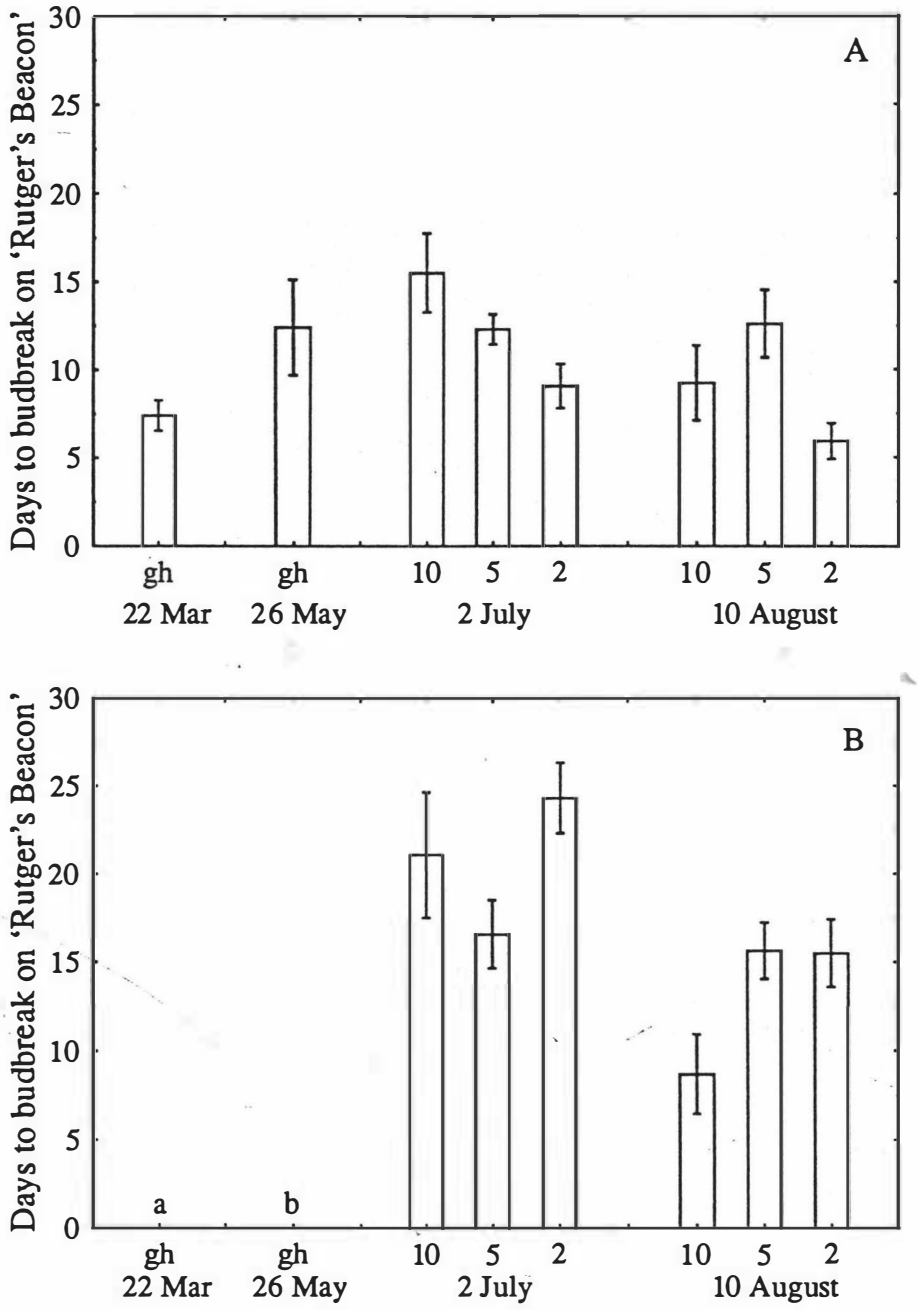
Time to budbreak and spread of budbreak

In this study, 'time to budbreak' refers to the time between transfer of plants to the 'autumn' or 'spring' growing temperature (20C or 12.5C) and commencement of exponential growth by the first spear to grow, as calculated using *Equation 4.2*. The spread of budbreak was assessed from the standard error of the mean time to budbreak as there were (generally) the same number of plants (n) in each treatment.

Budbreak occurred earlier (Fig.4.04) on plants at 20C than at 12.5C in 'autumn' and following each chilling treatment. Budbreak occurred at 20C throughout the experiments. Only four of the twenty plants transferred from the heated glasshouse (minimum temperature 13C) to 12.5C on 22 March grew in the following 49 days. None of the plants transferred from the heated glasshouse to 12.5C on 26 May grew during the experiment (Fig.4.04). Buds grew at 12.5C after the plants were stored at 10C or lower temperatures for 5 weeks i.e, after the plants had received at least 420 chilling units. Plants chilled at 5C for 5 weeks before transfer to 12.5C had less days to budbreak than plants chilled at 2C or 10C. Increasing the length of chilling to 10 weeks decreased the number of days to budbreak at 12.5C on plants chilled at 10C or 2C, but not on plants chilled at 5C.

There was an increase in the number of days to budbreak and in spread of budbreak on 'Rutger's Beacon' at 20C from 22 March to 26 May (Fig.4.04). Chilling for 5 weeks following 26 May had no effect on the number of days to budbreak on plants at 20C, although it did decrease the spread of budbreak. Chilling for a further 5 weeks after 2 July decreased the number of days to budbreak on 'Rutger's Beacon' plants chilled at 2C or 10C but not those chilled at 5C. Chilling at 2C produces the least spread in days to budbreak on plants chilled for 10 weeks before transfer to 20C.

The effect of natural chilling on number of days to budbreak in 'Rutger's Beacon' is presented in Fig.4.09, and the effect of the above chilling regimes on days to budbreak in 'Jersey Giant' and 'UC157' is presented in Fig.4.12a & b.



Note: a - data not shown as only 4 of 20 plants grew in 49 days
b - no plants grew in 115 days

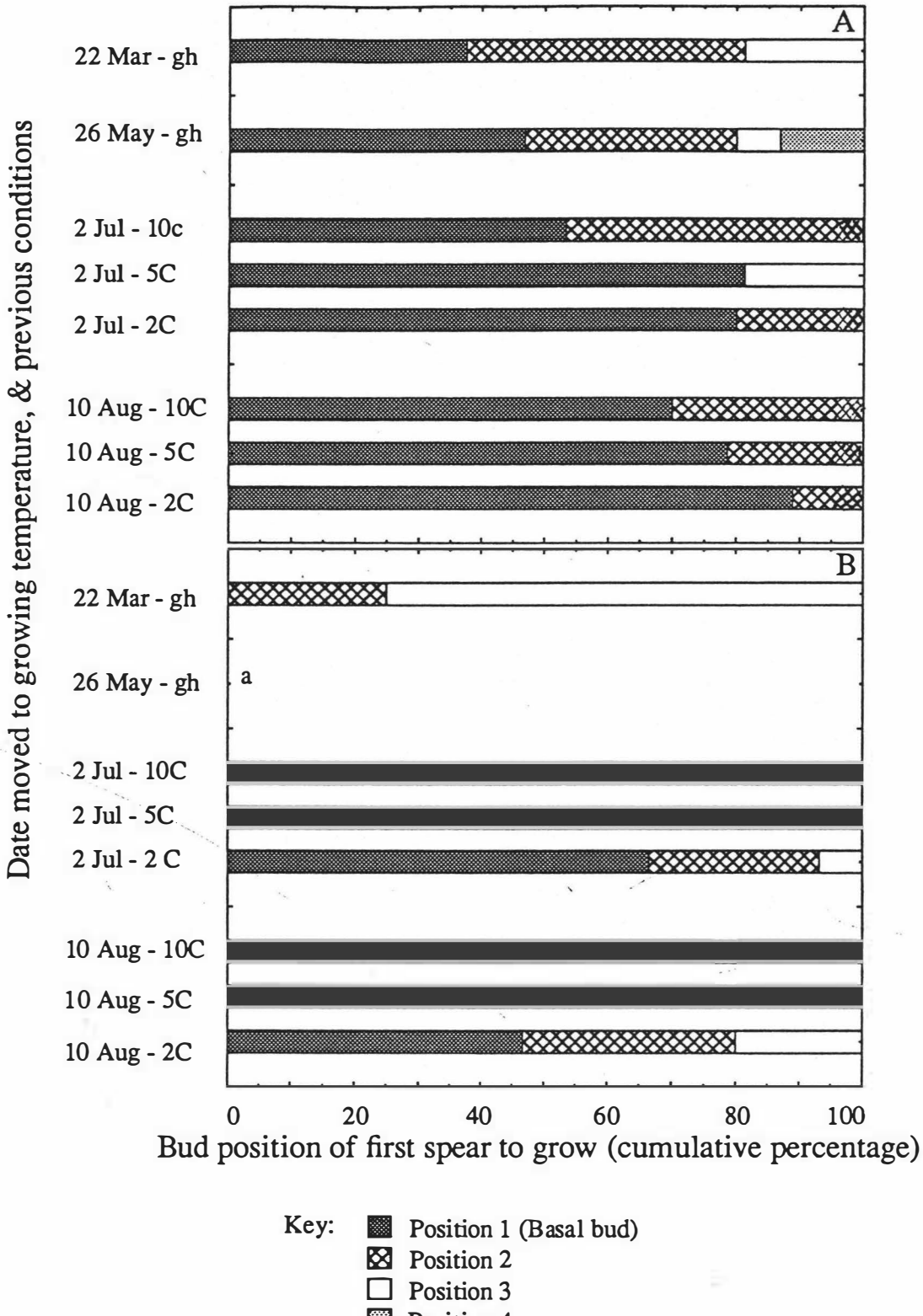
Figure 4.04: Mean number of days to budbreak by first spear to grow on each rhizome of 'Rutger's Beacon' at 20C (A) or 12.5C (B). Plants transferred to growing temperature on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

Position of the first bud to grow

Although most spears originated from the basal bud of a rhizome (Fig 4.01), 38% originated from younger buds. The percentage of first spears produced by each bud position changed following chilling on 'Rutger's Beacon' (Fig.4.05). The effect of chilling was significant at P=0.002 on plants grown at 20C, and at P<0.001 on plants grown at 12.5C in a Chi-squared analysis. Chilling at 2, 5 or 10C increased the percentage of initial spears originating from the basal bud. This was accompanied by a decrease in the number of younger buds producing the initial spear. The only exception to this appeared to be plants chilled at 2C before transfer to 12.5C which produced more spears from younger buds after 10 weeks of chilling than after 5 weeks of chilling. However, the effects of chilling temperature and length of chilling were not significant at P=0.05 (Chi-squared analysis, Table 4.06). This lack of significance may have been due to the small sample size: n=10 in all treatments except plants chilled at 2C or 10C for 10 weeks and transferred to 20C where n=5. The small sample size may also have prevented detection of differences due to plant sex or the growing temperature (Table 4.06).

None of the plants transferred to 12.5C on 26 May grew. Only four of the 20 plants transferred on 22 March grew, and budbreak occurred at Positions 2 and 3. Budbreak on plants transferred to 20C on 26 May occurred from a wider range of bud positions i.e, from younger buds, than on 23 March, but more initial spears originated from basal buds. However, these differences were not significant at P=0.05 (Table 4.06).

The effect of time and chilling temperature on the position of the first bud to grow in 'Jersey Giant' is presented in Fig.4.15.



Note: a - plants did not grow in 115 days of experiment

Figure 4.05: Effect of chilling on the position of the first bud to grow on 'Rutger's Beacon' at 20C (A) or 12.5C (B). Plants transferred to growing conditions on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks.

Table 4.06: Chi-squared analysis on the effects of various factors on the position of the first bud to grow following transfer to 12.5C or 20C.

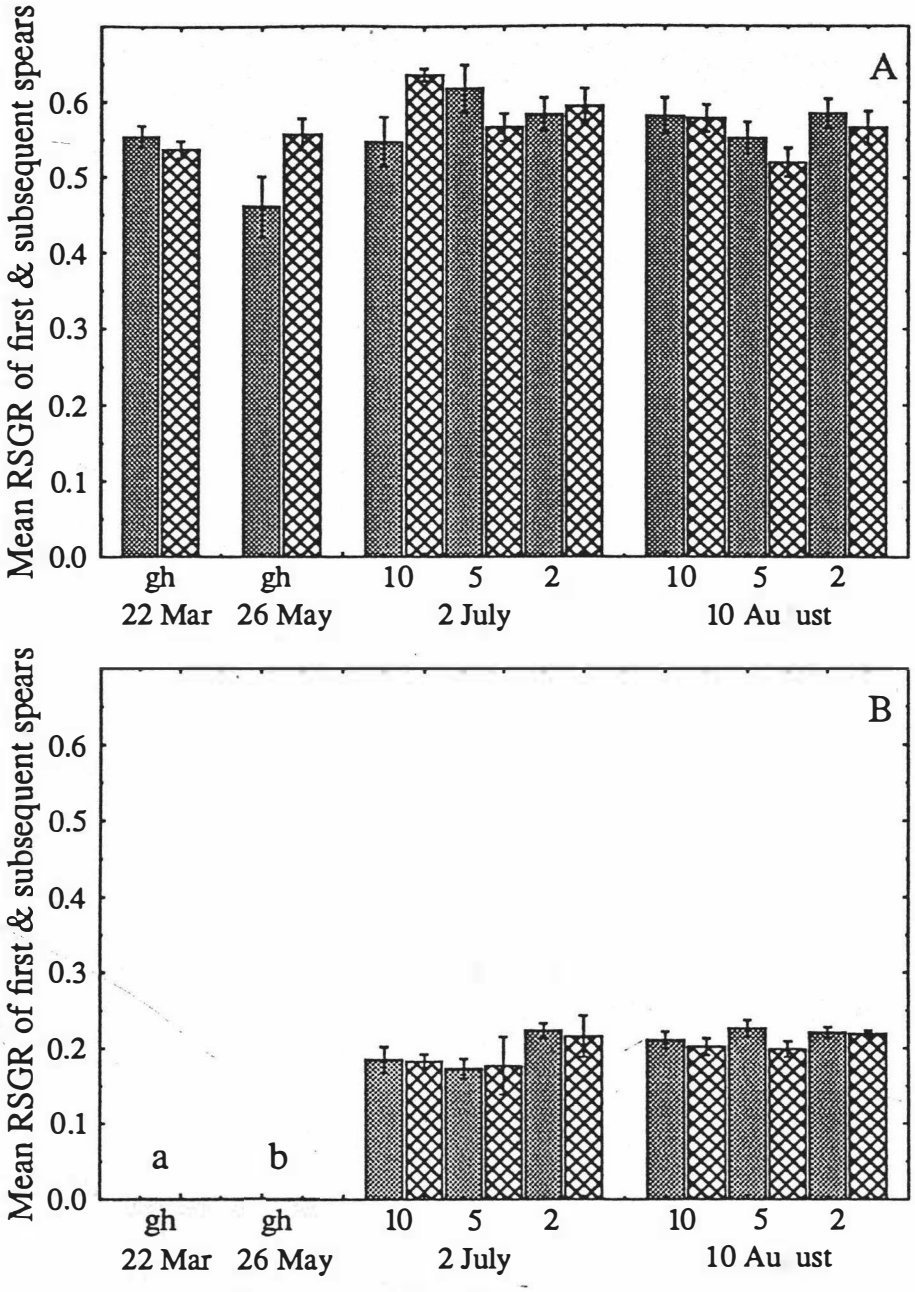
Factor	Probability that factor has a significant effect (Chi-squared analysis) at growing temperature of	
	12.5C	20C
Growing temperature	0.20	
Plant sex	0.10	0.16
Chilled *vs* unchilled plants	<0.001	0.002
Chilling temperature (chilled plants only)	0.16	0.33
Time of transfer:		
Chilled plants (5 or 10 weeks)	0.15	0.45
Unchilled plants (March or May)	-	0.33

Spear growth rates:

Spear growth rates were the final factor considered as a measure of chilling effectiveness in Experiments A and B. Spear height increased exponentially from budbreak to 200mm at both 12.5C and 20C when relative spear growth rate (RSGR) was calculated as described in Section 4.2.4. There was no evidence that spear growth at 12.5C followed two exponential curves as suggested by Nichols and Woolley (1985).

Ambient temperature had a large effect on RSGR with the RSGR at 20C approximately three times that at 12.5C (Fig.4.06a & b). Within each growing temperature, the RSGR's for spears from plants which had received different chilling treatments was similar. However, there were some differences in the RSGR of the first spear to grow.

At 20C, the RSGR of the first spear to grow was lowest on plants transferred on 26 May (Fig.4.06b). The RSGR of the first spear on 'Rutger's Beacon' plants transferred to 20C on 22 March was similar to that obtained after the plants had been chilled. Increasing the length of chilling at 5C from 5 to 10 weeks decreased the RSGR of the first spear



Note: a - data not shown as only 4 of 20 plants grew
b - no plants grew in 115 days

Figure 4.06: Mean RSGR of first and subsequent spears to grow on 'Rutger's Beacon' at 20C (A) or 12.5C (B). Plants transferred to growing conditions on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

to grow at 20C, but increased it at 12.5C. Length of chilling at 2C or 10C had no effect on RSGR of the first spear to grow.

The RSGR of the first spear was generally the same as the RSGR of subsequent spears, but at 20C, the RSGR of subsequent spears was higher than the first spear on plants transferred on 26 May and on plants chilled at 10C for 5 weeks.

The effect of natural chilling on the RSGR of 'Rutger's Beacon' is presented in Fig.4.10, and the effects of the above chilling regimes on RSGR of 'Jersey Giant' and 'Rutger's Beacon' is presented in Fig.4.13a & b.

Budbreak patterns in 'Rutger's Beacon'

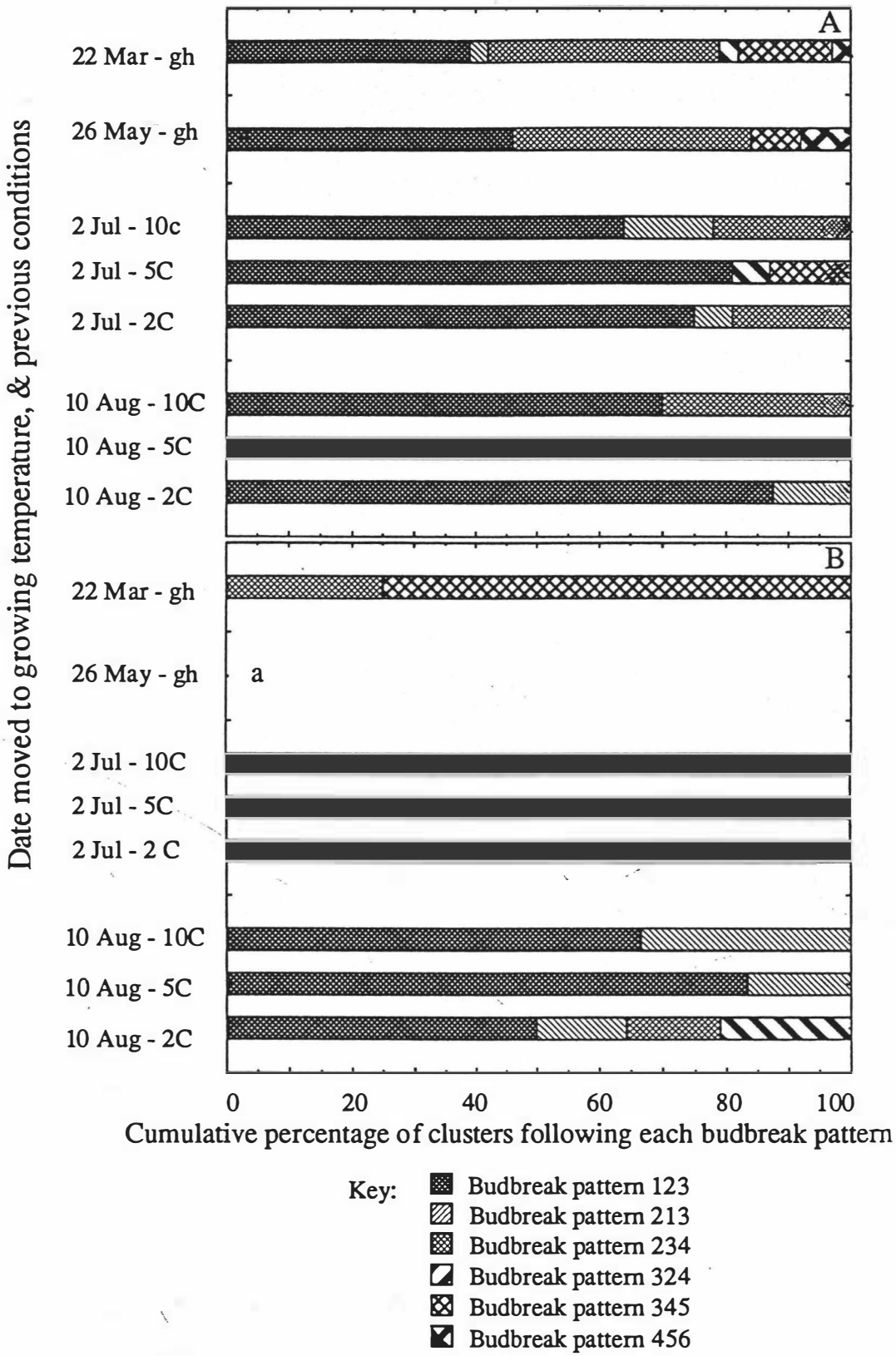
Budbreak pattern within a rhizome

Exponential growth of a spear generally commenced after the previous spear had been harvested (Fig.4.08a & b). The main exceptions to this occurred when the basal bud commenced growth after the bud in position 2 (e.g. Fig.4.08a spears 1 and 2) or when two spears commenced growth within 24 hours of each other (e.g. Fig.4.09a spears 3 and 4)

The major factor affecting budbreak pattern within a rhizome was whether or not the plants had been chilled (Table 4.07). The apparent lack of effect by chilling temperature, chilling time, plant sex and growing temperature on budbreak pattern when the data was analysed using Chi-squared analysis may be due to the small number (<16 per treatment) of rhizomes assessed in this experiment relative to the variation in budbreak pattern.

Budbreak progressed from older to younger buds on over 90% of the rhizomes which produced at least three spears (Fig.4.07a & b). This occurred regardless of which bud was the first to grow i.e, whether budbreak pattern was '1 2 3', '2 3 4' or '3 4 5' etc. The main exception to this occurred when the basal bud commenced growth after the bud in Position 2, i.e. when the budbreak pattern was '2 1 3' (Table 4.09).

There may be a lower occurrence of the '2 1 3' pattern on plants growing at 20C and on plants chilled at 5C but it was difficult to be certain from the small sample size (15 plants) (Table 4.09). Many plants produced less than three spears per rhizome in the 5



Note: a - plants did not grow in 115 days of experiment

Figure 4.07: Effect of chilling on cumulative percentage of rhizomes following each budbreak pattern on 'Rutger's Beacon' at 20C (A) or 12.5C (B). Data restricted to rhizomes which produced at least three spears. Buds numbered from the base of the dormant rhizome towards the apex, thus 1=basal bud. Plants transferred to growing conditions on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks.

Table 4.07: Chi-squared analysis on the effects of various factors on budbreak pattern within the first three spears to grow following transfer to 12.5C or 20C.

Factor	Probability that factor has a significant effect (Chi-squared analysis) at growing temperature of	
	12.5C	20C
Growing temperature	0.16	
Plant sex	0.50	0.48
Chilled *vs* unchilled plants	<0.001	0.001
Chilling temperature (chilled plants only)	0.32	0.22
Time of transfer:		
Chilled plants (5 or 10 weeks)	0.12	0.50
Unchilled plants (March or May)	-	0.60

weeks following transfer to 12.5C. A better comparison of budbreak patterns would have been possible if the same number or weight of spears had been harvested from plants at each temperature.

On most plants with the '2 1 3' budbreak pattern (Table 4.09), the basal bud began exponential growth before the spear from Position 2 was harvested. In ten of the 15 cases (67%), the first two spears were considered 'twins' i.e, the second spear commenced exponential growth and reached harvestable size within 1 day of the first spear at 20C, and within 2 days at 12.5C. This proportion was higher than in rhizomes which followed a '1 2 3' budbreak pattern where the first two spears were 'twins' on only 13% (15 of 114) of the rhizomes.

The mean RSGR of the first spear to grow was higher than or equal to the mean RSGR of the second spear to grow at both growing temperatures and on rhizomes with either the '1 2 3' or '2 1 3' budbreak pattern (Table 4.09).

The basal bud on the rhizomes with a '2 1 3' budbreak pattern may not be completely released from dormancy as the RSGR of the basal spear was lower if it was the second spear to grow than if it was the first spear to grow (Table 4.09). The difference at 12.5C was significant at P=0.5 (t test); however, the probability level for significance at 20C was near to 0.20. These results are physiologically sensible, but may be an anomaly caused by the small sample size.

The effect of natural chilling on the budbreak pattern within 'Rutger's Beacon' is presented in Fig.4.11.

Table 4.08: Mean relative spear growth rates (RSGR) of first two spears to grow on rhizomes of 'Rutger's Beacon' with budbreak pattern of '1 2 3' or '2 1 3'[a]. Standard errors of means in brackets.

	RSGR of first spear to grow	RSGR of second spear to grow	Number of plants
At growing temperature of 20C			
Budbreak pattern '1 2 3'	0.573 (0.009) [b]	0.563 (0.015) [c]	53
Budbreak pattern '2 1 3'	0.547 (0.054) [c]	0.546 (0.047) [b]	6
At growing temperature of 12.5C			
Budbreak pattern '1 2 3'	0.196 (0.008) [b]	0.182 (0.010) [c]	35
Budbreak pattern '2 1 3'	0.214 (0.010) [c]	0.181 (0.018) [b]	9

Notes:

a - buds and the spears which originate from them are numbered from the basal bud towards the apex of the dormant rhizome.

b - basal spear.

c - spear in Position 2.

Table 4.09: Time at which a spear commences exponential growth (T0), and subsequently reaches 200mm in length (T200) at 12.5C for the first two spears per rhizome when the second bud commences growth before the basal bud. Plants transferred to growing conditions on 22 March and 26 May were from the heated glasshouse (minimum temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks.

Case no.	Grow -ing temp.	Date trans- ferred	Chill temp. (C)	Second spear to grow: from basal bud			First spear to grow: from second bud		
				T0	T200	RSGR	T0	T200	RSGR
1	12.5	2 Jul	Nat	11.3	34.2	0.090	10.4	20.7	0.190
2	12.5	2 Jul	Nat	20.2	33.1	0.171	18.9	28.3	0.220
3	12.5	2 Jul	5	23.2	33.4	0.176	22.3	49.0	0.075
4	12.5	2 Jul	5	22.1	32.8	0.237	19.9	31.1	0.231
5	12.5	10 Aug	10	0.07	15.8	0.140	-6.3	7.90	0.152
6	12.5	10 Aug	10	5.7	14.7	0.213	4.9	14.0	0.216
7	12.5	10 Aug	10	14.5	28.6	0.156	3.7	13.1	0.224
8	12.5	10 Aug	2	10.6	25.0	0.172	10.5	22	0.204
9	12.5	10 Aug	2	12.8	24.8	0.182	12.1	21.3	0.234
10	20	22 Mar	gh	5.1	14.8	0.364	4.0	9.9	0.533
11	20	2 Jul	10	14.9	18.2	0.726	13.6	17.1	0.652
12	20	2 Jul	10	7.7	11.9	0.525	7.6	14.4	0.302
13	20	2 Jul	2	12.9	17	0.554	10.5	13.8	0.651
14	20	10 Aug	5	8.3	12.0	0.530	8.2	11.3	0.621
15	20	10 Aug	2	4.3	7.9	0.575	4.0	8.1	0.527

Budbreak pattern: interactions between rhizomes

On most of the 'Rutger's Beacon' plants, initiation of spear growth by the first and subsequent spears appeared to be independent of events on other rhizomes, e.g. Fig.4.08b. Any apparent synchronisation of budbreak between rhizomes was not maintained throughout the harvest.

Development of buds on axillary rhizomes was observed on a few plants at 20C e.g. Fig.4.08b. The RSGR of spears on the axillary rhizome was similar to spears on the parent rhizome.

Budbreak pattern: Two examples

Two plants of 'Rutger's Beacon' will now be used to illustrate the budbreak pattern within plants. Both plants were grown at 20C after chilling at 10C for 10 weeks and their budbreak patterns were typical of 'Rutger's Beacon' throughout this experiment. The plant illustrated in Fig.4.08b is one of the few in which budbreak occurred on an axillary rhizome.

Fig.4.08a illustrates budbreak in a plant with one main rhizome. On this plant, spears 1 and 2, and spears 3 and 4 were 'twins' - i.e, they commenced growth and were harvested within 24 hours of each other. Spear 4 appeared to inhibit the growth of spear 3, but the inhibition was very slight. Spear 7 also commenced growth before the preceding spear had been harvested. The RSGR decreased slightly from spear 1 to spear 8.

Fig.4.08b illustrates budbreak on a plant with two main rhizomes, labelled 'A' and 'B', two smaller rhizomes and buds in axillary positions on one of the main rhizomes. The budbreak pattern on the main rhizomes was independent. On both main rhizomes, the basal bud was the first to grow. The RSGR of the first spear was lower than subsequent spears on rhizome A but not on rhizome B.

Budbreak did not occur on the two small rhizomes; however, it did occur in two axillary positions on rhizome A. Budbreak by the basal bud on a developing axillary rhizome appeared to be inhibited by the bud 'above' it on the parent rhizome as the axillary bud in position A1.1 commenced growth after the adjacent spear in position A3 had been harvested; likewise the axillary bud A3.1 commenced growth after spear A5 had been

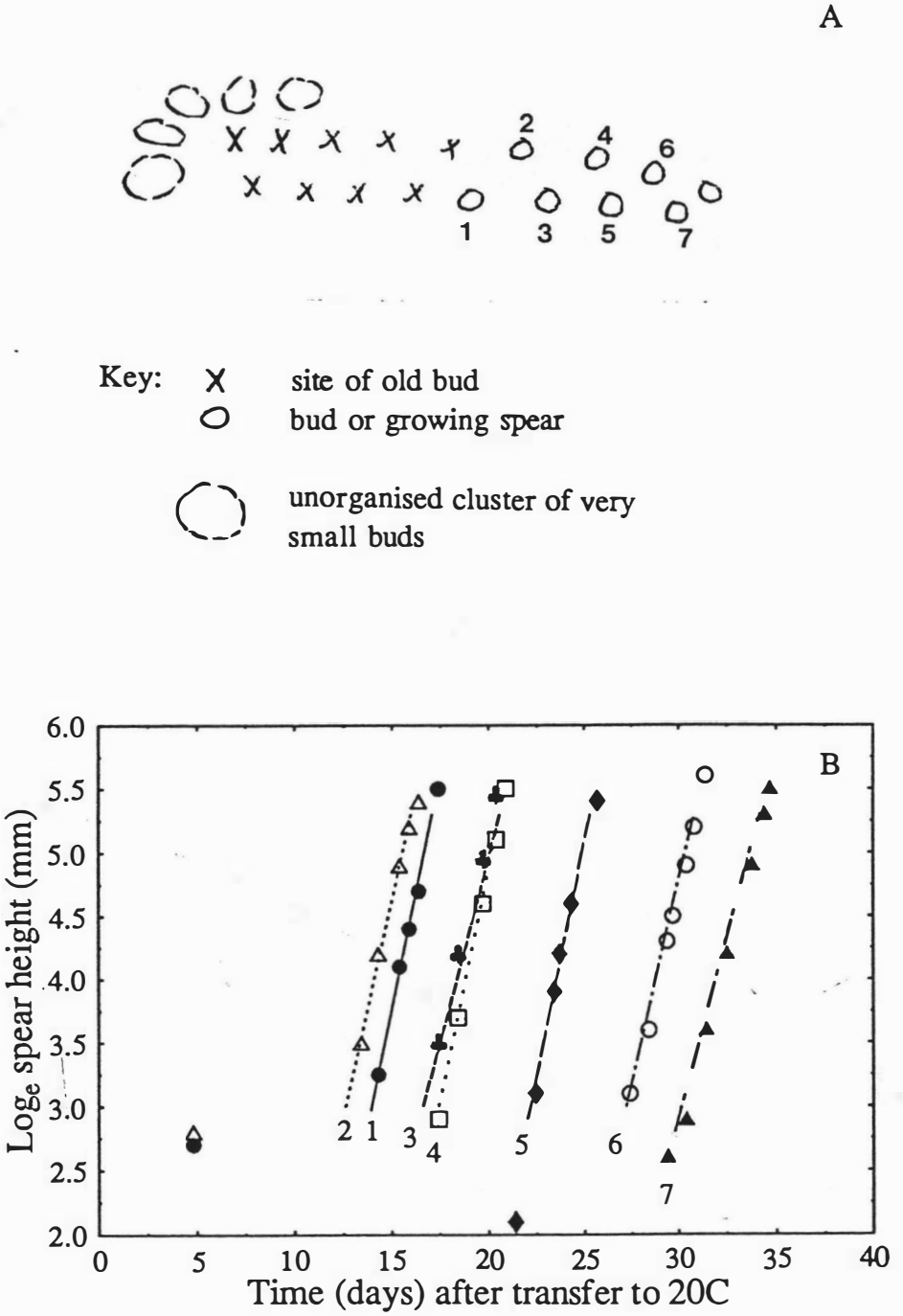
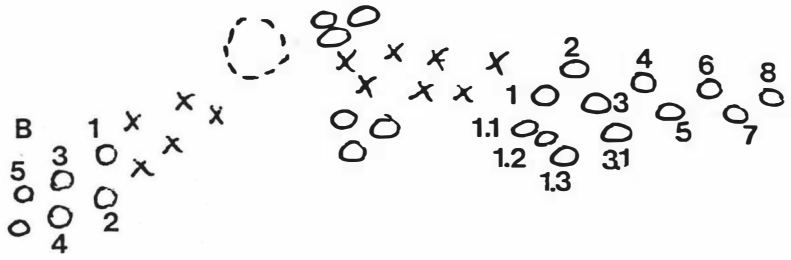


Figure 4.08a: Budbreak patterns in 'Rutger's Beacon' plant with one active bud cluster.
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear.

A



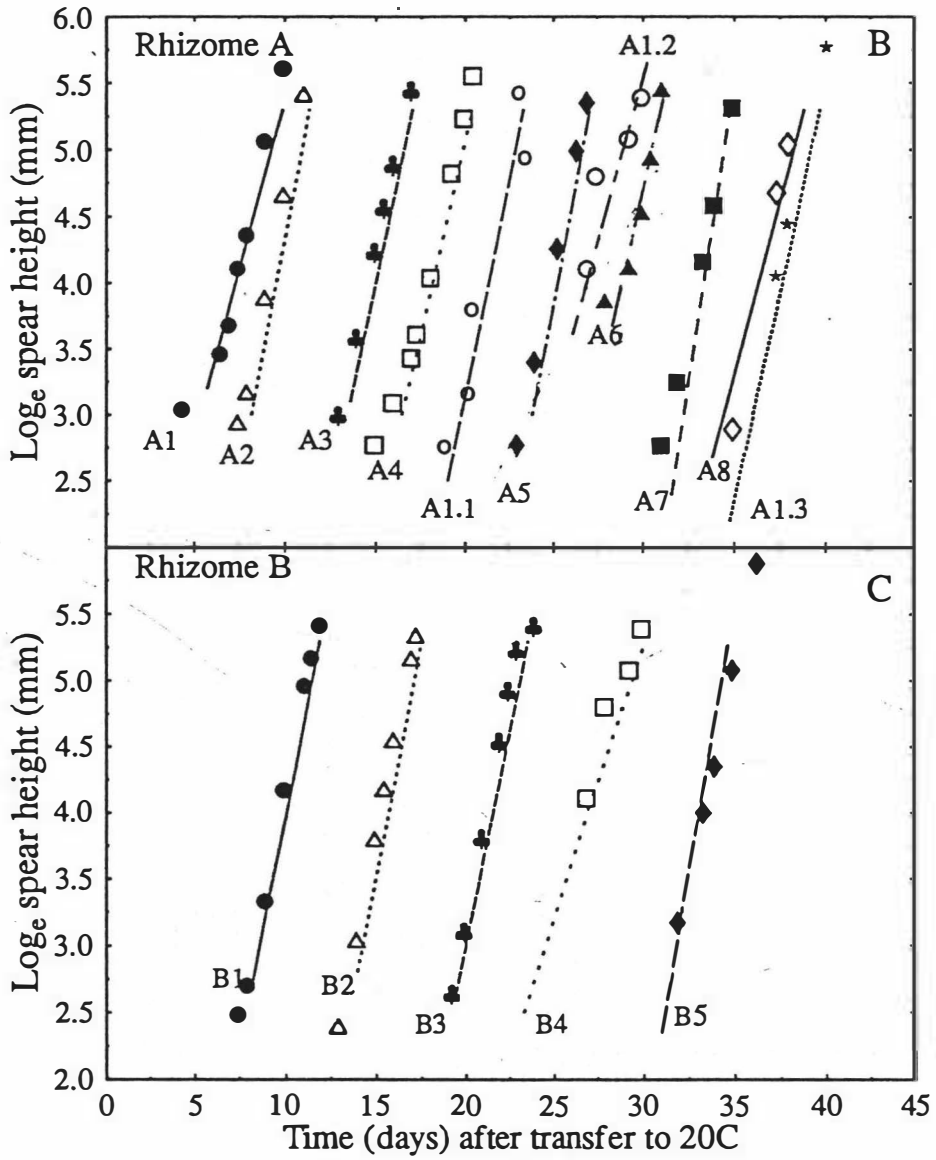


Figure 4.08b: Budbreak patterns in 'Rutger's Beacon' plant with two active bud clusters.
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear in rhizome A.
C: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear in rhizome B.

harvested. Spear A1.2 commenced growth before A1.1 was harvested, and it's growth was initially suppressed, apparently by A5. The physical relationship between A7 and A3.1 was similar to A5 and A1.2, but A7 has no effect on the growth of the axillary bud A3.1. However, buds on this axillary were not arranged in the typical zig-zag pattern and this may indicate that bud development was not normal.

Effect of photoperiod on spear growth

Photoperiod had no effect on either the number of days to budbreak or the RSGR of the first three spears on plants at 20C in the simulated autumn harvest (Experiment A) (Table 4.10). Effects on plants at 12.5C could not be determined as too few plants grew.

Table 4.10: Effect of photoperiod on time to budbreak (days) and relative spear growth rate (RSGR, mm/mm/day) of plants at 20C in a simulated autumn harvest. Effect of photoperiod not significant at P=0.05 in analysis of variance. Standard error of mean in brackets.

	Photoperiod (hours)	
	8	16
Days to budbreak	8.77 (1.44)	7.15 (1.61)
RSGR	0.534 (0.011)	0.511 (0.012)

Effect of plant sex on spear growth

The effect of plant sex on the number of days to budbreak and the RSGR of the first three spears was not significant at P=0.05 throughout the simulated autumn or spring harvests (Experiments A and B) (Table 4.11). However, the RSGR of spears on female plants was slightly higher (P=0.11) than on male plants. There was also a slight interaction (P=0.07) between growing temperature and plant sex in the number of days to budbreak. Budbreak on female plants was slightly slower than on male plants at 12.5C, but slightly faster than on male plants at 20C.

Table 4.11: Effect of plant sex on time to budbreak (days) and relative spear growth rate (RSGR, mm/mm/day) of plants at 12.5C or 20C in a simulated autumn and spring harvests. Standard error of mean is in brackets.

	Plant sex	
	Male	Female
Days to budbreak at 12.5C	18.57 (1.35)	21.35 (1.28)
Days to budbreak at 20.0C	13.01 (1.62)	10.66 (1.38)
RSGR at 12.5C	0.198 (0.009)	0.210 (0.010)
RSGR at 20.0C	0.551 (0.009)	0.569 (0.008)

4.3.3 SPEAR GROWTH BY RUTGER'S BEACON FOLLOWING NATURAL CHILLING: EXPERIMENT C

Plants of 'Rutger's Beacon' which had been exposed to natural chilling before 26 May were able to grow at 12.5C (Fig.4.09). These plants had received 267 chilling units before transfer to the 'spring' temperature. The number of days to budbreak decreased as the length of chilling increased, in a manner similar to plants chilled in constant temperatures (Fig.4.04).

The RSGR increased slightly as the length of natural chilling increased (Fig.4.10). The RSGR was more variable than on plants chilled at controlled temperatures. There were no consistent differences between the RSGR of the first and subsequent spears. There was no difference in the RSGR at 12.5C of plants exposed to natural chilling and plants exposed to controlled chilling temperatures.

There was a large number of plants with the '2 1 3' budbreak pattern when plants were transferred from natural chilling condition to 12.5C on 26 May (Fig.4.11). The proportion of plants with the '2 1 3' budbreak pattern decreased as the length of chilling increased. There was a low number of initial spears produced by the basal bud when the plants were transferred to 12.5C on 26 May and 2 July (Fig.4.11).

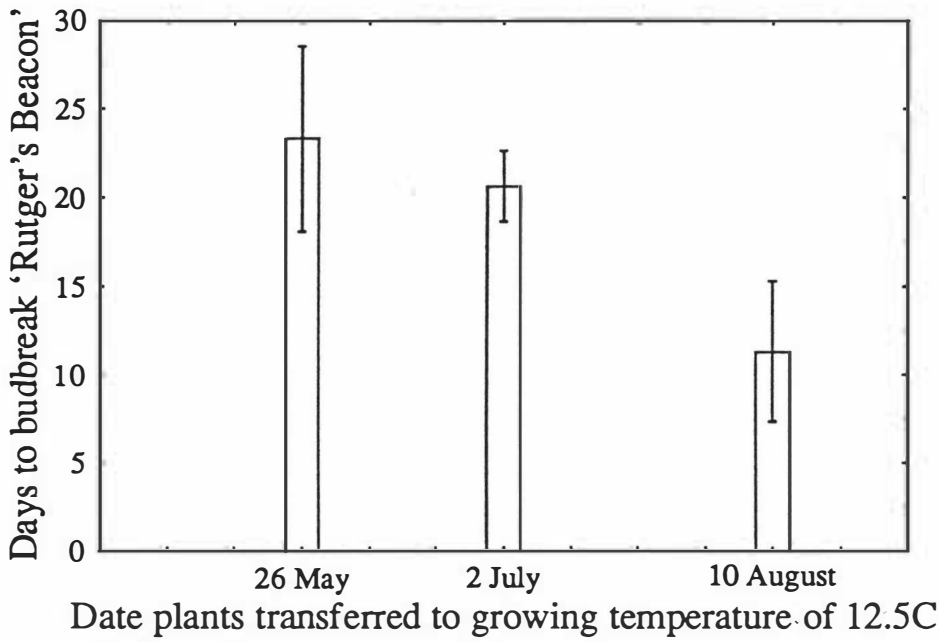


Figure 4.09: Mean number of days to budbreak by first spear to grow on each rhizome of 'Rutger's Beacon' transferred to 12.5C after chilling in natural conditions. Bars represent twice standard error of mean.

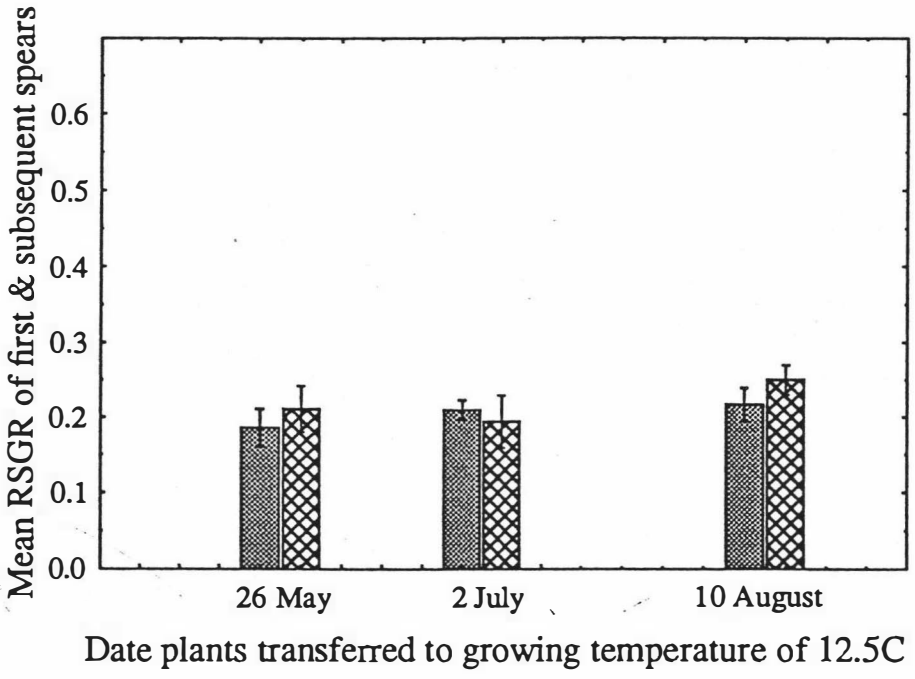


Figure 4.10: Mean RSGR of first and subsequent spears to grow on each rhizome of 'Rutger's Beacon' transferred to 12.5C after chilling in natural conditions. Bars represent twice standard error of mean.

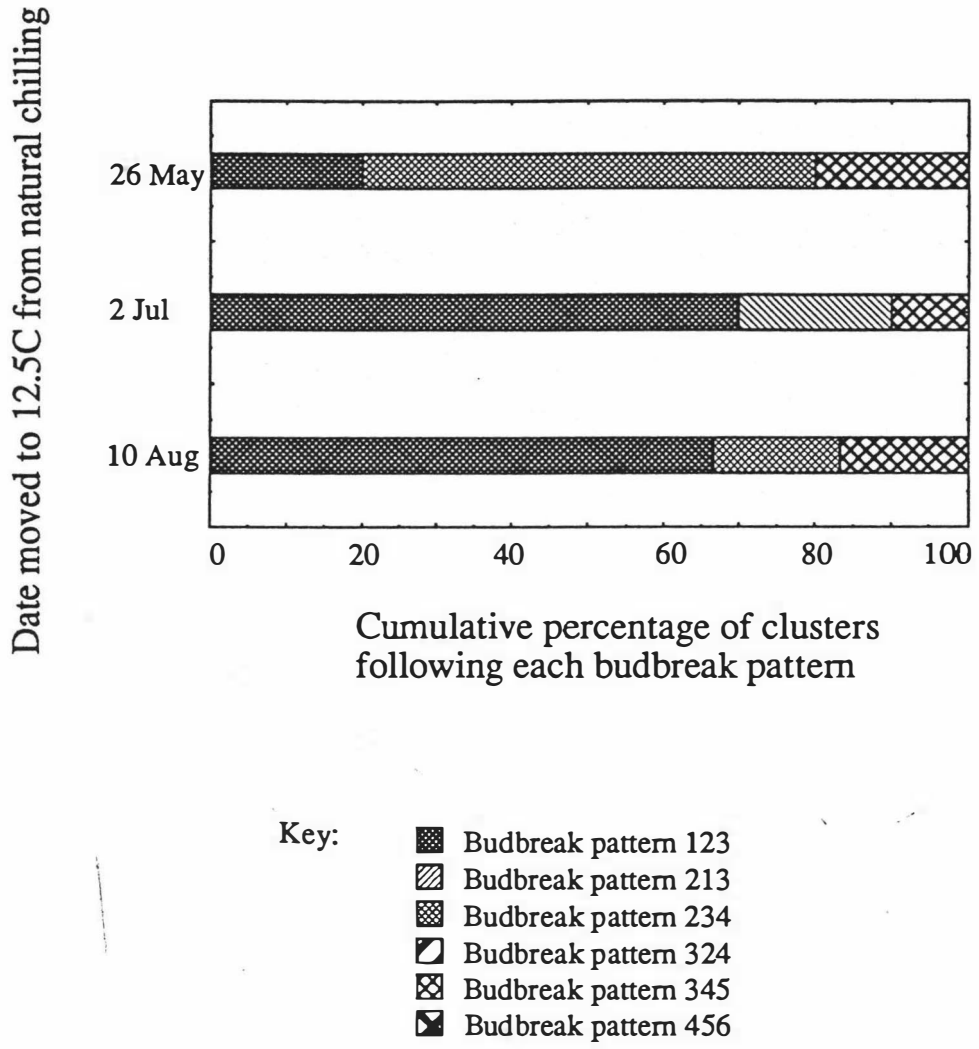


Figure 4.11: Effect of chilling in natural conditions on percentage of rhizomes following each budbreak pattern on 'Rutger's Beacon' at 12.5C. Data restricted to rhizomes which produced at least three spears. Buds numbered from the base of the dormant rhizome towards the apex, thus 1=basal bud.

4.3.4 SOME GENOTYPIC EFFECTS: EXPERIMENT D

Effect of internal dormancy on spear growth

Time to budbreak

Budbreak occurred earlier (Fig.4.12a & b) on plants of 'Jersey Giant' and 'UC157' at 20C than at 12.5C in 'spring' following each of the chilling treatments. As with 'Rutger's Beacon', budbreak in both cultivars occurred at 20C throughout the experiments. Budbreak on 'UC157' also occurred at 12.5C (Fig.3.13b) throughout the experiment; however 'Jersey Giant' transferred from the heated glasshouse (minimum temperature 13C) to 12.5C on 26 May did not grow during the experiment (Fig.4.12). Like 'Rutger's Beacon, buds on 'Jersey Giant' grew at 12.5C after the plants were stored at 10C or lower temperatures.

The number of days to budbreak is less for 'UC157' and 'Jersey Giant' than for 'Rutger's Beacon' (Fig.4.04). 'UC157' consistently produced spears earlier than 'Jersey Giant' of the same age (Fig.4.12).

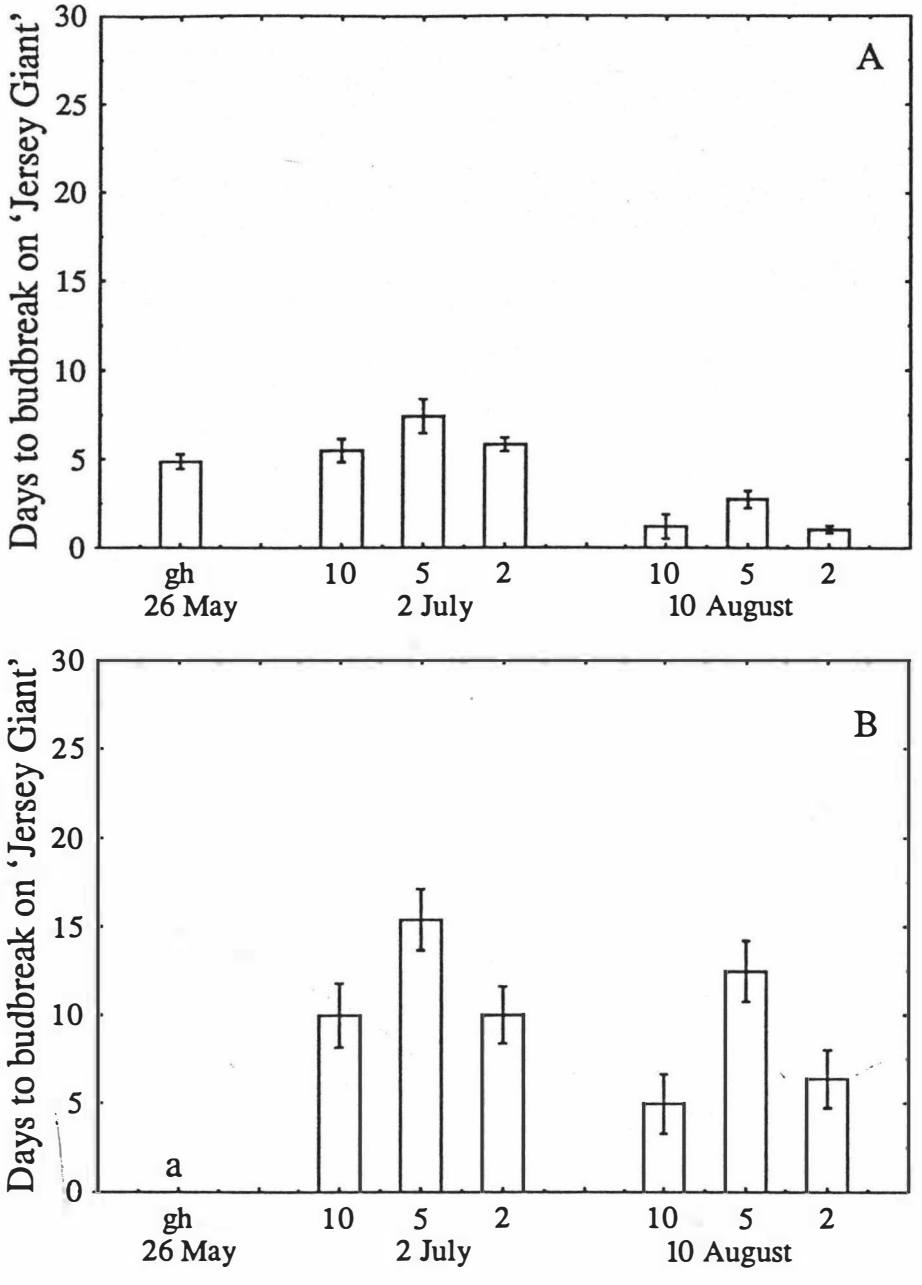
Chilling at 10, 5 or 2C for 5 weeks increased the number of days to budbreak at 12.5C on 'UC157'. However, the same chilling allowed budbreak to occur at 12.5C on 'Jersey Giant' which had not produced any spears when transferred on 26 May. Chilling for a further 5 weeks after 2 July decreased the number of days to budbreak on both 'UC157' and 'Jersey Giant'.

There were no consistent trends in the spread (i.e, SE) of budbreak on either 'Jersey Giant' or 'UC157'.

Spear growth rates

Spear height increased exponentially between 20 mm and 200 mm at both 12.5C and 20C on 'Jersey Giant' and 'UC157' when relative spear growth rate (RSGR) was calculated as described in Section 4.2.4. As with 'Rutger's Beacon', there was no evidence that spear growth at 12.5C followed two exponential curves.

The RSGR of the first spear of 'Jersey Giant' (Fig.4.13a) tends to be higher than 'Rutger's Beacon' (Fig.4.06a & b) and 'UC157' (Fig.4.13b), especially on plants transferred to growing conditions on 26 May and on plants chilled at 10C.



Note: a - no plants grew in 42 days

Figure 4.12a: Mean number of days to budbreak by first spear to grow on each rhizome of 'Jersey Giant' at 20C (A) or 12.5C (B). Plants transferred to growing temperature on 26 May were from a heated glasshouse (minimum temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

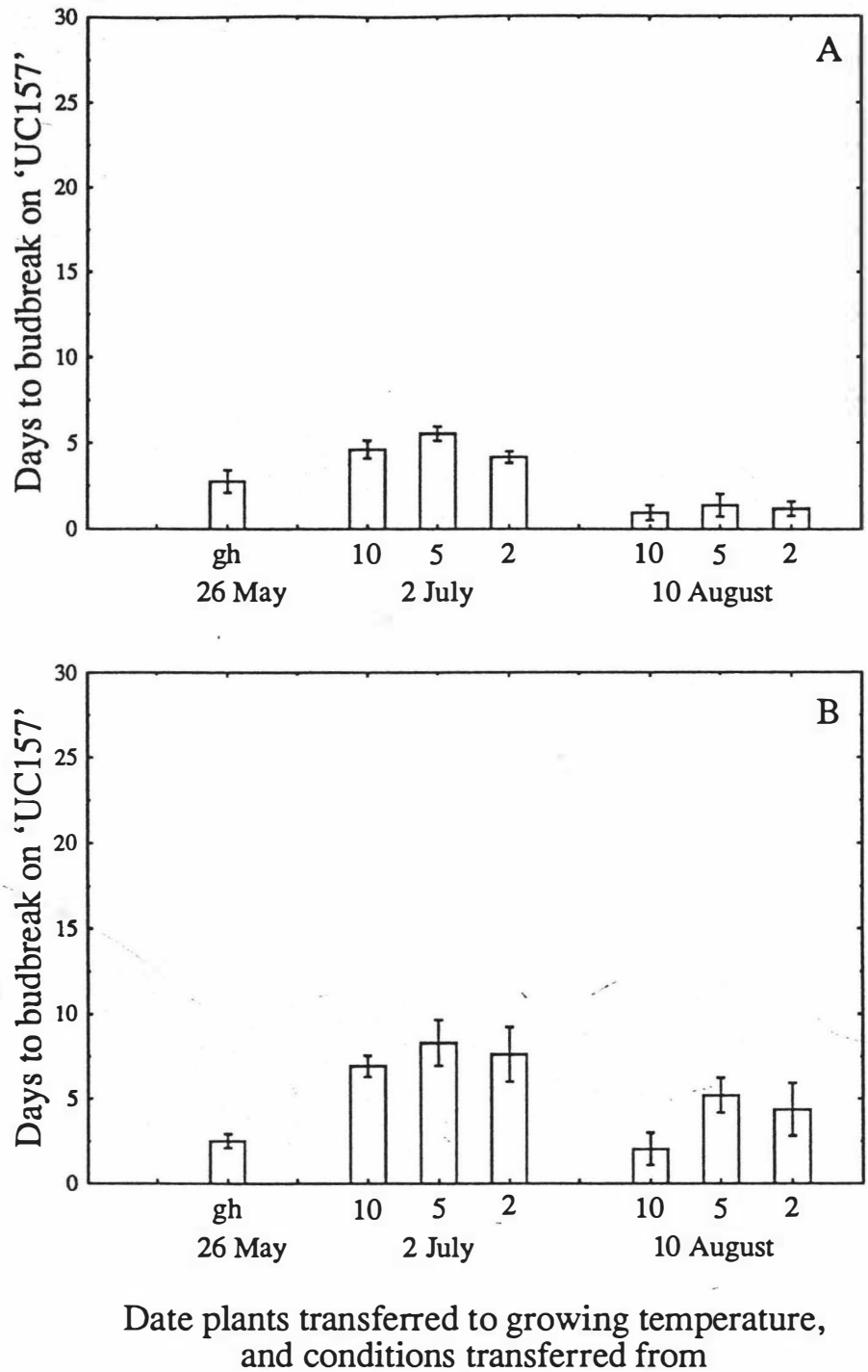


Figure 4.12b: Mean number of days to budbreak by first spear to grow on each plant of 'UC157' at 20C (A) or 12.5C (B). Plants transferred to growing temperature on 26 May were from a heated glasshouse (minimum temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

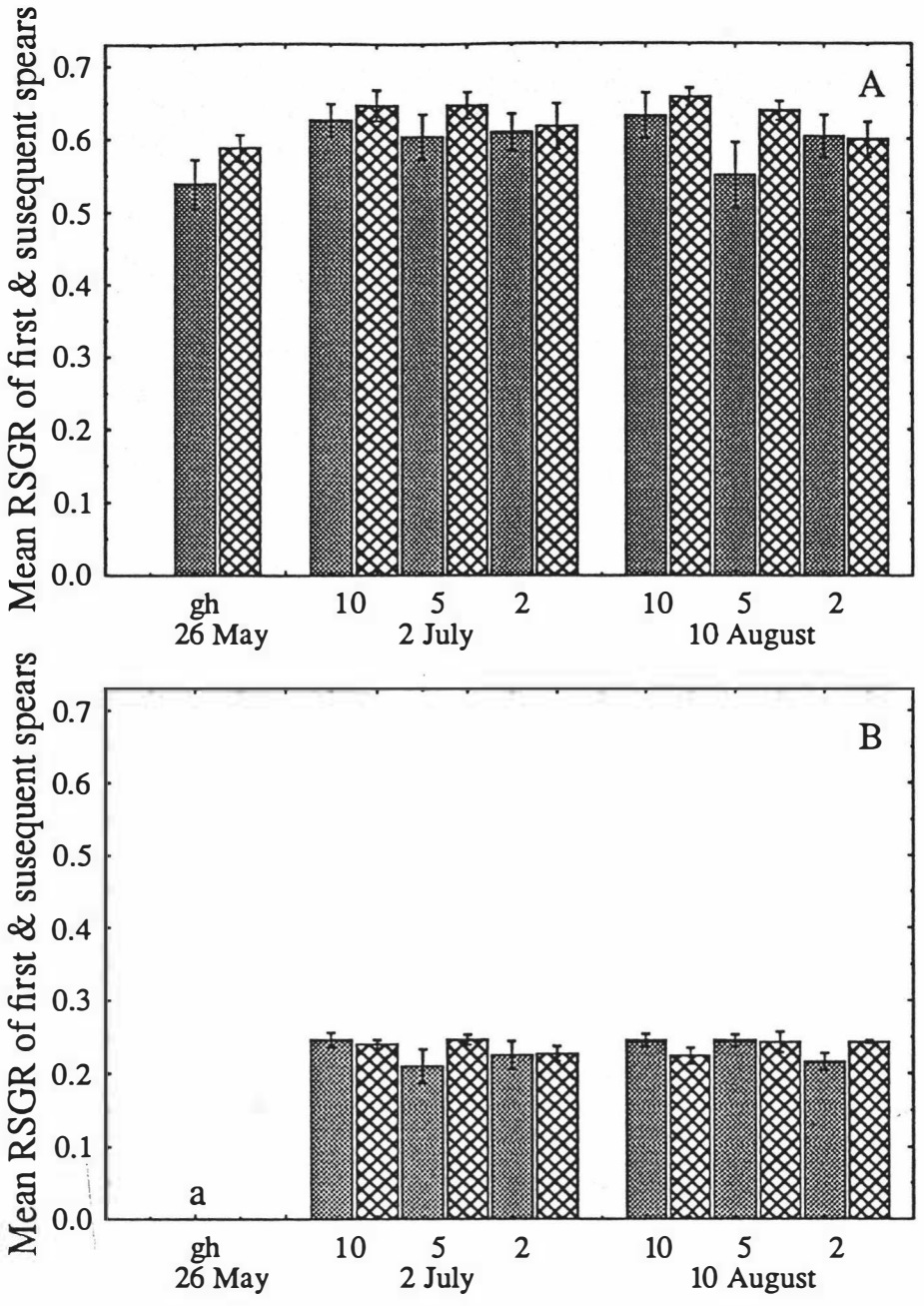


Figure 4.13a: Mean relative spear growth rate (RSGR) of first and subsequent spears to grow on 'Jersey Giant' at 20C (A) or 12.5C (B). Plants transferred to growing conditions on 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

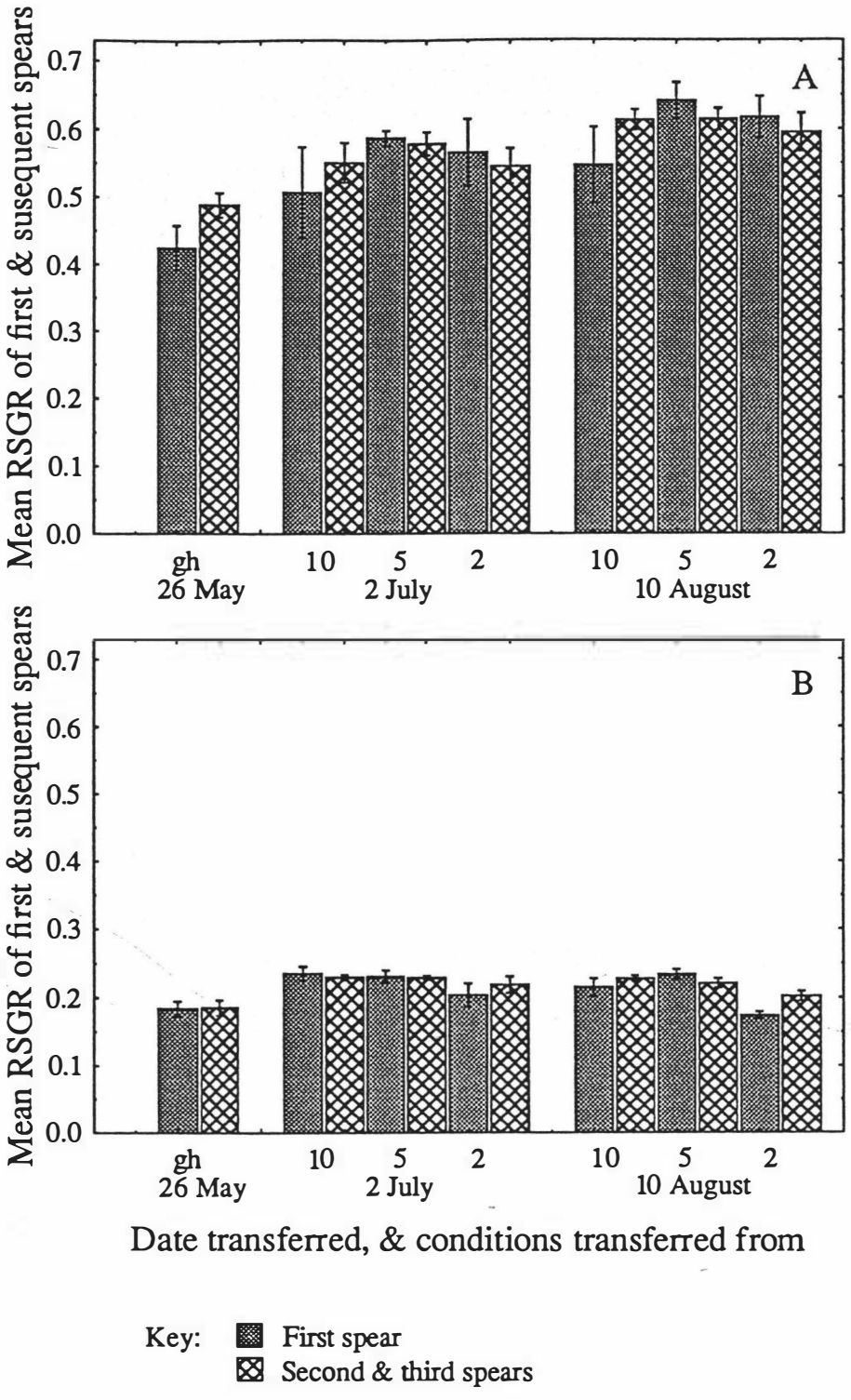


Figure 4.13b: Mean relative spear growth rate (RSGR) of first and subsequent spears to grow on 'UC157'at 20C (A) or 12.5C (B). Plants transferred to growing conditions on 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

Budbreak patterns

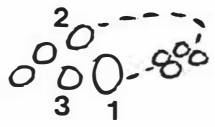
The plants of 'UC157' consisted of many ill defined rhizomes while those of 'Jersey Giant' consisted of a few clearly defined rhizomes (Table 4.12, Fig.4.14a & b). On 'Jersey Giant' there was no interaction between the rhizomes. The relationship between rhizomes, and the budbreak sequence on 'Jersey Giant' (e.g. Fig.4.14c&d) followed the same pattern as 'Rutger's Beacon'. The different rhizome arrangement of 'UC157' was accompanied by a different budbreak pattern: 'UC157' tended to produce spears in flushes, e.g. in Fig.4.14a, spears 1 to 4 inhibit the growth of spears 7 to 10, and in Fig.4.14b spears 1 to 5 inhibit the growth of spears 6 and 7.

The effect of chilling on the position of the first bud to grow in 'Jersey Giant' (Fig.4.15) was similar to the effect on 'Rutger's Beacon' (Fig.4.05). Chilling increased the proportion of basal buds which produced the first spear.

Table 4.12: Genotypic differences in mean number of active rhizomes per plant, 3 weeks after transfer to 20C or 5 weeks after transfer to 12.5C. Standard errors of means in brackets. Note that plants chilled for zero weeks do not have a chilling temperature.

Growing temperature (C)	Number of active rhizomes per plant when chilled for (weeks)						Chilling temperature (C)
	0		5		10		
	UC157	*Jersey Giant*	*UC157*	*Jersey Giant*	*UC157*	*Jersey Giant*	
20	5.8 (1.2)	1.5 (0.2)	6.7 (1.2)	1.5 (0.2)	4.8 (0.7)	1.5 (0.3)	10
			5.0 (0.9)	1.8 (0.4)	2.8 (0.5)	2.7 (0.6)	5
			5.0 (0.6)	2.0 (0.5)	3.7 (0.4)	2.7 (0.8)	2
12.5	5.2 (0.7)	0.0 (0.0)	4.5 (0.5)	1.2 (0.2)	4.0 (0.7)	1.2 (0.2)	10
			3.0 (0.8)	1.2 (0.2)	2.8 (0.2)	1.7 (0.2)	5
			3.2 (0.5)	1.3 (0.2)	3.8 (0.8)	1.8 (0.5)	2

A



Key: ✗ site of old bud

O bud or growing spear

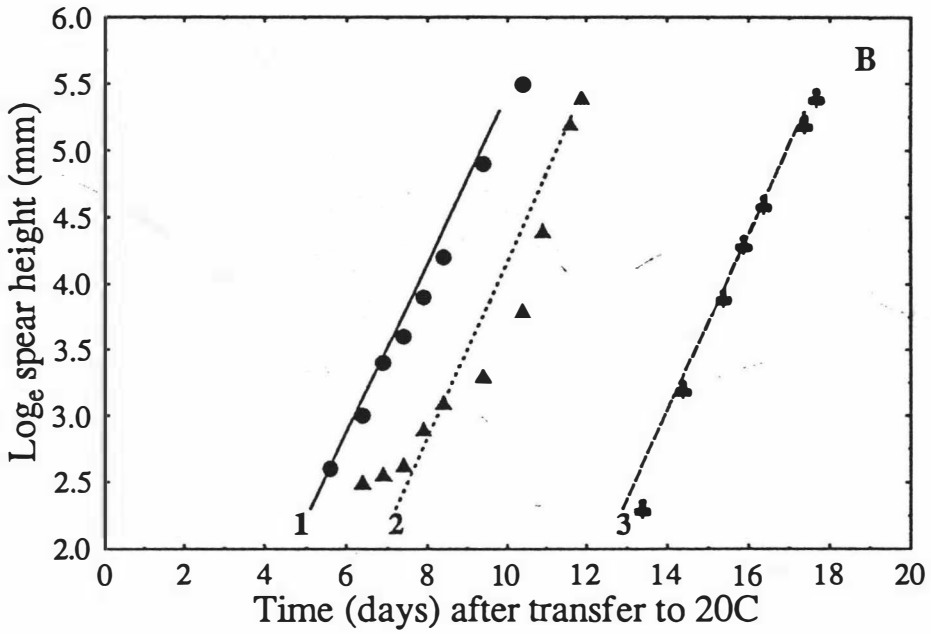


Figure 4.14a: Budbreak patterns in 'Jersey Giant' plant with one clearly defined bud cluster.
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear.

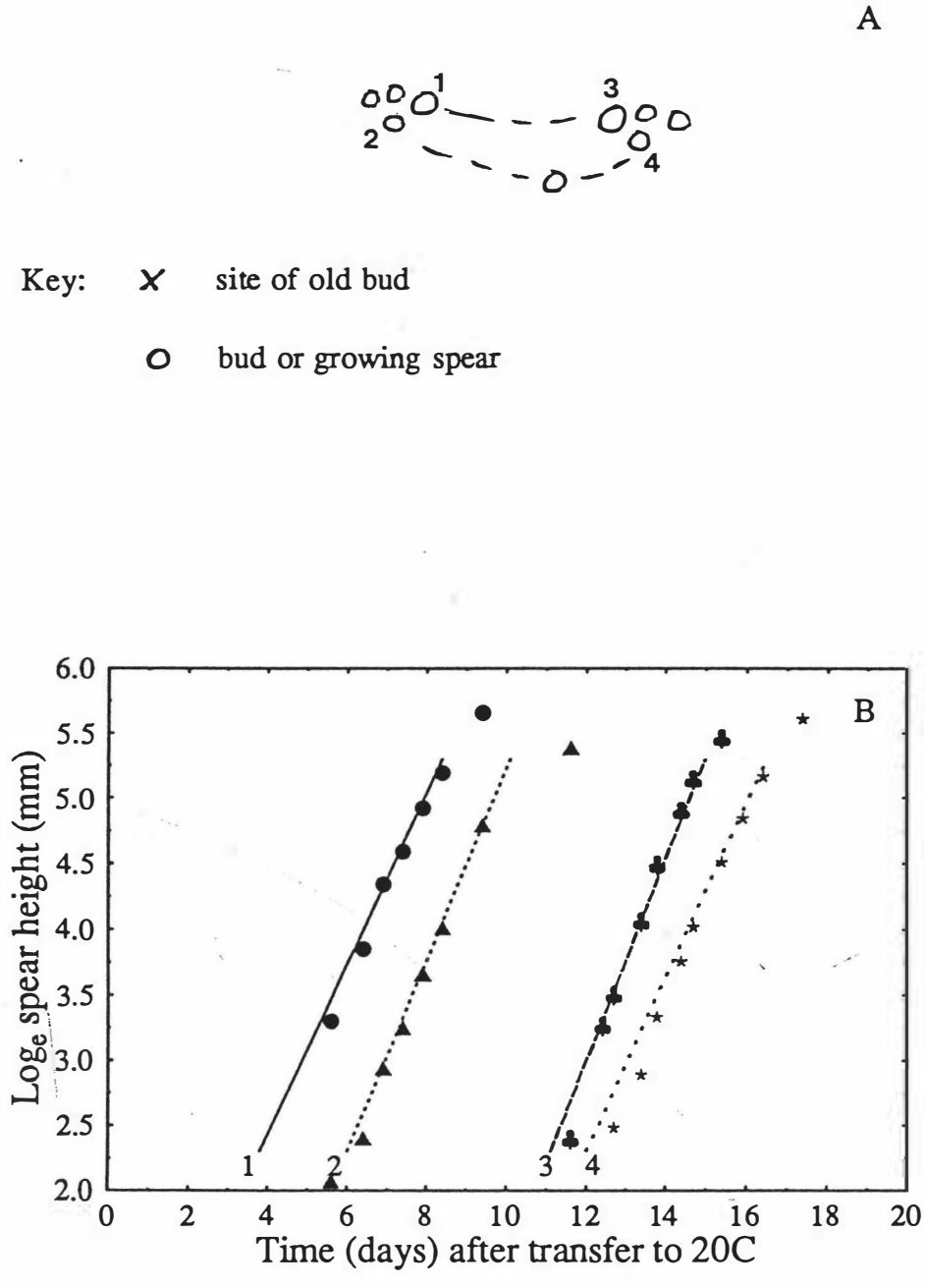


Figure 4.14b: Budbreak patterns in 'Jersey Giant' plant with two clearly defined bud clusters.
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear.

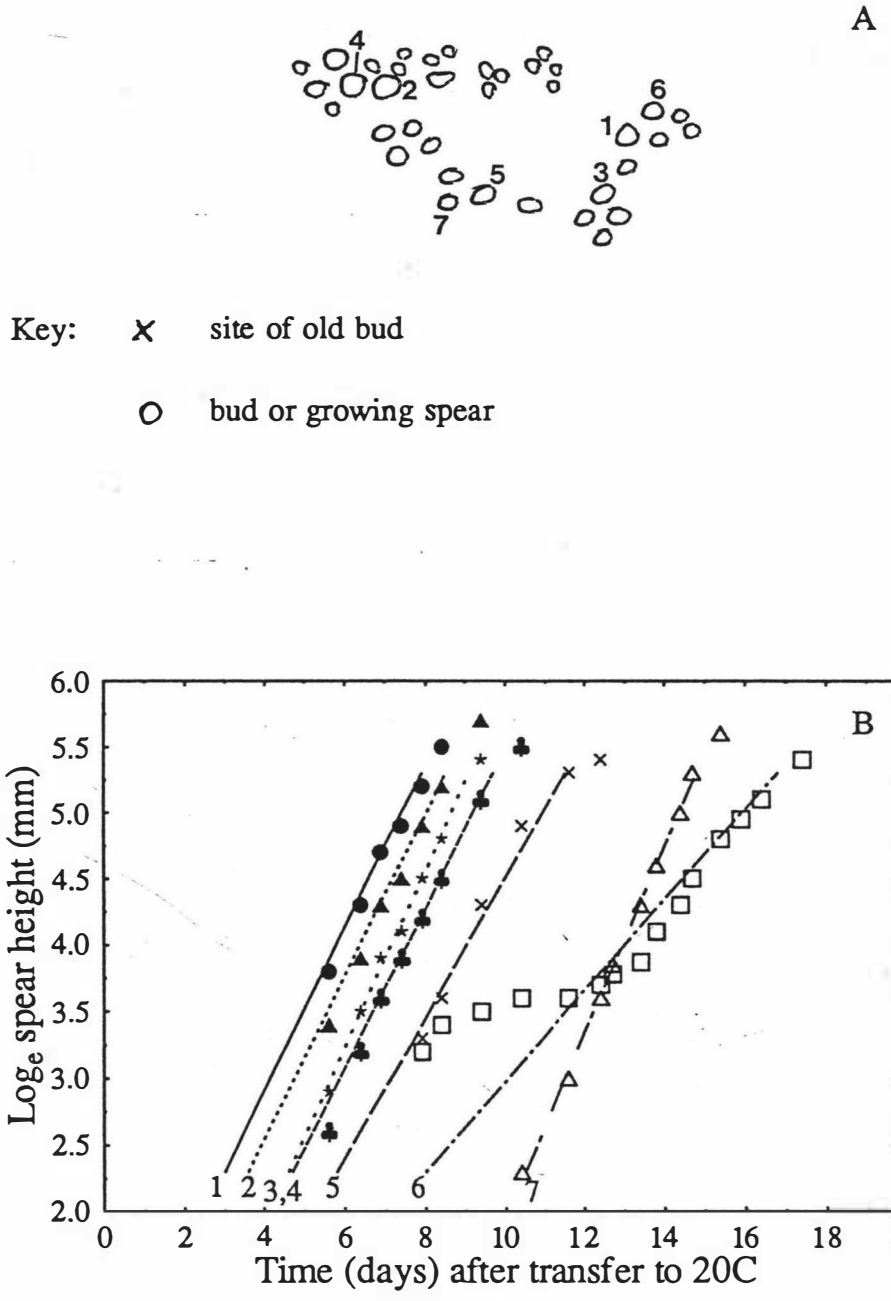


Figure 4.14c: Budbreak patterns in 'UC157' plant with several active bud clusters but no clearly defined rhizomes.
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear.

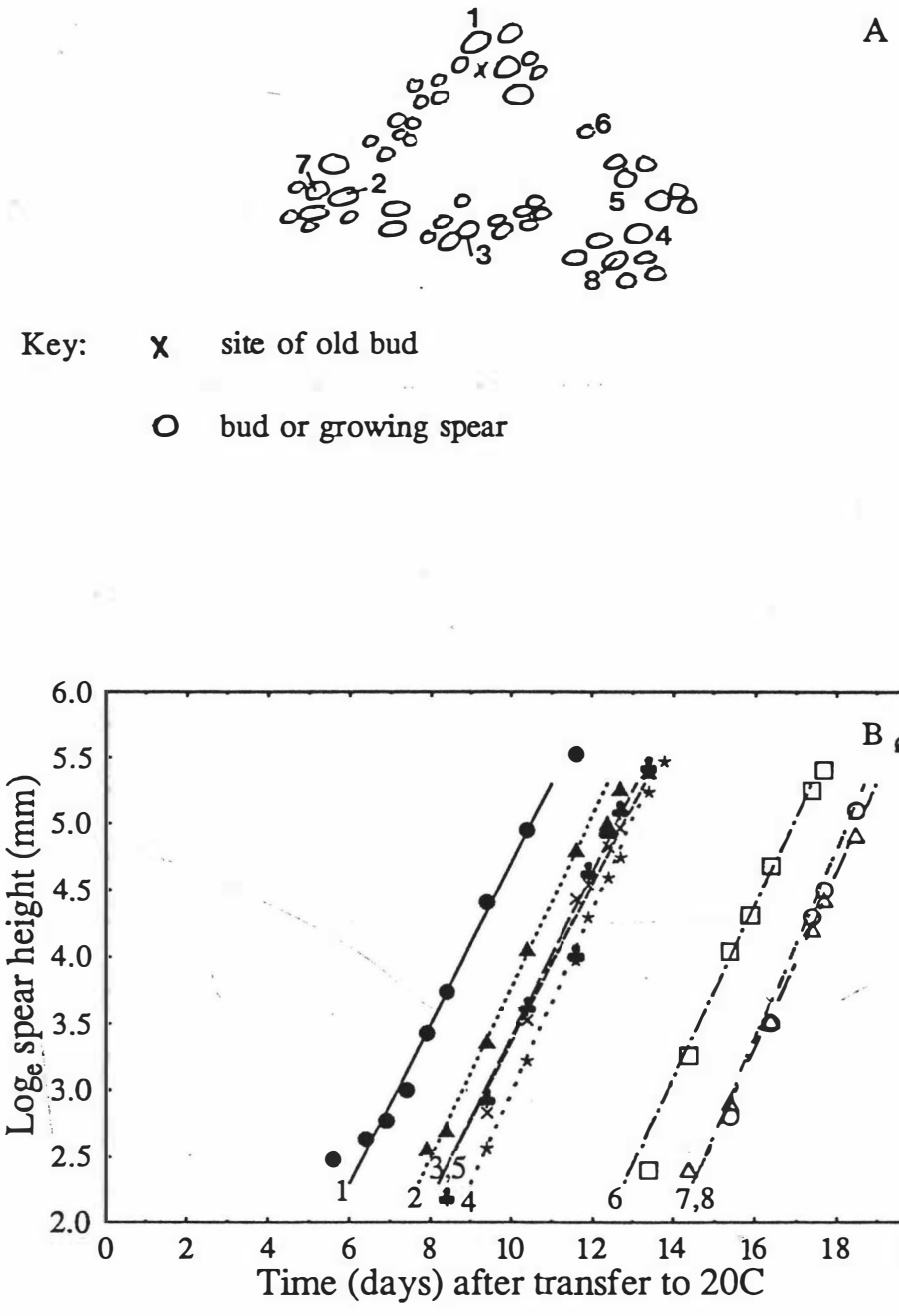
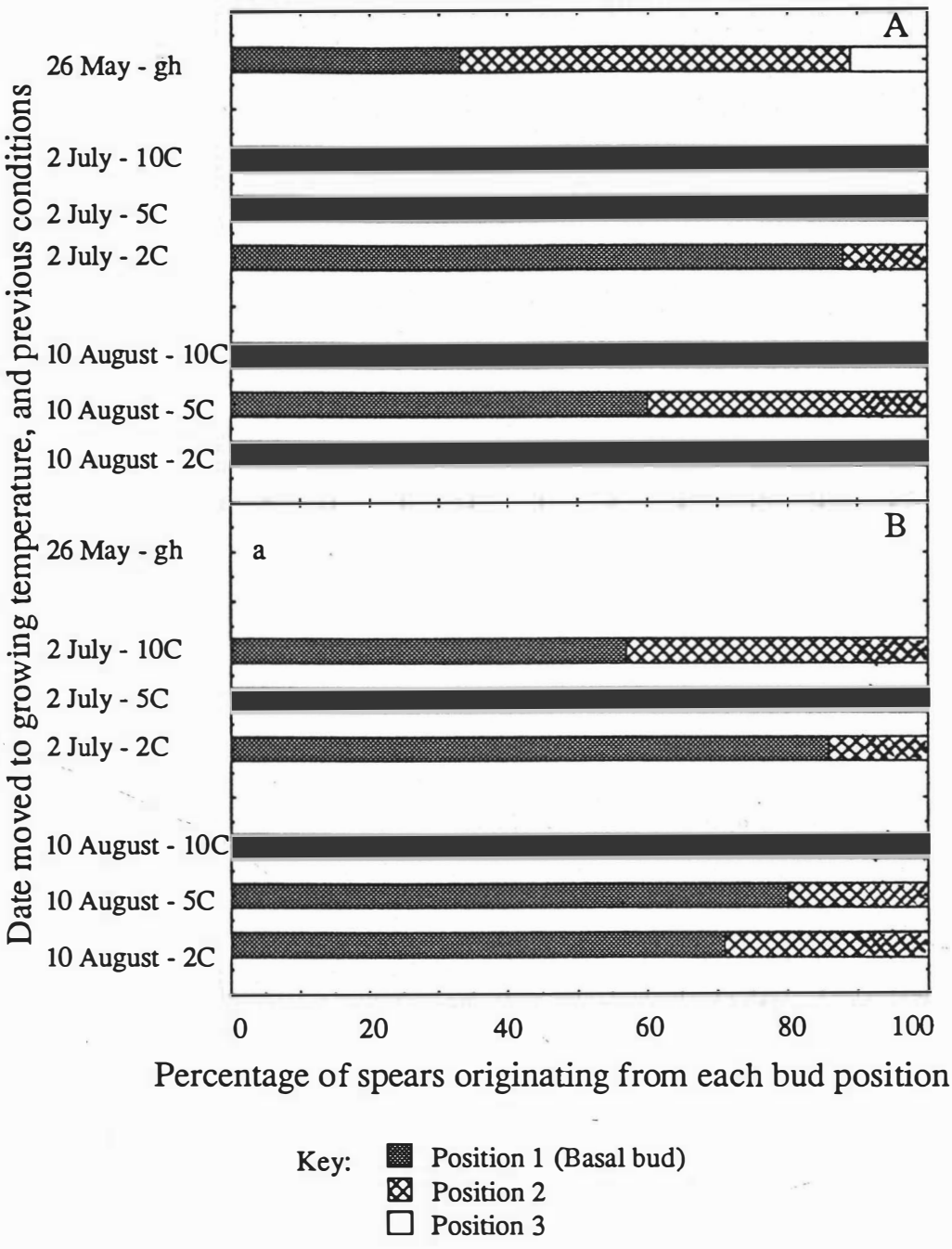


Figure 4.14d: Budbreak patterns in 'UC157' plant with several active bud clusters but no clearly defined rhizomes
A: rhizome map showing positions of buds which produced spears.
B: measurements of spear height and the regression line fitted to calculate relative spear growth rate (slope of line) of each spear.



Note: a - plants did not grow in 42 days of experiment

Figure 4.15: Effect of chilling on the position of the first bud to grow on 'Jersey Giant' at 20C (A) or 12.5C (B). Plants transferred to growing conditions on 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks.

4.4. DISCUSSION

4.4.1 A MODEL OF DORMANCY IN ASPARAGUS

Chilling markedly decreased the mean time to budbreak of the first spear to emerge after winter dormancy (Figs. 4.04 and 4.12), decreased the minimum temperature at which budbreak occurred (Figs. 4.04 and 4.12), and increased the RSGR of that spear (Fig.4.06). The position of the first spear to grow was also affected, becoming more basipetal in response to chilling (Fig.4.05). This indicates that chilling released winter dormancy which implies that a form of internal dormancy i.e, paradormancy or endodormancy, was operating. If ecodormancy had been operating during winter, chilling would not have changed the mean time to budbreak, or RSGR or position of the first spear to grow because temperature would have been the only factor limiting the initiation of growth. Also, the minimum temperature at which growth occurred would have been constant. These observations are similar to the effects of chilling on other meristems which undergo dormancy, including the buds of deciduous fruit trees (Vegis 1963, 1964; Saure 1985; Fuchigami and Nee 1987). It seems reasonable to assume, on the basis of this data, that similar mechanisms are operating in both asparagus and other plants which undergo internal dormancy. Therefore dormancy models developed using data from other plants should be able to be used for asparagus.

In Vegis's model, the temperature range at which budbreak occurs changes during dormancy. A similar process occurred in the three cultivars of asparagus in these experiments (Figs. 4.04 and 4.12). The minimum temperature for budbreak increased to above 12.5C in early winter (26 May) for 'Rutger's Beacon' and 'Jersey Giant'. The minimum decreased to near 10C in late winter (10 August) as a few plants of each cultivar evaluated had commenced growth while being stored at 10C (e.g. case 5, Table 4.09). However, genotypic differences seem to exist as the minimum for 'UC157' was below 12.5C throughout these experiments. Data from 20C did not clearly indicate whether the maximum temperature for budbreak changed during dormancy.

Although plants grew at 20C throughout the experiment, the ease of budbreak changed over time. The mean number of days to budbreak tended to be higher in early (May) and mid winter (July) than at the beginning (March) and end of dormancy (August) (Fig.4.04); and RSGR of first spear was lower in early winter than at other times (Fig.4.06). These data indicate that initiation of growth at 20C was most restricted in early winter, and that 5 weeks of chilling had little effect on the release of dormancy.

In Vegis's model, maximum dormancy occurs when the temperature range at which budbreak occurs is narrowest. Data obtained at 12.5C indicated that the minimum temperature for initiation of growth in asparagus was highest in early winter, and higher in mid autumn than in mid winter. Asparagus growth at 20C was most restricted in early winter, and more restricted in mid winter than mid autumn. Thus the development of dormancy in asparagus seemed to follow Vegis's (1964) statement that as plants approach deepest internal dormancy, the decrease in maximum temperature for growth occurs after the increase in minimum temperature. This could have resulted in a change in the optimum temperature for budbreak as illustrated in Fig.4.16, assuming that the optimum temperature is mid-way between the maximum and minimum temperatures. Thus, maximum restriction of growth at 12.5C preceded the maximum restriction of growth at 20C, and there was no clearly defined time of maximum internal dormancy when the temperature at which growth occurred was narrowest. Since asparagus appeared to follow Vegis's model in progressing through internal dormancy, the factors which induced dormancy were probably similar to those affecting deciduous fruit trees.

Photoperiod often induces internal dormancy, but it's effects on asparagus could not be determined from this study. The natural photoperiod had decreased from 15.7 hours in mid summer to 12.6 hours in late March (Francis 1972) before the plants were transferred to either an 8 or 16 hour photoperiod. This decrease in natural photoperiod may have induced internal dormancy. The experiment effectively tested the effect of the 'current' photoperiod, and ignored the conditions which the plants had already experienced. 'Current' photoperiod had no effect on spear growth in a simulated autumn harvest (Table 4.10). To determine the effect of photoperiod on internal dormancy, the experiment should either have commenced earlier in the year, or used supplementary lighting to extend photoperiod before 22 March.

The induction of internal dormancy by photoperiod can often be counteracted by warm temperatures. In this study, temperatures above 13C in late autumn-early winter prevented senescence of existing fern, but did not prevent the development of internal dormancy on plants with or without fern (Figs. 4.02 and 4.03).

In field grown asparagus, male plants tend to senesce later than females (Nichols pers. comm.) and tend to produce spears earlier in spring (Tiedjens 1924; Robbins and Jones 1925; Ellison and Schermerhorn 1958; Ellison et al. 1960). Plant sex had very little effect on growth in these experiments. However, there was a slight interaction between plant sex and 'spring' temperature, and male plants tended to produce spears earlier than female plants at 12.5C (Table 4.11).

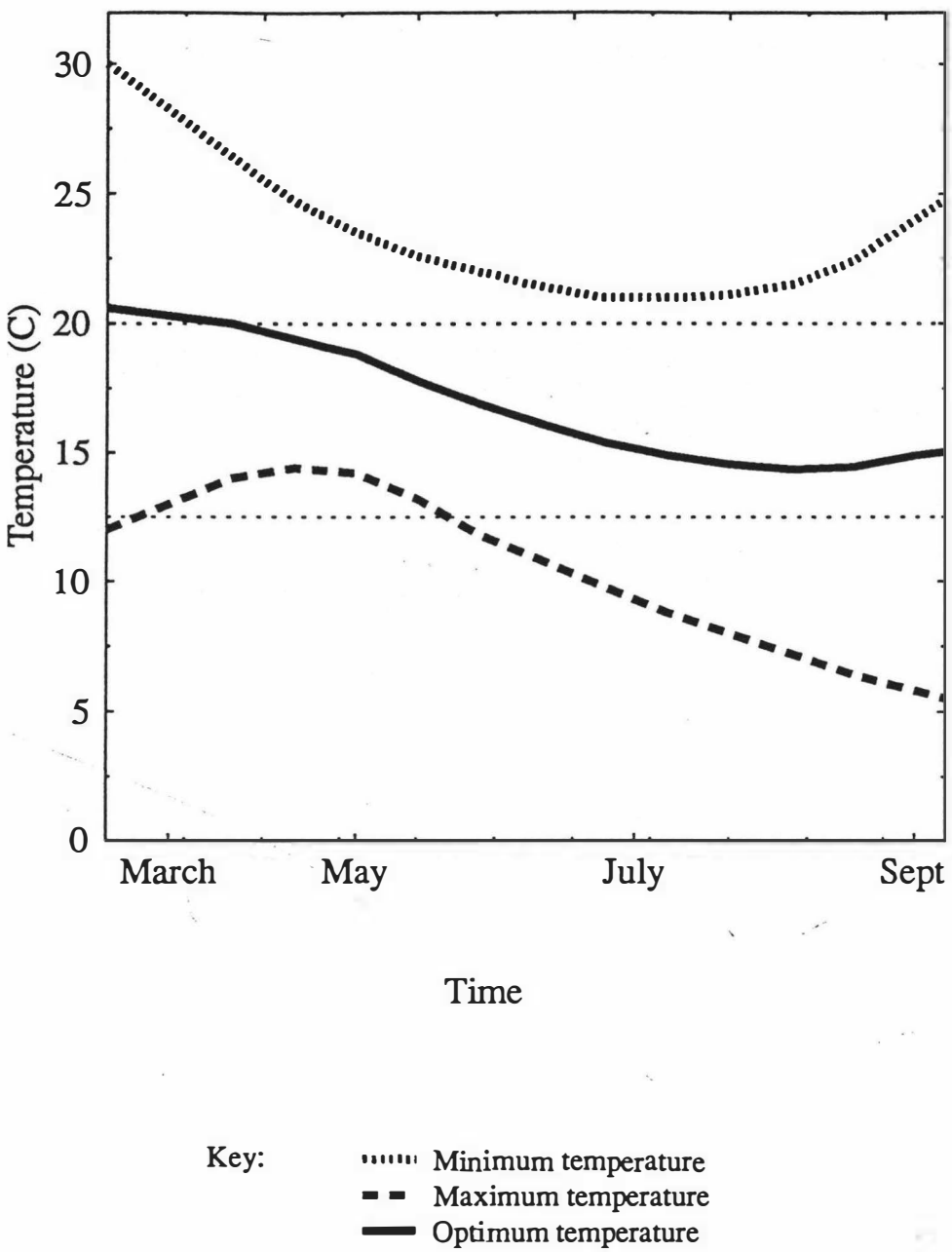


Figure 4.16: Modification of Vegis's model of internal dormancy to show a change in the optimum temperature for budbreak of asparagus caused by the changes in the minimum and maximum temperatures for budbreak being offset

There were differences between cultivars in dormancy development (Figs. 4.04 and 4.12). The minimum budbreak temperature for 'UC157' appeared to be lower than for 'Jersey Giant'. Alternatively, the time at which the minimum temperature response peaked may have varied between the cultivars. These results are comparable to low chill peach cultivars which are active later in autumn and earlier in spring than high chill cultivars (Bowen and Derickson 1978). Selection for weaker internal dormancy may be a consequence of 'UC157' being bred in a milder climate than 'Jersey Giant'.'UC157' was bred in California and 'Jersey Giant' in New Jersey. 'UC157' and 'Jersey Giant' both had less days to budbreak than 'Rutger's Beacon' (Figs. 4.12 and 4.04). This could be due to plant age as plants less than one year old emerge earlier than older plants in the field. However, in all three cultivars studied, dormancy developed according to Vegis's model.

The internal dormancy experienced by asparagus could be either endodormancy or paradormancy. It would be endodormancy if the bud meristem required chilling before growth occurred, and paradormancy if the bud scales (i.e, leaf tissue) responded to chilling and produced substances which subsequently released the meristems from dormancy. ABA may be involved as Matsubara (1980) found that maximum concentration in asparagus buds coincided with maximum dormancy. IAA and gibberellic acid may also be involved as IAA inhibits budbreak, and gibberellic acid promotes budbreak (Tiburcio 1961).

4.4.2 ESTIMATION OF CHILL UNITS REQUIRED TO REMOVE INTERNAL DORMANCY

Optimum chilling temperature

The temperature at which chilling effects commence in 'Rutger's Beacon' asparagus appeared to be close to 12.5C, similar to the temperature proposed for deciduous fruit trees in Utah (Table 4.02, Table 4.01). Plants kept at temperatures above 12.5C ± 0.5C from mid February onwards did not grow during this study (Fig.4.04) which suggested that chilling did not accumulate in these conditions. However plants stored at 10 ± 1C subsequently grew at 12.5C implying that chilling units had accumulated at 10C. However, it was difficult to determine which of the three chilling temperatures used in this experiment was closest to the optimum.

There were few significant and consistent differences in the chilling effectiveness of 2, 5 and 10C. Data from 'Rutger's Beacon' grown at 12.5C indicated that 5C may be the most effective temperature. After 5 weeks of chilling, the number of days to budbreak was the lowest (Fig.4.04), and the RSGR of the first spear was the highest (Fig.4.06) on plants chilled at 5C. Chilling at 2C or 10C for a further 5 weeks decreased the number of days to budbreak indicating that dormancy had not been completely released by 5 weeks of chilling at those temperatures. Although not statistically significant, budbreak occurred from the basal bud most frequently in plants chilled at 5C (Fig.4.05). Chilling at 10C may be more effective than 2C at releasing internal dormancy in asparagus as the number of days to budbreak after chilling at 10C is less than after chilling at 2C (Fig.4.04), and chilling at 2C appears to decrease budbreak by the basal bud (Fig 4.05). However, responses to chilling differed between plants grown at 12.5C and 20C.

On plants grown at 20C, chilling at 2C appeared to be the most effective in releasing the basal bud from internal dormancy (Fig.4.05), and produced the least spread in budbreak (Fig.4.04). The spears tended to have the highest RSGR (Fig.4.06), but budbreak was delayed compared with plants at 5 and 10C (Fig.4.04). Chilling at 10C before transfer to 20C was the least effective temperature for releasing the basal bud from dormancy when plants were grown at 20C (Fig.4.05). Plants chilled at 10C also required the longest number of days to budbreak (Fig.4.04) and produced spears with the lowest RSGR (Fig.4.06) after 5 weeks of chilling, but not after 10 weeks of chilling.

The data from 12.5C and 20C suggest that there was an interaction between chilling temperature and the temperature at which budbreak was subsequently assessed. At both 'growing' temperatures, the chill unit curve was very flat. Chill unit models generally show a clearly defined optimum, near to 6C (Richardson et al. 1974, Gilreath and Buchannan 1981, Shaltout and Unrath 1983). Some more recent reports indicate that buds with a lower chilling requirement show less response to temperature (Scalabrelli and Couvillon 1986). The difficulty in defining the optimum chilling temperature may also be due to a change in the optimum over time as del Real Laborde et al. (1990) has proposed for deciduous fruit trees. Differences between the buds on a rhizome may also contribute to the difficulties in defining the optimum temperature for chilling.

On asparagus, increasing the length of chilling and decreasing the chilling temperature tended to increase the proportion of first spears originating from the basal bud, except on plants chilled at 2C and subsequently grown at 12.5C (Fig.4.05). This indicated that the chilling requirement of buds increased from the apex towards the basal bud. This is similar to peaches where terminal vegetative buds require less chilling than lateral

vegetative and flower buds (Scalabrelli and Couvillon 1986). Chilling requirements also appeared to vary between cultivars.

The minimum temperature at which chilling effects commenced may have varied between asparagus cultivars. Like 'Rutger's Beacon' the temperature at which chilling effects commenced in 'Jersey Giant' appeared to be close to 12.5C. However, 'UC157' either accumulated chilling units at temperatures above 13C, or the minimum temperature for budbreak remained below 12.5C, as discussed previously. The effect of chilling on mean days to budbreak and RSGR of the first spear to grow were too small and inconsistent to determine the optimum chilling temperature for 'Jersey Giant' and 'UC157' (Fig.4.12a & b, Fig.4.13a & b).

Calculation of chill units

The number of chill units required to release the basal bud on 'Rutger's Beacon' asparagus from internal dormancy was calculated using the Utah model (Table 4.01), although there was some doubt that 6C was the optimum temperature for the accumulation of chilling units.

There was a relationship between the chilling units accumulated in either controlled or natural conditions and the percentage of first spears which were produced by the basal buds on plants grown at 12.5C (Fig.4.17) but not for plants grown at 20C. About 500 chill units were required for fifty percent of the first spears to be produced by the basal buds. This is considerably lower than the chilling units required for budbreak on many deciduous fruit trees, e.g, 720 to 1234 CU (Ashcroft et al. 1977), but is similar to the 450 and 550 CU required by two low-chill peach cultivars (Gilreath and Buchanan 1981). This is a further indication that the mechanisms controlling internal dormancy in asparagus are similar to those in deciduous fruit trees (with a low chilling requirement) even though they are very different plant types.

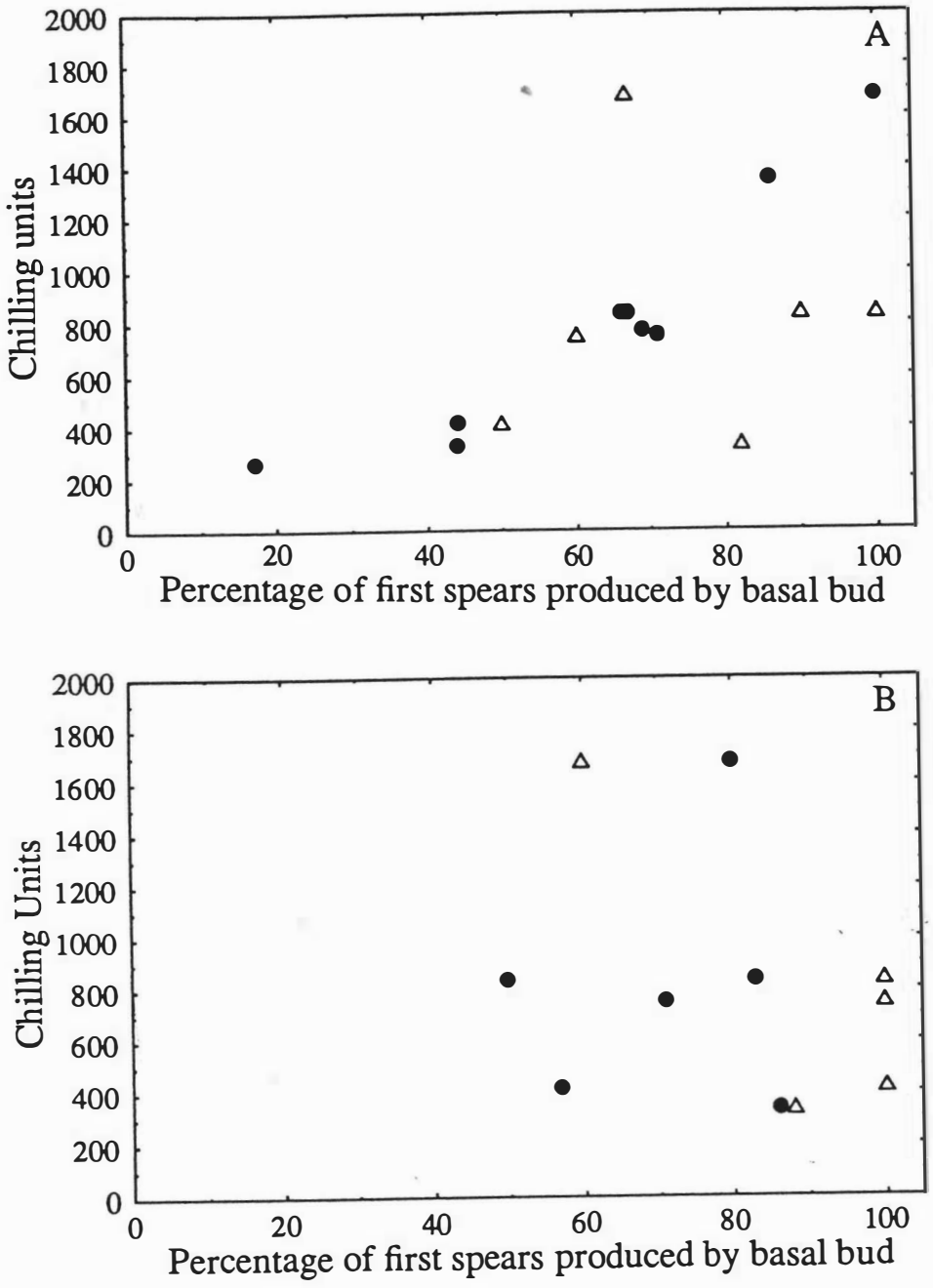


Figure 4.17: Effect of chilling units (Richardson et al. 1974) on percentage of first spears produced by the basal bud on a rhizome.
A: Rutger's Beacon
B: Jersey Giant

4.4.3 BUDBREAK PATTERNS AND THE EFFECTS OF CORRELATIVE INHIBITION

Budbreak pattern within a rhizome:

When all the buds on a 'Rutger's Beacon' rhizome had received adequate chilling, the basal bud grew first, followed by progressively younger buds (Fig.4.07a & b). Budbreak i.e, the beginning of exponential growth, generally did not begin until the previous spear had been harvested (Figs. 4.08 and 4.14). Thus, the growth of buds within a rhizome, other than the basal bud, was controlled by correlative inhibition.

However, correlative inhibition was weaker than in some species as more than one spear could grow at the same time. This occurred without any reduction in the RSGR of either spear (Fig.4.08). The simultaneous growth of more than one spear was generally confined to the growth of the first four spears on a rhizome which implied that correlative inhibition became stronger following the harvest of the first few spears on a rhizome.

Correlative inhibition appears to be weaker on plants with a '2 1 3' budbreak pattern than on plants with a '1 2 3' pattern. Rhizomes with a budbreak pattern of '2 1 3' were the main exceptions to budbreak proceeding from older to younger buds (Fig.4.07), and they had a higher proportion of the first two spears growing at the same time (Table 4.09). The '2 1 3' budbreak pattern was not an anomaly caused by experimental procedures (e.g. sudden decrease in temperature when plants were transferred to chilling conditions, or chilling at a constant temperature) as it occurred on plants which had been chilled in natural conditions.

A possible explanation for the '2 1 3' budbreak pattern is that the bud in position 1 (i.e, the basal bud) had not been completely released from internal dormancy: it began to grow but was not growing actively enough to inhibit budbreak by the bud in position 2. Thus exponential growth commenced in position 2 before position 1, and the inhibition exerted by the spear in position 2 was too weak to prevent budbreak at position 1. This explanation is supported by the lower RSGR of spears which developed from the basal bud when the budbreak pattern was '2 1 3' than when it was '1 2 3' (Table 4.09).

Development of axillary rhizomes

Budbreak on axillary rhizomes was observed on a few plants at 20C (e.g. Fig.4.08b). The RSGR of spears on the axillary rhizome was similar to spears on the parent rhizome. Budbreak did not occur on axillary rhizomes until several spears had been harvested from the parent rhizome. Budbreak did not occur on any axillary rhizomes at 12.5C, probably because too few spears were harvested.

Budbreak by the basal bud on a developing axillary rhizome appeared to be inhibited by buds and spears two nodes above the axil bearing the axillary (Fig.4.08b). Spears four nodes above the axillary also inhibited growth of spears in Position 2 on the axillary, but this did not always occur.

Interactions between main rhizomes

On most of the 'Rutger's Beacon' plants, initiation of spear growth by the first and subsequent spears on a main rhizome appeared to be independent of events on other main rhizomes (Fig.4.08a & b). Any apparent synchronisation of budbreak between rhizomes was not maintained throughout the harvest. Thus, the correlative inhibition within a rhizome of 'Rutger's Beacon' did not extend to buds on other rhizomes within the plant, unlike the results obtained on 'UC157' by Nichols and Woolley (1985). The plants used in their study were younger and thus had less distance and more live tissue between rhizomes which would permit the transport of materials such as carbohydrates and plant growth substances. Additionally, the arrangement of rhizomes in 'UC157' differs substantially from that of 'Rutger's Beacon' and 'Jersey Giant'.

Genotypic differences in budbreak pattern

In these experiments, there was no discernable interaction between the rhizomes on 'Rutger's Beacon' and 'Jersey Giant', while 'UC157' tended to produce spears in flushes e.g. in Fig.4.14a, Fig.4.14b. The budbreak pattern on 'UC157' was similar to that observed by Nichols and Woolley (1985) on the same cultivar. These differences in budbreak pattern could be due to differences in the strength of correlative inhibition.

Differences in the strength of correlative inhibition may also cause differences in rhizome morphology. 'Rutger's Beacon' and 'Jersey Giant' have relatively few, large, rhizomes with clearly defined bud axes while 'UC157' have a large number of small, poorly defined rhizomes (Fig.4.14a & b). Weak correlative inhibition in 'UC157' would

permit the growth of many lateral rhizomes, while strong correlative inhibition in 'Rutger's Beacon' and 'Jersey Giant' would allow very few lateral rhizomes to develop. Weak correlative inhibition in 'UC157' would also account for the 'concentrated spear initiation' and 'strong tendency to initiate ... three to five spears at a time' characteristic of this cultivar (Benson and Takatori 1968).

4.5 CONCLUSIONS

Data presented here indicate that asparagus winter dormancy is a type of internal dormancy and chilling is required to release the buds from dormancy. The optimum chilling temperature was difficult to determine from this data, but appears to be closer to 5C than to 10C or 2C for 'Rutger's Beacon' plants grown at 12.5C. The depth of dormancy can be determined from the growth of the basal bud as the chilling requirement of buds increases from the apex towards the basal bud. Asparagus appears to have a relatively low chilling requirement of approximately 500 chill units calculated according to the Utah model. This low chilling requirement is consistent with the very flat chilling response observed.

There are differences between genotypes in the depth of dormancy. The minimum temperature for budbreak increases to a level above 12.5C on 'Rutger's Beacon' and 'Jersey Giant', but is below 12.5C on 'UC157'. After chilling, the minimum temperature for budbreak is near to 10C.

Budbreak occurred at 20C throughout the experiment. The maximum temperature at which growth will occur may decrease during dormancy, but the data is not conclusive. Senescence is prevented if temperature remains above 13C. This indicates that budbreak may not be suppressed in conditions which are too warm for fern to senesce. Continued budbreak at 20C, and the low chilling requirement of asparagus ensures that the internal dormancy of asparagus does limit crop production by delaying or preventing budbreak in warmer climates as occurs with some deciduous fruit trees.

After dormancy has been released, the growth of buds is controlled by correlative inhibition. Genotypic differences in rhizome morphology appear to be related to correlative inhibition. 'Rutger's Beacon' and 'Jersey Giant' both have relatively few, large rhizomes and relatively strong correlative inhibition; 'UC157' has many small, poorly defined rhizomes, and relatively weak correlative inhibition.

CHAPTER FIVE

CARBOHYDRATE METABOLISM IN ASPARAGUS

5.1 INTRODUCTION

Maintaining adequate carbohydrate reserves in the crown of asparagus is considered to be essential for a vigourous, high yielding crop. However, insufficient information on carbohydrate content is available to establish a carbohydrate budget. The relationship between carbohydrate composition and crop vigour is also unknown. Crop management is based on grower's experience rather than on physiological principles.

The traditional time for harvesting asparagus in a temperate climate is spring, although harvesting in autumn is feasible. Autumn harvested crops can give yields comparable to spring, especially if the crop has a long growing season and ample moisture (Brasher 1956, Jasmin and Laliberte 1962, Garza 1970, Lin 1979, Takatori et al. 1970). However, yield is often less than that obtained from a spring harvest of the same length (Brasher 1956, Jasmin and Laliberte 1962, Takatori et al. 1970). Some autumn harvesting is carried out in New Zealand, and the yield is lower than obtained in spring (but the price is higher) (Nichols pers. comm.). Large scale autumn harvesting appears to be restricted to relatively warm climates where the fern grows for most of the year (Garza 1970). This indicates that carbohydrate reserves do influence vigour, especially in autumn harvested crops.

The experiments described in this chapter examine changes in storage carbohydrate content and composition during autumn, winter and spring.

5.1.1 SEASONAL CHANGES IN CARBOHYDRATE RESERVES OF ASPARAGUS

Seasonal changes in the carbohydrate concentration within the crowns of spring harvested plants are well documented (Scott et al. 1939; Taga et al. 1980; Shelton and Lacy 1980). However, interpretation of their data is limited as root mass has not been measured and thus changes in the carbohydrate pool size are unknown. Haynes (1987) has recorded changes in root mass on plants up to two years old, but these were not harvested. Major changes in carbohydrate concentration occur during and after spear harvest.

Although there is little change in crown dry weight during spear harvest, the crown carbohydrate concentration decreases (Scott et al. 1939, Shelton and Lacy 1980, Taga et al. 1980) as carbohydrate reserves were mobilised and partitioned between spear growth and new buds (Tiedjens 1926). Of the dry matter lost from the storage roots during harvest, one-third is transferred to the spears, and the remainder appears to be used in respiration and the growth of new buds (Scott et al. 1939). However, there was no reference to the production of new storage roots during the harvest period.

Crown carbohydrate concentration continues to decrease for 2-4 weeks following the end of a spring harvest (Scott et al. 1939; Shelton and Lacy 1980). No net storage of carbohydrates occurs until the first fern has expanded (Downton and Torokfalvy 1975; Lin and Hung 1978). The carbohydrate concentration is restored to pre-harvest levels within 6-12 weeks of the end of harvest, and then remains constant or decreases slightly before ferns senesce in autumn (Scott et al. 1939; Shelton and Lacy 1980, Taga et al. 1980). There is very little change in carbohydrate concentration between autumn and spring. Thus changes which occur during and immediately after spring harvest determine the status of the plants carbohydrate reserves.

The balance between harvest length and the time required to restore carbohydrate reserves to pre-harvest concentration varies, depending on the size of the plant, the length of the growing season, environmental factors such as temperature and drought, and the presence of growth limiting factors such as disease and competition. Extending spring harvest beyond the normal time may prevent the plant's carbohydrate reserves from being restored to preharvest level (Scott et al. 1939, Taga at el 1980, Shelton and Lacy 1980). This reduces plant vigour, and spear quality in future harvests (Takatori et al. 1970; Williams and Garthwaite 1973; Taga et al. 1980). It can also delay spear production in spring (Williams and Garthwaite 1973). The carbohydrate concentration in the crown during winter is lower in autumn harvested plants than in spring harvested plants (Scott et al. 1939), and this may also delay shoot growth in spring. Carbohydrate concentration appears to decrease more during fern establishment than during harvest (Scott et al. 1939). Fern establishment following dormancy in autumn harvested (Scott et al. 1939) or non-harvested plants (Haynes 1987) also utilises reserve carbohydrates.

Table 5.01 Summary of literature on asparagus carbohydrate reserves.

Fructan parameters			Plant size/age	Analytical Details		Reference
Max conc[a]	Min conc[a]	Fructan size [b]	Cultivar[c]	Extraction	Analysis[d]	
20% [e]	[e]	up to DP13	1 year MW 500W		HPLC	Kim and Sakiyama 1989b
70%	23%	most DP<9	5 l pots Lukullus	hot 80% ethanol + water	colori-metric	Martin and Hartmann 1990
55%	23%		5 l pots Lukullus	hot 80% ethanol + water	colori-metric	Martin and Hartmann 1990
21%	15%	most DP4 to DP6	3 l pots Brooks Imperial 84	hot 80% ethanol + water	HPLC	Pressman et al. 1989
54%	13%	-	Field grown Mellow-land Select		modified anthrone	McGrady and Tilt 1989
66%	27%	-	Field grown 0 to 2 years Limbras 10	hot water	modified anthrone	Haynes 1987
45%	11%	Max DP=21	Field grown 1 year MW	ethanol	modified anthrone	Shelton and Lacy 1980
73%	18%	-	Field grown 2 years MW	ethanol	modified anthrone	Shelton and Lacy 1980
60%	10%	-	Field grown 4-6 years			Taga et al. 1980
59%	13%	-	Field grown 3-5 years MW		AOAC standard	Scott et al 1939

Notes:

a - all concentrations expressed as a percentage of dry weight

b - DP= degree of polymerisation

c - MW = Mary Washington

d - HPLC - high performance liquid chromatography

e - estimate based on total of 4.5% fresh weight, and assuming a dry matter percentage of 22.5%. Minimum not calculated as there was little change in total carbohydrate.

In non-harvested plants, the increase in the carbohydrate concentration in the crown after fern establishment precedes an increase in root dry weight. Root carbohydrate concentration plateaus at a maximum at the same time as fern dry weight in late summer. Root dry weight increases until the fern senesces, and remains constant until late winter (Haynes 1987). Thus changes in root dry weight also appear to follow a seasonal cycle.

Data on seasonal changes in the crown carbohydrate concentration are difficult to compare as the analytical methods used vary and the plants differ in age, size and growing conditions (Table 5.01). A maximum concentration of 54 to 66 percent dry weight generally occurs after fern has established while a minimum of 10 to 27 percent dry weight occurs at the beginning of fern establishment.

There is little information on seasonal changes in the composition of carbohydrate reserves within asparagus. Carbohydrate reserves in asparagus are composed mainly of fructo-oligosaccharides or fructans (Shelton and Lacy 1980, Shiomi 1981a, Shiomi et al. 1976, 1979a). Low levels of starch have been reported (3-4% Scott et al. 1939, 2% Martin and Hartmann 1990) but other researchers have been unable to detect any starch in fern or in storage roots (Pressman et al. 1989). Most of the fructan present appears to have a degree of polymerisation (DP) between three and eight (Kim and Sakiyama 1989b, Martin and Hartmann 1990, Pressman et al. 1989). The maximum size reported is DP21 (Shelton and Lacy 1980).

5.1.2 FRUCTAN METABOLISM

Fructans are comprised primarily of fructose and are stored within the vacuole of plant cells (Wagner et al. 1983). The maximum number of fructose residues varies between species, ranging from about 10 for onion (*Allium cepa*) to over 100 in some grasses. Asparagus fructans contain 1 glucose residue, and a maximum of 20 fructose residues when sampled in spring-early summer (Shelton and Lacy 1980). The nine fructans so far characterised contain up to four fructose residues (Shiomi et al. 1976, 1979a; Shiomi 1981a).

Asparagus fructan synthesis is similar to the two stage model proposed by Edelman and Jefford (1968) which was based on studies of Jerusalem artichoke (*Helianthus tuberosus*) (Shiomi 1989). A trisaccharide is formed in the first step when the fructose residue of

one sucrose molecule is transferred to another sucrose molecule by the enzyme sucrose-sucrose fructosyl transferase (SST). Asparagus has two fructosyl transferase enzymes (FT) which then transfer fructose residues from a trisaccharide or a higher homolog 'donor' molecule onto another trisaccharide or fructan 'acceptor' molecule (Shiomi 1989). Thus fructans are synthesised by the repeated addition of single fructose residues.

$$\text{i.e. 1. G-F} + \text{G-F} \xrightarrow{\text{SST}} \text{G-F-F} + \text{G}$$

$$\text{2. G-F-F}_n + \text{G-F-F}_m \xrightarrow{\text{FT}} \text{G-F-F}_{n-1} + \text{G-F-F}_{m+1}$$

where m and n are less than the maximum DP for the species

A similar 2 step synthesis involving both SST and an FT occurs in other plants although there are differences in enzyme kinetics (Pollock and Cairns 1991).

Depolymerisation of fructans occurs with the progressive removal of single fructose residues. This is carried out by the fructosyl transferase involved in fructan synthesis. Hydrolase enzymes may also depolymerise fructans (Edelman and Jefford 1968).

Sucrose appears to be translocated in fructan storing plants as there is no evidence that fructans or their trisaccharide precursors, the kestoses (isomers of monofructosylsucrose), are translocated.

Fructans are the major carbohydrate reserves in about 12% of angiosperm plants (Hendry 1987) and up to 80% of plant dry weight can be comprised of fructan (Kandler and Hopf 1982, Meier and Reid 1982, Hendry 1987). The maximum concentration which has been recorded in asparagus is 73% of crown dry weight (see Table 5.01).

5.1.3 PHYSIOLOGICAL CHANGES ASSOCIATED WITH FRUCTAN METABOLISM

Although fructans can be present in very high concentrations, their role in plants is not clear. Their major function appears to be carbohydrate storage (Hendry 1987). They are the storage carbohydrates in species which are able to grow at lower spring temperatures than most non-fructan storing plants (Brocklebank and Hendry 1989). Fructans may be important in conferring frost or drought tolerance by allowing alteration of osmotic

potential (Kandler and Hopf 1982, Meier and Reid 1982) but this role is disputed (see Hendry 1987, Pollock and Cairns 1991).

In asparagus, fructans may be involved in frost tolerance, but are probably not involved in drought tolerance. Fructans are present in the roots and rhizome of asparagus, and may (Martin and Hartmann 1990) or may not (Pressman et al. 1989) occur in the fern. Short chain fructans (mostly DP4 to DP6) accumulate in storage roots as the fern senesces following transfer of plants to cool temperatures (Pressman et al. 1989). However, they are unlikely to contribute to drought tolerance as the storage root fructan concentration decreases when plants undergo sudden drought induced senescence (Pressman et al. 1989).

Many plants which store fructan are dormant over winter and utilise stored fructans to support vegetative or reproductive growth in spring (Cyr et al. 1990; Pollock and Cairns 1991). Fructan accumulation occurs when photosynthate production exceeds metabolic requirements, e.g. in low temperatures, in drought, or when nutrient supply is low (Pollock and Cairns 1991, Hendry 1987). Accumulation of fructans begins in late spring or summer, generally reaching a peak in autumn. The increase in fructan accumulation is generally accompanied by an increase in fructan size. Partial depolymerisation of fructans in autumn appears to occur in plants which do not retain their foliage overwinter (Cyr et al. 1990) but not in plants which continue growth during winter (Pollock and Jones 1979). Resumption of growth, or the increase in growth rate, in spring is preceded by depolymerisation of the stored fructans and a transient increase in monosaccharide and sucrose concentrations.

Fructan composition in asparagus crowns changed as spear growth commenced in spring. In the four days preceding 'sprouting', the concentration of sucrose and DP1 in the storage roots decreased while that of DP3 and DP4 increased slightly (Kim and Sakiyama 1989b). This probably indicates that DP1 and DP2 were metabolised or translocated out of the roots, while longer chain fructans were depolymerised. Within 2 days of sprouting, a concentration gradient developed along the length of the storage roots (Kim and Sakiyama 1989b) which indicates that fructans were preferentially mobilised from the apical end. Changes in the concentration of fructose (DP1) were much larger than changes in sucrose (DP2) or DP3-DP8, and occurred more rapidly at 20C than at 10C. Exogenously applied plant hormones such as gibberellic acid and indoleacetic acid appeared to have no effect on carbohydrate concentrations.

5.2 MATERIALS AND METHODS

The objective of this experiment was to relate changes in plant dry weight, size of the carbohydrate pool and fructan composition to physiological changes occurring within asparagus during autumn, winter and spring. Changes in plant dry weight and carbohydrate concentration were used to construct a carbohydrate budget. Fructan composition was related to changes in the depth of internal dormancy and the ability of the buds to grow.

As discussed in Chapter 4, asparagus plants sampled in early winter (26 May) were at a high level of dormancy, and the minimum temperature for growth was above 12.5C. The minimum temperature at which budbreak occurred decreased to a value below 12.5C after chilling at 10, 5 or 2C for five weeks. The optimum temperature for releasing internal dormancy appears to be 5C for plants grown at 12.5C, but there was little difference in the effectiveness of 2, 5 and 10C.

5.2.1 PLANT MATERIAL AND ENVIRONMENTAL CONDITIONS

The 'Rutger's Beacon' plants analysed in this experiment were grown and subjected to simulated autumn, winter and spring conditions as described for Experiments A and B in Chapter 4.2.2 to 4.2.5 and Tables 4.03 and 4.04. Plants were transferred to 12.5C or 20C in mid autumn (22 March), early winter (26 May) and in mid winter (2 July) or late winter (10 August) after five or ten weeks storage at 10, 5 or 2C, and the resulting spears harvested. Fern senescence was also examined following 22 March.

At the end of each part of the experiment, all plants were destructively sampled to determine crown morphological data and obtain samples for carbohydrate analysis. The plants were removed from their containers and the potting mix washed off using a high pressure hose. This also removed the feeding roots from the sample.

Each plant was dissected into underground stem, new roots, old roots and damaged or dying roots as described in Chapter 3. Rhizome length, root number, fresh weight and dry weight were recorded. Samples were dried in a vacuum oven (40C, 2 mm Hg) for at least 3 days and equilibrated at 20C, 55% RH for 24 hours before determining dry weight.

Samples for carbohydrate analysis were redried in the vacuum oven, and then ground in a Culatti hammer mill to pass a 1 mm screen. The samples were held in a forced draft oven (60C) for up to 12 hours immediately before grinding. Dried asparagus roots are very hydroscopic, and this prevented the samples adsorbing moisture between the vacuum oven and the hammer mill. The ground samples were frozen until required for carbohydrate analysis.

Data on fructan composition is presented for the main cluster on each plant; data on plant dry weight, concentration of total water soluble carbohydrate and size of the carbohydrate pool are presented on a per plant basis.

5.2.2 FRUCTAN ANALYSIS

The frozen, ground samples of storage roots were unsealed and placed in a freeze drier (40C, 2 mm Hg) for at least 48 hours to remove absorbed moisture; resealed; stored over silica gel at 2C and analysed within 7 days for concentration of water soluble carbohydrates or for fructan composition.

Analysis of water soluble carbohydrate concentration

'Water soluble carbohydrate' includes all fructans, plus monosaccharides (DP1) and disaccaharides (DP2).

Triplicate 100 mg samples were extracted in 5 ml reverse osmosis purified water at 90-95C for 15 minutes, then shaken at room temperature for 15 minutes. Samples were then centrifuged at 700 G in a bench-top centrifuge for 5 minutes and 1 ml of supernatant transferred to a second tube for hydrolysis.

The supernatant was hydrolysed by adding 500µl of 0.1M HCl and heating to 90-95C for 30 minutes. The samples were neutralised with 500µl 0.1M NaOH, and 1 ml of water added to bring the volume to 3 ml before testing the pH on Whatman pH 4-6 test paper. If necessary, the pH was corrected to pH 5-6 by adding 1M acid or alkali dropwise. 15 ml culture tubes sealed with teflon lined caps were used for the extraction and hydrolysis.

The hydrolysed samples were then filtered through pre-washed glass fibre discs (0.7 micron retention, Whatman GF/F) using a syringe and 13 mm filter holders before analysis by high performance liquid chromatography (HPLC).

All samples were analysed within 10 hours of extraction.

HPLC system

All components of the HPLC system were Water's brand. A 30µl sample was injected by autoinjector (Water's Intelligent Sample Processor (WISP) Model 710B) and was separated on a 'Dextropac' radially packed column. 'Dextropac' is a reversed phase column with C18 bonded silica, and has been developed to separate carbohydrate oligomers on the basis of their solubility in water (Kainuma et al. 1983). A Water's C18 guard column was used with the 'Dextropac' column.

The eluent used was water (reverse osmosis purified and degassed) at a flow rate of 2ml/min. The detector was a differential refractometer (Waters Model R401) set at 8x attenuation. The signal produced was processed by a Data Module Model 730 which provided retention time and peak area.

The carbohydrate concentration was calculated from the peak areas. A standard curve was determined daily using standards with 1.0-5.0 mg/ml fructose and 0.1-1.0 mg/ml sucrose. The standards were injected at least 3 times each day. A test sample was extracted in triplicate each day and the daily results adjusted accordingly.

The hydrolysis and HPLC conditions used were selected to give complete hydrolysis of the fructans to monosaccharide, and to minimise the size of the salt peak (Fig.5.01a). A higher concentration of acid produced a salt peak which emerged before the monosaccharide peak and sometimes fused with it. Sucrose was included in the standard to permit quantification of trace sucrose if hydrolysis was incomplete (Fig.5.01b).

Analysis of Fructan composition

A single 120 mg aliquot of each sample was extracted as described above. Two 1 ml aliquots of supernatant were hydrolysed and water soluble carbohydrate concentration determined as above. The remaining supernatant (2.5-3.0 ml) was filtered through glass fibre paper, and fructan composition determined by HPLC.

The HPLC system was as previously described with the following changes:
injection volume 50µl; attenuation 4x; concentration of standards: 0.5-2.0 mg/ml fructose and 0.5-2.0 mg/ml sucrose.

This system separated the fructans according to their degree of polymerisation (DP). The DP of peaks was determined by comparison with maltose mono- to hepta-saccharides (i.e, DP1 to DP7) (Sigma Chemicals) (Fig.5.02). Fructans up to DP9 could be separated (Fig.5.02) although the DP9 fraction was not quantified as it was not completely resolved. Data from fractions DP3 to DP8 have been combined and called 'short chain fructan' in the text. The quantity of fructan comprising DP9 and higher has been called 'long chain fructan' in the text and was calculated as the difference between the total water soluble carbohydrate and the sum of fractions DP1 to DP8.

All samples were analysed within 8 hours of extraction. Fructan composition was determined from a single extraction and a single injection of each sample. Sucrose standards were used to quantify the DP2, and fructose standards were used to quantify all fructans except DP2. This is based on the method of Kainuma et al. (1981) who used maltose (DP2) to quantify maltose oligosaccharides in similar conditions. His data demonstrated that peak areas produced using a differential refractometer following separation on a reversed phase column ranged from 1.06 for DP1 to 0.99 for DP6 maltose oligosaccharides. Using fructose as a standard may have slightly underestimated the concentration of trisaccharides and larger fructans. Pure fructans were not available for use as standards. Raffinose and stachyose were not used as standards as their retention times differed from the fructans, and their peak area:carbohydrate concentration ratio differed from fructose and sucrose.

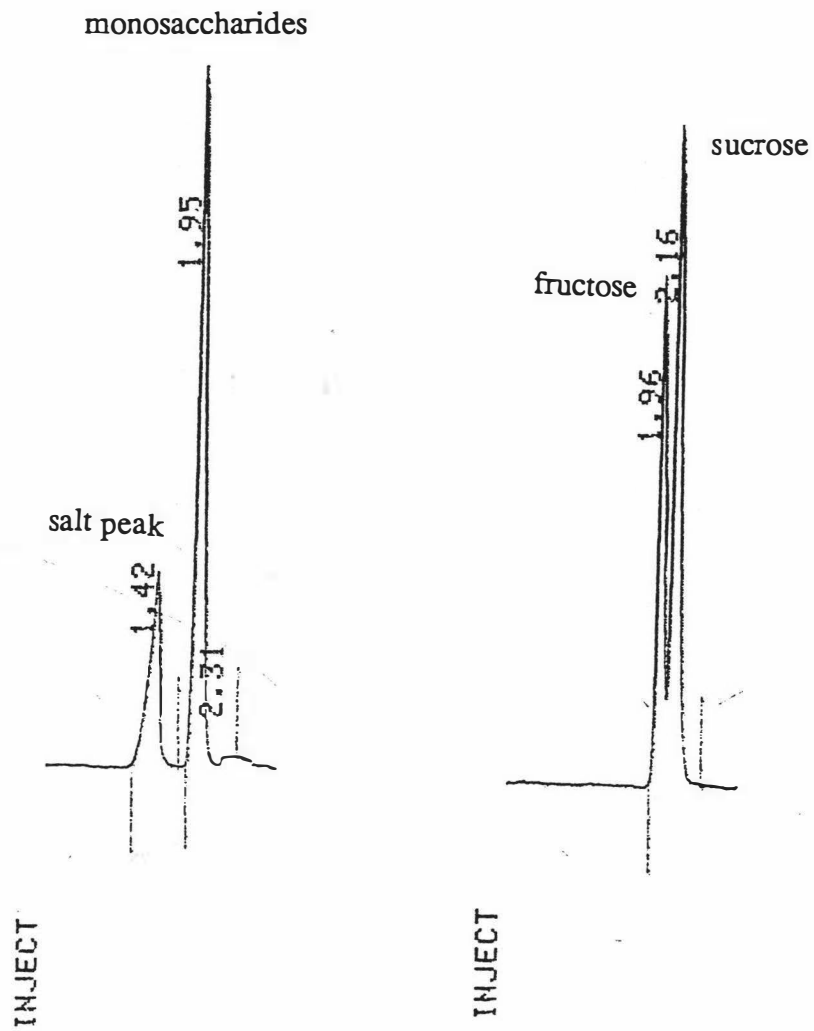


Figure 5.01: Chromatograms of
a) hydrolysed sample showing salt peak and monosaccharides
b) standard containing fructose and sucrose
Chromatographic conditions: column, 'Dextropac' (radially packed, reversed phase column with C18 bonded silica), with C18 guard column; eluent: water (reverse osmosis purified and degassed) at a flow rate of 2ml/min; detection by differential refractometer (Waters Model R401) set at 8x attenuation.

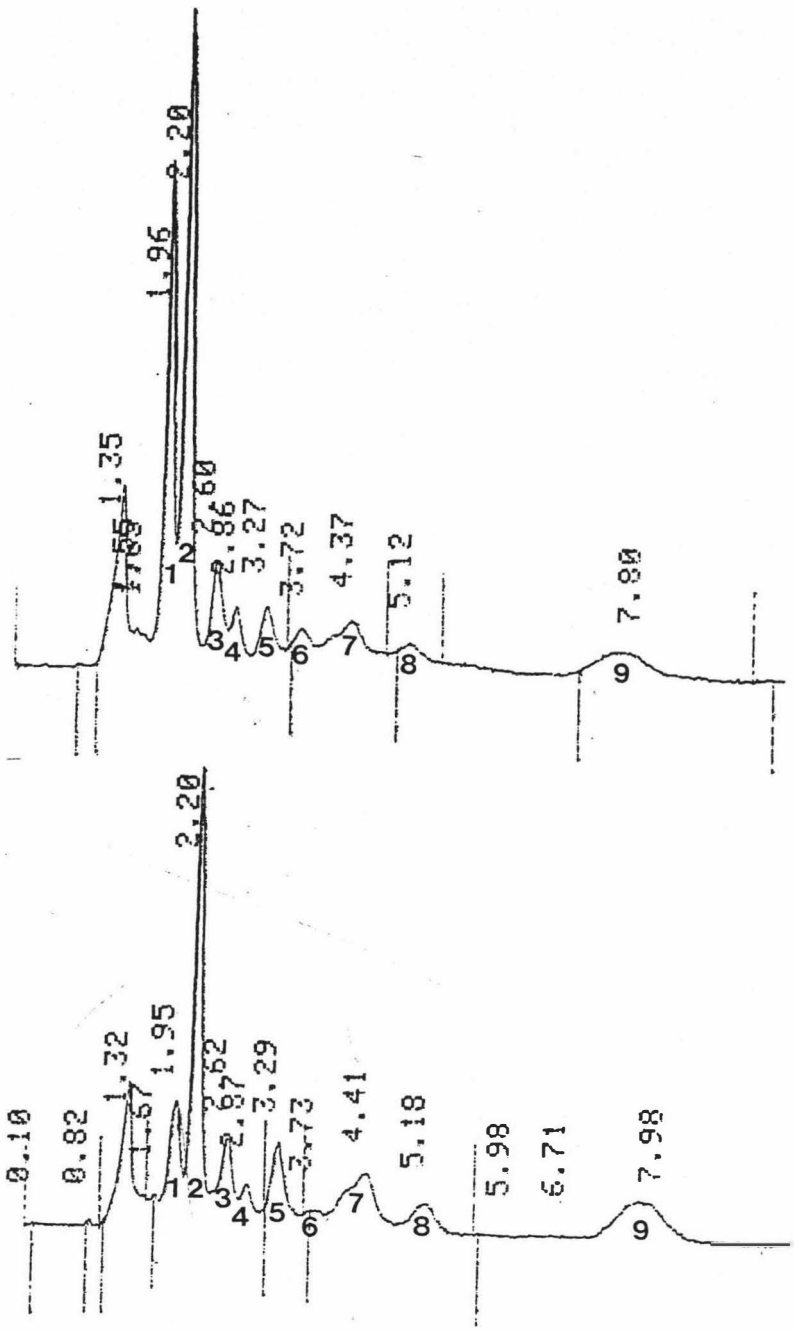


Figure 5.02: Chromatograms of non-hydrolysed extract from storage roots of asparagus. Numbers indicate degree of polymerisation of fructan.

a. Plant chilled at 10C for 10 weeks.

b. Plant chilled at 2C for 10 weeks.

Chromatographic conditions: column, 'Dextropac' (radially packed, reversed phase column with C18 bonded silica), with C18 guard column; eluent: water (reverse osmosis purified and degassed) at a flow rate of 2ml/min; detection by differential refractometer (Waters Model R401) set at 4x attenuation.

5.3 RESULTS

5.3.1 CHANGES DURING AUTUMN

Changes in plant dry weight, soluble carbohydrate concentration and size of soluble carbohydrate pool

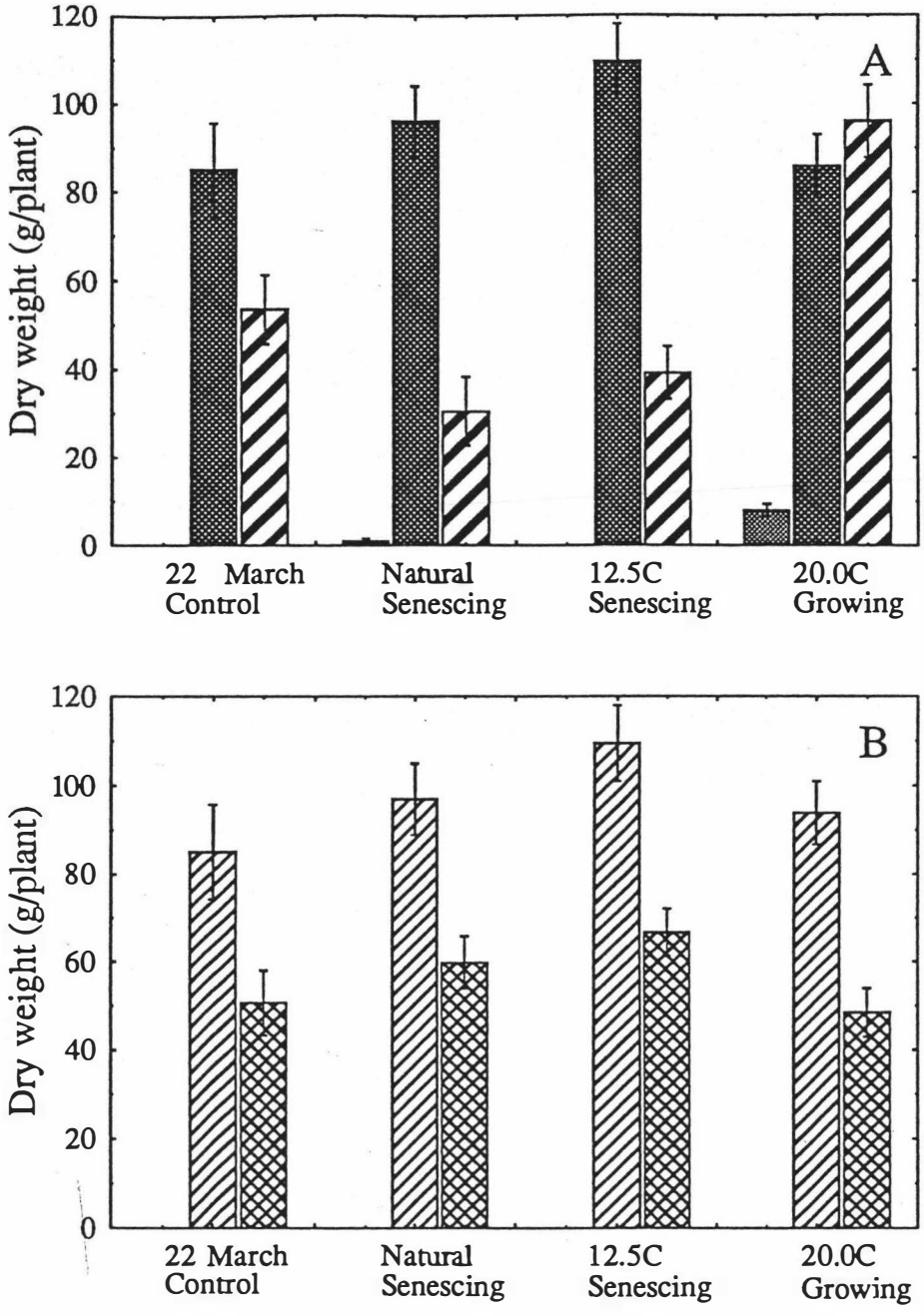
Plants transferred to natural conditions or 12.5C on 22 March senesced during the following 7 weeks (Fig.4.02). On these plants, crown dry weight increased slightly as fern dry weight decreased (Fig.5.03). However, there was no change in the carbohydrate content of the roots (Fig.5.04). Thus the increase in the carbohydrate pool (Fig.5.03) was due to an increase in crown dry weight.

Plants transferred to 20C on 22 March produced a flush of new fern growth in the following 7 weeks (Fig.4.02). This increased plant dry weight (Fig.5.03). The slight increase in crown dry weight was due to the presence of new roots after 7 weeks (Fig.5.03). This increase in crown dry weight was sufficient to counteract the decrease in carbohydrate concentration (Fig.5.04) and leave the size of the carbohydrate pool unchanged (Fig.5.03).

Changes in composition of water soluble carbohydrate

As plants senesced, the concentration of water soluble carbohydrate remained constant while that of DP2 increased and that of short chain fructans decreased slightly (Fig.5.04). These changes were more pronounced on the plants in natural conditions i.e, on the plants which were in cooler temperatures and had senesced more rapidly (Fig.4.02).

After 7 weeks of growth at 20C, the concentration of DP2 and short chain fructans was the same as at the beginning of the experiment (Fig.5.04). However, the concentration of DP1 had increased and that of long chain fructans decreased.

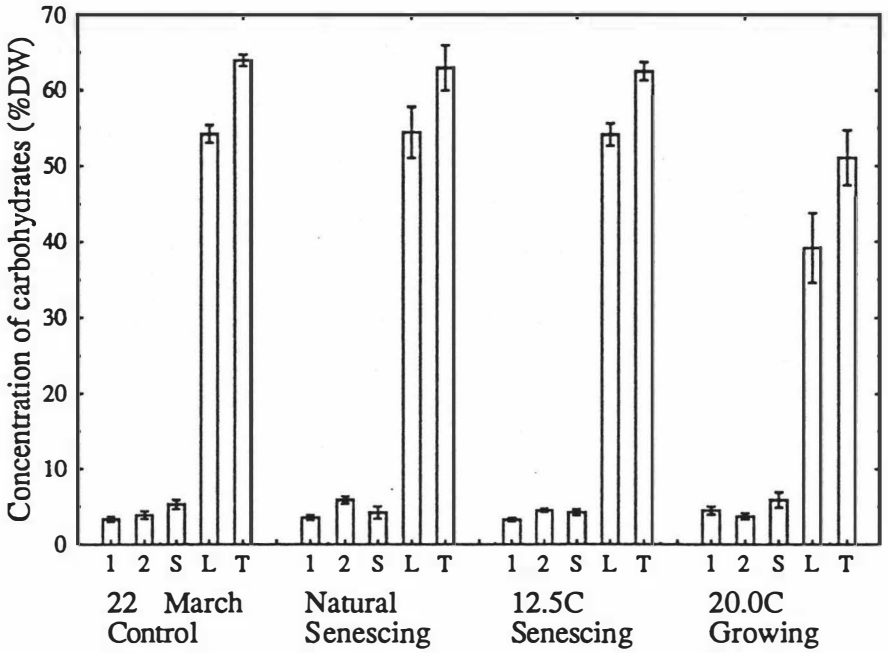


Dry weight of plant parts before & after simulated autumn

Key:
- Dry weight of new roots
- Dry weight of old roots
- Dry weight of fern
- Dry weight of crown
- Dry weight of crown carbohydrate pool

Figure 5.03: Plant dry weight and size of crown carbohydrate pool (g/plant) before and after simulated autumn. Plants were transferred from a heated glasshouse (minimum temperature 13C) on 22 March and sampled 7 weeks later. Plants transferred to natural conditions senesced faster than those at 12.5C. Plants transferred to 20C underwent a growth flush. Bars represent twice standard error of mean.

A: Dry weight of new roots, old roots and fern
B: Dry weight of crown and carbohydrate pool



Carbohydrate composition before and after simulated autumn

Key: 1 = DP1[1]; 2 = DP2; S = short chain fructans (DP3 to DP8); L = long chain fructans; T = total water soluble carbohydrate

Note 1: DP = degree of polymerisation

Figure 5.04: Composition of carbohydrates in storage roots before and after simulated autumn. Plants were transferred from a heated glasshouse (minimum temperature 13C) on 22 March and sampled 7 weeks later. Plants transferred to natural conditions senesced faster than those at 12.5C. Plants transferred to 20C underwent a growth flush. Data from 8 and 16 hour photperiods at 12.5C and 20C combined. Bars represent twice standard error of mean.

5.3.2 CHANGES DURING CHILLING

Changes in plant dry weight, soluble carbohydrate concentration and size of soluble carbohydrate pool

Crown dry weight and the carbohydrate pool increased by almost 50% in late autumn i.e, between 22 March and 26 May (Fig.5.05A) on plants held at a minimum temperature of 13C within a glasshouse. These plants did not produce any new ferns over this time, and thus maximised the photosynthate available for storage. Carbohydrate concentration was constant, and near to 65% which was the maximum level obtained in this experiment.

Plant dry weight generally decreased when the plants were dormant i.e, from 26 May to 10 August (Fig.5.05A). The loss in dry weight increased as the chilling temperature increased from 2C to 10C, and appeared to be greater in the second five weeks of chilling than in the first five weeks. The increase in dry weight after five weeks at 2C appears to be an anomaly in the data.

The concentration of carbohydrate in the roots also decreased during dormancy, especially in plants chilled at 10C (Fig.5.06). When combined with the decrease in plant dry weight, this resulted in a significant (P=0.05) decrease in the carbohydrate pool of plants chilled at 10C and 5C (Fig.5.05a).

Several plants in this experiment produced new roots during chilling, especially plants chilled at 2C. New roots comprised up to 8.3 % of crown dry weight (Table 5.02).

Changes in composition of water soluble carbohydrate

The composition of carbohydrates within the storage roots changed as plants progressed through internal dormancy (Fig.5.06). As dormancy increased between 22 March and 26 May, the concentration of DP1 and of short chain fructans decreased to the lowest levels detected in this experiment. The concentrations on 26 May were lower than in plants which were senescing (Fig.5.04).

Chilling asparagus plants at 10, 5 or 2C after they had been kept at temperatures above 13C stimulated an increase in the concentration of DP1, DP2 and short chain fructans

Table 5.02: Effect of chilling on dry weight and number of old and new roots on crowns of asparagus plants chilled at 2, 5 or 10C, starting on 26 May. Data for 5 and 10 weeks chilling combined. Standard error of means in brackets

	Before chilling	Temperature plants chilled at for 5 or 10 weeks (C)		
		10	5	2
Weight of old roots (g/plant)	122 (12)	102 (8)	112 (8)	122 (8)
Weight of new roots (g/plant)	nd	T	T	5.2 (0.7)
Number of old roots (per plant)	131 (14)	127 (10)	117 (10)	122 (10)
Number of new roots (per plant)	nd	T	3.8 (1.8)	11 (1.8)

Notes:
nd - not detected
T - trace

(Fig.5.06). The increase after five weeks of chilling was greater than in plants which senesced at 12.5C or in natural conditions for seven weeks (Fig.5.04).

After five weeks of chilling, the concentration of DP1, DP2 and short chain fructans was the same at each chilling temperature (Fig.5.06). When plants were chilled for a further five weeks, the concentration of total water soluble carbohydrate and long chain fructan continued to decrease. The concentration of DP1, DP2 and short chain fructans did not change at 10C, and increased slightly at 5C. However at 2C, the concentration of DP1 decreased, DP2 was unchanged, and that of short chain fructans almost doubled. Consequently, fructan composition differed with temperature after ten weeks of chilling. Plants chilled at 2C had lower concentration of DP1 and a relatively high concentration of short chain fructans, while plants chilled at 5C tended to have a higher concentration of DP2, and those chilled at 10C had a relatively low concentration of short chain fructans.

5.3.3 CHANGES DURING SPEAR HARVEST

Changes in plant dry weight, soluble carbohydrate concentration and size of soluble carbohydrate pool

As expected, crown dry weight and the size of the carbohydrate pool decrease during the harvest of asparagus spears (Fig.5.05b&c). Apparent increases in dry weight are not significant at P=0.05.

The temperature at which plants had been chilled had no effect on the weight of spears harvested, or on the crown dry weight or carbohydrate pool at the end of harvest (Fig.5.05). Therefore, the results from each chilling temperature have been pooled and presented in Table 5.03a & b.

The total dry weight of spears harvested after five or ten weeks of chilling tended to be higher at 20C than at 12.5C (Table 5.03): consequently crown dry weight, carbohydrate concentration and size of the carbohydrate pool tended to be lower at 20C than at 12.5C at the end of spear harvest.

Plants transferred to 12.5C on 26 May produced no spears in the following 16 weeks (e.g, Fig. 4.03). However, crown dry weight, carbohydrate concentration and size of water soluble carbohydrate pool all decreased slightly (Table 5.05, Fig.5.05). This loss in carbohydrate was less than in plants stored at 10C for ten weeks (Fig.5.05a), but similar to plants stored at 5C or 2C.

A water soluble carbohydrate budget

The carbohydrate budget in Table 5.03 examines the efficiency of carbohydrate use during spear harvest. Data obtained from plants chilled at 10, 5 or 2C was pooled. Standard errors of derived data, e.g. dry weight respired during harvest, were calculated using the method of Cochran and Cox (1957). Carbohydrate in the spears was calculated as 20% dry weight, based on King et al. (1988). The respiratory 'losses' of crown dry weight and carbohydrate have been calculated on a per plant basis, and for each gram of spear harvested.

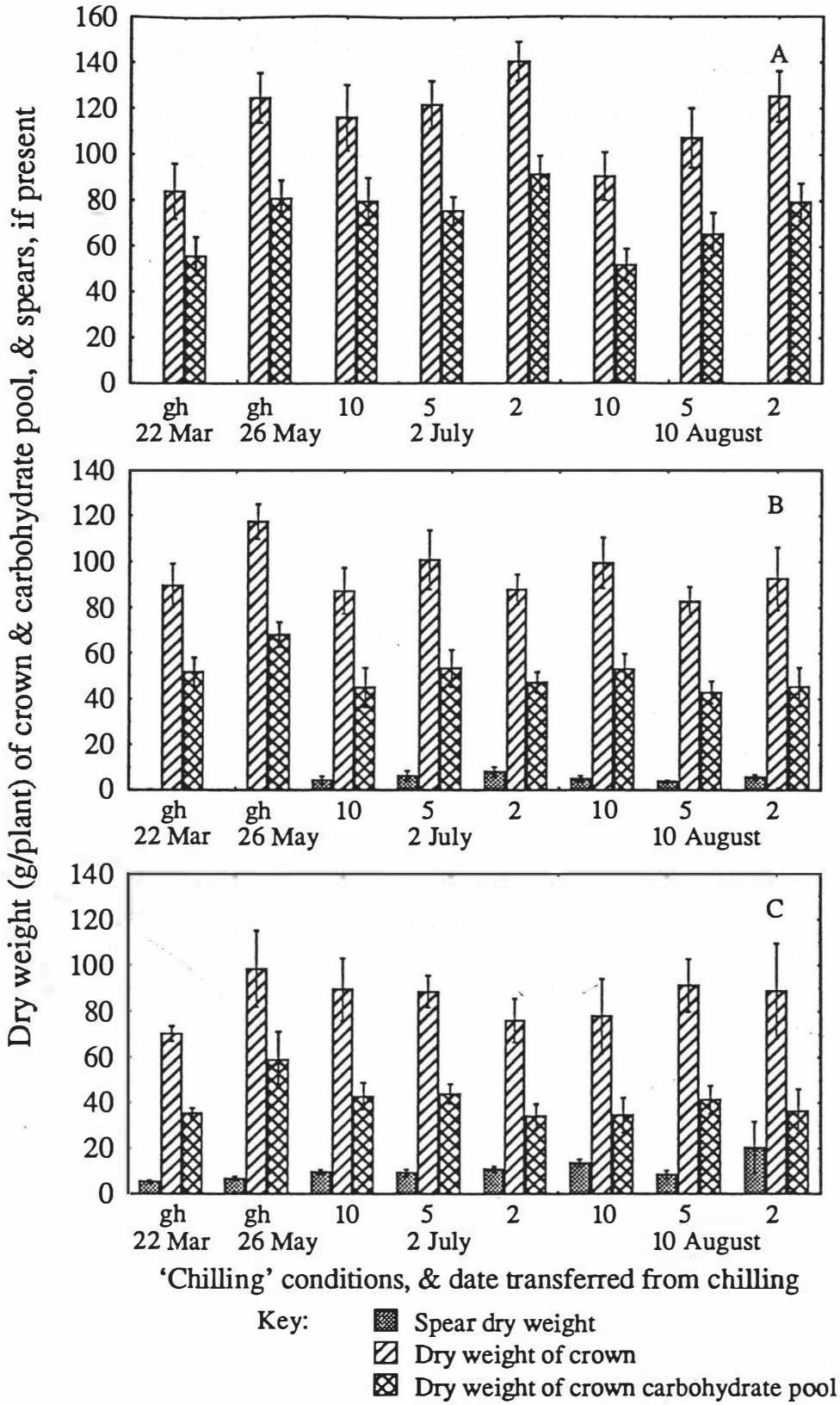
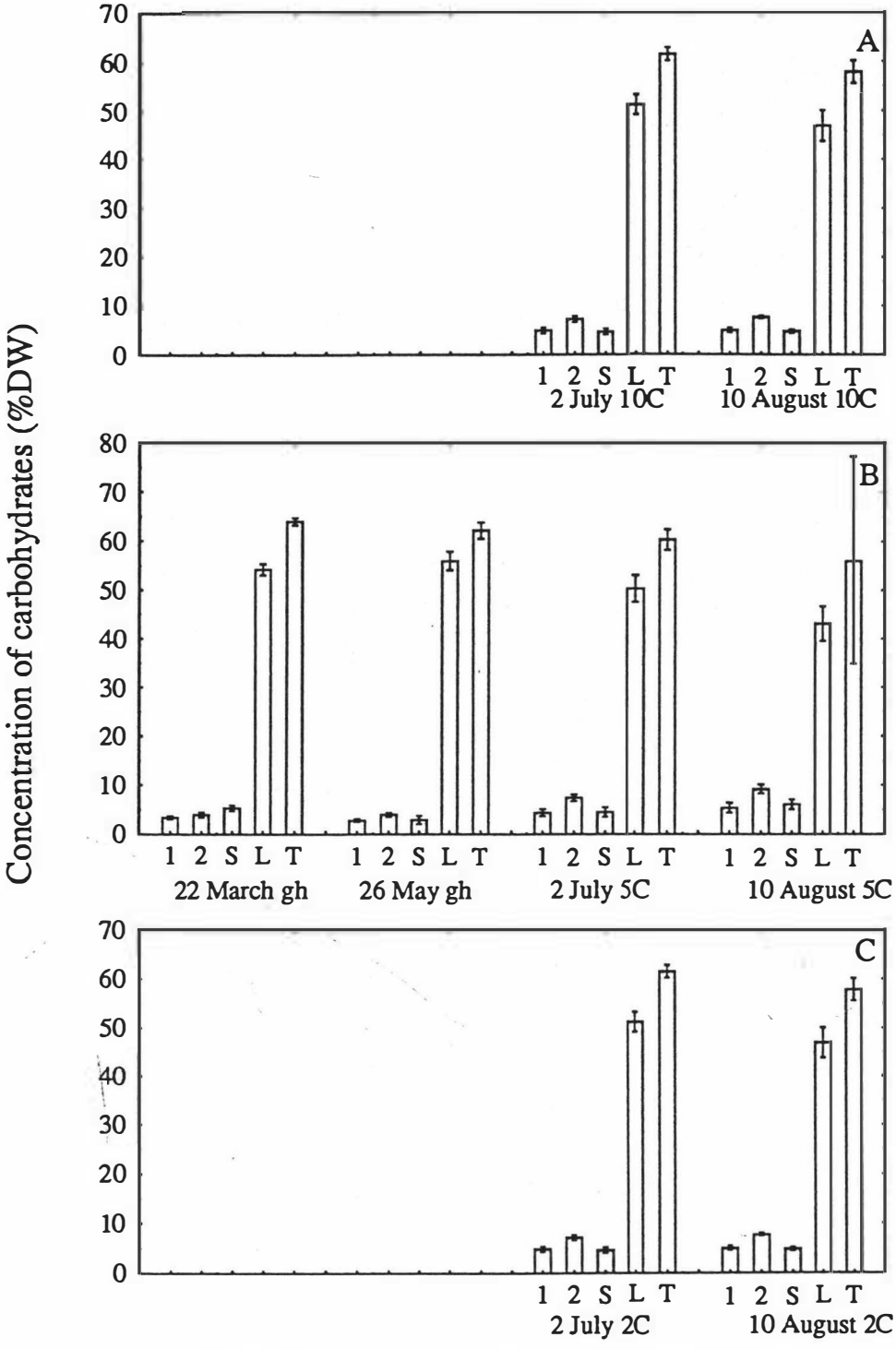


Figure 5.05: Crown and spear dry weight and size of carbohydrate pool (g/plant) during simulated winter and spear harvest at 12.5C or 20C. Plants were chilled at 2, 5, or 10C for 5 or 10 weeks during 'winter'. Plants transferred to growing conditions on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C) and produced very few or no spears at 12.5C; plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Bars represent twice error of means.
A: before spear harvest
B: after spear harvest at 12.5C
C: after spear harvest at 20C



Key: 1 = DP1[1]; 2 = DP2; S = short chain fructans (DP3 to DP8); L = long chain fructans; T = total water soluble carbohydrate

Note 1: DP = degree of polymerisation

Figure 5.06: Effect of chilling on concentration of carbohydrates (%DW) during simulated autumn and winter. Plants were chilled at 2, 5 or 10C for 5 or 10 weeks during 'winter'. Plants sampled on 22 March and 26 May were from a heated glasshouse (minimum temperature 13C); plants sampled on 2 July had been chilled for 5 weeks; plants sampled on 10 August had been chilled for 10 weeks. Bars represent twice standard error of mean.

Calculated respiratory losses during spear production varied considerably depending on the basis for the comparison i.e, dry weight, carbohydrate concentration, or size of carbohydrate pool, per plant or per spear (Table 5.03a & b). The loss in dry weight, expressed on a per plant or a per spear basis appeared to vary with the time at which plants were transferred to the growing temperature. Respiratory losses per gram of spear, whether expressed as dry weight or carbohydrate, were lowest in plants chilled for ten weeks.

Respiratory losses, on either a per plant or per spear basis, tended to be highest in plants transferred to growing conditions on 2 July i.e, after five weeks of chilling. The exception to this was the respiratory losses (dry weight and carbohydrate) per gram of spear dry weight in plants transferred to 12.5C on 22 March. These values were extremely high; however, very few plants produced spears, and the values included the respiratory losses from all plants in the sample.

Changes in carbohydrate composition

Harvesting at both 20C and 12.5C decreased the concentration of water soluble carbohydrates, and the concentration of all components except DP1 (Fig.5.07). There was no differences in the concentration of DP2 and short chain fructans between plants which produced spears, regardless of the 'spring' temperature or length of chilling.

The carbohydrate composition in plants harvested following 26 March differs from those harvested after chilling (Fig.5.07). Plants harvested at 20C in March had a relatively high concentration of DP2 and short chain fructans compared with plants harvested later in dormancy. Plants placed at 12.5C in March appeared to be very close to budbreak when they were sampled and they also have a higher concentration of short chain fructan.

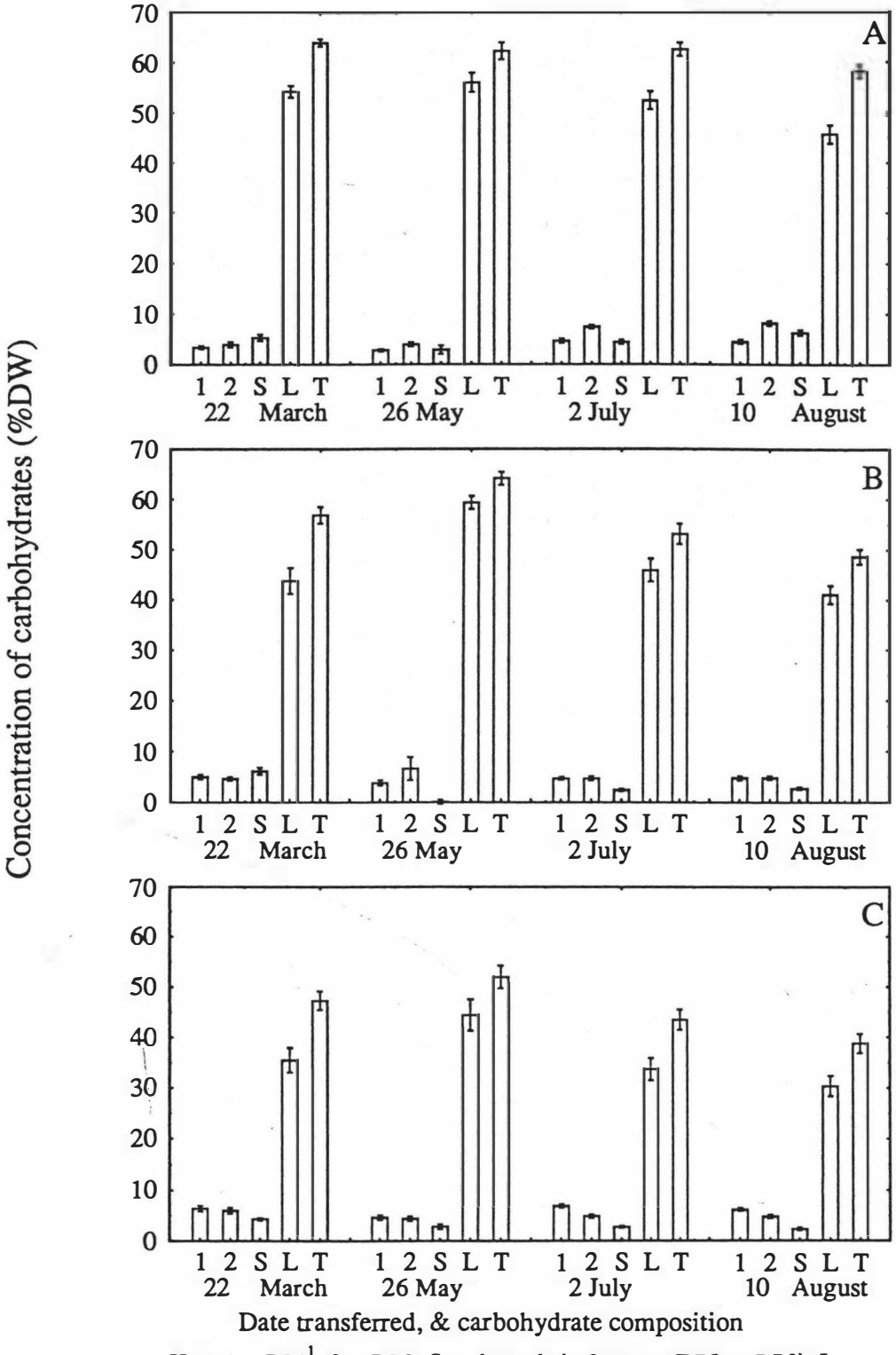
The composition of fructans in the roots of plants placed at 12.5C on 26 May changed during the following 16 weeks although the plants did not grow (Fig.5.07). The concentration of DP1 and DP2 increases, although not as much as on plants chilled at 10C and below for five weeks. There was also a marked decrease in the concentration of short chain fructans which did not occur on any other plants in this experiment.

Table 5.03a: Carbohydrate budget for plants harvested at 12.5C. Data from plants chilled at 2, 5 and 10C pooled. Standard errors of means in brackets.

	Date plants transferred to 12.5C			
	22 March	26 May	2 July	10 August
Before Harvest				
a. Crown dry weight (g/plant)	101.3 (14.8)	124.8 (10.8)	128.0 (9.0)	106.6 (7.5)
b. Carbohydrate concentration (% DW)	67.4 (0.9)	64.7 (1.6)	62.6 (1.3)	59.8 (1.4)
c. Carbohydrate pool (g/plant)	68.2 (9.9)	81.0 (7.9)	81.2 (6.9)	65.0 (5.6)
After harvest at 12.5C				
d. Crown dry weight (g/plant)	90.4 12.8)	117.3 (7.7)	90.5 (6.3)	90.5 (4.8)
e. Carbohydrate concentration (% DW)	57.6 (1.3)	58.1 (5.4)	50.0 (2.0)	50.3 (1.4)
f. Carbohydrate pool (g/plant)	52.5 (8.0)	68.2 (5.4)	46.0 (4.2)	46.2 (3.4)
g. Spear dry weight (g/plant)	0.19 (0.19)	0.0 (0.0)	8.00 (1.11)	5.03 (0.46)
h. Spear carbohydrate (g/plant)	0.04 (0.04)	0.0 (0.0)	1.60 (0.22)	1.00 (0.09)
Number of plants (n)	6	8	18	22
Dry weight respired during harvest = a-(d+g)	10.7 (4.6)	7.5 (5.6)	29.5 (7.5)	11.1 (6.3)
Carbohydrate respired during harvest =c-(f+h)	15.7 (3.3)	12.8 (7.0)	33.6 (5.5)	17.8 (4.8)
Dry weight respired per gram of spear	61.1 (74.7)	-	3.7 (1.2)	2.2 (1.4)
Carbohydrate respired per gram of spear	89.5 (99.9)	-	4.2 (0.7)	3.6 (1.2)
Days between transfer to 12.5C and end of 'harvest'	64	116	43	39
Mean days to budbreak		-	21	14

Table 5.03b: Carbohydrate budget for plants harvested at 20C. Data from plants chilled at 2, 5 and 10C pooled. Standard errors of means in brackets.

	Date plants transferred to 20C			
	22 March	26 May	2 July	10 August
Before Harvest				
a. Crown dry weight (g/plant)	84.0 (12.0)	122.4 (11.5)	130.2 (7.4)	98.8 (9.0)
b. Carbohydrate concentration (% DW)	64.9 (1.9)	64.7 (1.6)	63.0 (1.0)	59.3 (1.8)
c. Carbohydrate pool (g/plant)	55.6 (8.4)	79.5 (8.5)	83.0 (5.7)	60.0 (6.6)
After harvest at 20C				
i. Crown dry weight (g/plant)	70.5 (5.3)	98.4 (16.5)	84.3 (6.0)	91.0 (7.8)
j. Carbohydrate concentration (% DW)	50.7 (1.8)	57.2 (2.6)	47.3 (1.8)	43.5 (1.7)
k. Carbohydrate pool (g/plant)	36.1 (3.4)	59.0 (12.3)	40.2 (3.2)	40.1 (4.0)
l. Spear dry weight (g/plant)	4.79 (0.71)	6.66 (0.77)	9.80 (0.73)	10.4 (0.9)
m. Spear carbohydrate (g/plant)	0.96 (0.14)	1.33 (0.15)	1.96 (0.14)	2.09 (0.19)
Number of plants	10	8	23	15
Dry weight respired during harvest =a-(i+l)	8.6 (7.6)	17.3 7.2	36.1 (5.5)	8.1 (7.8)
Carbohydrate respired during harvest =b-(j+m)	18.5 (7.7)	19.2 (5.1)	40.8 (4.9)	23.3 (4.3)
Dry weight respired per gram of spear	1.8 (1.7)	2.6 (1.1)	3.7 (0.7)	0.78 (0.76)
Carbohydrate respired per gram of spear	3.9 (1.9)	2.9 (1.0)	4.2 (0.7)	2.2 (0.5)
Days between transfer to 20C and end of spear harvest	64	50	43	38
Mean days to budbreak	7	12	11	9



Key: 1 = DP1[1]; 2 = DP2; S = short chain fructans (DP3 to DP8); L = long chain fructans; T = total water soluble carbohydrate

Note 1: DP = degree of polymerisation

Figure 5.07: Effect of spear harvest at 12.5C or 20C on composition of carbohydrates in roots. Plants transferred to growing temperature on 22 March and 26 May were from a heated glasshouse (min. temperature 13C); plants transferred on 2 July had been chilled for 5 weeks; plants transferred on 10 August had been chilled for 10 weeks. Data from chilling temperatures of 2, 5 and 10C combined. Bars represent twice standard error of mean.
A: Concentration of carbohydrates before spear harvest
B: Concentration of carbohydrates after spear harvest at 12.5C
C: Concentration of carbohydrates after spear harvest at 20C

5.3.4 FRUCTAN METABOLISM

Changes during senescence, chilling and harvest which occurred in the concentration of each fructan below DP9 are highlighted in Table 5.04. The concentration of DP6 was below the limits which could be detected on the system used. The concentration of DP1 to DP5, plus DP7 and DP8 increased between 26 May and 10 August. Similarly, the concentration of each component decreased during spear harvest. The concentration of each of these fructans, except DP4, was higher on 10 August than in senesced plants on 6 May. The concentration of DP3 to DP8 combined was similar to that of DP1 and DP2 before spears were harvested (Table 5.04). Within the short chain fructans, the levels of DP3, DP5, DP7 and DP8 were higher than DP4 and DP6.

Correlations were calculated to indicate possible metabolic links between the various fructans. The concentration of DP1 has a very high negative correlation with the concentrations of long chain fructans (r=-0.79) and total carbohydrate (r=-0.69) (Table 5.05). The concentration of DP2 was positively correlated with the next largest fructan, DP3, (r=0.38, Table 5.05) and DP3 was correlated with DP4 (r=0.45), but the next 3 fractions (DP4, 5 and 6) were not significantly correlated with each other, and short chain fructans were not correlated with long chain fructans.

The concentration of each short chain fructan was positively correlated with that of the short chain fructans combined (Table 5.05). The correlations between DP1 and DP2, and between them and short chain fructans were relatively low.

5.3.5 SOME EFFECTS OF PLANT SEX

The crowns of male asparagus plants were larger than the crowns of female plants and therefore contained more carbohydrate before and after harvest although there was no difference in their carbohydrate concentration (Table 5.06) or composition (data not shown).

Although the weight and number of roots on male plants was higher than on female plants, the females had more new root than the males, both before and after spear harvest (Table 5.07). On both male and female plants, the number of new roots appeared to increase during harvest, although their dry weight decreased.

Table 5.04: Composition of crown fructans, crown dry weight and percentage dry matter in asparagus plants in simulated autumn, winter and spring. Standard errors of means in brackets. Plants sampled on 10 May had senesced in natural conditions; plants sampled on 26 May were from a heated glasshouse (minimum temperature 13C); plants sampled on 10 August had been chilled for 10 weeks; plants sampled on 25 September had been transferred to 'spring' temperatures on 10 August, after 10 weeks chilling. Data from chilling temperatures of 2, 5, and 10C combined.

Carbohydrate fraction	Effect of status of plants and date of sampling on carbohydrate concentration (% DW) and other measures of the size of the carbohydrate pool				
	Senesced at 12.5C (10 May)	Not chilled (T>13C) (26 May)	Chilled for 10 weeks (10 Aug)	Harvest at 12.5C after 10 wk chill (25 Sept)	Harvest at 20C after 10 wk chill (25 Sept)
DP1	3.33 (0.33)	2.81 (0.25)	4.48 (0.45)	4.75 (0.44)	6.10 (0.31)
DP2	4.52 (0.22)	3.95 (0.41)	8.20 (0.46)	4.75 (0.38)	4.73 (0.34)
DP3	0.80 (0.11)	0.49 (0.10)	1.54 (0.12)	0.11 (0.06)	0.04 (0.04)
DP4	0.93 (0.06)	0.13 (0.08)	0.48 (0.12)	0.0 (0.0)	0.0 (0.0)
DP5	0.81 (0.11)	0.63 (0.21)	1.61 (0.23)	1.06 (0.10)	0.98 (0.14)
DP6	nd	0.00 (0.07)	0.04 (0.05)	nd	nd
DP7	1.21 (0.13)	1.18 (0.27)	1.81 (0.20)	1.23 (0.24)	1.04 (0.13)
DP8	0.53 (0.06)	0.48 (0.19)	0.86 (0.11)	0.29 (0.12)	0.25 (0.12)
Short DP3-DP8	4.27 (0.37)	2.89 (0.87)	6.35 (0.55)	2.70 (0.27)	2.31 (0.30)
Long >DP8	53.99 (1.50)	55.92 (1.92)	45.71 (1.85)	41.11 (1.80)	30.33 (2.00)
Total	62.38 (1.25)	62.09 (1.68)	58.09 (12.63)	48.67 (1.46)	38.78 (1.94)
Other measures of carbohydrate reserves:					
Crown dry weight	124.32 (13.54)	104.54 (8.84)	92.65 (9.77)	45.90 (6.13)	45.62 (6.76)
% Dry matter	32.3 (1.4)	28.3 (0.8)	27.4 (0.7)	24.2 (0.6)	20.8 (0.7)

Note: nd = not detected

Table 5.05: Correlations between carbohydrate oligomers comprising the storage carbohydrate in asparagus roots and other measures of the size of the carbohydrate pool. (All correlations listed statistically significant at P=0.0001)

	DP2	DP3	DP4	DP5	DP6	DP7	DP8	Short	Long	Total	Crown DW	Percent DM	CHO pool
DP1									-0.79	-0.69	-0.57	-0.71	-0.60
DP2		0.38		0.38		0.29		0.38					
DP3			0.45	0.49		0.44	0.54	0.85		0.32	0.31		0.33
DP4							0.30	0.43		0.33	0.36	0.44	0.37
DP5						0.51	0.50	0.74					
DP6													
DP7							0.22	0.74					
DP8								0.68					
Short													
Long										0.96	0.67	0.80	0.74
Total											0.69	0.79	0.77
Crown DW												0.72	0.98
% DM													0.76

Table 5.06: Effect of plant sex on crown dry weight, crown carbohydrate pool (g/plant) and concentration of carbohydrate (% DW) during a simulated winter and subsequent spring harvest. Standard errors of means in brackets. Plants were sampled before transfer to 'spring' temperature on 26 May, 2 July and 10 August, and after 5 weeks of harvesting at 12.5C or 20C. Data from 12.5C and 20C combined.

	Before 'spring' harvest		After 'spring' harvest	
	Male	Female	Male	Female
Crown dry weight (g/plant)	132.9 (6.4)	99.6 (6.2)	98.1 (4.4)	84.0 (4.2)
Carbohydrate pool (g/plant)	82.7 (4.9)	62.7 (4.8)	51.1 (2.9)	42.3 (2.7)
Carbohydrate concentration (% DW)	62.0 (1.3)	61.1 (1.3)	50.4 (1.3)	49.7 (1.2)

Table 5.07: Effect of plant sex on weight (g/plant) and number of old and new roots following winter and subsequent spring harvest. Standard errors of means in brackets. Plants were sampled before transfer to 'spring' temperature on 26 May, 2 July and 10 August, and after 5 weeks of harvesting at 12.5C or 20C. Data from 12.5C and 20C combined.

	Before 'spring' harvest		After 'spring' harvest	
	Male	Female	Male	Female
Dry weight of old roots (g/plant)	132.1 (6.5)	97.0 (6.4)	111.8 (3.9)	87.7 (3.8)
Dry weight of new roots (g/plant)	0.9 (0.6)	2.5 (0.6)	0.8 (0.3)	1.6 (0.3)
Number of old roots	146.4 (7.8)	103.2 (7.7)	143.1 (4.6)	115.3 (4.5)
Number of new roots	2.5 (1.4)	5.4 (1.4)	4.8 (1.2)	7.1 (1.1)

5.4 DISCUSSION

Seasonal changes in the carbohydrate reserves of asparagus are associated with changes in plant growth, which in turn, is determined largely by environmental factors. The previous chapter discussed the effect of temperature on asparagus growth in autumn, winter and spring. This chapter discusses the associated changes in plant dry weight, and crown carbohydrate concentration, content, and composition.

5.4.1 CHANGES IN THE CARBOHYDRATE POOL

Changes in crown dry weight, carbohydrate concentration and carbohydrate pool size occurred concurrently during autumn, winter, and spring. The maximum carbohydrate concentration in this experiment was 65% (Figs.5.04, 5.06, 5.07). This level is similar to that reported elsewhere (Table 5.01), and may represent a physiological upper limit for the plants in this experiment. This level was reached before the fern senesced, and remained constant during senescence (Fig.5.04). Similar results have been interpreted as indicating that little change occurs in the quantity of carbohydrate reserves in autumn (Scott et al. 1939). However, in this experiment, crown dry weight increased, thus increasing the carbohydrate pool by 30% during senescence (Fig.5.03b). Haynes (1987) also reported an increase in crown dry weight in field grown plants in autumn. The changes which occurred during autumn differed from those during winter.

Crown dry weight, carbohydrate concentration and size of the carbohydrate pool generally decreased from early winter to late winter, i.e, while the plants were dormant (Figs.5.05, 5.06). These losses increased as the temperature increased from 2C to 10C, and appeared to be greater in late dormancy (mid winter to late winter) than near maximum dormancy (early to mid winter). This suggests that the rate of metabolism within the 'dormant' plants increased as dormancy was released, and as the temperature increased from 2C to 10C. This increase in metabolic rate appeared to include changes which must occur before budbreak as the efficiency at which crown dry weight and crown carbohydrate was used to produce spears appeared to change as the plants were released from internal dormancy (Table 5.03a & b). The changes which occurred during spear harvest appeared to be affected by the stage of dormancy which the plants had reached before transfer to 12.5C or 20C (Table 5.03 a & b).

Crown dry weight, carbohydrate concentration and size of the carbohydrate pool continued to decrease during spear harvest. The respiratory requirements per gram of spear appeared to decrease as the depth of dormancy at the time of transfer to growing

temperatures decreased. The decrease in respiratory requirements on plants chilled for ten weeks compared with plants chilled for five weeks before transfer to either 20C or 12.5C was associated with an apparent decrease in the depth of dormancy (Section 4.4.1). However, the respiratory losses which occurred before spear harvest have been combined with those which occurred during spear harvest in Table 5.03. If the respiratory requirements during spear harvest (i.e, after spear growth has commenced) were the same regardless of the plants' history, then the respiratory requirements between transfer to the growing temperature and budbreak were higher on plants transferred in mid winter than in plants transferred in late winter. Thus some process which must occur before budbreak by plants previously subject to internal dormancy appeared to occur after 2 July, either after plants were transferred to the growing temperatures, or between mid and late winter. This unknown process occurred after the decrease in the minimum temperature for budbreak which occurred between early and mid winter (Section 4.4.1).

The increase in respiratory requirements of plants transferred to 20C between early winter (26 May) and mid winter (2 July) could be due to plants being further from the optimum temperature for budbreak in mid winter than in early winter, as suggested in Section 4.4.2.

The relatively low amount of dry weight but relatively high amount of carbohydrate respired per gram of spear by plants at 20C on 22 March could be due to different metabolic processes occurring in plants entering dormancy, compared with plants being released from dormancy.

Scott et al. (1939, Table 14) reported losses in crown dry weight and crown carbohydrate of 2.95 g dry weight and 3.14 g carbohydrate per gram of spear (standard errors not given). These values were derived from spring harvested plants in natural conditions, and were within the range of values obtained in this experiment.

New roots were produced by plants during chilling and during spear harvest (Tables 5.02, 5.07). This has not been reported previously. During harvest, the number of new roots increased while their dry weight decreased. This implies that the initiation of new roots can occur when plants are depleting their carbohydrate reserves. Thus root initiation may be linked with shoot initiation rather than with the availability of carbohydrate for storage. It also suggests that the carbohydrate translocated to the new roots is readily remobilised.

5.4.2 FRUCTAN METABOLISM WITHIN ASPARAGUS

Composition of asparagus fructans

Most of the carbohydrate pool in plants in this experiment was long chain fructans i.e, DP>8 (e.g. Fig.5.04, 5.07). This differs from previous reports on fructan size distribution within asparagus crowns in which most of the carbohydrate was less than DP9 (Pressman et al. 1989; Kim and Sakiyama 1989b, Martin and Hartmann 1990). All long chain fructans present should have been extracted by Pressman et al., and Martin and Hartmann as both used water during the extraction process (Table 5.01). However, there were several other differences between the studies which could have contributed to differences in fructan size.

Each study used a different genotype: 'Rutger's Beacon'; 'Lucullus' (Martin and Hartmann 1990); 'Mary Washington 500W' (Kim and Sakiyama 1989b) and 'Brocks Imperial 84' (Pressman et al. 1989) but this is unlikely to cause differences of the magnitude recorded here. In other species, genotypes appear to vary in the distribution of fructan size, but not in the range of fructan sizes (Eagles 1967, Darbyshire and Henry 1981). Variations in plant size are more likely to have caused differences in fructan size, as has been reported in chicory (Bhatia et al. 1974).

The data in this experiment was obtained from larger plants than those used by Pressman et al. (1989), Martin and Hartmann (1990) or Kim and Sakiyama (1989b). The plants used here were in 10.8 litre containers, while those of Pressman at al., and Martin and Hartmann were in 3 and 5 litres respectively. Kim and Sakiyama used one year old plants. Like chicory, smaller asparagus plants may have smaller fructans. A decrease in the diameter of chicory from over 3.5cm to below 2.5cm decreased the maximum size of stored fructans from DP19 to DP13 (Bhatia et al. 1974). The larger asparagus crowns used in this study also had a higher concentration of water soluble carbohydrate.

In other species, the DP of stored fructans increases as the concentration of carbohydrate increases (Darbyshire and Henry 1981, Pollock and Jones 1979). The concentration of water soluble carbohydrates reported by Pressman et al. (1989) and Kim and Sakiyama (1989b) are approximately one third of the concentration in plants in this study. The values reported by Martin and Hartmann (1990) were an 'all season mean'. Although the maximum concentration they report is similar to this study, the mean will be lower as the minimum is lower (see Table 5.01) and maintained for several weeks. Thus the

higher concentration of carbohydrate reported here would also contribute to the larger size of the fructans.

Changes in the composition of asparagus fructans during senescence and dormancy

Changes in carbohydrate concentration and composition during senescence reported here are similar to those of Pressman et al. (1989), despite the differences in concentration and fructan size. In both studies, the concentration of DP2 appeared to increase in the first 40-50 days following transfer to cool conditions (12.5C constant and natural autumn temperatures, or 15/5C day/night) where the plants commenced senescence (Fig.5.04). The subsequent increase in carbohydrate concentration obtained as the fern senesced by Pressman et al. (1989) did not occur, possibly because plants in this experiment were already near a maximum physiological concentration for fructans, whereas the carbohydrate concentration in Pressman's plants were near to 20% which is very low (Table 5.01). Changes in the composition of asparagus fructans appeared to be linked to physiological changes which occurred during dormancy.

Plants which were entering dormancy required exposure to temperatures below 12.5C before the concentration of both DP2 and short chain fructans increased (Fig.5.04, 5.07). Plants which had been prevented from senescing by maintaining temperatures above 13C, and plants which senesced at 12.5C had a lower concentration of DP2 than plants which senesced naturally or were chilled at 10C and below. There was no evidence that long chain fructans depolymerised in senescing plants, or at 12.5C from early winter onwards. However, if the fern was removed in early dormancy (i.e, mid autumn), the concentration of DP1 increased as long chain fructans were depolymerised prior to budbreak (Fig.5.07b). This indicates that there is an interaction between stage of dormancy, presence and absence of fern, and temperature.

A further example of this interaction is the relationship between chilling temperature and changes in fructan composition. After five weeks of chilling, the fructan composition was the same at each chilling temperature (Fig.5.06). When the plants were chilled at 10C for a further five weeks, the concentration of DP1, DP2 and short chain fructans remained constant while the concentration of long chain fructans decreased. Consequently, the size of the carbohydrate pool also decreased (Fig.5.05a). This implies that there was an equilibrium between the rate at which fructans were depolymerised to DP1 and DP2 and the rate at which plants were utilising carbohydrate reserves at 10C. However, chilling at 5C and 2C tended to increase the level of DP1, DP2 and short chain fructans above that present after five weeks (Fig.5.06).

This difference in fructan composition appeared to be associated with a decrease with temperature in the metabolic rate of the plants. Plants at 5C and 2C utilised less carbohydrate during chilling than plants at 10C as indicated by their larger carbohydrate pool (Fig.5.05). This decrease in metabolic rate may also have restricted the breakdown of short chain fructans and thus decreased the level of DP1 and increased the level of short chain fructans at 2C (Fig.5.06). These variations in carbohydrate composition due to the length and temperature of chilling may have affected budbreak, especially near the minimum temperature for budbreak.

Budbreak was restricted in plants transferred to 12.5C on 22 March and 26 May (see Chapter 4). These plants had a lower concentration of DP1 than plants on which budbreak occurred (Fig.5.06). A minimum concentration of 4.0-4.5% dry weight of DP1 appears to be required before budbreak will occur at 12.5C. Plants transferred to 12.5C on 22 March were on the verge of budbreak when sampled, and their concentration of DP1 had reached 5% of dry weight. Budbreak did not occur on plants transferred on 26 May: DP1 had increased to only 3.8% of dry weight. The minimum of 4.0-4.5% may not apply to plants in late dormancy (i.e, 10 August). The concentration of DP1 on plants stored at 2C for ten weeks was below 5% dry weight and the number of days to budbreak was not depressed, although there was a decrease in the percentage of first spears originating from the basal bud. A low concentration of DP1 could restrict budbreak by limiting the energy available for an essential metabolic process, such as remobilisation of assimilate from the storage roots to the rhizome buds.

The minimum concentration of 4.5% of dry weight of DP1 may represent the energy required to load sucrose into the phloem prior to translocation. The low concentration of DP1 did not inhibit budbreak at 20C, possibly because sucrose is more readily metabolised to glucose at 20C than at 12.5C.

Another aspect of carbohydrate composition which is associated with the concentration of DP1 and budbreak is the concentration of DP3-DP8. In plants transferred to 12.5C in early winter (26 May), the short chain fructans were almost completely depolymerised to DP1 and DP2 in the following 16 weeks (Fig.5.07). Thus, blocking the breakdown of long chain fructans to short chain fructans may prevent an increase in the level of DP1, and thus prevent budbreak at 12.5C. A transient increase in the concentration of sucrose prior to growth in spring is a characteristic of fructan storing plants (Pollock and Cairns 1991).

Cold tolerance in asparagus has not been evaluated. This study, and Pressman's (1989), both investigate the effect of cool temperatures on fructan composition; they do not evaluate the resulting 'cold tolerance'. Short chain fructans appear unlikely to have a role in cold tolerance as their concentration did not increase in early dormancy. However, DP1 and DP2 may contribute to cold tolerance. Their combined concentration increased from 7.32% of dry weight on plants sampled on 22 March to 12.03% after five weeks of chilling (Fig.5.06). As in chicory and dandelion, the concentration of DP1 and DP2 was similar during vegetative growth, and the concentration of DP2 increased above that of DP1 during autumn and winter in an environment where the plants lost their foliage during winter (Cyr et al. 1990). This increase in DP2 was associated with depolymerisation of long chain fructans in chicory and dandelion. The apparent lack of depolymerisation of asparagus long chain fructans in early winter may be due to species differences, or alternatively to environmental differences. For example, slower senescence, in this experiment, may have allowed translocation into the crown to continue while the plants were developing cold tolerance. Thus the increase in sucrose concentration would be obtained from recently imported carbohydrate rather than from the reserves.

Changes in fructan composition during harvest

The reserve carbohydrates within asparagus were utilised much more rapidly during the harvest of spears than during dormancy (Table 5.03, Fig.5.05). As the reserve carbohydrates were utilised, both the dry weight of the crown and the concentration of carbohydrate decreased. The concentration of long chain fructans decreased as the total fructan decreased, but the concentration of DP1 increased (Fig.5.07, Table 5.05). DP1 would accumulate if the rate at which fructose was released during depolymerisation exceeded the rate at which it was converted to glucose and subsequently used as a respiratory substrate or in synthesis of sucrose. Thus, the concentration of DP1 would increase if long chain fructans were being continuously depolymerised, as appeared to happen during the harvest of asparagus spears.

The concentration of DP2 and short chain fructans was similar in all plants which had produced spears at either 12.5C or 20C, after chilling or following 26 May (Fig.5.07). This implies that there may be an equilibrium state for plants which are utilising their carbohydrate reserves.

5.4.3 OTHER ASPECTS OF FRUCTAN METABOLISM IN ASPARAGUS

The concentration of DP1 and DP2 appeared to be affected by different factors as they were poorly correlated (Table 5.05). Similarly, the concentration of DP1 and DP2 were poorly correlated with the concentration of short chain fructans and with each of DP3 to DP8. This may be related to their differing physiological functions. Short chain fructans are storage carbohydrates, possibly with a cold tolerance function; DP2 or sucrose is the translocated form of carbohydrate and may also have a cold tolerance function while the glucose component of DP1 is the respiratory substrate. These results give some indication of metabolic pathways as carbohydrate reserves are utilised during chilling and harvesting.

The increase in the concentration of short chain fructans which occurs during senescence and chilling was a result of an increase in the concentration of each of DP3 to DP8 (Table 5.04, 5.05). Similarly, during harvest the concentration of each component decreases.

Throughout this experiment, the concentration of short chain fructans was less than the concentration of DP1 and DP2 (Table 5.04, Figs 5.06, 5.07) on plants which were utilising storage carbohydrate. This implies that there was a very rapid turnover of short chain fructans when long chain fructans were being degraded, and probably indicates that the rates of depolymerisation and translocation are tightly regulated. However, Kim and Sakiyama (1989b) reported that, during budbreak, the concentration of DP1 and DP2 combined was 50-65% less than the DP3-DP8 combined. The reasons for this difference are not clear. Their data is not consistent with the increase in DP1 as total fructans decreased which occurred here. However, the concentration of total carbohydrate in their experiment was approximately 20%, which is considerably lower than in this experiment (Fig 5.07). Depolymerisation in plants with such a low carbohydrate content may differ.

In this experiment, the relative concentrations of the short chain fractions varied considerably (Table 5.04) with DP3, DP5 and DP7 being present at much higher concentrations than DP4, DP6 and DP8. The concentration of DP4 and DP6 were very low, especially in plants which were being harvested and rapidly utilising carbohydrate. This variation in concentration conflicts with that obtained by Kim and Sakiyama (1989b). In their plants, the concentration of DP3 and DP4 were the highest of the short chain fructans. The concentration of DP3 to DP8 became smaller as the size of the fructan increased. However, as previously stated, there were several differences between the two studies which were likely to affect fructans. The relative concentrations of

fructans is not related to fructan size in some grasses and cereals (Pollock and Cairns 1991).

5.4.4 SOME EFFECTS OF PLANT SEX

Differences in carbohydrate metabolism are not the reason for the well documented differences in the vigour and longevity of male and female plants. There was no difference between male and female plants in carbohydrate concentration or composition (Table 5.06). Male plants had a higher crown weight, and therefore a larger carbohydrate pool than female plants, both before and after spear harvest. The factors which affect crown size are probably related to the effects of berries on availability of assimilate for storage in the crowns (see Chapter 3).

Although the crowns of female plants are smaller than those of male plants, and they have less roots, the number and dry weight of new roots is higher than in male plants (Table 5.07). This is another example of differences in partitioning which are related to plant sex. However, the new roots comprised less than 3% of the weight of storage roots.

5.5 CONCLUSIONS

Temperature dependent changes in carbohydrate composition occurred during dormancy. This suggests that changes in carbohydrate composition determined when asparagus buds were released from internal dormancy. Alternatively, carbohydrate metabolism may be regulated by the same factors that permit budbreak, ensuring that plant growth and carbohydrate metabolism are integrated. Plant growth substances are important in releasing dormancy in deciduous fruit trees (Saure 1985), so their role in asparagus should not be dismissed, although no effects have been found as yet (Kim and Sakiyama 1989b).

Most of the fructans present in this experiment were long chain fructans, i.e, with a DP greater than eight. This differs from previous reports, but is likely to be closer to the field situation as plants in this experiment were larger and had a higher water soluble carbohydrate concentration than the plants used by previous researchers (Pressman et al.; Kim and Sakiyama 1989b; Martin and Hartmann 1990).

The plants reached a maximum carbohydrate concentration of 65% before the fern senesced. However, the size of the crown carbohydrate pool increased by 30% during senescence due to an increase in dry weight. This indicates that it is essential to collect data on both crown dry weight and carbohydrate concentration when examining the carbohydrate balance.

The concentration of DP2 increased during senescence which indicated that sucrose may have a role in cold tolerance in aspa gus. Chilling at 10C and below also increased the concentration of DP1 and short chain fructans. Maintaining the temperature above 13C prevented senescence, and prevented an increase in the concentration of DP1, DP2 and short chain fructans.

There appeared to be a process associated with the release of dormancy in asparagus which had a high respiratory requirement. This process occurred during the second five weeks of chilling, i.e, in late winter. If plants were transferred to the growing conditions before dormancy was released, then the process appeared to occur before the beginning of budbreak as the respiratory requirement per gram of spear harvested was higher in such plants.

This process essential to the release of internal dormancy was associated with the ability to depolymerise long chain fructan. Depolymerisation of long chain fructans occurred in plants at 2C to 10C between mid and late winter. Depolymerisation did not occur in plants transferred to 12.5C in early winter. The ability to metabolise DP1, DP2 and short chain fructans may also have changed as plants were released from dormancy. In late dormancy, the minimum temperature for budbreak of asparagus decreased to near 10C. At 10C, the size of the fructan pool decreased as carbohydrates were metabolised; however, the concentration of DP1, DP2 and short chain fructans increased at 5C and 2C. Plants may require a minimum DP1 concentration of 4-4.5% dry weight before budbreak will occur at 12.5C.

The rates of depolymerisation and translocation appeared to be tightly regulated as there appeared to be a rapid turnover of short chain fructans and DP2 in plants which were being harvested, as indicated by their near constant concentration. However, the concentration of DP1 increased during spear harvest. This indicates that either there was an inverse relationship between the concentration of DP1 and long chain fructans, or the rate at which fructose was metabolised lagged behind the release of fructose by depolymerisation. Data on the ratio of fructose to glucose within the DP1 fraction would give further insight into fructan metabolism.

There were no differences between male and female plants in their carbohydrate concentration or composition. However, male plants had a higher crown dry weight than females, and thus a larger carbohydrate pool.

CHAPTER SIX

A MODEL OF SEASONAL CHANGES IN GROWTH AND CARBOHYDRATE METABOLISM

Data presented in the previous four chapters indicate that asparagus growth is controlled, to a large extent, by temperature. This data, and the temperature regimes which occur at Palmerston North, New Zealand, were used to develop a simple model of seasonal changes in the accumulation and utilisation of the plant's carbohydrate reserves. Changes in plant dry weight, crown carbohydrate concentration and size of the crown carbohydrate pool were incorporated into the model. This model is more descriptive than that of Lampert et al. (1980), but also considers more physiological interactions. Although it is largely empirical, it endeavours to consider interactions which do, or which may, occur in carbohydrate production and utilisation.

6.1. DEFINING THE BASIC UNIT FOR CARBOHYDRATE MOVEMENT AND ALLOCATION.

The basic physiological unit for carbohydrate production and allocation in asparagus cultivars such as 'Rutger's Beacon' is a rhizome and any axillary rhizomes which have not developed ferns (Section 3.4.1). There is limited movement of carbohydrate reserves to other major rhizomes during vegetative growth but there is no apparent interaction between major rhizomes during spear harvest (Section 4.4.6) indicating similarly limited movement of plant growth substances. However, the extent of carbohydrate movement, and the size of the basic physiological unit within different cultivars is likely to vary with rhizome morphology. Rhizome development appears to be determined by the strength of correlative inhibition within the cultivar.

Axillary rhizomes generally develop from the axils of buds which produced the previous season ferns (Section 3.3.2) in cultivars such as 'Rutger's Beacon'. During spear harvest, they can also develop from the axils of harvested spears (Section 4.4.6). Thus, the development of axillary rhizomes is controlled by correlative inhibition in which the shoots, (fern or spears), arising from the older buds strongly inhibit development of their axillary buds, as well as younger buds on the same axis. Consequently, cultivars such as 'Rutger's Beacon' and 'Jersey Giant' have few large rhizomes with a clearly defined bud axis.

'UC157' is a cultivar with many, small bud clusters which appear to result from relatively weak inhibition of axillary buds (Section 4.4.6). This cultivar tends to produce spears in 'flushes' i.e, spears develop from several clusters at the same time, and these spears strongly inhibit budbreak by other spears until they are harvested (Section 4.4.6; Benson and Takatori 1968; Nichols and Woolley 1985). Therefore, whatever controls budbreak appears to have unrestricted movement between rhizomes. This indicates that there may also be unrestricted movement of assimilates between rhizomes, and thus the entire plant may comprise the fundamental unit for carbohydrate production and allocation. However, 'UC157' was not studied in detail in this thesis, and the model presented here was developed using 'Rutger's Beacon'.

6.2 A MODEL FOR CARBOHYDRATE PRODUCTION AND UTILISATION IN HARVESTED ASPARAGUS PLANTS

6.2.1 THE MODEL

An outline

This model of the seasonal changes in carbohydrate production and allocation was developed using male plants of 'Rutger's Beacon'. Most of the changes in carbohydrate content are expressed on a per week basis.

Seasonal changes in plant growth were divided into six phases, based on visible differences, as follows:

1. Senescence
2. Dormancy
3. Spear harvest
4. Carbohydrate depletion
5. Carbohydrate restoration
6. Pre-senescence

Timing of the phases of growth and the mean temperature within each phase has been derived from local observations of plant growth and Palmerston North temperature data (Table 6.01). Thus, the model describes growth in a temperate climate. It also assumes that growth is generally not restricted by nutrients or by water supply. In this model, the growth of plant tissues is generally controlled by the amount of tissue and/or carbohydrate reserve present.

Table 6.01: Temperate climate in which asparagus growth was modelled: temperature normals and daylength. Data from DSIR, Palmerston North, latitude 40 23S, longitude 175 37E (meterological station E05363).

Month	Daily temperature (C)				Day-length (hrs) [c]
	Mean [a]	Mean [a] Maximum	Mean [a] Minimum	Mean [b] Soil (10cm)	
Jan	17.7	22.3	13.1	18.5	15.3
Feb	18.1	22.9	13.2	18.1	14.2
Mar	16.8	21.4	12.2	16.3	12.9
Apr	14.1	18.4	9.7	13.2	11.5
May	11.2	15.2	7.1	10.1	10.5
Jun	8.8	12.8	4.8	7.7	9.8
Jul	8.1	12.1	4.0	6.7	10.1
Aug	9.3	13.3	5.2	7.6	11.0
Sep	11.0	15.0	6.9	9.9	12.4
Oct	12.6	16.8	8.4	12.5	13.8
Nov	14.4	18.7	10.1	15.1	15.1
Dec	16.4	20.8	11.9	17.3	15.6
Year	13.2	17.5	8.9	12.8	

Notes:

a - 1951-1980

b - 1939-1980

c - Francis 1972

Plant tissues are divided into two categories: shoots i.e, spears or ferns, and crown i.e, rhizome and storage roots. Feeding roots have been excluded from the model. They comprise less than 3.5% of crown dry weight in field grown plants (Haynes 1987). Upper and lower limits for crown carbohydrate concentration have been set at 65% and 10% respectively, based on data presented here (Figs.5.04 & 5.07) and elsewhere (see Table 5.01).

Phase 1: Senescence

The senescence of ferns commences when plants are subjected to temperatures below 13C (Section 4.4.1). The minimum temperature at which budbreak occurs increases before any visible sign of senescence (Section 4.4.1, Hughes unpub. data; Nichols pers. comm; Dufault 1990) indicating that internal dormancy is induced before ferns begin to yellow.

During senescence, fern dry weight decreases while crown dry weight increases (Fig. 3.14; Fig. 5.03). The data records changes over different time periods, and indicates that the rate of change varies with time, reaching a maximum in late April. This has been simplified in the model.

The model assumes that fern and crown dry weight change at a constant rate for 4 weeks during senescence. Fern dry weight decreases at 15% per week, and crown dry weight increases at 8% per week. The carbohydrate concentration remains constant at 65%.

Phase 2: Dormancy

In Palmerston North, asparagus plants appear to be dormant for 20 weeks. Asparagus requires about 500 chilling units to release 50% of the basal buds from internal dormancy and permit growth at 12.5C (Section 4.4.2). Chilling commences at temperatures between 12.5C and 10C (Section 4.4.1). In Palmerston North, chilling will commence in late April-early May and be complete mid-late June. Thus budbreak in July and August is suppressed by ecodormancy i.e, cold temperatures.

Table 6.02: A model of asparagus growth in a temperature climate: summary of phenology, agronomic practises and status of carbohydrate reserves associated with spring harvest of a mature crop.

Phase	Mean temp. (C)	Date commences	Weeks after 1 Jan	Phenology, agronomic practices and carbohydrate (CHO) status
1 Senescence	12.7	1 Apr	13	Internal dormancy induced. Minimum temperature for budbreak above 12.5C. Senescence commences, visible by mid April.
2 Dormancy	9.7	29 Apr mid-late May 8 Jul	17 27	Senescence complete; maximum crown weight & CHO concentration. Plant at maximum internal dormancy. Chilling complete. Budbreak will occur at 12.5C
3 Harvest	13.5	16 Sept 30 Sept	37 39	Budbreak in field consistent. Minimum temperature for budbreak 10C Harvest commences - crown weight & CHO concentration decrease.
4 Carbohydrate depletion	16.4	9 Dec	49	Harvest ceases. Rapid decrease in crown weight and CHO concentration as first ferns develop.
5 Carbohydrate restoration	17.9	1 Jan	0	Rapid fern growth, relatively slow root growth. Crown dry weight and CHO concentration increases.
6 Pre-senescence	16.8	4 Mar	9	Slow fern growth; maximum crown CHO concentration; crown dry weight increases rapidly.

There is a respiratory cost associated with winter dormancy. The decreases in crown dry weight and carbohydrate concentration which occurred at 10C and 5C (Fig. 5.05a) were high compared with data on field grown plants (Fig.3.14; Haynes 1987). However, there does appear to be a respiratory cost involved with some process which must occur before budbreak occurs (Section 5.4.1). This process has been included in the 'dormant' phase of the model, and mean values for plants chilled for 10 weeks used in the model.

The model assumes that during the 20 weeks of dormancy, crowns will lose 14% of their dry weight (Fig.3.14) and the concentration of storage carbohydrates (i.e, water soluble carbohydrates) decreases to 60% (Table 5.03).

Phase 3: Harvest

The time at which budbreak occurs varies between plants. Budbreak tended to be earlier on larger plants (data not presented). In the field, sporadic budbreak may occur during late July and August. Budbreak normally becomes consistent in mid September when the soil temperature at the rhizome is close to 10C (Table 6.01).

Commercial harvesting normally commences in late September-early October in Palmerston North (Nichols, pers. comm.). Harvesting generally ceases in the first week of December, when quality declines and the cannery ceases processing the crop. Some growers harvest until Christmas or mid November, in alternate years, without adversely affecting crop vigour (Nichols pers. comm.). The model assumes that plants are harvested for 10 weeks, commencing 2 weeks after budbreak in mid September.

The model derives changes in crown dry weight and carbohydrate content from the dry weight of harvested spears. The number of spears harvested is calculated from the RSGR during harvest. The mean daily temperature during harvest is 13.5C. RSGR increases only slightly between 10C and 15C (Nichols and Woolley 1985); therefore I have estimated the RSGR at 13.5C as 0.255 mm/mm/day. The mean RSGR for spears grown at 12.5C was 0.204 mm/mm/day (Table 4.09).

The minimum saleable spear length is 18 cm. However, spears are cut about ground level and the 18 cm does not represent the total spear height. The model assumes that each spear has 5 cm below ground. Thus the minimum harvestable spear height, measured from the rhizome, is 23 cm.

If spears are harvested daily, a spear which is just below harvestable height will be 29.7 cm high (measured from the rhizome) 24 hours later (Equation 4.1). Thus the mean harvestable height is 26.3 cm. Spears require 9.24 days to reach this height, assuming a spear growth rate of 0.255 mm/mm/day and a spear diameter of 12.5mm. Thus in the field, a spear would be harvested from each active rhizome every 9-10 days.

If growth of the next spear on a rhizome begins immediately after harvest, eight spears will be harvested from each rhizome of a mature plant during a ten week (70 day) harvest. Based on data in this thesis, the mean dry weight of a harvested spear is 1.8g. Thus the total dry weight transferred from the crown to 8 spears during a 10 week harvest will be 14.4 g. Using the respiratory factors calculated for plants chilled for 10 weeks and subsequently grown at 12.5C (Table 5.03a), this represents a loss of 31.7 g dry weight and 51.8 g carbohydrate from the crown of each plant harvested. This probably over-estimates carbohydrate utilisation during harvest as spear growth rate decreases as carbohydrate concentration decreases (Kim and Sakiyama 1989b), and the time lag between harvest and growth of the next spear may increase (Tiedjens 1926).

Phase 4: Carbohydrate depletion

In other studies, the crown carbohydrate concentration decreased more during fern re-establishment than during spear harvest (Scott et al. 1939; Taga et al. 1980; Shelton and Lacy 1980). Each fern is a carbohydrate sink until the cladodes have expanded (Downton and Torokfalvy 1975; Lin and Hung 1978), thus fern production will utilise crown carbohydrate reserves until the first fern produces enough photoassimilate to supply the developing second fern.

These studies provide data on carbohydrate movement over 3.3 weeks between the end of harvest and establishment of at least one fern per plant (Fig 3.14). Crown dry weight decreased by 45%; fern dry weight was 92% of the decrease in crown dry weight, and equal to the decrease in the carbohydrate pool. During this time, the crown carbohydrate concentration decreased from 58% to 17% of dry weight. Carbohydrate movement of a similar magnitude was reported by Haynes (1987) in non-harvested plants, although it occurred over a longer time in early spring.

The model assumes that 4 weeks is required before the ferns begin exporting photoassimilate to the crown. Until then, they are a carbohydrate sink. Crown dry weight decreases at 15% per week. At the end of that 4 weeks, fern dry weight is 90% of the decrease in crown dry weight, and equal to the decrease in the carbohydrate pool, unless this pushes the crown carbohydrate concentration below 10%. If crown carbohydrate concentration reaches 10%, then fern dry weight equals the decrease in the carbohydrate pool. Thus the dry weight of the first ferns depends on crown dry weight and carbohydrate concentration at the end of harvest.

Phase 5: Carbohydrate restoration

During this phase of growth, the fern dry weight increases rapidly and reaches a maximum about the same time as crown carbohydrate concentration. Root dry weight increases after the crown carbohydrate concentration is restored to it's maximum level of 65%. This sequence occurs in data presented by Haynes (1987) and Fisher (1982), and is consistent with data reported in Chapter 3.

Dry weight parameters for this phase are based on data in Figure 3.14. Changes in carbohydrate concentration are based on Haynes (1987). Fern dry weight increases by 15% per week for 8 weeks. During this time, the dry weight of the carbohydrate pool increases by 0.07g/g fern dry weight per week. Crown dry weight is constant until the crown carbohydrate concentration reaches a physiological upper limit of 60-65%, and then increases to maintain the concentration at 65%.

Data from Chapter 2 has not been used in this part of the model as it was derived from the growth of young asparagus plants in their first year of growth. Plants which have been harvested have a large amount of crown tissue present when fern growth commences after spear harvest. This will change the root:shoot ratio, and may have a significant effect on the RGR and/or photosynthetic rates.

Phase 6: Pre-senescence

Carbohydrate partitioning between fern and crown changes as the plants reach maximum fern dry weight (Section 3.4.3; Haynes 1987; Fisher 1982). The crown is now a stronger sink than the fern.

The model assumes that fern dry weight is constant until senescence begins. The rate at which the crown carbohydrate pool expands increases to 0.1g dw/g fern dw per week. Crown dry weight increases to maintain the carbohydrate concentration at 65%.

6.2.2 EVALUATING THE MODEL

The model developed above was applied to a rhizome of 100g dry weight on 1 May i.e, following senescence, and which is harvested for 4 weeks in the following spring (Table 6.03). This is equivalent to a field grown plant in it's third year of growth (Haynes

1987). The model was also applied to a plant with a 200g rhizome, and which is harvested for a full season of 10 weeks (Table 6.04).

Most of the values generated using the model appear to be realistic. The time required to restore crown carbohydrate concentration to preharvest levels is similar to that reported in field grown plants (Scott et al. 1939; Taga et al. 1980; Shelton and Lacy 1980). In both cases, the dry weight of fern present at the end of 'carbohydrate depletion' was limited by crown carbohydrate concentration reaching 10% of dry weight (Table 6.03, 6.04). This is at the lower limit of values reported for field grown plants and indicates that the depletion of carbohydrate reserves in the model during harvest may be too high. However, the model can still be used to consider long term plant growth.

Crown dry weight increased by 30% in both examples (Tables 6.03, 6.04). This increase in size is necessary in a young plant (Table 6.03) to develop a carbohydrate pool large enough to sustain a full harvest of 10 weeks. In the mature plant (Table 6.04), the increase in dry weight would probably go into the growth of axillary rhizomes which will become independent units. Observations in this study indicate that at least one growing season is required for an axillary to develop into an independent rhizome (Section 3.4.1).

Table 6.03: Carbohydrate status at end of each phase of growth of a male plant with a 100g rhizome. The plant was harvested for 4 weeks in spring.

Growth phase	Fern DW g/rhizome	Crown DW g/rhizome	Crown carbohydrate concentration % DW	Crown carbohydrate g/rhizome
1 Senescence	-	100.0	65.0	65.0
2 Dormancy	-	86.0	60.0	51.6
3 Harvest (4 weeks)	3.6	77.2	50.1	38.6
4 Carbohydrate depletion	34.6	40.3	10.0	4.0
5 Carbohydrate restoration	121.9	78.0	65.0	50.7
6 Pre-senescence	121.9	96.5	65.0	62.7
1 Senescence	63.6	131.3	65.0	85.3

Note: Maximum carbohydrate concentration of 65% reached after 6 weeks of 'carbohydrate restoration'.

Table 6.04: Carbohydrate status at end of each phase of growth of a male plant with a 200g rhizome. The plant was harvested for 10 weeks in spring.

Growth phase	Shoot DW g/rhizome	Crown DW g/rhizome	Crown carbohydrate concentration % DW	Crown carbohydrate g/rhizome
1 Senescence	-	200.0	65.0	130.0
2 Dormancy	-	172.0	60.0	103.2
3 Harvest (10 weeks)	12.6	143.0	40.4	57.8
4 Carbohydrate depletion	50.3	74.6	10.0	7.5
5 Carbohydrate restoration	153.9	97.2	65.0	63.2
6 Pre-senescence	153.9	192.0	65.0	124.8
1 Senescence	80.3	261.2	65.0	169.8

Note: Maximum carbohydrate concentration of 65% reached after 7 weeks of 'carbohydrate restoration'.

6.2.3 INCORPORATING MALE-FEMALE DIFFERENCES

Data presented in this thesis indicate that berry production by female plants is the key difference between male and female plants in the production and utilisation of carbohydrate reserves (Sections 3.4.3; 3.4.7; 4.3.2; 5.3.4). As stated earlier, the model considers tissues in male plants to be either shoots or crown. These tissues function as either a source or a sink, depending on the growth stage of the plants. The berries on female plants differ from these tissues in that they function only as a carbohydrate sink.

There are no differences between male and female plants during dormancy and harvest, except perhaps the time of budbreak. The little data available does not indicate any differences in carbohydrate physiology during senescence, or during post-harvest fern establishment. Field observations indicate that senescence generally begins earlier in female plants than in male plants, and that fern establishment after harvest may be slower in female plants. Neither of these factors have been incorporated into the model.

The model assumes that the presence of berries will begin to affect carbohydrate translocation during the phase of 'carbohydrate restoration'. Flowers may form during 'carbohydrate depletion', but are not a major sink.

New ferns are a very weak sink on female plants when compared with male plants (Section 3.4.3). Therefore the increase in fern dry weight during Phase 4, carbohydrate depletion, was set at 5% per week, compared with 15% in male plants. Photosynthate production was set at 0.1g/g fern dry weight per week which is higher than in male plants (Section 3.4.7). This is split equally between berries and the crown which may underestimate the sink strength of the berries (Section 3.4.3). However, sink strength and mobilising ability change over time, and data from a detailed time course is not available.

Data in this thesis indicates that fern production in female plants increases after berries develop on the first 1-2 fern (Section 3.4.3). Thus fern growth occurs later in the growing season than in male plants. The model assumes that fern continues to grow at 5% per week during the pre-senescence phase, and that no assimilate is translocated to the berries during this phase. All of the exported photosynthate (0.1g DW/g fern dry weight per week) is translocated to the crown.

The model was evaluated for a female plant with a 200g rhizome at the end of senescence (Table 6.05). In this example (Table 6.05), crown dry weight decreased by 30% during one season. The reduced longevity of female plants in the field indicate that the plant utilises more carbohydrates than is produced, as occurs in this model. However, a 30% reduction appears rather high. There are three possible explanations of this.

Firstly, the values for carbohydrate utilisation during harvest are too high. A similar problem was identified when the model was run on male plants.

Secondly, female plants have smaller crowns than male, and thus a smaller carbohydrate pool (Section 5.3.5). This may reduce carbohydrate utilisation in the field by delaying budbreak in spring (Table 4.09). Variation in crown size would also contribute to variation in the time of budbreak within male plants.

Thirdly, the increased photosynthetic efficiency of female plants is higher than stated in the model (Section 3.4.7).

Table 6.05: Carbohydrate status at end of each phase of growth of a female plant with a 200g rhizome. The plant was harvested for 10 weeks in spring

Growth phase	Shoot DW g/rhizome	Berry DW g/rhizome	Crown DW g/rhizome	Crown carbohydrate concentration % DW	Crown carbohydrate g/rhizome
1 Senescence	-	-	200.0	65.0	130.0
2 Dormancy	-	-	172.0	60.0	103.2
3 Harvest	12.6	-	143.0	40.4	57.8
4 Carbohydrate depletion	50.3	-	74.6	10.0	7.5
5 Carbohydrate restoration	74.3	25.2	74.6	43.8	32.7
6 Pre-senescence	90.0	25.2	102.0	65.0	66.3
1 Senescence	47.0	25.2	139.0	65.0	90.2

Note: Maximum carbohydrate concentration of 65% reached after 2 weeks of slow fern growth

6.3 ADVANTAGES AND LIMITATIONS OF THE MODEL

The model indicates that simulations of asparagus growth can be based on carbohydrate accumulation and utilisation, and the changing source-sink relationships of shoots and the crown. Improved data on the effect of temperature on several plant processes would allow this model to be developed and applied to other locations. At present, temperature is used only in an indirect way, to determine the times at which internal dormancy is induced, released, and when a harvestable spring growth flush commences at Palmerston North.

The induction of internal dormancy and commencement of a harvestable flush of spear growth in spring are both controlled by temperature, but the data available does not cover a wide enough temperature range for this to be incorporated into the model. Data on induction of senescence, and the chilling units required to release dormancy have been included in the model, but require confirmation.

A major advantage of this proposal is that it models carbohydrate allocation and utilisation within one integrated unit i.e, a single rhizome, rather than a complete plant. At present, the model does not include the initiation of axillary rhizomes and their development into independent units. The development of axillaries could be included in the model by limiting the size of the main rhizome and establishing another carbohydrate sink. However, more information is required on factors controlling the initiation of axillary rhizomes and their transition into independent units. Another advantage of this model is that it incorporates the effects of plant sex on plant growth.

Plant sex has no effect on carbohydrate metabolism during dormancy or during a spring harvest, and the model used for male plants predicted changes which occurred during these phases. The changes which occur during fern growth have been established, although a more detailed time-course would be useful. However, the model assumes that plant sex also has no effect on senescence, or on 'carbohydrate depletion' following spear harvest when field observations indicate that there may be some differences during these phases. The model also assumes the plant sex has no effect on the time of budbreak. This is consistent with the data presented in this thesis (Table 4.09), but at variance with field observations (e.g. Robbins and Jones 1925; Ellison and Schermerhorn 1958; Ellison et al. 1960).

Variation in crown size may contribute to variation in the time of budbreak, both within male plants, and between male and female plants. Smaller plants tended to produce spears after larger plants (data not presented), and female plants tended to produce spears later than male plants (Table 4.09). As plants age, the differences in crown dry weight between male and female plants will increase, as will any effect of crown size on time of budbreak.

A weakness of the model is that the rate of carbohydrate utilisation during harvest may be too high. However, in the present model, the values used during the 'carbohydrate restoration' phase appear to compensate for this. As noted above, the values used in this phase may also be too high.

The model assumes that the rate of spear production is constant. This assumption applies to the first few spears produced by a rhizome (Sections 4.3.2 & 4.3.4), but may not apply throughout the harvest period. Spear elongation changes from exponential to linear as carbohydrate reserves decrease (Kim and Sakiyama 1989a), but this change has not been related to the quantity of carbohydrate reserves present. The time lag between

harvest and budbreak by the next spear may also increase (Tiedjens 1926). Thus the rate of spear production in the model may need to be reduced.

The other factor which contributes to carbohydrate utilisation during harvest is the respiratory requirements of spear production. The model uses data from 12.5C which is lower than the mean temperature in the later half of the harvest season.

CHAPTER SEVEN

GENERAL CONCLUSIONS

7.1 EFFECTS OF TEMPERATURE ON GROWTH OF ASPARAGUS

This thesis demonstrates that asparagus is well adapted to survive in temperate climates. In fact, most of the visible, seasonal changes in growth are induced by or dependent on changing temperature regimes. Senescence of ferns in autumn is temperature dependent (Section 4.3.1); crowns must be chilled to break the internal dormancy which occurs during winter (Section 4.4.1); the minimum temperature at which budbreak occurs changes during dormancy (Section 4.4.1); changes in the composition of carbohydrate reserves, which appear to permit budbreak, are temperature dependent (Section 5.4.2); and the rate of spear growth is very dependent on temperature (Section 4.3.2), as is fern and crown growth (Section 2.3.2). The changing temperature regimes which occur at Palmerston North, New Zealand, and data on the effects of temperature on asparagus growth were combined to develop a model of seasonal changes in the accumulation and utilisation of the carbohydrate reserves in both male (Section 6.2.1) and female (Section 6.2.3) plants.

The model indicates that simulations of asparagus growth can be based on carbohydrate accumulation and utilisation, and the changing source-sink relationships of shoots and the crown (Sections 6.2.2, 6.2.3). Improved data on the effect of temperature on several plant processes would allow this model to be developed and applied to other locations. At present, temperature is used only in an indirect way, to determine the times at which internal dormancy is induced, released, and when a harvestable spring growth flush commences at Palmerston North.

Plant processes which contribute to the accumulation and utilisation of carbohydrates are also temperature dependent and need improved understanding before they are incorporated into this model, e.g. the relationship between temperature, RSGR and the respiratory requirements of spear production; the effects of temperature on the growth of fern and roots, and on photosynthetic efficiency. Despite its limitations, the model provides a framework to assist focusing of future research.

Much of the data in this thesis was obtained at constant temperatures. This has allowed broad effects to established, e.g. that winter dormancy is a type of internal dormancy

which is released by chilling. Clarification of the processes occurring in the field will require investigation of the effects of alternating temperatures

7.2. AGRONOMIC IMPLICATIONS

Internal dormancy

Data presented here provide conclusive proof that asparagus winter dormancy is a type of internal dormancy and chilling is required to release it (Section 4.4.1). The internal dormancy of asparagus is not a problem in warmer climates in the way which occurs with deciduous fruit trees as budbreak is not suppressed in conditions which are too warm for fern to senesce. The low level of chilling required (Section 4.4.2) appears to ensure that asparagus can grow in climates where internal dormancy is induced and winter temperatures are rarely below 12.5C.

Temperature dependent changes in carbohydrate composition occur during dormancy (Section 5.4.2). Changes in carbohydrate composition may determine when asparagus buds are released from internal dormancy. However, it is more likely that carbohydrate metabolism is regulated by the same factors that permit budbreak, ensuring that plant growth and carbohydrate metabolism are integrated. Plant growth substances are important in releasing dormancy in deciduous fruit trees, so may have a role in asparagus.

Genotypic differences

This thesis has confirmed earlier work which indicated that agronomic differences between cultivars (e.g. yield, earliness) are not related to the overall growth of the plant (Section 2.4.1), but may be related to differences in partitioning between roots and shoots (Section 2.4.2). Other physiological differences which also contribute to observed agronomic differences are length of internal dormancy (Section 4.4.1), which affects time at a spring harvest commences and length of fern growth, and strength of correlative inhibition which affects rhizome development and spear production (Section 4.4.6).

Male-female differences

Differences in carbohydrate metabolism are not the reason for the well documented agronomic differences between male and female plants. There were no differences between male and female plants in their carbohydrate concentration or composition during dormancy or harvest (Section 5.3.5). However, the male plants had a higher crown dry weight, and thus a larger carbohydrate pool to support shoot growth. Differences in the size of the crown and the carbohydrate pool are due to the strong sink effect of berries which 'diverts' assimilates away from both fern and crown (Section 3.3.5). This reduces photosynthetic tissue (fern dry weight) and carbohydrate storage (crown dry weight), although it may increase photosynthetic efficiency.

7.3 METHODS

A major difficulty in analysing the data presented in this thesis was variability in the morphology of the plants. Smaller plants may be easier to obtain and less variable, but I cannot recommend them as at least some physiological responses differ from older plants, e.g depth of dormancy. Plants with large, well developed rhizomes are easier to work with as a rhizome and it's attached axillaries form an integrated unit for carbohydrate production and allocation, and the relationships between rhizomes are clearer than on plants with many, small rhizomes.

Plant morphology would be easier to determine if the plants were growing in some form of hydroponic system, e.g. coarse sand, or fine pumice and nutrient solution. This may also permit progressive measurements of the same plants, e.g. root number, root weight, or repeated samplings, e.g. root dry matter which would further reduce variation in the data.

It is important that variations in plant morphology do not influence experiments on source-sink relationships or carbohydrate budgets. Minimising variation in such experiments would also allow better discrimination between seasonal changes and effects of plant size and morphology. Plant morphology should be treated as a separate variable in source-sink studies.

However, in growth analysis, or other studies of genotypic differences, plants of the same morphology cannot be selected without biasing the sample of plants studied. Replication should be increased to at least six single plant replicates as genotypic differences tend to be small relative to the effects of temperature.

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