The kinetics of spear growth and asparagus productivity: control by environmental and internal factors

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2006

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The kinetics of spear growth and asparagus productivity: control by environmental and internal factors

A thesis presented in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
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ABSTRACT

Studies on asparagus growth in relation to yield were undertaken in environmentally controlled growth cabinets and in greenhouses. Bud production during the annual growth cycle was also investigated in the field. Growth cabinet experiments showed that increasing the temperature had a significant effect on bud break and relative spear growth rate (RSGR), but although prior chilling had a significant effect on the length of time to bud break at 10°C and 15°C, the effect on RSGR was not so clear. The cytokinin-active compound, *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU), and the naturally occurring cytokinin, zeatin riboside (ZR) significantly stimulated spear elongation. However, spear leaf scale removal reduced spear elongation in the absence and presence of CPPU. CPPU only stimulated spear growth when spear leaf scales were present, indicating that other plant hormones may interact with cytokinins in promoting elongation. The importance of spear growth rate to yield was discussed.

In greenhouse experiments, CPPU applied as a foliar spray at 10 or 20 mg L^{-1} was effective in producing longer and thicker cladodes that might be associated with increased photosynthetic rate. However, photosynthetic rate was unaffected by 10 mg L^{-1} CPPU treatment. Repeated CPPU applications to foliage reduced net assimilation rate (NAR) compared to untreated controls as determined by growth analysis studies. In asparagus plants, it was difficult to collect xylem sap and further experiments were undertaken with *Capsicum annuum*. The root exudate of CPPU-treated plants significantly decreased hypocotyl length in the lettuce gibberellin bioassay, suggesting that CPPU blocks gibberellin biosynthesis in roots. However, the application of GA_3 to shoots did not reverse growth suppression caused by CPPU-treated roots.

Bud production, both in growth cabinets and in open field plantings, started to occur during the spear harvest period in contrast to previously accepted views. During harvest three to four additional buds per cluster were produced in cabinet-grown plants and an average of 51 buds per m^2 in field plantings. These results confirm that new bud initiation and development starts to occur during spear harvest, as well as during fern growth and establishment.

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EXTENDED SUMMARY

Studies on asparagus growth were undertaken both in environmentally controlled growth cabinets and in greenhouses. A field experiment was also carried out to investigate bud production during the annual growth cycle. Two main experiments were carried out in the growth cabinets; one studied the effects of length of chilling and growing temperature on dormancy of crown buds and subsequent rates of spear growth, the other studied the effects of plant hormones and spear leaf scales on spear growth.

In growth cabinet experiments, one main experiment was the effect of different chilling periods at several different growing temperatures on bud break, and relative spear growth rate (RSGR) of asparagus. In the first experiment, twenty-two-month asparagus non-chilled plants of 'Apollo' and 'Desto' were placed into controlled growth cabinets at one of five temperatures (15°C, 20°C, 25°C, 30°C, and 35°C) for two months. In the second and third experiment, two and half year plants of 'Dariana' and five-year-old plants of 'Apollo' were placed in a coolstore at 5°C for 0, 3 and 6 weeks. After the specified chilling time, the pots were placed into controlled growth cabinets at one of five temperatures (10°C, 15°C, 20°C, 25°C, and 30°C) for 'Apollo' and three temperatures (20°C, 25°C, and 30°C) for 'Dariana' in dark conditions. The results showed that increasing the temperature had a significant effect on the time to bud break, but at 15°C and below chilling had a significant effect on the timing of bud break. Chilling facilitated commercial production by hastening bud break at lower temperatures. The RSGR increased with increasing temperature from 10°C to 30°C, but the effect of prior chilling on RSGR was not clear, possibly because control plants received some chilling due to insufficient heating in the glasshouse. The growth of spears was mainly exponential (constant RSGR) from 20°C to 30°C. At 15°C, 56.9% of the spears showed exponential growth and 8.1% linear growth. At 10°C and 35°C less than 50% showed exponential growth. These results confirm that at 10°C and 35°C spear growth tended to be linear while between 15°C and 30°C spears grew exponentially. Thus growing temperatures are important factors for affecting RSGR and time to bud break, but the effect on bud break and RSGR may be modified by the amount of prior chilling.

The other main experiment investigated the effect of plant growth regulators and spear leaf scales on spear elongation in asparagus. Spears of uniform size were immersed completely in indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA_3) or *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) in test tubes. IAA and GA_3 slightly stimulated, and ABA inhibited, elongation of specific regions of the shoot, but overall the effects on total spear elongation were small. In contrast CPPU and ZR significantly stimulated spear elongation. Increasing CPPU concentration from 0 to 10 mg L^{-1} increased the elongation rate of spears. CPPU may influence spear length by stimulating the biosynthesis of native cytokinins or other plant growth promoter hormones as the naturally occurring cytokinin, ZR, was also found to stimulate spear elongation. However, spear leaf scale removal reduced spear elongation in the absence and presence of CPPU. CPPU only stimulated spear growth when spear leaf scales were present, indicating that other plant hormones may interact with cytokinins in promoting elongation.

In greenhouse experiments, the effect of CPPU application to foliage (foliar spray and foliar dip), soil drench and roots (crown soaking) on fern numbers, cladophyll length and diameter, root number and bud production was investigated. CPPU applied as a foliar spray, foliar dip and root soak decreased fern numbers, but drenching asparagus crowns with CPPU at 10 mg L^{-1} was effective in stimulating fern numbers, which may increase canopy size and photosynthetic surface area. The increase in fern might be expected to improve crown activity (root dry weight, bud number, root number, and total bud numbers). However, CPPU-treated plants did not show increased crown activity. CPPU applied as a foliar spray was effective in producing longer and thicker cladodes, features often associated with increased photosynthetic rates. However, photosynthetic rates were unaffected by CPPU foliar spray.

It was observed that the cladodes of new ferns were of normal size, and that therefore repeat foliar sprays of CPPU were required to increase the diameter and length of cladodes of subsequent ferns. Repeated CPPU foliar sprays significantly increased cladophyll surface area and cladophyll dry weight, but decreased root dry matter and the number of buds. Despite the higher cladophyll surface area, CPPU did not increase photosynthetic efficiency as measured by relative growth rate (RGR) and net assimilation rate (NAR).

Soaking asparagus crowns in 10 mg L^{-1} CPPU decreased fern height, root dry weight and crown buds of asparagus, suggesting that CPPU applied as a crown soak may affect plant hormones in roots such as gibberellins. Because of difficulty in collecting xylem sap from asparagus plants, further experiments were undertaken with pepper plants. Root applied CPPU at 10 mg L^{-1} significantly decreased plant height, probably by inhibiting the activity of the sup-apical region of shoot, reduced internode number and length at 2 and 4 weeks after treatment, and suppressed root growth. Leaf water potential of the 10 mg L^{-1} CPPU-treated plants was lower than untreated controls, suggesting the smaller root systems of CPPU-treated plants may limit water uptake. Xylem sap collected from the roots of CPPU-treated *Capsicum* plants had less detectable gibberellin, possibly associated with blocking of gibberellin biosynthesis. However, GA_3 foliar spray at 100 mg L^{-1} did not reverse growth suppression caused by the CPPU treatments of pepper roots.

Bud production both in growth cabinets and in open field plantings occurred during the spear harvest period. Under the growth cabinet conditions, during spear harvest new buds were produced but new buds per cluster were not influenced by temperatures between 15°C to 30°C, while at 10°C bud production decreased. Three to four additional new buds were produced per cluster during the spear harvest period. Thus, as well as slowing spear growth, temperatures below 15°C may limit the production of new buds, and thus subsequent yield. In field plantings an average 51 buds per m^2 were produced during the spear harvest period. These results confirm that bud initiation and development occurs during harvest season as well as during the summer.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
AD	Apical dominance
ANOVA	Analysis of variance
ATA	Auxin transport autoinhibition
BA	Benzyladenine
CCC	Chlormequat chloride
CI	Correlative inhibition
CP	Chilling period
CPPU	*N*-(2-chloro-4-pyridyl)-*N'*-phenylurea
CRD	Completely randomized design
CV	Cultivar
CVs	Cultivars
EC	Electrical conductivity
GA_n	Gibberellic $acid_n$ - n denotes the acid number
GAs	Gibberellic acids
GS	Stomatal conductance
IAA	Indole-3-acetic acid
iRGR	Instantaneous relative growth rate
LAI	Leaf area index
LAR	Leaf area ratio
LSD	Least significant difference
LWR	Leaf weight ratio
NAA	α-naphthaleneacetic acid
NAR	Net assimilation rate
Pn	Net photosynthetic rate
$\overline{R}$	Mean relative growth rate
RGR	Relative growth rate
RSGR	Relative spear growth rate
SAS	SAS system for statistical analysis
SLA	Specific leaf area
SLW	Specific leaf weight
ZR	Zeatin riboside

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Asparagus (*Asparagus officinalis* L.) production in many countries is increasing because of high consumer demand (Benson, 1999). Global asparagus production increased from 140,000 hectares to 218,335 hectares between 1990-1999, respectively (Nichols, 1990; Benson, 1999). Increasing consumption of white and green asparagus for both domestic and export markets probably caused the area in asparagus production to increase (Benson, 2002). Estimated world total production areas in 2005 were 225,095 hectares, of which 43% were white and 57% green spears (Benson, 2006).

In 2004, the area of asparagus in New Zealand was 1300 hectares. Some 5,000 tonnes were produced, with 2,423 tonnes exported. Total exports in 2005 were 2,536 tonnes, of which 917 tonnes was fresh and 1,619 tonnes was processed (frozen and canned). Total exports in 2005 were worth 5.3 million dollars for fresh asparagus and 7.0 million dollars for processed asparagus (http://www.vegefed.co.nz). Asparagus production from 2000 to 2005 has fluctuated, but currently asparagus production has tended to decrease. The reason of for this includes plant age, disease, and carbohydrate depletion of old plants. However, New Zealand asparagus growers have a major goal of increasing the annual spear yield of the crop. Although the planting of new, high-yielding cultivars will greatly assist this objective, further progress will be made by elucidating the yield physiology of asparagus in relation to growing temperatures, chilling, plant growth hormones, photosynthesis, storage roots in the crown, and bud number.

This thesis consists of three inter-related studies that affect asparagus yield:

(1) Growth rates of the spears developing in spring from over-wintering buds;

(2) Photosynthetic rates and accumulation of dry weight in the crown;

(3) Factors affecting bud numbers in relation to temperature and *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) treatments.

Finally because of limitations in the collection of xylem sap from asparagus, some studies were undertaken on the response of pepper plants to CPPU.

1.2 Botany and morphology of Asparagus

Asparagus (*Asparagus officinalis* L.) is a perennial vegetable belonging to the family Liliaceae (Tutin et al., 1980; Nonnecke, 1989) and is grown commercially in a wide range of environments including temperate regions, humid tropics, and arid tropics. Asparagus is a dioecious plant with approximately 150 species (Drost, 1997). Among these species, only *Asparagus officinalis* L. is grown for its edible spears (Nonnecke, 1989).

Asparagus plants consist of two main parts: underground (referred to as the crown) and above ground (referred to as the fern). The crown consists of underground, horizontal stems (rhizomes) and fleshy storage and fibrous feeding roots (Figure 1.1). The rhizome consists of bud clusters with each bud cluster containing several large and many smaller buds (Blasberg, 1932; Nichols, 1988). Each bud cluster has its own connection with fleshy roots (storage roots). The fleshy roots originating from the base of the rhizomes act as carbohydrate storage organs for up to six years (Scott et al., 1939), while fibrous roots are primarily nutrient and water absorbing organs (Nonnecke, 1989; Nichols, 1990), and tend to live for only about one year (Reijmerink, 1973).

The fern consists of spears, stems, cladodes, and berries (female plant only). The asparagus plant is produced for its succulent fleshy spears, which in temperate climates, emerge after a dormant winter period. The edible part of asparagus is referred to as spears. Spears grow rapidly and if they are not harvested become fern. Each fern has a central stem that form many lateral branches. Cladophylls (cladodes) are needle-like structures, which grow in whorls at nodes on stems and branches, subtended by a scale leaf that represents the true leaf (Blasberg, 1932).

Photosynthesis occurs mainly in the cladodes, although photosynthesis ocurrs in all green tissue (Downton and Torokfalvy, 1975; Lin and Hung, 1978; Faville et al., 1999; Guo et al., 2002). Asparagus, unlike most vegetable crops, is a perennial plant and the photosynthetic rate does not directly contribute to spear yield until the subsequent growing season (Guo et al., 2002). Assimilate produced in cladodes by photosynthesis after fern growth and development is initially translocated into the underground storage roots and then utilized for spear growth and vegetative growth during the following year (Robb, 1984; Haynes, 1987; Pressman et al., 1993). However, while vigorous fern growth is essential for carbohydrate production for storage roots and subsequent utilization for spear growth in the harvest season, excessive late fern growth can deplete carbohydrate resources from storage roots thus decreasing asparagus yield. Proper fern management avoiding excessive fern growth is the key to providing the best seasonal balance between carbohydrate accumulation into roots and efficient utilization of root reserves to maximize spear yield.

Flowers from female plants have rudimentary stamens while male plants have flowers with rudimentary ovaries (Lazarte and Palser, 1979). Female plants show little variation in flower morphology, but male plants exhibit variations both within and among plants for the development of ovaries and stamens. Female plants have only female flowers, and these plants produce the berries. Berries can comprise over 25% of plant dry weight on female plants with berries (Robbins and Jones, 1923; Hughes, 1992). In commercial production, male plants produce higher yields than female plants (Abe and Kameya, 1986; Ellison et al., 1990; Sinton and Wilson, 1999; Seong et al., 2001) and have greater longevity in the field (Lazarte and Garrison, 1980). The lower yield of female plants may be due to berry and seed production (Robb, 1984; Hughes, 1992). Male plants have larger root systems, accumulate more storage carbohydrates and produce more buds compared to female plants (Sinton and Wilson, 1999). It is therefore important to distinguish between male plants and female plants in relation to asparagus potential production. All-male hybrids such as 'Jersey Giant' have become very popular in commercial production because of their potentially higher yield.

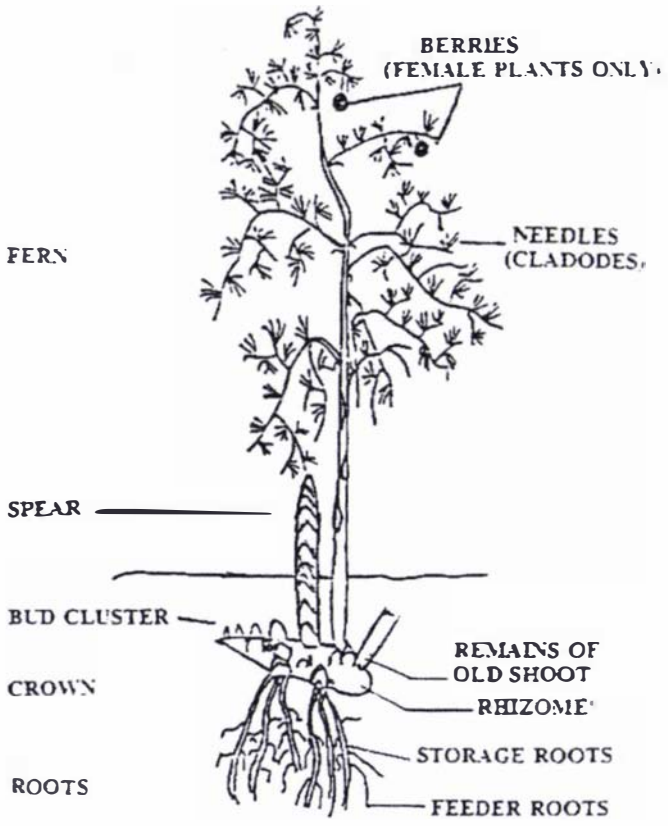

Figure 1.1 The structure of asparagus: underground and above ground (After Nichols, 1996).

1.3 Physiology of asparagus in relation to phenology and yield

1.3.1 Phenology

ln temperate climates asparagus plants have a clear annual cycle of bud break and spear harvest in spring, fern growth and accumulation of carbohydrates in storage roots during the summer and early autumn, and dormancy in winter (Shelton and Lacy, 1980; Robb, 1984; Haynes, 1987; Pressman et al., 1993; Drost, 1997; Woolley et al., 1999). Bud break and elongation rates in spring and early summer are an important physiological attribute of asparagus spears in relation to yield (Chapter 3).

1.3.1.1 Dormancy

The function of vegetative dormancy is to enable perennial plants to adapt to extreme environmental conditions (freezing winter temperatures, water stress, or high temperature stress) by remaining inactive or resistant to extreme environmental conditions. Deciduous fruit trees and other temperate woody perennial plants undergo a period of dormancy or rest over the winter months under natural conditions. lt is generally known that the main factor in breaking bud dormancy in such plants is low temperature and that inadequate chilling delays bud break (Arora et al., 2003; Bonhomme et al., 2005), shoot growth and flowering in the spring (Jacobs et al., 2002). Understanding the mechanism controlling the induction and release of winter dormancy with regard to bud break is important in many woody perennial plants (Arora et al., 2003; Horvath et al., 2003; Cook et al., 2005; Heide and Prestrud, 2005) and may be important in influencing asparagus yield.

1.3.1.1.1 The induction and maintenance of dormancy

ln temperate climates, fern senesces in autumn and plants enter dormancy during the winter. The rhizomes of asparagus undergo a period of dormancy or rest over the winter months. The concept of dormancy varies. Lang (1987) has defined dormancy as 'the temporary suspension of visible growth of any plant structure containing a meristem'. The suspension of visible growth during the winter may be imposed on a plant by external factors such as low temperatures, or it may be due to internal factors such as

plant growth regulators. Lang et al. (1987) further categorized dormancy into ecodormancy, paradormancy, endodormancy (Figure 1.2).

Ecodormancy is imposed directly by external environmental factors such as cold, drought stress, nutrient deficiencies or high temperatures so that conditions and in suitable for growth. Ecodormancy can be imposed on asparagus plants by drought stress or cold stress. In both cases it is unclear whether this imposed dormancy (ecodormancy) leads to internal dormancy (paradormancy or endodormancy).

Paradormancy is regulated by factors that are within the plant, but are external to the dormant structure. For example bud scales, and sometimes subtending true leaves, may impose dormancy on the bud meristem. This dormancy may not be released until a specific environmental signal is perceived, for example chilling in the case of innate winter dormancy (see Section 1.3.1.1.3), or the organ imposing dormancy is removed. In the terminology discussed by Lang et al. (1987) apical dominance is classified as a form of paradormancy but since both the mechanism of apical dominance, and the function is different from other forms of dormancy it will be treated separately (see Section 1.3.1.2). Whether a form of winter dormancy involving paradormancy exists in asparagus is unknown, but paradormancy often leads to endodormancy.

Endodormancy occurs when growth is inhibited by physiological factors within the affected structure e.g. meristems that require chilling or a specific photoperiod to release them from dormancy. Horvarth et al. (2003) defined endodormancy as based on physiological changes internal to the buds that prevent or stimulate bud growth during seasonal transitions of environmental conditions. Low temperature plays an important role in the induction and breaking of endodormancy in temperate, woody perennials (Vegis, 1964; Saure, 1985; Fuchigami and Nee, 1987; Heide, 1993; Dokoozlian, 1999; Heide and Prestrud, 2005). Thus we have the interesting situation that cold temperatures deepen winter dormancy (usually in association with day length) but later cold temperatures release dormancy. Great care is thus required in determining the cold requirements of dormant plants (Heide and Prestrud, 2005). That is when does the imposition of dormancy end and the breaking of dormancy begin?

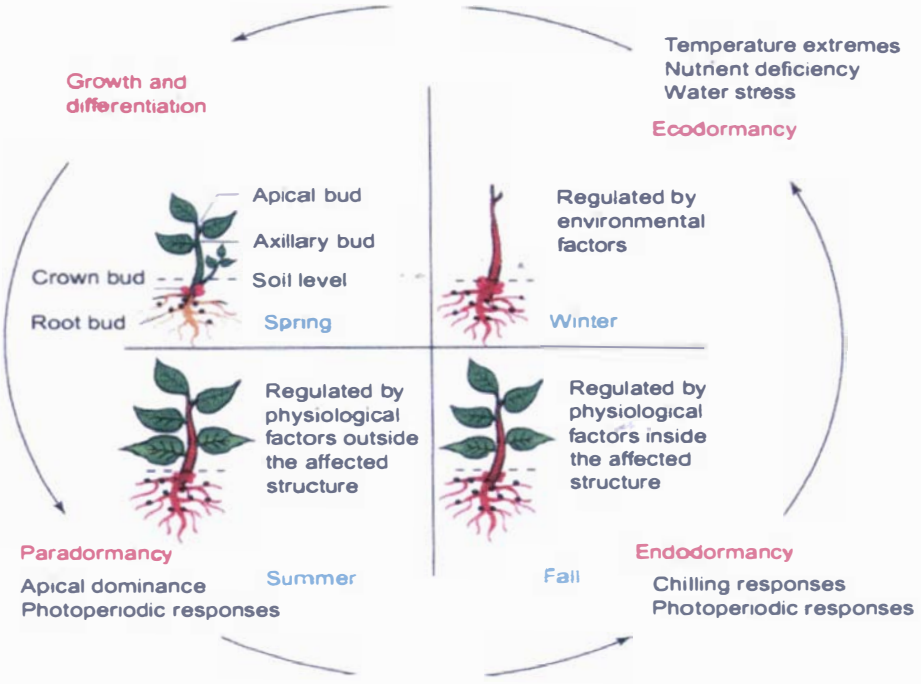

Figure 1.2 Diagram of signals and four seasons response to the different types of dormancy (cited in Horvath et al., 2003).

1.3.1.1.2 The release of dormancy

Low temperature (chilling) is the major environmental factor to release buds of many temperate plants from dormancy. After dormancy has been induced, a prolonged period of chilling is required to release bud dormancy and allow vegetative growth and flower production in the spring (Heide and Prestrud, 2005; Warmund and Krumme, 2005). Heide and Prestrud (2005) examined effective chilling temperatures in apples and pears. They indicated that a temperature of 6°C was most effective and 12°C only marginally effective for dormancy release as per the normal Richardson (Utah) chill unit model (Richardson et al., 1974; Richardson et al., 1986) and see Section 1.3.1.1.4.

The minimum temperatures for bud break in asparagus in spring have been estimated as 4.4°C (Bouwkamp and McCully, 1975), 5.6°C (Lampert et al., 1980), 5.8°C (Culpepper and Moon, 1939a) and 7.2°C (Blumenfield et al., 1961). This variation probably reflects cultivar differences. 'Pacific 2000', a late cultivar, releases buds at crown

temperatures above 13°C whereas 'JWC1', an early cultivar, releases buds at 8.5°C (Falloon personal communication). In asparagus, prior chilling markedly decreased the mean days to bud break and increased the relative spear growth rate (RSGR) of the first spear for 'UC 157' after winter dormancy (Hughes, 1992). These observations indicated that chilling released winter dormancy, which implied that some form of internal dormancy i.e, paradormancy or endodormancy was operating. A low temperature treatment of 5°C for 3 weeks was effective for breaking dormancy of asparagus and allowed considerable bud break to occur at 12.5°C compared to non-chilled plants (Hughes, 1992; Seong et al., 2002).

1.3.1.1.3 Hormonal control of innate winter dormancy

The definition of innate bud dormancy is when no visible growth occurs in a viable organ, even in conditions suitable for growth, until a specific environmental release signal (e.g chilling) is received. Innate dormancy may be either paradormancy or endodormancy but is quite distinct from apical dominance, as discussed earlier.

Seeley and Powell (1981) found a progressive decrease in free abscisic acid (ABA) content in buds from autumn to spring; thus ABA appears to be associated with the regulatory mechanism of dormancy. The highest level of free ABA occurs in autumn, approximately at the onset of winter dormancy, and it is lowest just before bud burst, suggesting that ABA plays an important role in the induction and maintenance of winter dormancy.

There is a reciprocal relationship between ABA and gibberellins in the control of dormancy in plants. ABA concentrations may increase as dormancy is induced, and reduce as bud dormancy is released, while gibberellins show the opposite pattern (Wareing and Phillips, 1970; Matsubara, 1980; Lee et al., 2002; El-Sese and Mohamed, 2003; Sankaran et al., 2004). For birch plants, there is evidence that buds resume growth under long days, whether the leaves are present or absent. When the buds are exposed to long days and the leaves to short days, the buds fail to grow. On the other hand, if the buds are exposed to long days and the leaves to short days, the buds remain dormant (Wareing and Phillips, 1970). They suggested that leaves produced growth inhibitors under short days and these growth substances may be translocated to buds.

ABA increased considerably in leaves and buds when bud dormancy occurred under short days (Wareing and Ryback, 1970). ABA levels tended to increase but GA content of leaves excised from plants decreased when plants were transferred from long to short days (Kumar and Wareing, 1974). Thus, induction of dormancy is controlled by the relative levels of endogenous ABA and gibberellins. More recent evidence using mutant and transgenic seeds of *Arabidopsis thaliana* and tomato shows that ABA is required for dormancy induction and GA releases dormancy and promotes germination (Ni and Bradford, 1993; Debeaujon and Koornneef, 2000; Steber and McCourt, 2001; Kucera et al., 2005).

Gibberellic acid promoted bud break of *Actinidia chinensis* Planch only after a certain amount of chilling (Lionakis and Schwabe, 1984). Thus, the induction of bud dormancy by short days may be due to high ABA levels (Wareing and Phillips, 1970), but for breaking of dormancy some process, other than just gibberellins, may be involved since early chilling in many species cannot be reversed by gibberellic acid (GA_3). To be effective application of GA_3 usually requires a small amount of initial chilling to release bud dormancy. Evidence amassed over many years (Wang et al., 1997a; Xin et al., 1998; White et al., 2000; White and Rivin, 2000; Horvath et al., 2002; Horvath et al., 2003; XiangDong and Harberd, 2003; Anderson et al., 2005) suggests that ABA may repress genes associated with gibberellin biosynthesis, and may also block gibberellin action if present in sufficiently high concentrations.

1.3.1.1.4 Chill units of perennial fruit and vegetables

Deciduous fruit trees require a period of low temperatures to stimulate bud break, allow rapid shoot growth and produce flowers in the spring (Dokoozlian, 1999; Jacobs et al., 2002; Arora et al., 2003; Cook et al., 2005). The amount of chilling required can be calculated as chilling hours (number of hours below a specific temperature-usually 7.2° C) or as chill units.

One chill unit is defined as 1.0 hour at the most effective chilling temperature. Richardson et al. (1974) published an early Utah chilling model for peach flowers in which temperature between 2.5 and 9.1°C equaled 1 chill unit. The more recent Richardson chill unit model (Richardson et al., 1986) in which one chill unit was

defined as one hour at 6°C, is widely used in New Zealand. Fuchigami and Nee (1987) indicated that a temperatures of 5°C were most effective for most temperate woody perennials in order to satisfy their cold requirements, but could range from 3.5°C to 10° C. For raspberries, temperatures below 5.6°C are effective in accumulating one chilling hour (Dale et al., 2003). Recently, Warmund and Krumme (2005) proposed a blackberry chilling unit model in which temperatures between 0 to 9.1°C = 1 unit and those above 18°C = –1 chill unit. They proposed that chilling hours or units calculated using models that varied in the time when chilling hours began accumulating (chilling inception) and in the range of temperatures that induced chilling. Negative chilling unit, which reversed the effect of chilling, accumulated at warm temperatures and prolonged internal dormancy (Erez et al., 1990).

In asparagus prior chilling may facilitate commercial production by hastening bud break and spear growth rates at lower temperature. Hughes (1992) proposed that if sufficient chilling was given the minimum temperature for rapid bud break was around 12.5°C for 'Rutgers Beacon' and 'Jersey Giant' and around 10°C for 'UC 157'. The optimum chilling temperature appeared to be closer to 5°C than to 10°C or 2°C for 'Rutgers Beacon' plants later grown at 12.5°C.

Following chilling temperature below 5°C for 250, 500, 750, 1,000, 1,250, 1,500 or 1750 hours, asparagus plants ('Excel') were exposed to 15°C to measure the degree of bud burst (Seong et al., 2002). They reported that dormancy tended to begin in early December and was most pronounced during late December to early January in South Korea. This corresponded with accumulated hours of 1000 to 1250 hours under the open field conditions. However, Hughes (1992) reported that asparagus plants appeared to have relatively low chilling requirement of about 500 chill units as calculated by the Utah chill unit model. This is considerably lower than the chill units required for bud break of 'Excel' in South Korea, e.g. 1000 to 1250 chill units (Seong et al., 2002), and is similar to low chill cultivars of some fruit trees, e.g, 450 and 550 chill unit needed by two low-chill cultivars (Gilreath and Buchanan, 1981). Seong et al. (2002) also reported that chilling plants of 'Excel' significantly increased total and marketable yields compared to non-chilled plants under greenhouse conditions. Thus prior chilling may increase marketable yield under some conditions.

1.3.1.2 Correlative inhibition (CI)

Apical dominance (AD) is a special case of correlative inhibition (Hillman, 1984) and can be defined as the control exerted by the shoot apex (Cline, 1994, 1997; Mori and Tanaka, 2004) or young shoot tissues (Hosokawa et al., 1990) over the growth of lateral buds. The primary source of the bud-inhibiting effect is the growing shoot apex (Tamas, 1995), which if damaged or removed, removes apical dominance and the lateral buds rapidly grow out (Phillips, 1975; Cline, 1994). In contrast, in some plants the main source of the bud-inhibiting effect is not the growing shoot apex, but young leaves [e.g in *Phaseolus vulgaris* L, (White et al., 1975)], and expanding leaves [*Euphorbia pulcherrima* L, (Weiss and Shillo, 1988)]. Hosokawa et al. (1990) reported that the apical 13 cm of the shoot, including the young leaves, had a strong inhibitory influence on the outgrowth of lateral buds of *Ipomoea nil* compared to stem apex (consisting of the terminal 0.5 cm of the shoot).

Apical dominance is dependent at least in part on endogenous ratios of auxin to cytokinin in the tissues surrounding the bud primordia (Hall, 1973; Davies, 1987). Apically derived auxin is transported in a basipetally polar manner from the dominant apical region to the lower lateral buds and inhibit their outgrowth, probably through an interaction with cytokinins (Cline, 1994, 1997; Chatfield et al., 2000; Horvath et al., 2003). The release of apical dominance following decapitation of the shoot apex is suppressed by the treatment of the decapitated shoot stump with exogenous auxin (Thimann, 1937). Low auxin in an apical region is correlated with the release of apical dominance (Cline, 1997). The application of auxin transport inhibitor on a stem promotes axillary bud outgrowth. Bangerth et al. (2000) reported that auxin transport autoinhibition (ATA) is sufficient to impose growth inhibition on lateral buds in the dominated organ. They also reported that a mutual interaction between auxin production in the shoots and cytokinin production in the roots plays an important part in the regulation of correlative dominance phenomena, with auxins interacting mainly with root derived cytokinins which counteract the inhibitory effect of indole-3-acetic acid (IAA) (Woolley and Wareing, 1972). However, Cline (1994) and Beveridge et al. (2000) found that other unidentified signals, produced in the roots and stem, may also have a significant effect on shoot outgrowth as well as auxin and cytokinin. Thus,

although auxins and cytokinins play an important role, the complete signaling mechanism regulating apical dominance is unknown.

In asparagus the main rhizome and secondary rhizomes represent a compressed stem in which buds are arranged in clusters. Each bud in a cluster can develop into a spear and, if not harvested, into a fern. Tiedjens (1926) proposed that a growing spear delayed the bud break of neighbouring buds, often until that bud was harvested. Tiedjens (1926) and Kretschmer and Hartmann (1979) referred to this as apical dominance. However, unlike apical dominance in most other plants, in asparagus the growing spear is repressing the adjacent buds above (Daningsih, 2005). Nichols and Woolley (1985) therefore defined this as correlative inhibition. Thus, in asparagus, delay in bud break and/or slow spear growth, due to the presence of other spears, should be referred to as correlative inhibition rather than apical dominance.

Bud break tends to start at one of the two basal buds on a rhizome and progresses towards the tip of the rhizome (Hughes, 1992). Distal budburst is inhibited via correlative effects of the proximal spears on the buds toward the tip of the rhizome, i.e. paradormancy in the terminology of Lang et al. (1987). It is therefore likely that completely satisfying the chilling requirement of the bud will enable faster bud growth, early harvesting of the spear and allow subsequent shoots (spears or buds) to develop at a faster rate.

Since high ABA levels have been associated with rapid spear elongation (Kojima et al., 1993) it is possible that ABA from elongating spears inhibits neighbouring buds. Thus normally only one bud per bud cluster grows at a time (Nichols and Woolley, 1985).

An inhibition period between the growing spear and the next spear has been attributed to correlative inhibition (Daningsih, 2005). In addition elongation of the subsequent spear is often slow if the previous spear has not been harvested. On the other hand, if an inhibition period does not exist, spears elongate simultaneously without any correlative inhibition effect. When several spears from different bud clusters on a crown grew at the same time, they grew at a similar rate, but the growth of other spears in the same clusters was severely inhibited (Nichols and Woolley, 1985).

Spear growth rate depends mainly on temperature (Nichols and Woolley, 1985). As a fast growing spear will be harvested earlier than slowing growing spear, Daningsih (2005) suggested that the faster the spear grows, the sooner the release from correlative inhibition occurs. Thus in warm temperature not only will spear growth rates be faster the length of the inhibition period will be shorter. Drost (1997) suggested that spears allowed to grow beyond marketable size (> 20 cm) were particularly effective at inhibiting adjacent buds. This could cause significant yield reductions in a time-limited harvest season.

Applying plant growth regulators may stimulate fern production during the growing season by releasing correlative inhibition. Tiburcio (1961) found that GA_3 drenches at 10 mg L^{-1} improved the number of new ferns and promoted rate of spear growth, suggesting that GA_3 may reduce correlative inhibition. Benzyladenine (BA) as a foliar spray at 300 mg $litre^{-1}$ promoted shoot emergence of three asparagus cultivars (Mahotiere et al., 1993). Uesugi et al. (1995) similarly reported that a single BA foliar spray in autumn promoted spear sprouting during the period after application, but not during the following spring in cv 'Welcome'.

1.3.2 Yield

1.3.2.1 CH_2O level

Spear production in a spring harvest in a temperate climate depends on utilizing stored assimilates in the root system, particularly soluble carbohydrate (Wilson et al., 1999b). The amount of stored soluble carbohydrates depends on the total root biomass and the soluble carbohydrate concentration in the storage roots. The amount of soluble carbohydrates fluctuate during the annual growth cycle of asparagus (Figure 1.3), and is complicated by changes in storage root mass and changes in carbohydrate concentration associated with fern and spear growth during the year (Shelton and Lacy, 1980; Haynes, 1987; Pressman et al., 1993).

There are four main stages of carbohydrate turnover in asparagus during the year (Figure 1.3). 1) winter dormancy stage, 2) spear harvest stage, 3) fern renewal stage, and 4) carbohydrate accumulation stage (Nichols, 1996). During the winter dormancy

stage there may be little or no reduction of soluble carbohydrate (Haynes, 1987) or, in warm climates more than 30% (Pressman et al., 1993). Any loss of carbohydrate during the winter dormancy stage was primarily due to root maintenance respiration (Danckwerts and Gordon, 1989).

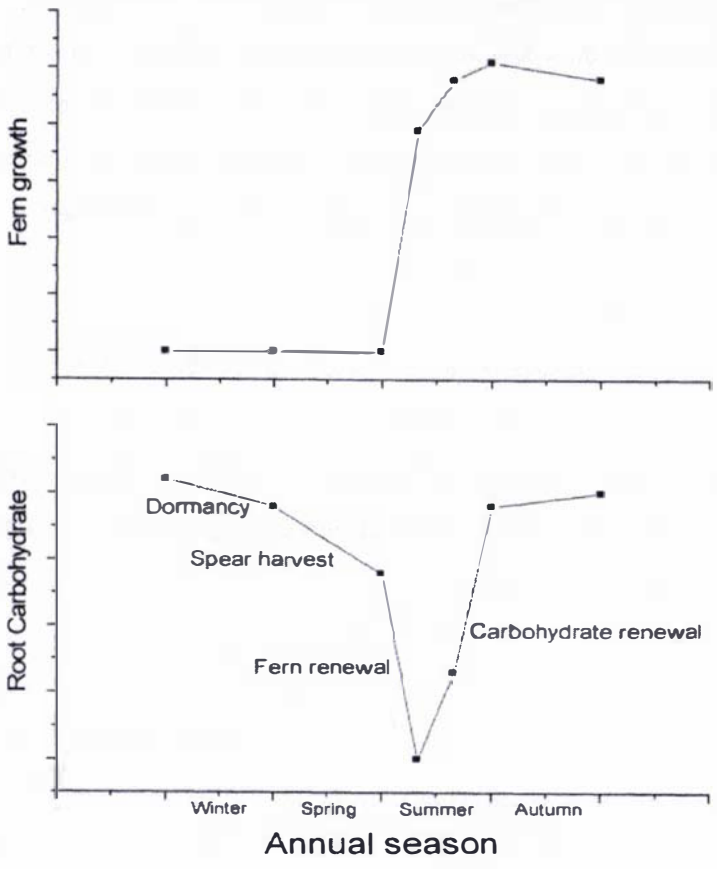

Figure 1.3 Fern growth and root carbohydrate during asparagus annual growth cycle in temperate regions (cited in Daningsih, 2005).

Spear harvest starts in early spring after release of bud dormancy provided temperatures are adequate. Carbohydrate levels in the storage roots are reduced during the spear harvest stage but production does not normally use large quantities of carbohydrates. Prolonged and excessive harvest during the spear production stage can however deplete carbohydrate resources of the storage roots, reduce the plants potential to recharge these reserves during the carbohydrate accumulation stage and thereby decrease long-term yield (Drost, 1997). In the cool temperate climate of Poland, the normal harvest period for asparagus lasts from late April or early May until the end of June. Knaflewski and

Krzesinski (2002) reported that hastening the beginning of harvest with low plastic tunnels increased the yield provided enough carbohydrate resources were available, but when harvest was forced in July it decreased the subsequent yield due to a reduced recharge period. A ten-year field trial was carried out at Ingelheim, Germany (Latitude 49° 58′ 50 N, Longitude 8° 4′ 25 E) to investigate the influence of harvest duration (from four to nine weeks per season, with the end of harvest varying 25 May to 29 June) on yield of white asparagus (Paschold et al., 2002). They found that total spear yield over several seasons was highest when the harvest was eight weeks long, but if the harvest was either one week longer, or any shorter than eight weeks, spear yield decreased. The cumulative yield of grade 1 spears (diameter 16-26 mm) was highest from the nine week harvest in the early years, but the spear yields decreased faster in later years than for the eight week harvest. The yields of grade 1 were similar with six, seven or eight week harvests, and yield declines were less than with the nine week harvest. That is the shortened harvest season preserved carbohydrate levels in the storage roots. The fact that the yield of grade 1 spears was similar for six, seven and eight weeks harvest can be easily explained by the results of Daningsih (2005) who reported that the yield of grade 1 spears is limited by the number of large buds, which are the buds that tend to be harvested first. Wilson et al. (1999b) reported that extended harvest period treatments severely depleted root systems at the end of season and produced lower spear yields the following season. In New Zealand, asparagus growers traditionally harvest from mid-September to mid-December. Some growers harvest until Christmas to meet high public demand (Drost and Wilson, 2003). Extending harvest may reduce future yield because of problems in establishing a good canopy and length of recharge period (carbohydrates). Paschold et al. (2002) suggested that measurement of soluble carbohydrate in the root system would help growers determine the best time to stop the harvest on a crop-by-crop basis. This paper followed the ideas of the Aspire NZ system (Wilson et al., 2000, 2002a).

Carbohydrate content in the storage roots drops rapidly between the end of the harvest and fern establishment (fern renewal stage). Following fern renewal stage, after ferns are established, carbohydrates increase rapidly and should reach pre-harvest levels during carbohydrate accumulation stage (Haynes, 1987; Pressman et al., 1993; Drost, 1997). Photosynthetic rate increases as the canopy establishes in summer and then decreases as the fern senesces during autumn in temperate climates (Lin and Hung,

1978; Haynes, 1987). As well as replenishing stored carbohydrates before winter dormancy, this stage is also reported to be the major period for production of new buds.

1.3.2.2 Bud number and size

Spear yield in asparagus depends on carbohydrate resources available (Wilson et al., 1999b) together with the number of buds (Woolley et al., 2006) which determines the number of potential spears. It is generally known that bud initiation and production tends to start after fern development and establishment in the summer until the autumn (Tiedjens, 1924; Robb, 1984; Haynes, 1987). Furthermore, Wilson et al. (1999b) demonstrated that a few buds may possibly start to form toward the end of the harvest period, but more recently Woolley et al. (2004) and Daningsih (2005) have found that considerable numbers of buds form during the harvest period, as well as after fern establishment. Their reports suggested that as many as 20 to 30 percent of the buds produced in a normal commercial planting were formed during the harvest period.

The initiation of new buds is under both genetic control (Tiedjens, 1924, 1926; Blasberg, 1932), and environment influence (Drost and Wilcox-Lee, 1990; Drost and Wilcox-Lee, 1997a, 1997b; Daningsih et al., 2004) and relies on carbohydrate reserves stored during the previous season (Shelton and Lacy, 1980). Water stress has been reported to decrease bud size and number (Drost and Wilcox-Lee, 1997a, 1997b). In a hydroponics growing system, Daningsih et al. (2004) reported that the highest number of roots, buds and ferns were produced in a medium with an electrical conductivity (EC) of between 4 to 8 mS cm^{-1}.

Bud initiation is correlated with vigorous fern growth (Tiedjens, 1926). High yield is correlated with many large fern (Ellison and Scheer, 1959; Ellison et al., 1960). For asparagus seedlings, shoot initiation predominated over bud production early in the season (Dufault and Greig, 1983). Fisher (1982) reported that in seedlings, buds are initiated throughout the season until the time of peak shoot number in young seedling plants. He suggested that the growth of the new shoot influences directly or indirectly bud initiation. As asparagus grow, partitioning of carbohydrate into the crown increase at the expense of the fern (Benson and Takatori, 1980; Dufault and Greig, 1983; Haynes, 1987; Hughes et al., 1990). A large crown is favored as it produces greater fern

biomass, more storage carbohydrate, and initiates more buds (Drost, 1997). Wilson and Sinton (2004) reported that large crowns had twice as many buds and roots as the small ones. Crowns with high carbohydrate levels and bud numbers will usually produce high yields in the current harvest season.

1.3.2.3 Spear elongation rates

1.3.2.3.1 Temperature

Asparagus yields depend on the growth rate of spears developing from buds formed in the previous season. The elongation of spears was most rapid in the zone just below the spear tip (Culpepper and Moon, 1939a, 1939b; Lampert et al., 1980). This zone was more sensitive to temperature than the rest of the spear (Culpepper and Moon, 1939a). The overall elongation rate of spears increased with increasing temperature from 10°C to 30°C (Culpepper and Moon, 1939a; Nichols and Woolley, 1985; Kim et al., 1989). The elongation rate of the spear may be linear (Figure 1.4A), exponential (Figures 1.4B and 1.4C) or two phases of exponential (Figure 1.4D), depending on internal or environmental conditions. Spears grew linearly when storage resources were exhausted (Kim and Sakiyama, 1989). However, spear growth appeared to be exponential when spears were 10 to 200 mm long (Nichols and Woolley, 1985) and when the storage roots were fully charged with carbohydrate (Kim and Sakiyama, 1989). Thus, temperature is a major factor for spear growth rates if plants are not limited by other resources, such as carbohydrates.

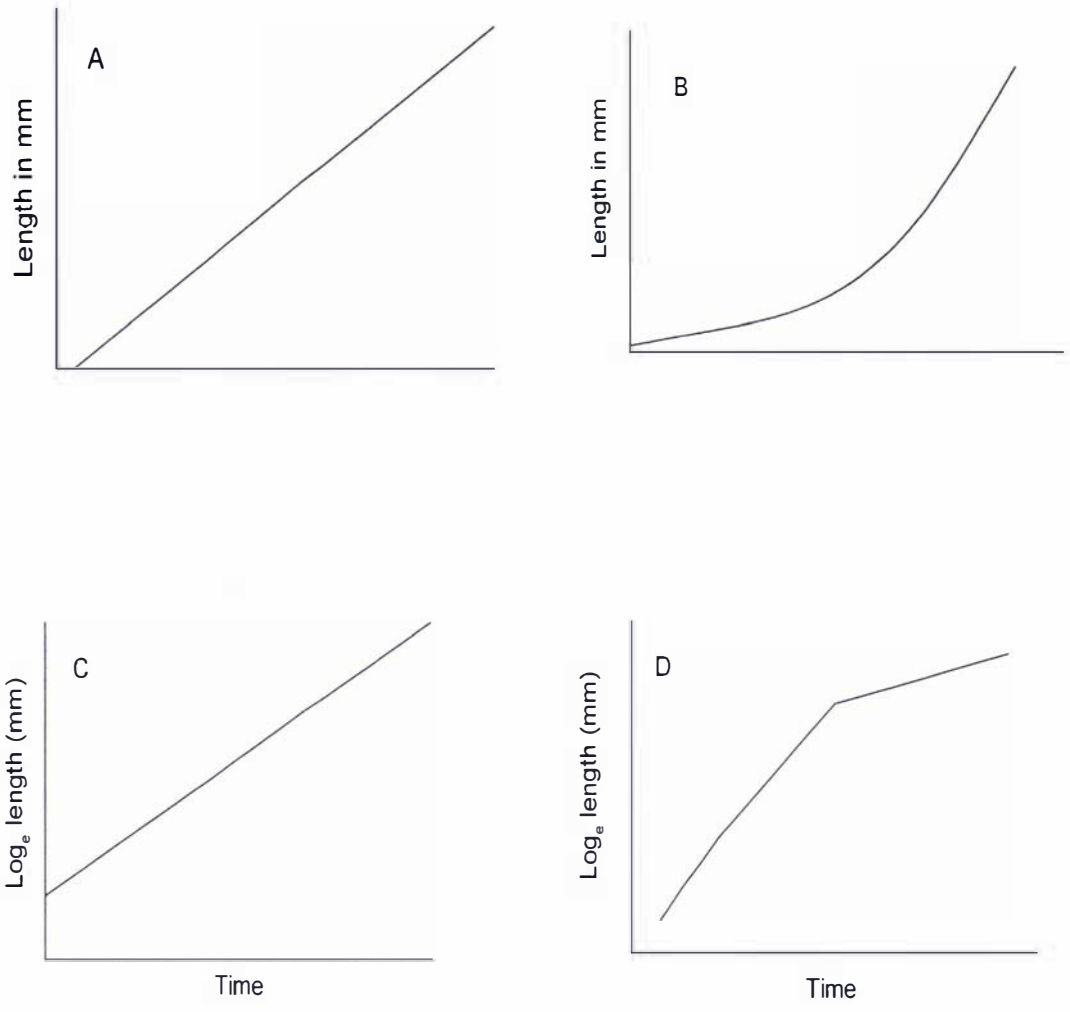

Figure 1.4 Typical patterns of spear growth over time, linear (A), exponential (B, C) or two phases of exponential (D).

1.3.2.3.2 Spear growth in relation to hormones

Plant growth substances have been reported to stimulate spear growth rates. Application of α-naphthaleneacetic acid (NAA) to decapitated spears stimulated growth of those spears initially 10 - 15 cm tall, but had little effect on 5 cm spears (Dedolph et al., 1963). GA_3 drenches at 10 mg L^{-1} stimulated the elongation rate of spears compared to control plants. Exogenous application of GA_3 improved growth rate of spears (Drost, 1997). Kojima et al. (1993) and Kojima and Sakurai (1994) analysed differences in hormone levels such as ABA and IAA along the spear from spear tip to spear butt but did not ascertain the role of these hormones on spear growth rate. The synthetic cytokinin-active compound, CPPU, has been widely researched (Fellman et al., 1987; Woolley et al., 1992; Biasi et al., 1993; Cruz-Castillo et al., 2002). CPPU increases fruit size and promotes growth in some tissues by stimulating cell division and cell expansion (Fellman et al., 1987; Woolley et al., 1992; Kojima et al., 1993; Cruz-Castillo et al., 2002). Thus, plant growth regulators may influence cell division and elongation of spears and thus their growth rates.

1.3.2.3.3 Cell division and elongation

The rate of plant growth is determined by the rate of cell division and/or elongation. Spear growth can be substantially increased at high temperatures, which may stimulate cell elongation and/or cell division in the spear tip and elongation zone (Figure 1.5). Cell division occurs in the apical meristem of spear tip. The cell elongation zone appears to be immediately below the spear tip and is about 20 mm long, according to Kojima et al. (1993).

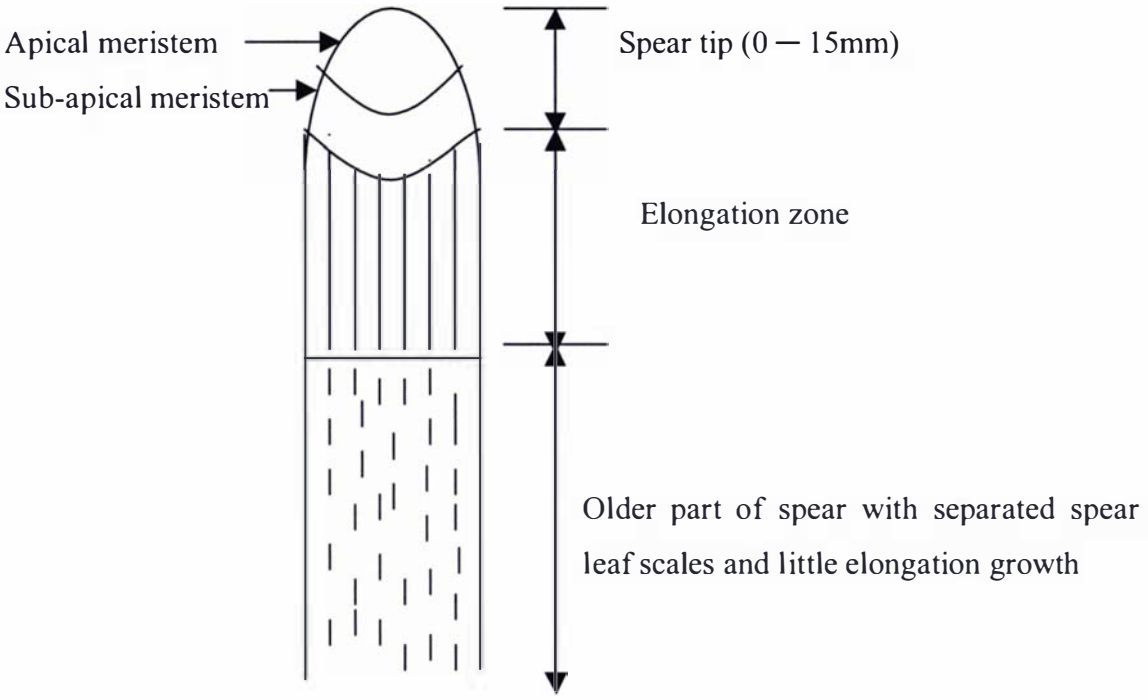

Figure 1.5 Diagram of spear showing the position of spear tip, elongation zone and rest of the spear.

1.3.2.3.4 Apical region and sub-apical meristem

The apical meristem is responsible for most of the organogenic phenomena normally associated with shoot morphogenesis, while the subapical meristem is the major site of cell division of cells contributing to stem elongation (Sachs, 1965; Brown and Sommer, 1992). Shoot elongation can be promoted by exogenous GA_1, GA_3, GA_4 or GA_9 (Little and Pharis, 1995; Wang et al., 1997b; Hansen et al., 1999; Sommer et al., 1999; Little and MacDonald, 2003) and reduced by applying inhibitors of GA biosynthesis (Suzuki et al., 2004). GA_3 was effective in stimulating meristem activity and mitotic activity in neoformed internodes of dwarf sweetgum trees (Sommer et al., 1999), leading to greater numbers of cells in the internode, but also stimulated the elongation of internode cells (Wareing and Phillips, 1981; Daykin et al., 1997). In contrast, Suzuki et al. (2004) reported that paclobutrazol, a potent inhibitor of gibberellin synthesis, decreased etiolated and non-etiolated longitudinal shoot growth, but had no effect on apical meristem activity. Sachs et al. (1960) reported that treating tall chrysanthemums with

chlormequat chloride (CCC) caused them to be dwarfed, but their apical meristem was not affected.

1.3.2.4 Root growth

The crown of an asparagus plant consists of an underground rhizome, fleshy storage roots, and fibrous feeding roots. The large adventitious roots (fleshy storage roots) come directly off the rhizome while the other lateral roots (referred to as fibrous feeder roots) usually developed for the storage root (Blasberg, 1932; Mullendore, 1935).

A reduction of root dry weight and carbohydrates occurs until fern expansion (Dufault and Greig, 1983; Haynes, 1987; Drost and Wilcox-Lee, 1997a, 1997b) as stored carbohydrates are required to provide for the development of spear and the new seasons fern. Assimilate produced in cladodes by photosynthesis during fern growth and development is then translocated into the underground storage roots and then utilized for spear growth and vegetative growth during the next season (Robb, 1984; Haynes, 1987; Pressman et al., 1993). Subsequently increase in root mass and fructans in the storage roots are important determinants of crown activity and yield the following season.

1.3.2.5 Photosynthetic rate and net assimilation rate

All green tissues on asparagus are able to photosynthesize, but photosynthesis occurs mainly in the cladodes, which constitutes the bulk of foliar portion of the fern (Downton and Torokfalvy, 1975; Lin and Hung, 1978). Photosynthesis is the primary energy-storing process of plants. Light energy is stored as chemical energy in organic compounds. The raw materials of photosynthesis are carbon dioxide and water; the products are carbohydrates and oxygen (O_2).

It is important to distinguish between net photosynthesis (Pn) and NAR. The efficiency of an individual leaf is measured by its net photosynthetic rate at a particular light intensity, where:

Net photosynthesis (Pn) = Gross photosynthesis – (dark respiration + photorespiration).

NAR is measured as the net gain in dry weight of the plant unit of leaf area per unit time (total photosynthesis of the plant - total respiration of the plant). Net assimilation rates increase as the canopy establishes in summer and then decreases as the fern senesces during later autumn and winter in temperate climates (Lin and Hung, 1978; Haynes, 1987).

Genotypic variability in photosynthetic rate has been reported in asparagus plants (Bai and Kelly, 1999; Faville et al., 1999; Guo et al., 2002). For example, the higher yielding genotypes, 'Franklim' and 'Hart-3', had higher photosynthetic rates (27.8 μmol m^{-2} s^{-1} and 24.1 μmol m^{-2} s^{-1}, respectively) and the lowest yield genotype, '86Sam3', had the lowest photosynthetic rate (15.7 μmol m^{-2} s^{-1}) (Bai and Kelly, 1999). Photosynthetic rates of the high yielding cultivar 'ASP-69' and low yielding cultivar 'ASP-03' were 8.94 ± 0.38 μmol m^{-2} s^{-1} and 6.50 ± 0.38 μmol m^{-2} s^{-1} respectively (Guo et al., 2002). The high yielding cultivar, 'Karapiro', had a higher photosynthetic rate than the lower yielding cultivar 'UC-157' (Faville et al., 1999). Such variation in photosynthesis among genotypes is derived from physiological differences in fern respiration, carboxylation efficiency and stomatal limitation (Woolley et al., 1999) or is related to genotypic differences in cladophyll diameter, content of photosynthetic biochemical constituents, and mesophyll resistance to CO_2 diffusion (Faville et al., 1999).

Guo et al. (2002) reported that seasonal patterns of photosynthetic parameters (net photosynthesis and stomatal conductance) were strongly correlated with cladophyll developmental stage for both high yield and low yielding cultivars. They reported that 'ASP-69' and 'ASP-03' had high rates of net photosynthesis as fully expanded cladodes measured in mid-summer (February) but photosynthetic rates in both cultivars significantly decreased as cladodes senesced in April. They further showed that cladode diameter in the 'ASP-69' were significantly greater than in 'ASP-03'. Thick and heavy cladodes contained higher amounts of photosynthetic mesophyll cells that enhanced photochemical activity (Hills, 1986). Thus cladophyll properties appear to be important in determining maximum photosynthesis between cultivars. The close relationship between cladophyll thickness and photosynthetic capacity could have potential for selecting high yielding cultivars with greater photosynthetic rate. In addition some

growth regulators, such as CPPU may increase cladophyll diameter and length, increase photosynthetic rate, and subsequently enhance yield performance in the following season.

1.3.2.6 Growth analysis

Growth analysis has widely been used to study plant growth over time (Gardner et al., 1985). The following growth analysis terms used in this thesis are defined below.

Relative growth rate (RGR) or Specific growth rate (Hunt, 1978) can be defined as gain in weight per unit weight of plant per unit time which can be used to compare the performance of plants of widely different size.

Mean relative growth rate ($\overline{R}$) can be calculated as:

$$\overline{R}_{1-2} = \frac{Log_e W_2 - Log_e W_1}{T_2 - T_1}$$

That is the average relative growth rate between two harvests, 1 and 2, can be calculated as the difference between the natural logs of the dry weights divided by the time interval between the harvests.

In modern growth analysis it is more common to calculate the instantaneous relative growth rate (iRGR) rather than the mean RGR ($\overline{R}$). This is done by fitting a curve to the Log_e for the growth data getting the equation of the curve and then differentiating to find the slope (the iRGR) at any point in time. The instantaneous RGR, calculated using the curve fitting approach can be used together with leaf area ratio to determine the net assimilation rate.

Leaf area ratio (LAR) is the ratio of total leaf area to whole plant dry weight. This is simply the area of leaf to the total dry weight of the plant. In a broad sense, LAR represents the ratio of photosynthetic to respiring material within the plant (i.e. the 'leafiness of the plant'). In order to calculate net assimilation rate the instantaneous value must be calculated.

Net assimilation rate (NAR) is a measure of the ability of the leaves to produce a net gain in weight per unit area of leaf. It is therefore more correctly referred to as a unit leaf rate (ULR). If the RGR and LAR have been obtained NAR can be calculated: NAR = RGR/LAR (all these are instantaneous values) and represents total photosynthesis of the plant minus total respiration.

Specific leaf area (SLA) is the mean area of leaf per unit weight of leaf (a measure of either leaf density or relative thickness). Kebede et al. (1994) observed a positive correlation between SLA and leaf thickness in two *Lycopersicon* species. On the contrary, Van Arendonk and Poorter (1994) showed that the low SLA of slow-growing species is mainly explained by higher contents of lignin, total nonstructural carbohydrates, and organic acids, expresses per unit leaf area. They found no differences in leaf thickness among fast- and slow-growing species. However, the leaf density (dry matter per unit volume) of the slow-growing low-SLA species was significantly higher than that of fast-growing high-SLA species.

Leaf weight ratio [LWR = dry weight of leaves (LW)/dry weight of whole plant (W)] is an index of the leafiness of the plant on a weight basis.

RGR = NAR x LAR.

LAR = SLA x LWR

So RGR ($g\ g^{-1}\ week^{-1}$) = NAR ($g\ m^{-2}\ week^{-1}$) x SLA ($m^2\ g^{-1}$) x LWR ($g\ g^{-1}$).

1.3.2.7 Root-shoot relationships

1.3.2.7.1 Root-shoot communication

Roots are the main site of water uptake and mineral nutrition. Plant water uptake can be influenced by high electrical conductivity (EC) of the nutrient solution in hydroponics system (Ehret and Ho, 1986), indicating increasing osmotic potential. The electrical conductivity of a nutrient solution influences the growth of asparagus seedlings. Daningsih et al. (2004) reported that an EC from 4 to 8 mS cm^{-1} was the optimal range for asparagus growth.

Although the coarse control of root-shoot ratios of plants is dependent on environmental conditions regulating supply of carbohydrates from the shoot to the root, or water and all mineral ions from the root to the shoot, fine control is under hormonal control.

Roots are key factors regulating shoot growth through the supply of hormones such as gibberellins, cytokinins and ABA. Carmi et al. (1983) suggested that retarded shoot growth may be due to lack of hormones, such as gibberellins and cytokinins produced in the roots. Reduction in shoot growth can affect hormonal levels (Carmi and Heuer, 1981) and produce an imbalance in growth regulators (Dubik et al., 1990), especially root-sourced ABA. Root-synthesized ABA is involved in regulating the shoot growth of plants grown under root restriction conditions (Hurley and Rowarth, 1999). Ternesi et al. (1994) reported that ABA content in xylem sap was seven times higher in the sap of root confined plants. It is therefore likely that root sourced chemical signals such as gibberellin, cytokinins and ABA are involved in co-ordinating root-shoot relationships under favourable or unfavourable conditions.

1.3.2.7.2 Root-shoot allometric relationship

Root:shoot ratio is generally understood to be the relationship between dry weight partitioning to the root and to the shoot. High and low yielding genotypes of asparagus showed significant difference between the root:shoot ratio irrespective of plant size. High yielding genotypes had a higher crown weights and were considered to partition more assimilate into crown than low yielding genotypes (Benson and Takatori, 1980).

Environmental factors such as temperature (Hughes et al., 1990), daylength (Sudjatmiko et al., 1997; Woolley et al., 1999), water stress (Wilcox-Lee and Drost, 1990) and mineral nutrition (Fisher and Benson, 1983; Precheur and Maynard, 1983) also affect root:shoot ratio.

Root:shoot ratios change with plant size as the plants grow or with differences in plant size between genotypes. In contrast the allometric relationship between root and shoot stays constant for any particular development stage.

For example, for asparagus, Hughes et al. (1990) reported that the allometric relationship between root and shoot overcame the effect of harvest date. The allometric relationship is calculated using the regression of the natural Log_e of root dry weight against the natural Log_e of shoot dry weight.

The function to transform natural logarithms of both sides of the equation and to fit as linear regression equation is:

$$Log_e Y = Log_e a + b\ Log_e X$$

where:

Log_e a is a constant.

b is the allometric constant (the slope)

Log_e Y is natural logarithm of shoot dry weight

Log_e X is natural logarithm of root dry weight

The allometric constant (b) values depend on relative partitioning of dry matter to roots and shoots over time. If 'b' is greater than 1.0 then more dry matter is partitioning to the shoots, whereas if 'b' is less than one more dry matter is partitioned to the root.

1.4 Rationale for thesis and use of CPPU

In most countries there is an increased consumer demand for asparagus but competition between producer countries is high. In New Zealand labour costs are high, but the competitive ability of New Zealand growers could be improved by increasing yield of high quality spears per hectare. Benson (2006) reported that average yields in Thailand and Peru are 15 and 14.1 tonne ha^{-1}, while in New Zealand the average is only 3.7 tonne ha^{-1}. A major goal of New Zealand asparagus growers is to increase yields of high quality spears in order to remain competitive.

In this thesis a number of factors that influence yield was investigated (Figure 1.6). Factors controlling spear growth rates and bud numbers were investigated in Chapters 2 and 3, and factors affecting vegetative growth and possibly photosynthesis in Chapters 4 to 6.

The synthetic cytokinin-like compound (CPPU) was used extensively and results reported in Chapters 4 to 6, as it was known to affect both vegetative and reproductive growth of plants. For example, CPPU promotes shoot growth (Sugiyama et al., 1993; Cruz-Castillo, 1998), increases fruit size (Greene, 1989; Lawes et al., 1992; NeSmith, 2002), and stimulates cell division and expansion in several crops (Lewis et al., 1996; Yu et al., 2001). Applying BA and CPPU to explants in tissue culture stimulated axillary bud break in roses (*Rosa hybrida* L. cvs. 'Madelon and Motrea') (Kapchina-Toteva et al., 2000). Kapchina-Toteva and Stoyanova (2003) reported that the application of cytokinins [kinetin (Kin) and CPPU] stimulated the bud break in *Gypsophila* stems in tissue culture when the medium contained 1.0 μM CPPU.

Applying CPPU to asparagus foliage and roots has not been previously reported in the literature, but was widely used in this thesis. Because of problems in collecting root exudates of asparagus some experimental work was also carried out on two cultivars of *Capsicum annuum* (Chapters 7 and 8).

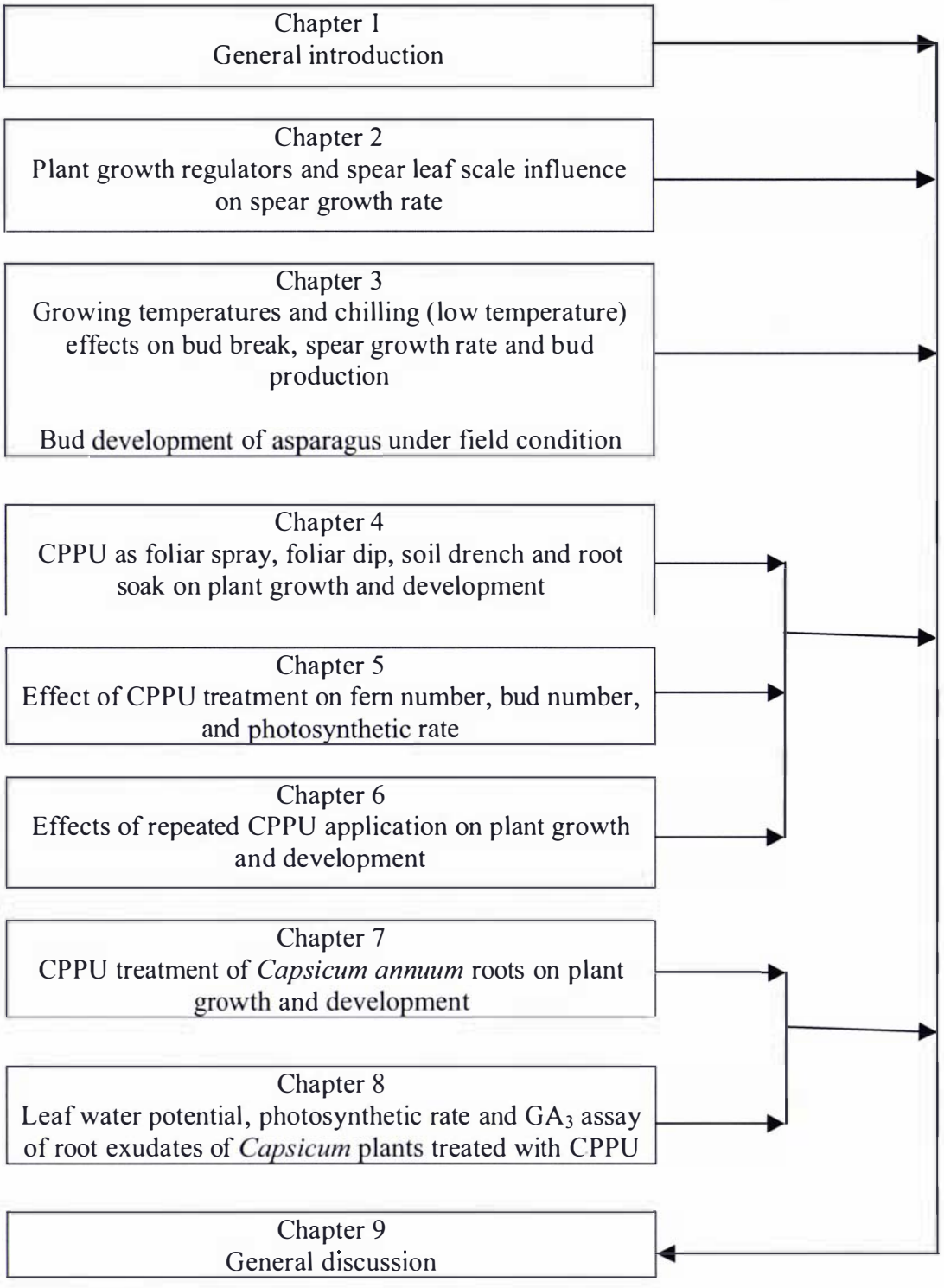

Figure 1.6 Structure and outline of this thesis.

CHAPTER 2

EFFECT OF PLANT GROWTH REGULATORS AND SPEAR BUD SCALES ON GROWTH OF *ASPARAGUS OFFICINALIS* SPEARS

Abstract

The effect of plant growth regulators and spear leaf scales on spear elongation in asparagus was investigated. Spears of uniform size were immersed completely in indole-3-acetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA_3) or *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) in test tubes. IAA and GA_3 slightly stimulated, and ABA inhibited, elongation of specific regions of the shoot, but overall the effects on total spear elongation were small. In contrast the cytokinin-active compound, CPPU, significantly increased spear length. Increasing CPPU concentration from 0 to 10 mg L^{-1} increased the elongation rate of spears. CPPU may influence spear length by stimulating the biosynthesis of native cytokinins or other plant growth promoter hormones as the naturally occurring cytokinin, zeatin riboside (ZR), was also found to stimulate spear elongation. In particular, CPPU greatly stimulated the growth of the first and second segments below the spear tip, and removal of spear leaf scales inhibited this growth. CPPU did not reverse the growth suppression caused by removing spear leaf scales suggesting that other factors may interact with cytokinins in promoting elongation.

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Effect of plant growth regulators and spear bud scales on growth of *Asparagus officinalis* spears.

Yang-Gyu Ku and D. J. Woolley

2.1 Introduction

Elongation rate of spears is an important factor determining final yield of asparagus in many temperate climatic conditions (see Chapter 3). Elongation rate depends on the number of expanding cells that are present and their rate of longitudinal elongation. Cell division occurs in the apical meristem of the spear tip (Culpepper and Moon, 1939b). As cell division continues the older cells are gradually swept into the zone of elongation below the tip and, finally, into a zone below the elongation zone comprised of non-elongating, lignified, and vascular tissues (Lill et al., 1990). Kojima et al. (1993) found that cell division was confined to the top 0-15 mm of the spear (the spear tip), while the zone of elongation was 15-35 mm below the apex.

The elongation zone of the spear is only a small percentage of the total length for long spears, but spears grow exponentially until they are about 150 - 200 mm in length. Therefore it follows that, in order to maintain exponential growth, either the elongation zone must accelerate its activity as the spear grows (Nichols and Woolley, 1985) or the size of the zone of elongation must increase. One possible explanation is that a signal, perhaps hormonal in nature, originates from lower parts of the spear and stimulates the elongating zone or the region of cell division in the apical meristem of the spear, resulting in cells being swept more quickly into the elongation zone. Thus, it is hypothesized here that plant hormones, possibly from the spear leaf scales (Woolley, D. J., unpublished data), may be the signal from the older parts of the spear that accelerates the activity of the elongation zone and thus results in exponential growth. However, there have been few reports directly showing stimulation of spear growth rates by plant hormones. Application of NAA to decapitated spears (apical 25 mm) promoted growth of those spears initially 10 - 15 cm tall, but had little effect on 5 cm spears (Dedolph et al., 1963). Tiburcio (1961) showed that GA_3 drenches at 10 mg L^{-1} stimulated the elongation rate of spears, while Kojima et al. (1993) and Kojima and Sakurai (1994) analysed differences in hormone levels such as IAA and ABA along the spear but did not investigate the effect of these hormones on spear growth rate. The cytokinin-active compound, CPPU, is well known to promote growth in some tissues by stimulating cell division and cell expansion (Fellman et al., 1987; Woolley et al., 1992; Cruz-Castillo et al., 2002). Therefore the objectives of this study were to determine which hormone group(s) stimulates spear growth, their possible relationships to spear growth rate and

whether they may provide a signal, possibly originating from the spear leaf scales, to produce exponential elongation in organs in which only a small region appears to be elongating.

2.2 Materials and methods

All spears harvested for use in the experiments were cut at ground level and were ten to twenty mm longer than required for the specific treatment and trimmed to the required length at the start of the experiment. Spear diameters were 9.0 ± 1.0 cm. Elongation rates of spears growing under the same conditions show little correlation with spear diameter (Nichols and Woolley, 1985) and thus small variations in spear diameter are unlikely to contribute significant variability to the elongation rates. All harvested spears were immediately placed in high humidity in polyethylene bags and used for the elongation studies within two hours of harvest.

2.2.1 Experiment 1

Spears were harvested from six-year-old 'Jersey Giant' asparagus plants grown in a commercial planting at Bulls (latitude 40.2° S, longitude 175.4° E), New Zealand. Spears were harvested randomly and transported from field to lab for investigation. After trimming to 80 mm length spears were immersed completely for one hour in aqueous solutions of 10 mg liter^{-1} concentration of IAA, ABA, GA_3 or CPPU (Sitofex EC 2.0%; SKW, Trostberg, Germany) in test tubes. Control spears were submerged in distilled water for one hour. Before immersion, spears were marked at 20 mm intervals using a fine point permanent marker and divided into four segments from spear tip to base. Thereafter, segments were identified as tip (0-20 mm), first segment (20-40 mm), second segment (40-60 mm) and third segment (60-80 mm) from the top to the base. Each segment length was measured once a day over 102 hours. The experiment was a completely randomised design with five replications (spears) per treatment.

2.2.2 Experiment 2

Spears (80 mm length) were completely immersed in one of the following solutions contained in test tubes: control (water), 0.5 mg L^{-1} CPPU, 1 mg L^{-1} CPPU, and 10 mg L^{-1} CPPU. Spears were dipped in each solution for 30 minutes, 60 minutes or 90 minutes. Spear length was measured every day over 96 hours. Plant material and measurements of segment length were as described in Experiment 1 (Section 2.2.1). The experiment had a 4 x 3 factorial arrangement of treatments in a completely randomised design with five replications per treatment.

2.2.3 Experiment 3

Spears of asparagus 'UC-157' were harvested from the field at the Plant Growth Unit, Massey University, Palmerston North, New Zealand and re-trimmed prior to start of experiment to 80 mm. Spear leaf scales were removed carefully from the basal 60 mm using a sharp razor blade. Spears with bud scales removed and intact spears were submerged completely in aqueous solutions of CPPU (10 mg L^{-1}) or water for thirty minutes. The experiment was a completely randomized design with four replications (spears) per treatment.

2.2.4 Experiment 4

Spears of asparagus 'Rutgers Beacon' were harvested from the field at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. Spears were trimmed to 100 mm and placed in 50 ml beakers with the basal 3 cm in the following solutions: control (water), 0.1 M sucrose, and 10 mg L^{-1} ZR plus or minus 0.1 M sucrose. The experiment was a completely randomized design with eight replications (spears) per treatment.

For all experiments except Experiment 4 (Section 2.2.4), each spear was transferred after treatment into 50 ml beakers with the basal 2 to 3 cm in a single labelled jar of distilled water. Spears for all experiments were placed in a controlled environment growth cabinet at a constant day/night temperature of 25°C, with a 12-hour photoperiod. Spear length was measured every day from the base of the spear to the tip of the growing point with a flexible ruler. The fresh weight of each spear was determined and stem diameter measurements were made 1 cm above the spear base using digital calipers (Mitutoyo, Digmatic, Japan).

2.2.5 Data analysis

Significant effects of treatments were identified by analyzing data using GLM procedure of SAS software version 8.2 (SAS Institute, Cary, North Carolina, USA). The least significant difference (LSD) at $P < 0.05$ was used to separate means in Experiments 1 (Section 2.2.1) and 4 (Section 2.2.4), and Duncan's multiple range test at $P < 0.05$ was used for Experiment 3 (Section 2.2.3). For Experiment 2 (Section 2.2.2), mean separations were done by orthogonal polynomial contrasts to test whether there was a significant linear or curvilinear relationship between the length or fresh weight and concentration.

2.3 Results

2.3.1 Experiment 1

The length of spears treated with CPPU was significantly greater than those treated with the other plant growth regulators. CPPU at a concentration of 10 mg L^{-1} stimulated a greater spear growth rate than the control and those treated with IAA, GA_3 or ABA, but in all cases growth ceased after 30 hours (Figure 2.1). The other hormones had no significant effect on final spear length, although spears dipped in ABA appeared to be slightly shorter than the others, while those dipped in IAA or GA_3 were slightly longer.

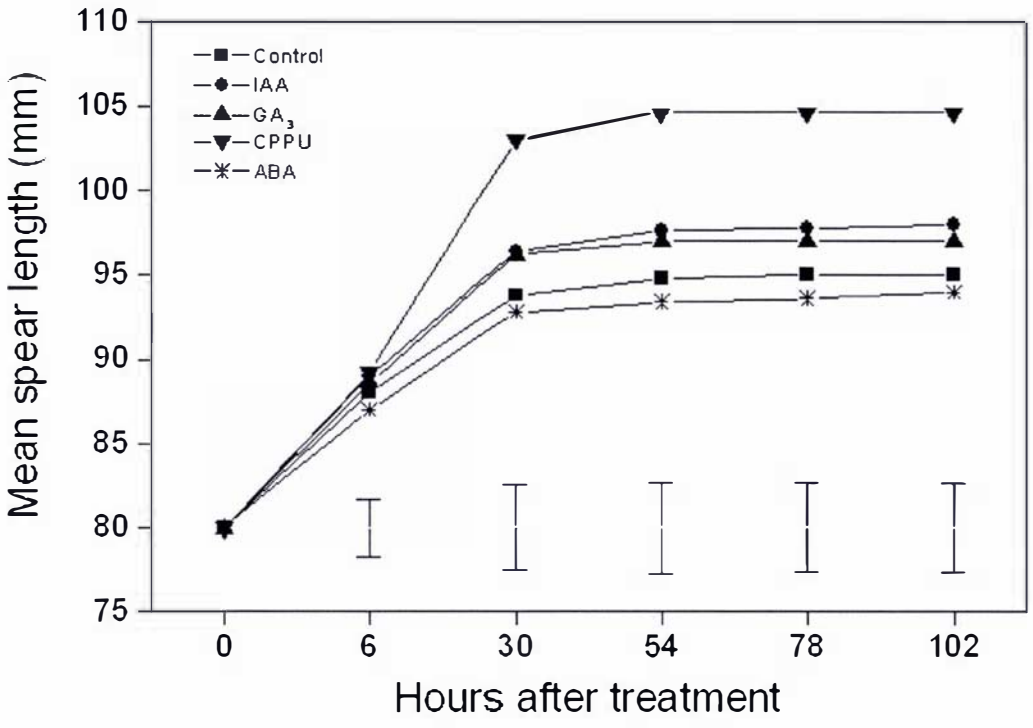

Figure 2.1 Effect of plant growth regulators on spear length of 'Jersey Giant' over time. Each point is the mean of five spears per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

The region of greatest growth in all treatments was the second segment from the spear tip except for the CPPU treatment in which growth rates of the segments two and three were similar. Treatment with CPPU stimulated elongation of all segments more than the other plant growth regulators (Figure 2.2). IAA and GA_3 significantly increased the length of the second segment from the spear tip, but ABA slightly decreased the length of the first segment.

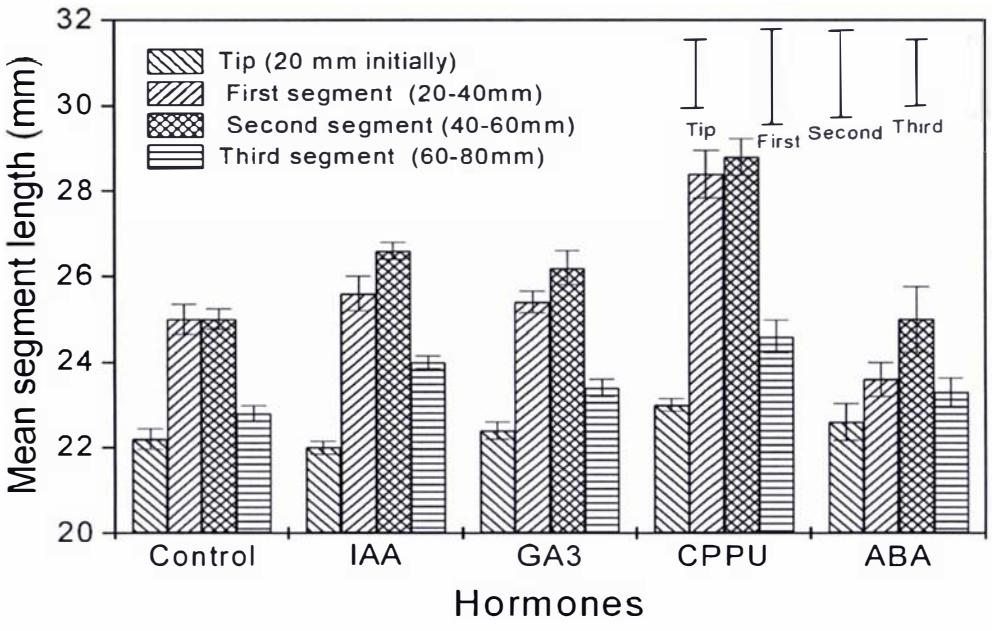

Figure 2.2 The effect of plant growth regulators on mean segment length of 'Jersey Giant' spears over 102 hours (initial length of each segment = 20 mm). Vertical bars represent the standard error of mean (n = 5). The LSD for each segment is shown above (right corner).

2.3.2 Experiment 2

Mean spear length and fresh weight of the spears treated with CPPU increased with increasing CPPU concentration (Table 2.1). The lowest concentration applied, 0.5 mg L^{-1} CPPU, resulted in higher rates of spear elongation and fresh weight increase than the controls. Increase in length had a significant quadratic component over the concentration range studied. Stem diameter growth rate was not influenced by CPPU concentrations. There was no effect of time of immersion on spear length, weight, and diameter (data not shown).

Table 2.1 Effects of CPPU on spear growth, fresh weight and stem diameter of 'Jersey Giant' over 96 hours.

Concentration of CPPU (mg L^{-1})	Length[z] (mm)	Increase in fresh weight (g)	Increase in diameter (mm)
0 (control)	94.0c	1.00c	0.58a
0.5	97.6b	1.35b	0.59a
1	100.7ab	1.67a	0.72a
10	103.6a	1.70a	0.71a
Significance[y]	L*** Q***	L***Q***	ns

[z]Each value is the mean of fifteen spears.
[y]Treatment effects were not significant (ns) or significant at $P \leq 0.001$(***).
Means sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

The greatest increase in segment length in all CPPU concentrations was the first segment from the spear tip. Increasing CPPU concentrations from 0 mg L^{-1} to 10 mg L^{-1} significantly increased elongation rates of the first and second segments (Figure 2.3). Spears treated with 10 mg L^{-1} CPPU had longer spear tips and third segment region than the controls, but those treated with other CPPU concentrations were similar to the controls.

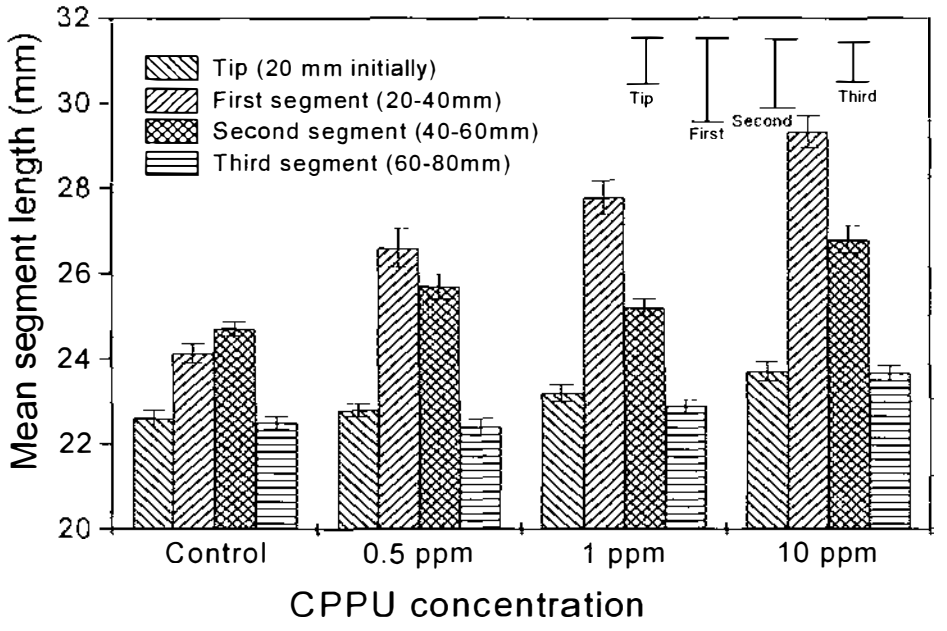

Figure 2.3 The effect of CPPU concentration on mean segment length of 'Jersey Giant' spears over 96 hours (initial length of each segment = 20 mm). Vertical bars represent the standard error of means (n = 15). The LSD for each segment is shown above (right corner) (Experiment 2).

2.3.3 Experiment 3

The effects of CPPU and spear leaf scale removal on spear length were very significant ($P < 0.008$), and the interaction between their effects was also significant ($P < 0.02$) (Table 2.2). Spears treated with CPPU that had intact spear leaf scales had significantly increased spear length compared to the control spears. Spear leaf scale removal did not significantly affect spear length of the controls when measured four days after removal, but CPPU only stimulated spear growth in the presence of spear leaf scales.

Table 2.2 The effect of CPPU and the removal of spear leaf scales on spear length of 'UC-157' over 96 hours.

Treatment		Spear length [z] (mm)
CPPU	Spear leaf scale	
+	+	111.6a
-	+	95.0b
+	-	91.8b
-	-	90.0b
Significance[y]		
CPPU soak		**
Spear leaf scale		***
CPPU soak x spear leaf scale		*

[z]Each value is the mean of four spears.
[y]Treatment effects were significant at $P \leq 0.05(^{*})$ or $P \leq 0.01(^{**})$ or $P \leq 0.001(^{***})$.
Means sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

2.3.4 Experiment 4

Growth of spears treated with ZR + sucrose was significantly higher than the control (Figure 2.4). The response to ZR alone was almost significant at the 5% level but sucrose alone produced little or no stimulation of growth compared to the controls.

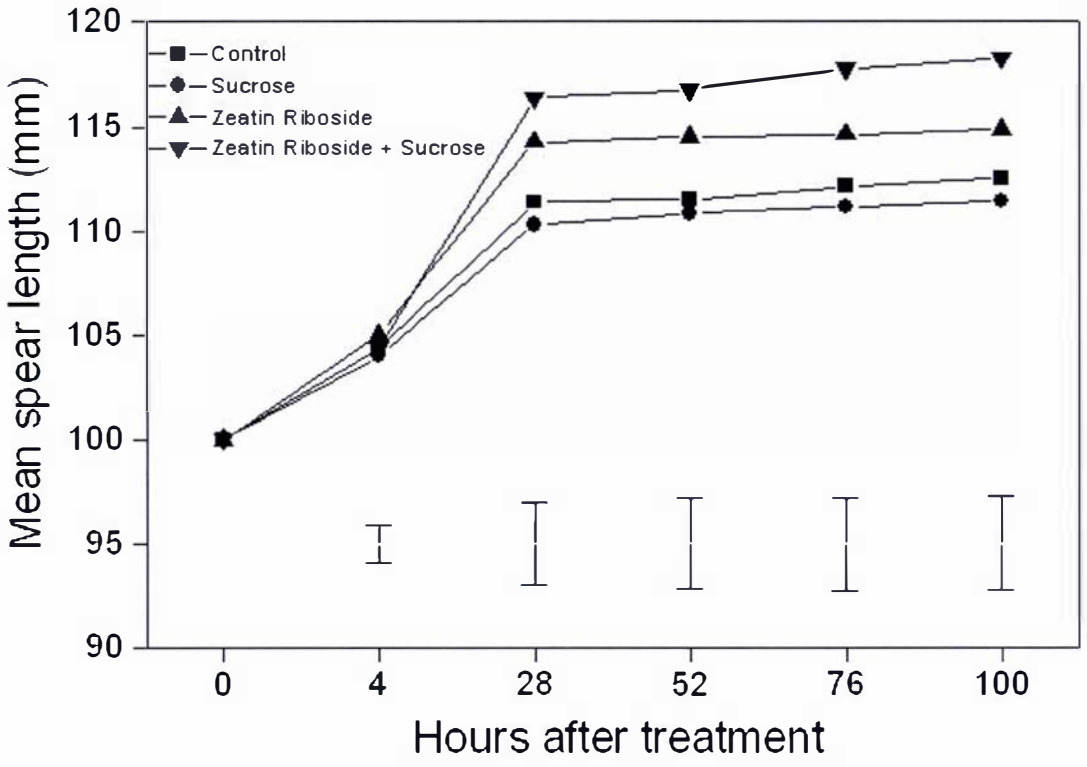

Figure 2.4 The effect of sucrose and zeatin riboside on spear length of 'Rutgers Beacon' spears over 100 hours grown at 25°C. Each point is the mean of eight spears per treatment. Vertical bars represent the LSD at $P \leq 0.05$ (Experiment 4).

2.4 Discussion

These experiments showed clearly that cytokinins such as CPPU and ZR were effective in stimulating spear elongation (Figures 2.1 and 2.4). The elongation rate of spears increased with increasing CPPU concentration (Table 2.1). It is well known that CPPU is transported slowly or is immobile within plants (Morris et al., 1986; Tartarini et al., 1993) but in these experiments only limited mobility was required as the whole spear was soaked.

The second and third segments from the tip elongated significantly when treated with CPPU, GA_3 and IAA, but GA_3 and IAA had no significant effect on the tip or first segment compared with the control (Figure 2.2). It is suggested that although cells in segment two gradually cease growth and become non-elongating tissue, IAA and in particular CPPU, can extend the zone of elongation beyond 60 mm. This was confirmed by the results of increasing CPPU concentrations in which elongation rates of the first and second segments from the tip were significantly greater than the control spears (Figure 2.3). All segments in 10 mg L^{-1} CPPU were longer than the control spears, indicating that CPPU can extend the zone of elongation along whole spears. Thus, these results disagree with those of Culpepper and Moon (1939b) and Kojima et al. (1993) that the elongation zone is confined to a region only 20 mm long. In the present work a region 40 mm below the tip elongated and some elongation occurred even further from the apex, especially in tissue treated with hormone. However, it is clear that elongation slows considerably as cells are swept from the segment two to segment three, while segment one is strongly promoted by CPPU but inhibited by ABA. However, growth ceased after about 30 hours possibly due to lack of hormones or other resources from the roots (Figure 2.1). Growth could have been limited by lack of carbohydrates from the root system but this seems unlikely as supplying sucrose to the detached spears did not prolong growth (Figure 2.4).

Woolley et al. (1992) found that CPPU stimulated both cell expansion and cell division in fruit tissue, but in asparagus stem tissue there was no additional stimulation in size of the apical bud, suggesting that cell division was not involved, and that cell elongation, not only in the 20 mm below the apical bud but also in the next two segments, was the

major response. Thus, length of spears was produced by cell elongation, not cell division, and elongation of detached spears ceased very quickly (Figure 2.1).

The relationship between spear elongation and endogenous hormones is unclear. IAA concentration showed a declining gradient from spear tip to the butt region, regardless of spear length, suggesting that IAA possibly promoted the accumulation of assimilates in the tip (Kojima and Sakurai, 1994). Green 'Jersey Giant' spears had higher concentrations of IAA than white spears (Makus and Guinn, 1992) and showed greater elongation. Thus, IAA may have promoted spear elongation (Kojima, 2002). However, in the present work IAA did not significantly promote over-all spear growth, although there was a small stimulation of segment three (Figure 2.2). It is possible that endogenous IAA levels were already optimal (non-limiting). ABA suppressed the elongation of the first segment below the tip in the comparison with other plant hormones (Figure 2.2). ABA may directly inhibit growth or inhibit accumulation of carbohydrate in the growth region as has been found in wheat (Hall and McWha, 1981), bean (Van Volkenburgh and Davies, 1983), and sugar beet (Daie, 1986).

Spear leaf scale removal significantly decreased spear elongation in the presence of CPPU; in the absence of CPPU the spear leaf scale effect was not significant at the 5% level. In intact plants, spear leaf scale removal below the zone of elongation has been shown to cause a transient decrease in spear elongation that can be reduced by GA_3 (Woolley, D. J. unpublished data). It therefore seems likely that elongation of the spear is controlled by complex interactions between plant hormones from the spear leaf scales and shoot tissues, and cytokinins from the root tissues. The stimulation of spear growth by CPPU in detached spears supports the hypothesis that cytokinins from the root system may be involved in stimulating spear growth (Table 2.2). CPPU may stimulate biosynthesis of natural cytokinins (Mok et al., 1979; Woolley et al., 2006). Given the importance of spear elongation rate in determining yield in asparagus crops, particularly in relation to temperature (see Chapter 3), further experiments on hormonal interaction in controlling growth in spears, plus or minus spear leaf scales, with or without roots is warranted.

With respect to the mechanism of exponential growth, when only a limited zone of elongation is present, as in asparagus spears, the present results suggest that the

increased number of spear leaf scales present as the spears grow, together with an increase in the length of the elongation zone, may provide at least a partial explanation. Further explanation of the physiological basis for exponential growth in asparagus spears may provide valuable insights into increasing yield, as spear growth rates in temperate climates in the field often limit yield.

2.5 Summary

The cytokinin-active compound, CPPU, and a naturally occurring cytokinin, ZR, increased elongation rate of spears, suggesting that cytokinins may be important in stimulating the rapid growth of spears. CPPU only increased elongation in the presence of spear leaf scales suggesting that other factors may interact with cytokinins in promoting spear elongation. Only small responses were found to IAA, GA_3 or ABA.

CHAPTER 3

THE EFFECT OF CHILLING DURATION AND TEMPERATURE ON ASPARAGUS SPEAR GROWTH

Abstract

The aim of this study was to investigate the effect of different chilling periods at several different growing temperatures on bud break, relative spear growth rate (RSGR), and bud production of asparagus. Five experiments were carried out in the environmentally controlled growth cabinets and in a field experiment. The field experiment was carried out to investigate bud production during the annual growth cycle. The growth cabinet experiment was divided into three main experiments. In the first experiment, twenty-two-month asparagus non-chilled plants of 'Apollo' and 'Desto' were placed into controlled growth cabinets at one of five temperatures (15°C, 20°C, 25°C, 30°C, and 35°C) for two months.

Part of this chapter was presented as a poster at the International Asparagus Symposium, Netherlands, June 2005 and has been published in ***Acta Horticulturae*** (in press) with the following title and authors:

The Effect of Chilling Duration and Temperature on Asparagus Spear Growth

Yang Gyu. Ku, D. J. Woolley and M. A. Nichols

Part of this chapter has been submitted for publication to ***The Journal of Horticultural Science and Biotechnology*** (in review) under the following title and authors:

Temperature effects on dormancy, bud break and spear growth in asparagus (*Asparagus officinalis* L)

Yang Gyu. Ku, D. J. Woolley, A. R. Hughes and M. A. Nichols

In the second and third experiment, two and half year plants of 'Dariana' and five-year-old plants of 'Apollo' and were placed in a coolstore at 5°C for 0, 3 and 6 weeks. After the specified chilling time, the pots were placed into controlled growth cabinets at one of five temperatures (10°C, 15°C, 20°C, 25°C, and 30°C) for 'Apollo' and three temperatures (20°C, 25°C, and 30°C) for 'Dariana' in dark conditions.

The results showed that increasing the temperature from 10°C to 30°C had a significant effect on the time to bud break, but at 15°C and below chilling had a significant effect on the timing of bud break. The RSGR increased with increasing temperature from 10°C to 30°C, but the effect of prior chilling on RSGR differed between experiments. The growth of spears was mainly exponential (constant RSGR) from 20°C to 30°C. At 15°C, 56.9% of the spears showed exponential growth and 8.1% linear growth. At 10°C and 35°C less than 50% showed exponential growth. New buds were produced in both controlled environmental conditions, and in open field conditions during spear harvest. In summary, growing temperatures are important factors for spear production and growth, affecting RSGR, time to bud break and new bud initiation, but the effect may be modified by the amount of prior chilling.

3.1 Introduction

The dynamics of bud break and subsequent spear growth are strongly controlled by environmental factors such as temperature. The rate at which spears grow relates closely to the growing temperature (Tiedjens, 1924; Culpepper and Moon, 1939a; Blumenfield et al., 1961; Nichols and Woolley, 1985; Kim et al., 1989; McCormick and Geddes, 1996; Wilson et al., 1999a). Spears grew exponentially with time from 10°C to 30°C (Culpepper and Moon, 1939a; Blumenfield et al., 1961; Nichols and Woolley, 1985). Nichols and Woolley (1985) reported that spear growth from 10°C to 30°C showed an exponential pattern when spears are 10 to 200 mm long thus giving a constant RSGR the size of which depended on temperature. However, their data for 10°C suggested that growth may not be truly exponential (Figure 6 of Nichols and Woolley, 1985). Kim and Sakiyama (1989) reported that spears grew exponentially at root temperatures from 10°C to 30°C. They also found that elongation rates of spear were exponential while storage reserves were fully recharged in storage roots, but grew

linearly as the root resources were depleted. In contrast, Dean (1999) has suggested linear patterns at 18°C, 24°C, and 30°C.

Chilling also influences bud break and subsequent spear growth rates in asparagus cultivars. In temperate climates such as New Zealand, under natural conditions, asparagus plant appears to undergo a period of dormancy from late autumn until the end of winter, when temperatures are often less than 10°C. Hughes (1992) reported that chilling markedly decreased the mean time to bud break in spring. A low temperature treatment of 5°C for 3 weeks was the most effective treatment for breaking dormancy of asparagus (Hughes, 1992; Seong et al., 2002). Spear growth rate appeared to depend not only on the growing temperature but, at least in some cultivars, on the amount of chilling received during the previous winter (Hughes, 1992).

There is limited information concerning the relationship between temperatures and chilling on spear growth rate and bud production. Knowledge of the interactions between the chilling duration and the growing temperature on asparagus spear growth rate and bud production are required, as the effect of winter chilling and growing temperature are important factors for spear growth rate and early yield. The objective of the experiment was to investigate in more detail the effects of winter chilling and several temperature regimes on asparagus spear growth rates, rates of bud break and bud production in a controlled environment under dark conditions.

3.2 Materials and methods

Four experiments were undertaken in controlled climate cabinets at the Plant Growth Unit, Massey University, Palmerston North (latitude 40.2° S, longitude 175.4° E), New Zealand during 2000-2001 year. The first and second experiments were conducted from December 2000 to April 2001. The third and fourth experiments were conducted from May to December 2001. The fifth experiment was carried out on bud development in the field.

3.2.1 Experiment 1

3.2.1.1 Plant materials and temperatures

Twenty-two-month old asparagus plants (cvs 'Apollo' and 'Desto') were grown in a greenhouse in plastic pots (15 cm diameter x 39 cm high) filled with 50:50 v/v mixture of pumice and peat, and slow release osmocote fertiliser (15 N, 4.8 P, 10.8 K, 1.2 Mg, 3.0 S, 0.4 Ca plus trace elements) (200g of long term and 100g of short term: 300g / 100 liters). Plants were dried off to impose dormancy before the experiment started. Dead asparagus ferns were removed and the pots were placed in controlled climate cabinets at five temperatures (15°C, 20°C, 25°C, 30°C and 35°C) under dark conditions. The plants were irrigated by watering daily as required.

Dataloggers (Micro Logger, Dallas Semiconductor, USA) were used to measure air temperature every half-hour throughout the experiment. However, owing to temperature fluctuations in the climate cabinets the respective temperatures were 14.3 ± 0.7, 19.2 ± 0.9, 24.7 ± 0.4, 30.3 ± 1.5, 36.9 ± 1.8.

3.2.1.2 Measurements of spear height

The buds on the rhizome were exposed by removing the growing medium after the top of the crown was covered with the medium. The buds on the rhizome were exposed in order to measure spear height from the time of bud break. The height from soil surface to the spear tip was measured by using a flexible ruler at 12 hour or 24 hour intervals. The spears were harvested when spear height was between 200 and 250 mm.

3.2.1.3 Relative spear growth rate (RSGR)

RSGR was determined as the natural logarithm of spear height plotted against time (age) (Nichols and Woolley, 1985; Yen et al., 1996). The slope of the line represents the RSGR.

3.2.1.4 Data analysis

This experiment was a factorial arrangement of treatments comprising four temperatures and two cultivars. There were 2 plants of 'Apollo' and 3 plants of 'Desto' per treatment. To obtain RSGRs, four spears were measured over time for each plant. Each spear was well separated in time of initiation and position from other spears on the same plant to avoid inter-spear inhibition that can occur within a cluster (Daningsih, 2005). Analysis of variance (ANOVA) was employed to analyse data for main and interaction effects of the two factors (temperature and cultivar) on the RSGR using SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA).

3.2.2 Experiment 2

Three-year-old asparagus plants 'Apollo' were grown at 30 liter pots filled with a standard bark-peat mix (50:50), and slow release osmocote fertiliser (15 N, 4.8 P, 10.8 K, 1.2 Mg, 3.0 S, 0.4 Ca plus trace elements) (200g of long term and 100g of short term: 300g / 100 liters) in the glasshouse and then dried off to impose dormancy. Dead asparagus ferns were removed and two plants were chilled in a coolstore at 5°C for two weeks. Non-chilled plants remained at 15°C without chilling period under dark conditions. Non-chilled plant and chilled plant were then watered and grown in controlled climate rooms at 15°C under dark conditions.

3.2.3 Experiment 3

Two and half year old asparagus plants 'Dariana' were grown in 20 liter plastic containers and then dried off to impose dormancy. Dead asparagus ferns were removed and plants were placed in the coolstore at 5°C for either 0, 3, or 6 weeks. After the specified chilling time, the pots were placed in growth cabinets at one of three temperatures (20°C, 25°C or 30°C) in dark conditions. For 'Dariana' plants, the experiment had a 3 x 3 factorial arrangement of treatment in a completely randomized design with three replications per treatment. The growing media was as described in Section 3.2.2. Measurements of spear height and RSGR were as described in Section 3.2.1.2 and Section 3.2.1.3, respectively.

3.2.4 Experiment 4

3.2.4.1 Plant material and experimental conditions

Five-year-old asparagus plants 'Apollo' were used. All ferns were removed and plants remained in the glasshouse. For about 2 hour each night for about seven days night temperature fell just below 10°C due to insufficient heating. After counting the buds on each crown, plants were re-potted into 30 liter plastic containers with a potting mix of bark-peat (17 ± 1 cm planting depth). The growing media is as described in Section 3.2.2.

Fifteen plants remained at 15°C under dark conditions (non-chilled control plants), 15 plants were placed in a coolstore at 5°C for 3 weeks and 15 plants were placed under these conditions for 6 weeks. After the specified chilling time, nine plants (three plants each for non-chilled, 3 weeks and 6 weeks chilling) were placed at one of five temperatures (10°C, 15°C, 20°C, 25°C, and 30°C) in darkness. The plants were irrigated by hand to initiate growth and then as needed to prevent water stress, but fertilisers were not supplied during the harvest period. Dataloggers (Micro Logger, Dallas Semiconductor, USA) were used to measure air temperature every half-hour throughout the experiment. Owing to temperature fluctuations in temperature controlled rooms, respective mean temperatures were 9.7 ± 0.6, 15.6 ± 0.7, 19.3 ± 0.9, 24.7 ± 0.5, and 30.3 ± 1.6.

3.2.4.2 Measurements of spear height, bud break and bud numbers

The number of days to bud break of the first bud per plant was recorded. Soil adhering to crown was removed using compressed air. The number of buds per cluster and the number of clusters per crown was counted before and after spear harvest. Buds were counted when their diameter was > 2mm. Bud positions and size within clusters were mapped for each crown. Measurements of spear height and RSGR were as described in Section 3.2.1.2 and Section 3.2.1.3, respectively.

3.2.4.3 Data analysis

The experiment was a 3 x 5 factorial in a completely randomized design with three replications per treatment. Data was analyzed using the general linear model procedure of SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). Mean separations were done by Duncan's multiple range test at $P \leq 0.05$.

3.2.5 Experiment 5

The experiment was undertaken at commercial planting of asparagus at Bulls, Manawatu Region, New Zealand. Asparagus plants 'Jersey Giant' were used. Soil adhering to crowns was removed carefully using a water jet. An 80 cm grid with 20 cm intervals was laid. Bud position and size were mapped with crowns within this area and number of buds counted. After buds were counted, crowns within this area (80 cm x 80 cm) were covered with perlite for easy removal in order to observe bud production later. Soil was then placed over the perlite to form a similar level to the whole block. Buds were mapped and number of buds counted before spear harvest (6th September 2002), after spear harvest (23rd December 2002) and in the following winter (6 July 2003). Number of buds, spears and cut spears were recorded. Ferns were counted during autumn (2nd May 2003).

3.3 Results

3.3.1 Experiment 1

At 15°C, two cultivars, 'Apollo' and 'Desto' produced 2 to 3 spears per plant, and only three to four spears per cultivar showed exponential growth. Therefore, this data were omitted from the analysis. For temperature from 20°C to 35°C growth was exponential with no significant interaction between temperature and cultivar. The main effect of temperature was highly significant ($P \leq 0.0001$). There was also evidence ($P \leq 0.05$) to indicate a significant difference in spear growth rate between cultivars (Figure 3.1).

With no winter chilling the optimum temperature for spear growth was around 27°C for 'Apollo' and 'Desto'. At 25°C and 30°C, there were no significant differences between cultivars, but at 20°C and 35°C 'Desto' had a significantly higher RSGR than 'Apollo' (Figure 3.1).

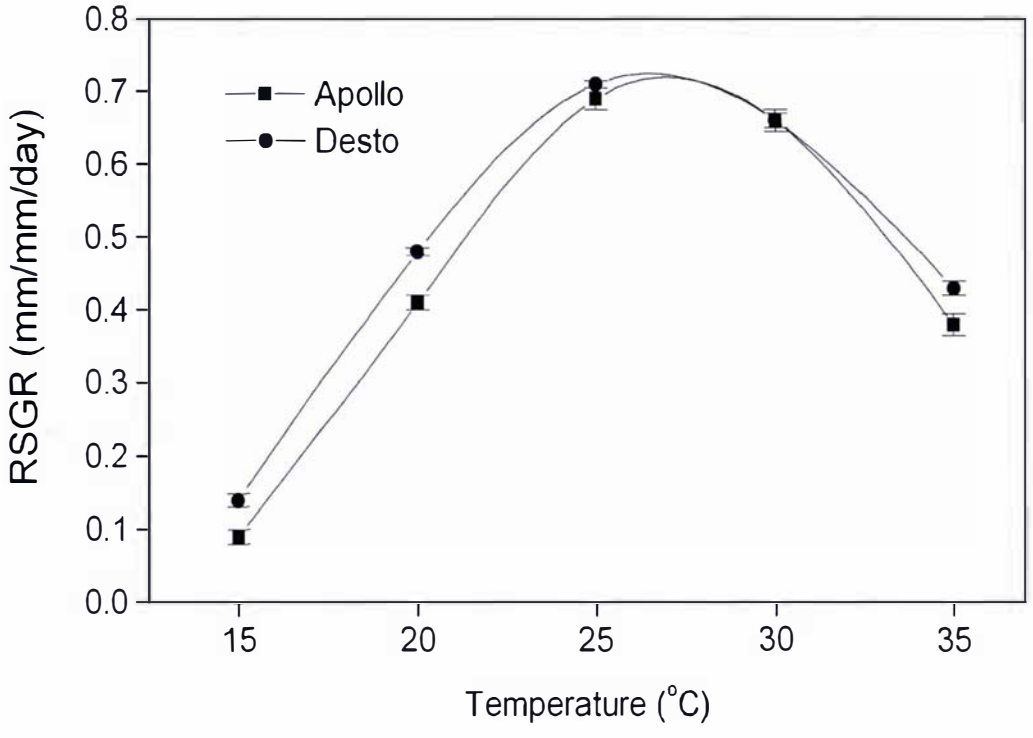

Figure 3.1 The effect of temperature on relative spear growth rate (RSGR) of 'Apollo' and 'Desto' cultivars. Bars represent the standard error of the mean.

At 20°C, 25°C, and 30°C, most of spears appeared to show exponential growth (74% to 80%) and linear growth (2% to 11%). However, at 35°C, 46.4% of the spears showed linear growth and 36.2% exponential growth (Table 3.1).

Table 3.1 The number and percentage of spear growth patterns of two asparagus cultivars at four temperatures.

Temperature (°C)	Cultivar	Linear		Exponential		Others[y]	
		Number	%	Number	%	Number	%
20	Apollo	1	7.6	6	46.2	6	46.2
	Desto	1	1.7	51	87.9	6	10.4
	Total	2	2.8	57	80.3	12	16.9
25	Apollo	2	8.3	17	70.8	5	20.9
	Desto	2	2.9	55	78.6	13	18.5
	Total	4	4.3	72	76.6	18	19.1
30	Apollo	2	4.3	42	89.4	3	6.3
	Desto	10	17.5	35	61.4	12	21.1
	Total	12	11.5	77	74.0	15	14.5
35	Apollo	8	38.1	8	38.1	5	23.8
	Desto	24	50.0	17	35.4	7	14.6
	Total	32	46.4	25	36.2	12	17.4

[y]Not clearly linear or exponential.

3.3.2 Experiment 2

The growth of spears for chilled plants was faster than those from non-chilled plants. At 15°C the growth rate of spears was linear for non-chilled plants but exponential for chilled plants (Figure 3.2).

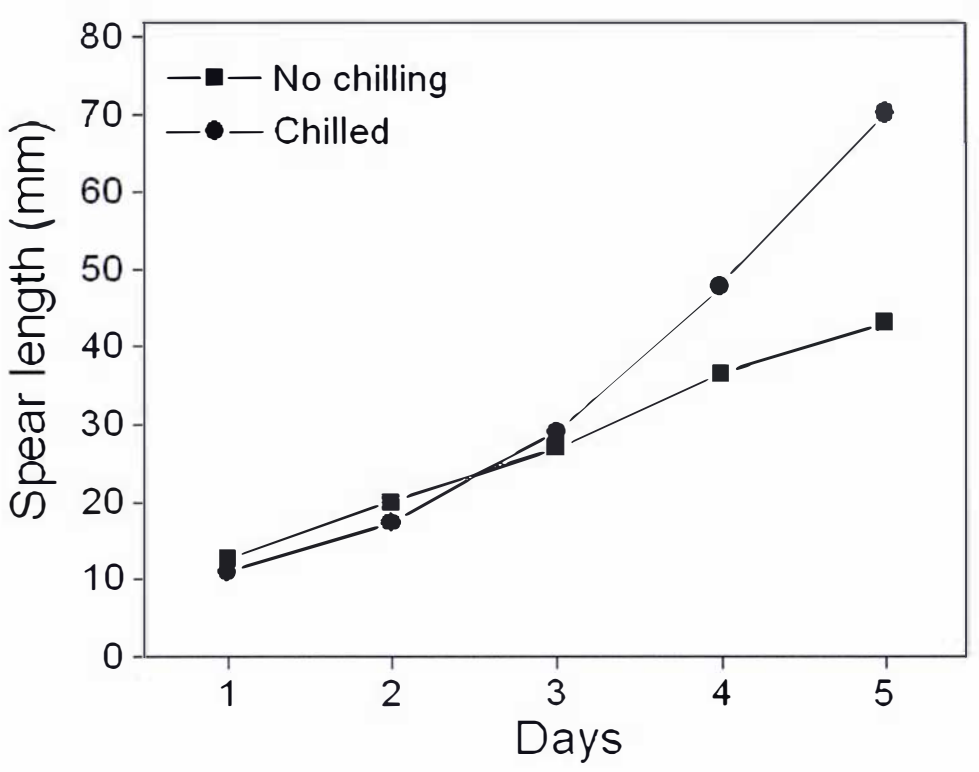

Figure 3.2 The effect of chilling on subsequent spear growth rates of 'Apollo' plants at 15°C. Plants were chilled in a coolstore at 5°C for two weeks and transferred to 15°C under dark conditions. Control plants remained at 15°C without chilling under dark condition.

3.3.3 Experiment 3

Increasing temperatures from 20°C to 30°C significantly decreased mean days to bud break (Figure 3.3). At 30°C, the six week chilling treatment had significantly slower bud break than those of the control plants and the 3 week chilling treatment, although the differences were small. Bud break commenced after 11 days at 20°C and 8 days at 25°C, but the variance was much greater at 20°C. At 30°C the break was 6 days with a very small variance. Chilling had no significant effect on the number of days to bud break on plants at 20°C and 25°C.

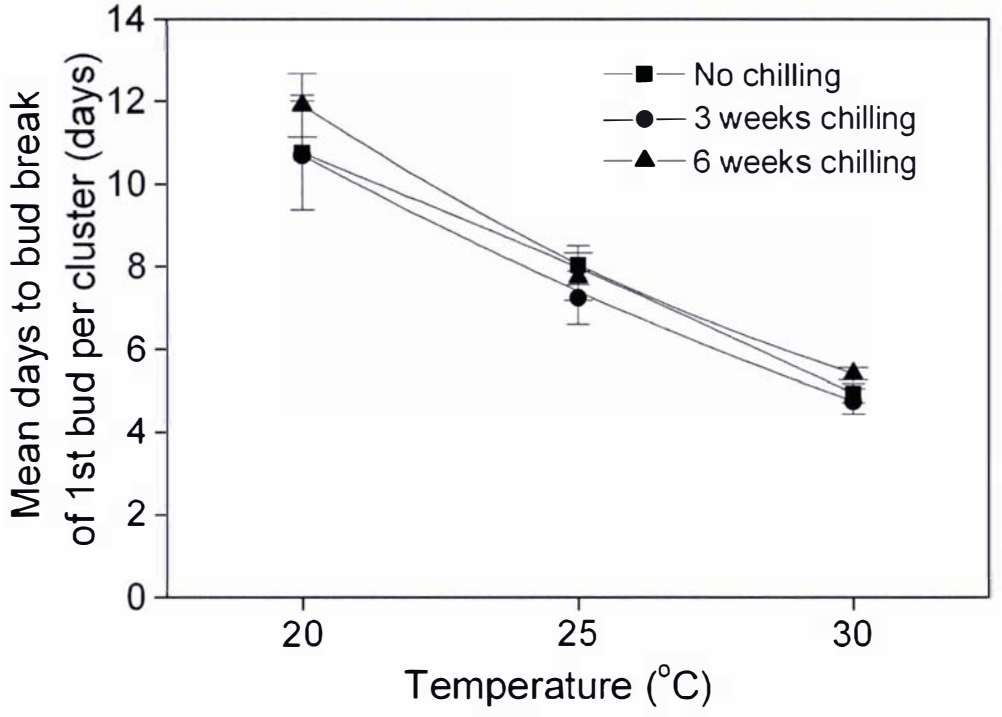

Figure 3.3 Relationship between growing temperature and chilling on mean days to bud break of 1st bud per cluster for 'Dariana' grown at 20°C, 25°C, and 30°C. Control: no chilling. Treated plants were chilled for either 3 or 6 weeks at 5°C. Bars represent the standard error of the mean.

The interaction between temperature and chilling treatments was not significant. Chilling had no significant effect on RSGR. However, the main effect of temperature was highly significant ($P \leq 0.001$). RSGR increased over temperature range 20°C to 30°C (Figure 3.4). Plants treated by withholding chilling, then grown at 30°C, had higher RSGR than plants exposed to either chilling treatments. At 30°C increasing the length of chilling to 6 weeks reduced the RSGR compared to 3 weeks chilling. Plants grown at 20 and 25°C showed no significant differences in RSGR between non-chilled plants and chilling treatments.

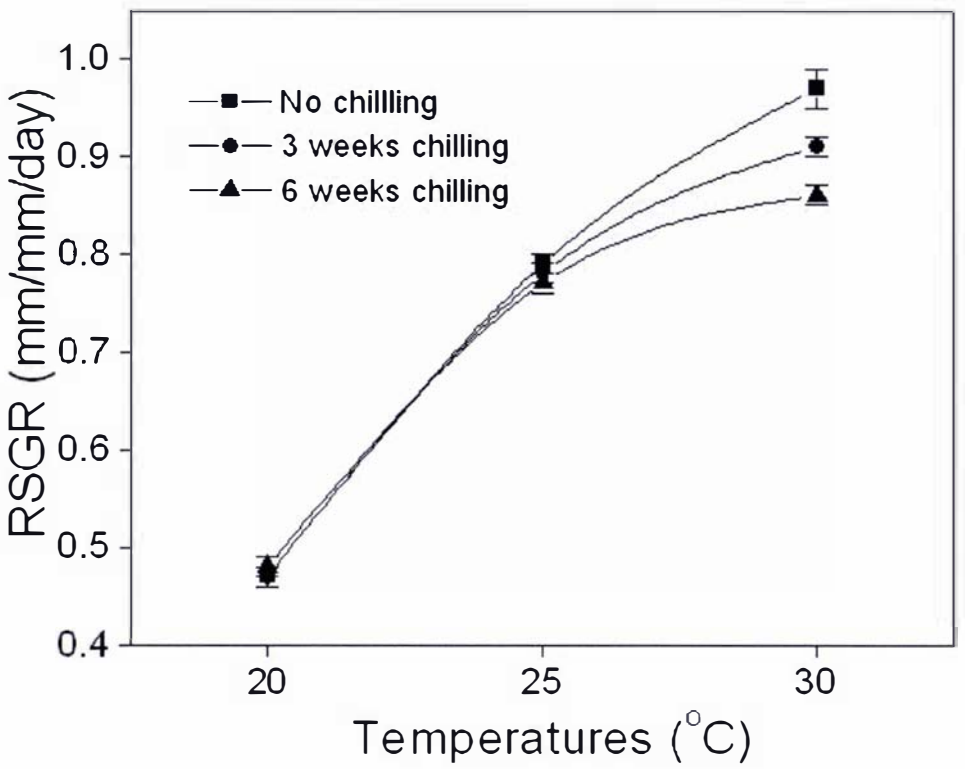

Figure 3.4 Effect of three temperatures and three chilling treatments on RSGR of 'Dariana' cultivar grown under dark condition. Control: no chilling, or plant chilled for 3 or 6 weeks at 5°C. Bars represent the standard error of the mean.

3.3.4 Experiment 4

Increasing temperatures from 10°C to 30°C decreased the mean numbers of days to bud break of 'Apollo' plants (Figure 3.5). Growing temperatures of 20°C and above resulted in bud break within 7 to 11 days but at temperatures of 15°C and below bud break took approximately one to two months. Prior chilling promoted bud break at 10°C and 15°C but had no effect above 20°C. Growing temperatures of 20°C appeared to represent a cross-over point at which chilling may have slightly increased days to bud break.

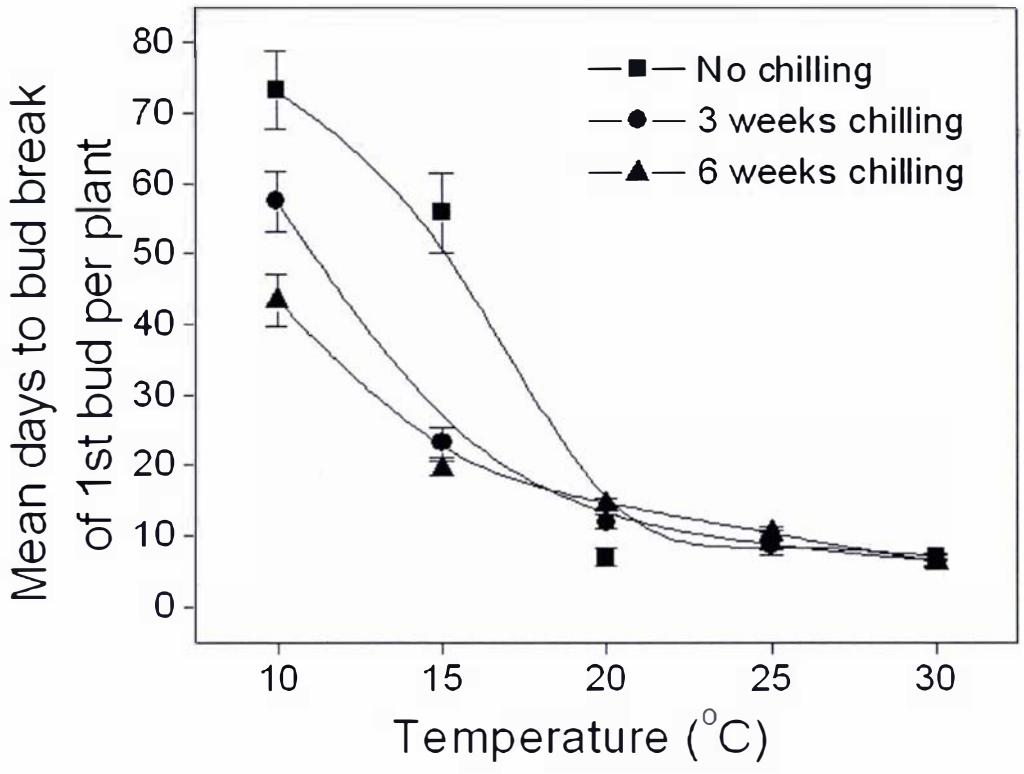

Figure 3.5 Effect of temperature and chilling on bud break of 'Apollo' plants. Each point is the mean of three plants. Bars represent the standard error of the mean.

The RSGR of each cluster per plant increased greatly, and almost linearly, with increasing temperature between 10°C and 30°C, irrespective of chilling treatment (Figure 3.6). The RSGR at 30°C was approximately two fold that at 20°C and nine fold that at 10°C. At 30°C, non-chilled plants had higher RSGRs compared to chilling treatment. However, chilling had no effect on subsequent spear growth at 25°C and below (Figure 3.6).

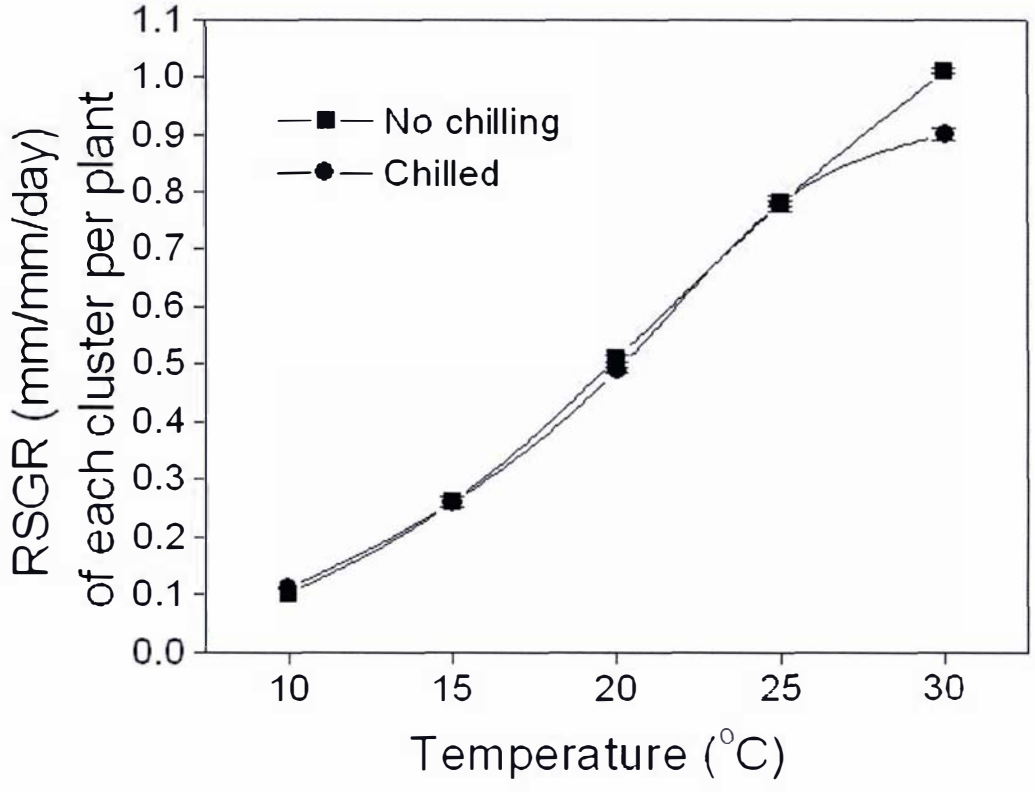

Figure 3.6 Effect of temperature and chilling pre-treatment at 5°C (3 and 6 week combined) on RSGR of 'Apollo' plants in the dark. Bars represent the standard error of the mean (within symbol).

The growth of spears between 20 and 30°C mainly showed exponential growth. At 15°C the most common growth curve was exponential (56.8%) and only 8.1% of the spears had constant linear growth rates (Table 3.2). However, at 10°C spears elongating linearly or exponentially showed a similar percentage, linear (20.7%) and exponential (20%). At 10°C there was shown two phases of exponential growth (47.4%), but at 15°C and above was indicated less than 25% (Table 3.2).

Table 3.2 Growth pattern of 'Apollo' spears grown at five temperatures and three chilling treatments.

Temperature (°C)	Chilling (weeks)	Total	Linear		Exponential		Two exponential [y]		Mix[x]	
			No.	%	No.	%	No.	%	No.	%
10	0	34	1	2.9	10	29.4	21	61.8	2	5.9
	3	44	11	25.0	1	2.3	26	59.1	6	13.6
	6	57	16	28.1	16	28.1	17	29.8	8	14.0
Total		135	28	20.7	27	20.0	64	47.4	16	11.7
15	0	17	3	17.6	8	47.0	5	29.4	1	5.8
	3	64	6	9.3	38	59.3	12	18.7	8	10.9
	6	79	4	5.0	45	56.9	21	25.3	9	11.3
Total		160	13	8.1	91	56.8	38	23.1	18	10.6
20	0	61	0	0.0	50	82.0	10	16.4	1	1.6
	3	75	0	0.0	56	74.7	16	21.3	3	4.0
	6	44	3	6.8	25	56.8	15	34.1	1	2.3
Total		180	3	1.7	131	72.8	41	22.8	5	2.8
25	0	54	0	0.0	44	81.5	7	13.0	3	5.6
	3	62	0	0.0	52	87.1	3	4.8	5	8.1
	6	81	0	0.0	81	85.2	8	9.9	4	4.9
Total		197	0	0.0	167	84.8	18	9.1	12	6.1
30	0	54	0	0.0	43	79.6	9	26.7	2	3.7
	3	56	1	1.8	45	80.4	7	12.5	3	5.4
	6	60	1	1.7	47	78.3	7	11.7	5	8.3
Total		170	2	1.2	135	79.4	23	13.5	10	5.9

[y] Two phases of exponential growth. [x] Not clearly linear or exponential.

New buds were formed during the harvest season. Buds produced during the harvest season could be distinguished by their white color from buds produced in the previous season, which were dark brown (Figure 3.7).

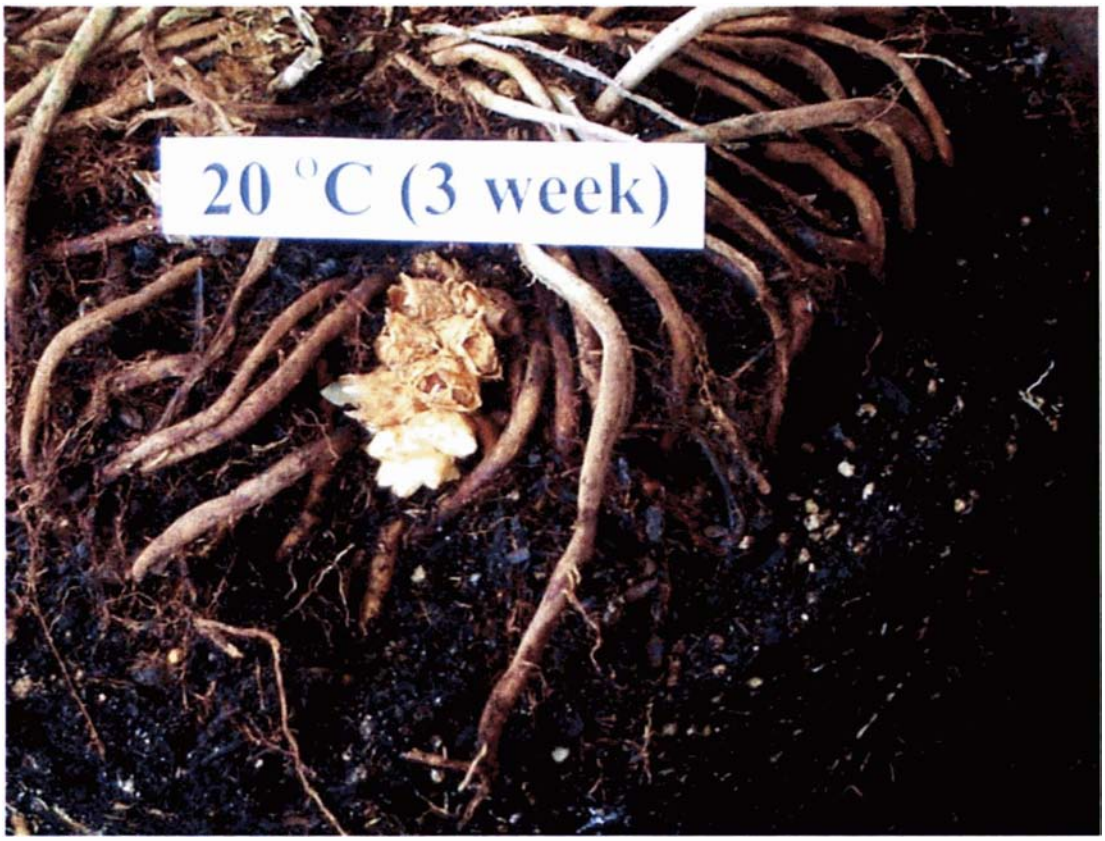

Figure 3.7 Asparagus plants cv. 'Apollo' incubated at 20°C after a 3 week chilling pre-treatment. Dark brown buds were developed in the previous season and white buds developed during the harvest period.

Some of the buds that developed during the harvest period grew into spears that could be harvested within the time period of the experiment (Figure 3.8).

Figure 3.8 "New" bud (cv. Apollo) producing a marketable spear. Large buds behind the spear, small buds in front.

The numbers of new buds per cluster were not significantly affected by growing temperature between 15 and 30°C and by chilling treatment (Table 3.3). At 10°C, the number of new buds per cluster decreased.

Table 3.3 The number of new buds (cv. Apollo) per cluster produced at five growing temperature regimes and three chilling treatments.

Chilling period (CP)	New bud numbers per cluster[z]					
	Temperature (°C) (T)					
	10	15	20	25	30	CP mean
Control	3.2	3.8	4.8	4.1	6.1	4.4a
3 weeks	3.1	5.0	3.5	4.5	4.4	4.1a
6 weeks	2.9	4.7	3.1	4.6	3.4	3.7a
T mean	3.1b	4.5a	3.8ab	4.4a	4.6a	
Significance[y]						
T		*				
CP		ns				
T x CP		ns				

[z]All values are mean of three to five clusters per plant.
[y]Treatment effects were not significant (ns) or significant at $P \leq 0.05$(*).
Within the column and row, means sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

3.3.5 Experiment 5

Spear harvest in the field produced an average of 140 spears per m^2. Approximately 51 new buds were produced during the spear harvest period. The total difference between bud numbers before and after spear harvest indicated the plant produced additional buds during the harvest period. However, 34% of old buds did not develop (Table 3.4).

Table 3.4 Comparison bud counts before and after spear harvest period of 'Jersey Giant' in the field. Old buds represent buds from previous season, new buds formed during the spear harvest.

Measurements	Old buds	Spear	Cut spear	Dead	Total	Differences* After-before	New buds
Before harvest	235 ± 25	26 ± 9	0	0	262 ± 31		
After harvest	84 ± 20	3 ± 1	140 ± 9	29 ± 12	307 ± 26	45 ± 6	51 ± 14

The area measured in the field was 0.64 m^2 but has been converted in the table to a per m^2 basis. * The assumption is made that all spears produced from old buds. ± represents the standard error of the mean.

3.4 Discussion

Low temperature (chilling) is the main environmental factor for bud dormancy release in many plants in temperate zones. The minimum temperature at which bud break occurs varies as plants progress through dormancy and shoot growth is both delayed and reduced if insufficient chilling is received (Vegis, 1964; McColl, 1986; Guo et al., 1995; Arora et al., 2003). Prior chilling markedly decreased mean time to bud break of 'Apollo' at 10°C and 15°C (Figure 3.5). Harvesting of a crop normally commences in early September in New Zealand when mean daily temperatures are below 15°C (Hughes, 1992), thus lack of chilling during a warmer winter could significantly delay bud break in spring. Chilling decreased the mean days to bud break of 'Rutgers Beacon' and 'Jersey Giant' at 12.5°C but had little effect on 'UC 157' (Hughes, 1992). At 10°C, bud break occurred earlier on plants chilled for 6 weeks than on those chilled for only 3 weeks (Figure 3.5).

With no prior chilling, the growth of spears at 15°C was slower than for chilled plants (Figure 3.2). The RSGR of the first spear of chilled plants at 12.5°C was higher than for non-chilled plants of 'Jersey Giant', but prior chilling of 'Rutgers Beacon' and 'UC 157' had no effect on RSGR (Hughes, 1992). RSGR of 'Dariana' (Figure 3.4) and 'Apollo' (Figure 3.6) were not affected by prior chilling, except for a decrease at 30°C. It should be noted that as RSGR is a relative, rather than absolute, growth rate, very small numerical differences can lead to large differences in the number of days a spear takes to reach harvestable size (18-23 cm).

These results suggest that endodormancy or paradormancy in asparagus have characteristics similar to the widely studied dormancy phenomena found in temperate, woody perennial plants (Lang et al., 1987). Delay in bud break is typical of plants in which dormancy has been incompletely broken (Guo et al., 1995). Typical examples are provided by studies on grapevines (McColl, 1986) and apple and peach trees (Arora et al., 2003). When chilling is inadequate, it would appear that asparagus has a form of dormancy in which growth at lower temperatures is restricted by lack of chilling, typical of the dormancy response highlighted by Vegis (1964). Thus in regions where growing temperatures are normally high no effect of lack of winter chilling would be

experienced (Figures 3.4 and 3.6), but cool springs following relatively warm winters could result in very slow initial spear growth rates (Hughes, 1992).

One of the problems of studying the effect of chilling on dormancy is that cool temperatures are often an inducer of dormancy, together with day length, but are also the main environmental release signal once the plant starts to accumulate chill units (Seeley, 1996; Allan, 2004; Warmund and Krumme, 2005). Thus in the present experiments cool temperature may have initially thrown buds deeper into dormancy, but subsequently stimulated breaking of dormancy. Thus the mechanisms that induce and release dormancy in some asparagus cultivars appear similar to those operating in many temperate perennial plants (Saure, 1985; Fuchigami and Nee, 1987; Heide, 1993; Dokoozlian, 1999; Petri and Leite, 2004; Heide and Prestrud, 2005) with an optimum chilling temperature around 5°C and a widening of the temperature range (Vegis, 1964) over which maximum growth for a given temperature will occur as the level of dormancy is reduced.

Temperature had a large effect on RSGR. The growth of asparagus spears increases with increasing temperature (Culpepper and Moon, 1939a; Blumenfield et al., 1961; Robb, 1984; Nichols and Woolley, 1985; Kim et al., 1989; Yen et al., 1996; Dean, 1999). The optimum temperature for spear growth was around 27°C for 'Apollo' and 'Desto' plants (Figure 3.1) and at 30°C for 'Dariana' and 'Apollo' plants (Figures 3.4 and 3.6). These results support those of Yen et al. (1996) in which 28°C was shown as the most favorable temperature for asparagus growth. The optimum temperature for spear growth was 25°C to 30°C (Robb, 1984) and 30°C (Dean, 1999), but below or above these temperatures plant growth was slow (Kim et al., 1989; Yen et al., 1996; Dean, 1999). Hence, 'Apollo' and 'Desto' cultivars represent a poor adaptation to high temperatures of 35°C. Yen et al. (1996) reported that 'Larac' represents a poor adaptation to the high temperatures of 36°C, while 'Brocks' and 'UC 157' are better adapted to high temperatures of 33°C and 36°C. From the present results 'Apollo' appeared to have a similar temperature response curve to 'Larac', rather than 'Brocks' and 'UC 157'. The cultivar 'Desto' growth rates were significantly higher than that of 'Apollo' at 20°C and 35°C and appears to be intermediate between cultivars adapted to

warmer growing temperatures such as 'Brocks' and those adapted to lower temperatures such as 'Larac'.

Nichols and Woolley (1985) reported that the growth of 'UC 157' spears from 10°C to 30°C appeared to be exponential, but Hughes (1992) found that spear growth of 'Rutgers Beacon' plants at 12.5°C did not always show an exponential curve. At 10°C, only 20% of the 'Apollo' spears showed exponential growth and 20.7% linear growth, with the remaining 59.3% showing an unclear growth pattern (Table 3.2). At 35°C, 46% of the 'Apollo' and 'Desto' spears showed linear growth and 36.2% exponential growth. However, from 15 to 30°C spears grew exponentially (Tables 3.1 and 3.2). Thus, at 10°C and 35°C, elongation rates of 'Apollo' and 'Desto' spears probably tended to be linear while between 15°C and 30°C spears grew exponentially, if not limited by some other factor or correlative inhibition by other spears (Nichols and Woolley, 1985; Daningsih, 2005). These results suggest that when plants are not limited by other resources such as carbohydrates in the crown, temperature strongly influences the pattern of spear growth, either linear or exponential.

Nichols and Woolley (1985) showed that there was a linear decrease in the relative growth rate of spears sequentially from different bud clusters, and this effect remained until the earlier emerging spears were removed. They suggested that when several spears from different bud clusters grew at the same time, they grew at a similar rate, but the growth of other spears on those crowns was severely inhibited. At 10 and 15°C, the growth of spears strongly decreased compared to higher temperatures (Figure 3.6). Thus, at lower temperatures the growth of both current spear and subsequent spear was slow. The length of the harvesting season varies with the age of the plants and the climate in which they are growing (Robb, 1984). Most New Zealand asparagus growers tend to harvest mid September to late December then establish vigorous fern growth to recharge the resources by fern photosynthetic activity and export of CH_2O to the root system (Findlay and Ryan, 1974). Thus, a slow growing spear at lower temperatures not only takes a long time to reach harvestable size, but also delays the initiation of growth of the next spear. This will subsequently affect final asparagus yield in a limited harvest period (McCormick and Geddes, 1996; Yen et al., 1996).

Asparagus yield depends on the amount of stored carbohydrate in the storage root and the number of buds on the crown. Haynes (1987) and Robb (1984) reported that bud initiation and formation mostly occurred after spear harvest. Tiedjens (1926) reported that few buds are formed during harvest season while Wilson et al (1999b) demonstrated that a few buds may appear toward the end of the harvest period. However, in an other study, between 20 and 30 percent of the buds produced in a normal commercial planting near Bulls (New Zealand) were formed during the harvest period (Woolley et al., 2004; Daningsih, 2005) and in the present work also (white) buds were produced during the harvest period (Figure 3.7). New bud formation during spear harvest was 3 to 4 buds more per cluster at higher temperatures. In field plantings, new buds were initiated during the spear harvest period (Table 3.4). Mean new buds formed average 51 buds / m^2. However, 48% of old buds remained dormant or died. Small buds produced on the crown may contribute to yield in the current harvest season or continue growing to become moderately mature fern, while the remaining buds will contribute to the following seasons yield (Figure 3.8; Table 3.4). Prior chilling had no significant effect on bud production (Table 3.3).

The present study indicated that production of visible buds (new bud production) was not influenced by temperatures between 15°C to 30°C, but decreased at 10°C (Table 3.3). It is possible that bud development was directly inhibited at 10°C compared to 15°C and above. Wilson et al. (1999b) reported that asparagus yield depends on the availability of carbohydrates in the storage roots during spear harvest but Woolley et al. (2006) have shown that under some circumstances, the number of buds may limit yield. Thus as well as slowing spear growth temperatures below 15°C may limit the production of new buds, and subsequent yield. These results confirm that visible new buds are produced during the harvest season as well as during fern establishment.

3.5 Summary

In temperate climates, asparagus plants grown in the field undergo a period of dormancy over the winter months. Increasing temperatures from 10°C to 30°C decreased the mean number of days to bud break of 'Apollo' plants. Prior chilling at 5° C for 3 or 6 weeks decreased the mean days to bud break of 'Apollo' plants at 10°C. Extending chilling length to 6 weeks decreased mean days to bud break 'Apollo' at 10° C. Thus, asparagus plants tend to require chilling at 5°C for about 500 to 1000 hours.

RSGR increased with increasing temperature from 10°C to 30°C. Prior chilling had no effect on RSGR, except for a decrease at 30°C. Prior chilling did not have an effect on subsequent spear growth rates, possibly because temperatures fell below 10°C for one or two hours per day for a week, due to insufficient heating, therefore, all plants received some chilling prior to the experiment (Section 3.3.4; Figure 3.6). The relative maximum spear growth rate was achieved at 30°C with non chilled plants for 'Apollo' and 'Dariana' plants. Thus, growing temperatures are important factors for spear growth, affecting RSGR, but the effect may be modified by the amount of prior chilling.

At 10 and 35 °C, spears appeared to show linear pattern of growth while between 15 and 30°C spears displayed an exponential pattern of growth. Chilling had no effect on bud production, but temperature had a significant effect on bud production. New bud numbers were not affected by temperatures (between 15 and 30°C). At 10°C new bud numbers decreased. Thus as well as slowing spear growth temperatures below 15°C may limit the production of new buds. Also in open field experiment new buds were produced about 50 buds/ m^2. These results confirm that new bud production starts to form during the spear harvest as well as fern growth and establishment.

CHAPTER 4

EFFECT OF CPPU TREATMENTS ON ASPARAGUS GROWTH AND DEVELOPMENT

Abstract

This research investigates the effect of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) application to foliage (foliar spray and foliar dip), soil drench and roots (crown soaking) on fern numbers, fern height, axillary bud break, cladophyll length and diameter, root number and bud production. CPPU applied as a foliar spray, foliar dip and root soak decreased fern numbers, but drenching asparagus crowns with CPPU at 10 mg L^{-1} was effective in stimulating fern numbers of asparagus seedlings. CPPU as a foliar spray or dip stimulated the height of initial ferns, but decreased the height of subsequent ferns. Root applied at 10 mg L^{-1} decreased both initial and subsequent fern heights. All CPPU treatments significantly enhanced axillary bud break and stimulated axillary shoot length compared to the control. CPPU was effective in promoting cladophyll diameter and length but did not influence the ratio of root mass: crown bud numbers and asparagus yield in the subsequent harvest season.

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Response of Asparagus (*Asparagus officinalis*) growth to *N*-(2-Chloro-4-Pyridyl)-*N*-Phenylurea application.

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4.1 Introduction

Bud break on the bud cluster of asparagus (*Asparagus officinalis* L.) is strongly controlled by apical dominance — defined as correlative inhibition by (Nichols and Woolley, 1985). Applying plant growth regulators may stimulate fern production during the growing season by releasing correlative inhibition. Tiburcio (1961) found that gibberellic acid (GA_3) drenches at 10 mg L^{-1} increased the number of new ferns and promoted the rate of spear growth, suggesting that GA_3 may reduce correlative inhibition. Mahotiere (1976) found that dipping the crowns in 750 to 1000 mg L^{-1} of (2-chloroethyl) phosphonic acid (ethephon) stimulated fern numbers per plant. Dikegulac-sodium (Atrinal) applied as a foliar spray (300-500 mg L^{-1}) and as crown soaking (300 mg L^{-1}) was also found to promote the emergence of asparagus ferns (Mahotiere et al., 1988; Mahotiere et al., 1989).

Applying benzyladenine (BA) directly to the foliage of asparagus plants promoted spear shoot emergence in three asparagus cultivars (Mahotiere et al., 1993). Uesugi et al. (1995) also found that BA as a foliar spray in autumn promoted additional spear production in cv 'Welcome'. Although BA as a foliar spray promoted fern production, a crown soak at 200 mg L^{-1} did not promote fern numbers (Mahotiere et al., 1993). It is therefore clear that the effect of plant growth regulators that influence correlative inhibition is dependant on the site of application.

It is well known that the synthetic cytokinin compound, CPPU, has strong growth regulator activity. CPPU was reported to positively improve plant growth of several crops including apple (Greene, 1989; Sugiyama et al., 1993), coffee (Cruz-Castillo, 1998), kiwifruit (Lawes et al., 1992), and azaleas (Fellman et al., 1987). Cytokinins have been reported to release apical dominance and these chemicals can stimulate the growth of lateral buds in several crops (Bell et al., 1997). Exogenous BA and CPPU applied to explants in tissue culture stimulated the axillary bud break in roses (Kapchina-Toteva et al., 2000; Singh and Syamal, 2001), and pineapple (Adaniya et al., 2004). Kapchina-Toteva and Stoyanova (2003) reported that the application of cytokinins (kinetin and CPPU) stimulated bud break in *Gypsophila* stems in tissue culture when the medium contained 1.0 μM CPPU.

Increasing cladophyll diameter and specific leaf weight are potential important determinants of photosynthesis, subsequently enhancing yield performance in the harvest season. Photosynthetic capacity was positively with correlated specific leaf weight (SLW) and cladophyll diameter (Bai and Kelly, 1999; Faville et al., 1999; Guo et al., 2002). CPPU applied as a foliar spray to developing fern can promote cladode size (Woolley, D. J, unpublished data). It is hypothesized that CPPU as a foliar spray and foliar dip may alter cladophyll development so that thicker and longer cladodes are produced. The impacts of applying CPPU to asparagus as a foliar spray, foliar dip, soil drench or as a crown soak has not previously been reported. The purpose of this research, therefore, was to investigate the effects of CPPU, as a foliar spray, foliar dip, soil drench, or crown soaking, on asparagus fern numbers, fern height, axillary bud break, cladophyll length and diameter, and bud production.

4.2 Materials and methods

4.2.1 Experiment 1

Asparagus officinalis L. 'Jersey Giant' seeds were germinated and grown in plastic cell trays (45 ml volume) containing a sterile medium composed of vermiculite. Four-week-old seedlings were transplanted into plastic bags (12 cm diameter x 22 cm high) containing a bark-peat-pumice mix (1:1:1), agricultural lime (100g / 100L), dolomite (300g / 100L), and slow release osmocote (16 N-3.5 P-10.8 K-1.2 Mg-0.02 B-0.05 Cu-0.4 Fe-0.06 Mn-0.02 Mo-0.15 Zn) (200g of long term and 100g of short term: 300g / 100L). The seedlings were grown for two months in a glasshouse and 126 uniform seedlings selected. The 42 seedlings of each spray treatment (0, 10 or 20 mg L^{-1}) were sprayed to runoff with CPPU (Sitofex EC 2.5%; SKW, Trostberg, Germany). Control plants were sprayed with tap water. Half the plants in each spray treatment were root drenched with the aqueous solutions of 10 mg L^{-1} CPPU (200 ml / pot).

The treatments were thus as follows:

1. Control + 0 mg L^{-1} foliar spray
2. Control + 10 mg L^{-1} foliar spray
3. Control + 20 mg L^{-1} foliar spray
4. Root drenching at 10 mg L^{-1} (200 ml / pot) + 0 mg L^{-1} foliar spray
5. Root drenching at 10 mg L^{-1} (200 ml / pot) + 10 mg L^{-1} foliar spray
6. Root drenching at 10 mg L^{-1} (200 ml / pot) + 20 mg L^{-1} foliar spray

The pots were placed on glasshouse benches. The experiment was a randomized complete block design with a factorial arrangement of three foliar sprays and two root drenching CPPU treatments with the three blocks with twenty one plants per treatment. The glasshouse was ventilated at 25°C during the day and heated to 17°C at night. The seedlings received natural radiation and were irrigated as required.

4.2.2 Experiment 2

Four-month-old 'Jersey Giant' seedlings were used for the experiment. Fourteen seedlings of uniform size with four ferns were selected. The roots of 7 of the seedlings were soaked for one and half hours in an aqueous solution of CPPU at 10 mg L^{-1}. Control plants were soaked with tap water. Asparagus seedlings were then repotted and transferred to a glasshouse. Plant culture, glasshouse temperature and water irrigation were as described in Section 4.2.1. The experiment was a completely randomized design and each treatment comprised seven plants.

4.2.3 Experiment 3

This experiment was designed to further evaluate the effect of foliar spray (applied to one fern), foliar dip and crown soaking treatments on fern numbers, lateral bud production, cladophyll length and diameter, and crown morphology. Seeds of asparagus 'Jersey Giant' were germinated on a heated bench in glasshouse, heated to 16°C and fan ventilated at 25°C. Four-week-old seedlings of uniform size with one fern were

transplanted into plastic bags (12 cm diameter x 22 cm high). The CPPU treatments were as follows:

1. Control: seedling sprayed with tap water only
2. Foliar spray: seedlings sprayed to runoff with CPPU at a concentration of 10 mg L^{-1} (≈ 6ml per each plant)
3. Foliar dip: ferns immersed in 10 mg L^{-1} CPPU aqueous solution for five minutes
4. Crown soak: seedling crowns soaked for two hours in aqueous solution of CPPU at 10 mg L^{-1}

After soaking the crowns, seedlings were repotted. The pots were placed on glasshouse benches and arranged in a randomized complete block design consisting of four blocks with four plants of each treatment per block. The growing media, glasshouse temperature and water irrigation were as described in Section 4.2.1.

4.2.4 Measurements of plant growth

Before the application of CPPU treatments, initial fern height and fern numbers were obtained. Fern numbers were recorded weekly after CPPU treatment. The fern present at the time of CPPU application were denoted "initial fern" and ferns formed subsequent to CPPU treatment were defined as "subsequent fern". Fern height of both initial and subsequent fern was measured weekly from the potting mix surface to tip using a ruler. Axillary shoot numbers were recorded. The tallest axillary shoot length was selected and measured. Stem diameter of asparagus fern was measured at 2.5 cm above the ground using digital calipers (Mitutoyo, Digmatic). At harvest, in addition, fern fresh weight and dry weight were also obtained for the fern present at the time of CPPU application (initial ferns). Ferns formed subsequent to treatments were also measured for fern height, and stem diameter (subsequent ferns). The fresh weight of these ferns was obtained. Crowns were thoroughly washed with mild water pressure and number of buds, bud clusters and storage roots were counted. The fresh weight of crowns and ferns was obtained and then oven dried at 60°C to a constant dry weight.

4.2.5 Measurements of cladophyll length and width

Cladophyll length (L) was determined, and the diameter (D) of the middle part of the cladophyll measured with a calibrated eye-piece micrometer in a compound microscope (OLYMPUS, Tokyo) eight weeks after CPPU treatment. In addition, cladode surface area was estimated, using the method of Guo et al. (2002) in which surface area was calculated according to the equation:

$$\text{Area} = \sum_{n} (L \times D \times \pi)$$

where L is the length of the cladodes, D is cladode diameter at the mid point and n is the number of cladodes used.

4.2.6 Data analysis

Data were collected and analyzed using the procedure GLM of SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). For Experiments 1 (Section 4.2.1) and 3 (Section 4.2.3) mean separations were done by Duncan's multiple range at $P \leq 0.05$. For Experiment 2 (Section 4.2.2), mean separations were done by TTEST (Section 4.2.2) at $P \leq 0.05$.

4.3 Results

4.3.1 Experiment 1

Up to 8 weeks after CPPU treatment, fern numbers of CPPU-treated plants were significantly less than those of control plants (Table 4.1). However from 9 weeks onwards, control plants and plants that had been treated with 10 mg L^{-1} CPPU were not significantly different for fern numbers. Compared to control plants, at six weeks after treatment, CPPU foliar spray at 10 and 20 mg L^{-1} decreased the fern numbers by 12% and 18%, respectively. There was no significant interaction between foliar sprays and drench treatments.

Drenching asparagus crowns with CPPU at 10 mg L^{-1} was effective in stimulating fern numbers of 'Jersey Giant' asparagus seedlings from 3 to 7 weeks after treatment (Table 4.1). CPPU drench had increased the fern numbers by 11% over controls 6 weeks after treatment, but the effect was only 6% and not significant by week 10.

Table 4.1 Effect of CPPU foliar sprays and drench on fern production of 'Jersey Giant' plants.

	Initial shoot (no.)	Fern numbers per plant Weeks after treatments										
		1	2	3	4	5	6	7	8	9	10	12
Spray (mg liter^{-1})												
0	4.0[z]a	5.1a	5.6a	6.6a	7.5a	8.2a	9.0a	9.8a	10.3a	11.0a	11.7a	12.4a
10	4.0a	4.5b	4.9b	5.4b	6.3b	7.3b	7.9b	8.9b	9.5b	10.4ab	11.2ab	12.2a
20	4.0a	4.6b	4.9b	5.3b	6.0b	6.7c	7.4b	8.3b	9.0b	9.7b	10.5b	11.5a
Drenching (200ml/pot)												
Plus	4.0[y]a	4.7a	5.2a	6.1a	6.9a	7.7a	8.6a	9.5a	10.0a	10.7a	11.5a	12.4a
Minus	4.0a	4.7a	5.0a	5.5b	6.3b	7.0b	7.7b	8.5b	9.2b	10.0b	10.8a	11.7a
ANOVA[x]												
Spray	ns	**	**	***	***	**	**	*	*	*	ns	ns
Drenching	ns	ns	ns	*	*	*	**	*	ns	ns	ns	ns
S x D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[z]Each value is mean of forty two plants per each spray treatment. [y]Each value is mean of sixty three plants per root drenching treatment. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$. [x]Treatment effects were not significant (ns) or significant at the $P \leq 0.05$ (*), or $P \leq 0.01$ (**), or $P \leq 0.001$ (***) levels

A CPPU spray was effective in increasing initial fern height, but with no additional response to the CPPU drench (Figure 4.1). The responses to 10 and 20 mg L^{-1} CPPU were similar.

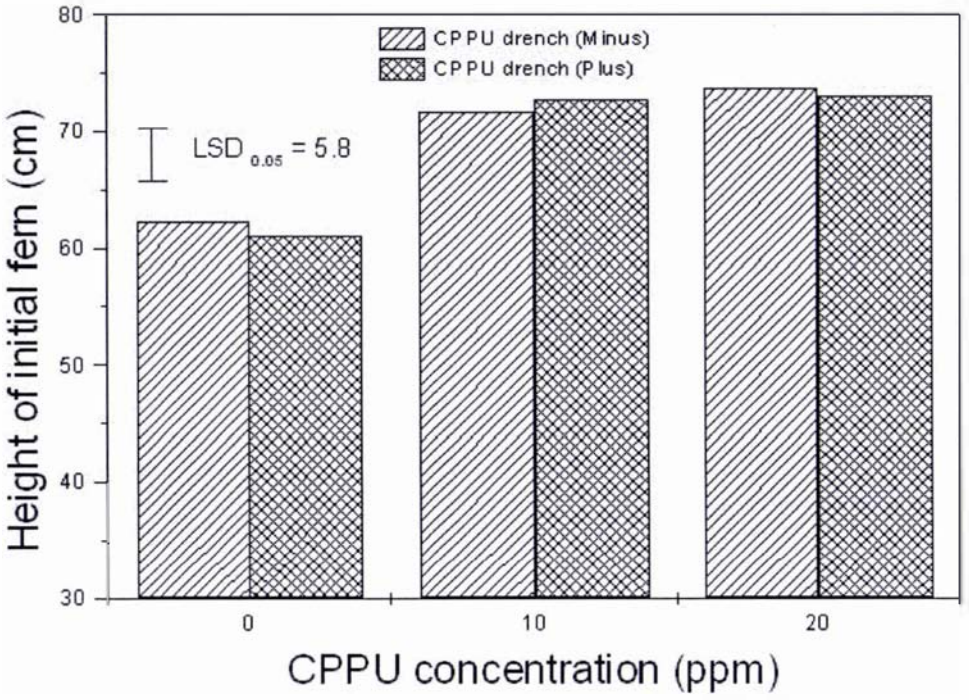

Figure 4.1 Effect of foliar spray and drench of CPPU on height of initial ferns that were initially CPPU-treated at week 12. Vertical bar represents the LSD at $P \leq 0.05$.

In contrast to the effect of CPPU on the initial fern, fern formed subsequent to a CPPU spray were reduced in height, and drenching produced a further decrease (Figure 4.2).

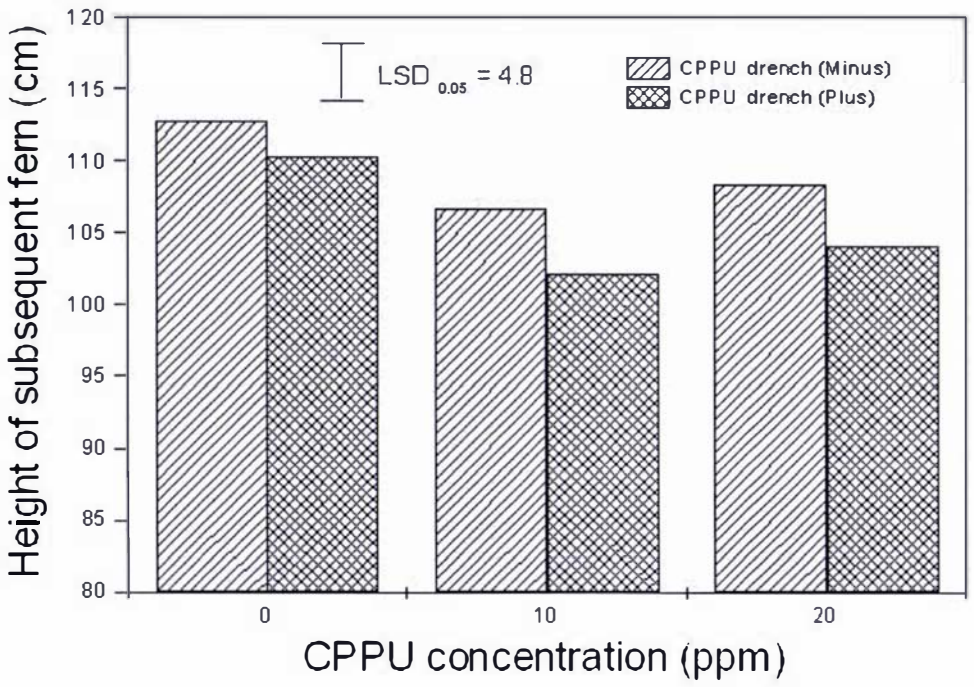

Figure 4.2 Effect of foliar spray and drench of CPPU on the longest height of subsequent ferns at week 12. Vertical bar represents the LSD at $P \leq 0.05$.

CPPU sprays were effective in stimulating initial stem diameter measurements, but ferns formed subsequent to a CPPU spray were reduced in stem diameter at 20 mg L^{-1} but not 10 mg L^{-1} (Table 4.2). CPPU drench did not affect stem diameter (data not shown).

Table 4.2 Effect of CPPU sprays on stem diameter of 'Jersey Giant' plants at week 12.

CPPU concentrations (mg L^{-1})	Stem diameter measurements (mm)[y]	
	Initial ferns	Subsequent ferns
0	1.09b	3.73a
10	1.19a	3.46ab
20	1.21a	3.32b

[y]All values are mean of forty two plants per treatment. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Applying CPPU directly to asparagus ferns produced significantly more axillary shoots. Control plants produced few axillary shoots. The application of CPPU to foliage appeared to overcome apical dominance within 2 weeks after CPPU treatment (Figure 4.3). CPPU drench treatment did not affect lateral shoot numbers. Axillary buds were not stimulated on subsequent ferns.

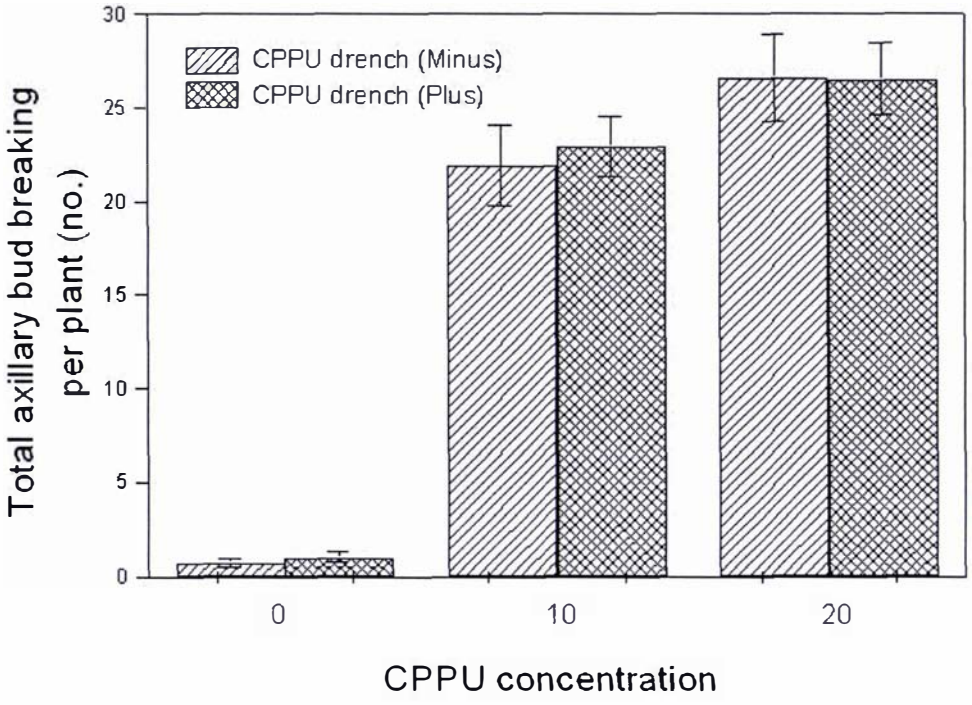

Figure 4.3 Effect of CPPU foliar spray and CPPU drench on lateral bud breaking of 'Jersey Giant' plants 2 weeks after treatments. Bar represents the standard error of mean.

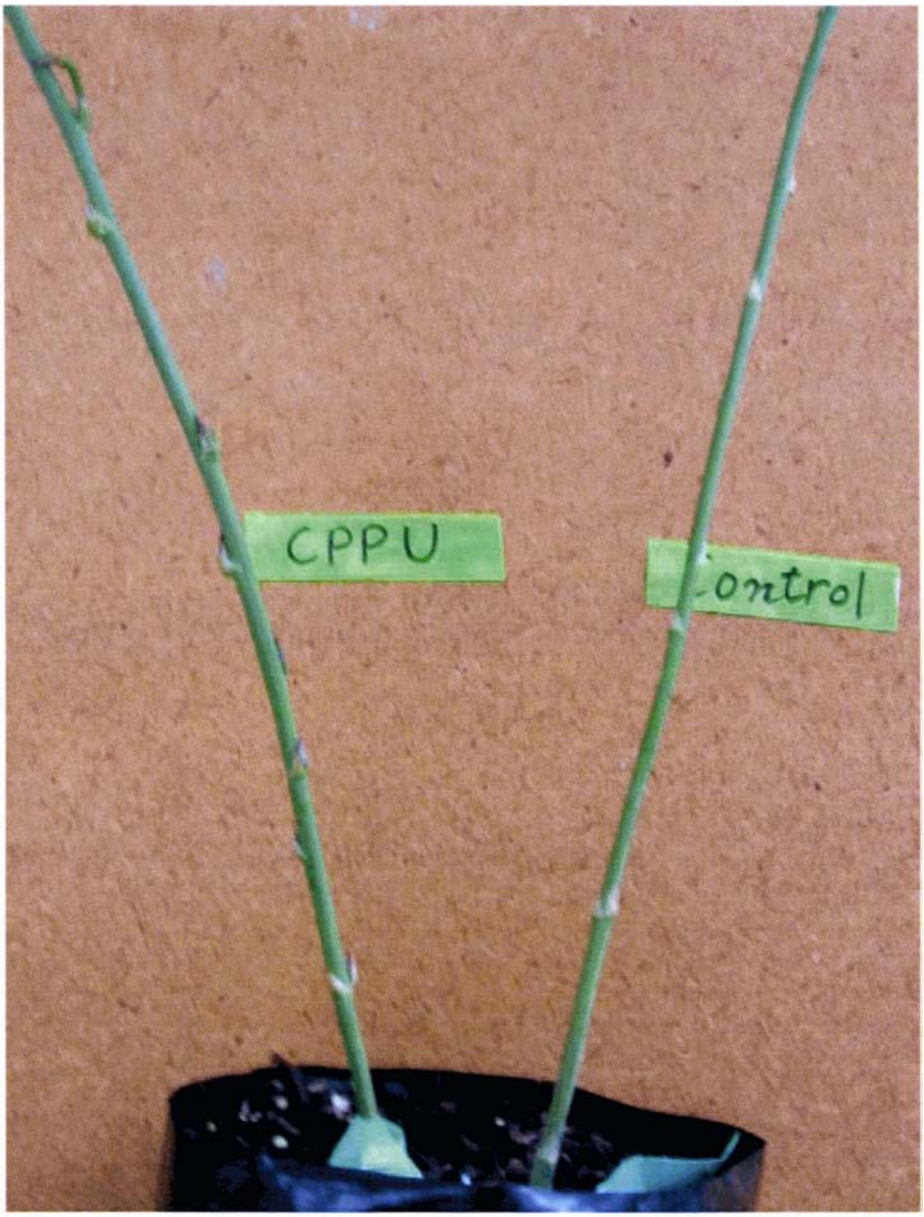

Figure 4.4 Effect of CPPU spray on basal axillary bud break of 'Jersey Giant' plants.

At harvest, fresh and dry fern weights of the CPPU sprayed plants were significantly higher than those of controls (Table 4.3). However, root fresh and dry weights of the CPPU-treated plants at 20 mg L^{-1} were significantly less than those of control plants. The CPPU sprayed plants had a significantly decreased root to shoot ratio compared to control plants, but the crown bud number was not affected. CPPU soil drench significantly promoted fern fresh weight but decreased dry weight. CPPU soil drench did not significantly affect root fresh and dry weights, crown bud numbers and root to shoot ratio (Table 4.3). There was no significant interactions between CPPU spray and drench treatments.

Table 4.3 Effect of CPPU application to young asparagus on growth parameters emergence of asparagus seedlings at week 12.

	Fresh wt (g)		Dry wt (g)		Total dry wt (g)	Bud (no.)	Root (no.)	Root: shoot ratio
	Shoot	Root	Shoot	Root				
Spray (mg liter^{-1})								
0	70.9b	164.1a	18.0b	41.6a	59.62a	24.0a	79.4a	2.3a
10	78.4a	151.8ab	20.1a	37.6ab	57.82a	24.3a	74.2ab	1.8b
20	77.7a	148.2b	19.5a	36.2b	55.86a	23.5a	67.8b	1.8b
Drenching (200ml/pot)								
Plus	78.5a	156.9a	18.5b	37.9a	56.4a	23.9a	75.1a	2.0a
Minus	72.8b	152.5a	19.8a	39.1a	58.9a	24.0a	72.5a	1.9a
ANOVA[x]								
Spray	*	*	*	*	ns	ns	*	*
Drenching	*	ns	*	ns	ns	ns	ns	ns
S x D	ns	ns	ns	ns	ns	ns	ns	ns

Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$. [x]Treatment effects were not significant (ns) or significant at the $P \leq 0.05$ (*) level.

4.3.2 Experiment 2

Soaking asparagus crowns in 10 mg L^{-1} CPPU significantly decreased fern numbers per plant from 5 weeks after CPPU treatment (Table 4.4). By eight weeks CPPU-treated plants had produced only 4.5 new ferns compared to 7.5 for the controls.

Table 4.4 Effect of CPPU soaking of asparagus crowns on fern numbers of 'Jersey Giant' plants.

Treatments	Initial fern (no.)	Fern numbers per plant[z]							
		Days after CPPU treatment							
		7	14	21	28	35	42	49	56
Control	4.3a	5.6a	6.1a	7.0a	8.4a	9.0a	9.4a	11.3a	11.9a
CPPU	4.6a	5.4a	5.9a	7.0a	7.6a	8.0b	8.0b	8.6b	9.1b

[z]All values are the mean of seven plants per treatment. Means within columns sharing the same letter are not significantly different by *t* test at $P \leq 0.05$.

Fern height was significantly suppressed by CPPU soaking treatment. From 2 to 5 weeks after the CPPU soaking treatment, the elongation of ferns with CPPU-treated of asparagus crowns was severely depressed compared to control plants (Table 4.5). Ferns of the control plants emerged earlier and grew faster than those of CPPU-treated plants. By six weeks after CPPU treatment, there was no significant effect on fern height and the effect of CPPU was no longer significant.

Table 4.5 Effect of CPPU soaking of asparagus crowns on length of the longest fern of 'Jersey Giant' plants.

Treatments	Initial fern ht	Fern height per plant (cm)[z]							
		Days after CPPU treatment							
		7	14	21	28	35	42	49	56
Control	49.6a	59.4a	66.4a	76.0a	86.4a	93.1a	104.0a	101.7a	105.1a
CPPU	49.1a	53.2a	56.9b	59.7b	68.4b	78.7b	88.4a	92.7a	94.7a

[z]All values are the mean of seven plants per treatment. Means within columns sharing the same letter are not significantly different by *t* test at $P \leq 0.05$.

Soaking crowns in 10 mg L^{-1} CPPU markedly increased the release of both lower and upper axillary buds compared to the controls (Figure 4.5A). The axillary shoot length of the CPPU-treated plants was significantly higher than those of control plants (Figure 4.5B).

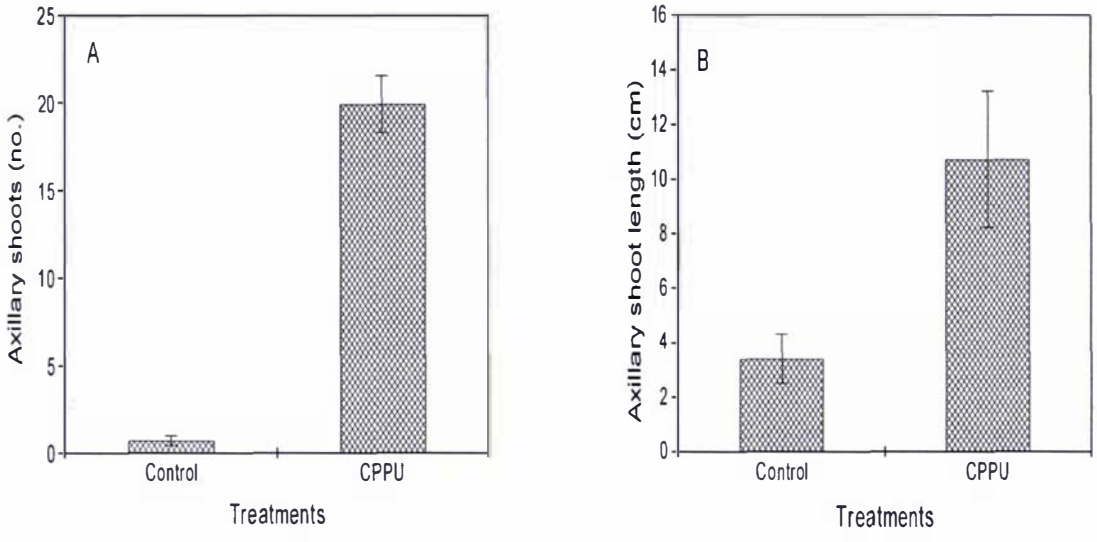

Figure 4.5 Effect of CPPU soaking of asparagus crowns on number of axillary buds growing (A) and axillary shoot lengths (B) at week 3. Vertical bars represent the standard error of means (n = 7).

Stem diameter of both initial ferns and subsequent ferns was not significantly affected by the CPPU treatment. Numbers of buds, total buds (buds + ferns), clusters and roots were not significantly affected by the CPPU treatment (Table 4.6) even though root mass was reduced (Table 4.7).

Table 4.6 Effect of CPPU soaking of asparagus crowns on stem diameter, bud number, fern+bud number, cluster number and root number.

	Stem dia[z](mm)		Bud	Ferns+ buds	Cluster	Root
	Initial	Subsequent	(no)	(no)	(no)	(no)
Control	1.3a	2.4a	14.7a	26.6a	2.8a	42.4a
CPPU	1.4a	2.2a	14.6a	23.7a	2.8a	38.1a

[z]All values are the mean of seven plants per treatment. Means within columns sharing the same letter are not significantly different by *t* test at $P \leq 0.05$.

Fresh and dry masses of the initial ferns of CPPU-treated plants were significantly higher ($P \leq 0.05$) than those of control plants (Table 4.7). In contrast, fresh and dry masses of the subsequent ferns of CPPU-treated plants were lower than those of control plants. CPPU increased the fresh and dry masses of initial ferns by 32% and 29%, but decreased the fresh and dry masses of subsequent ferns by 49% and 51% compared to control plants. CPPU decreased root fresh and dry masses by 35% and 39%, respectively. Total dry weight of the CPPU-treated plants was decreased by 38 % compared to the controls.

Table 4.7 Effect of CPPU soaking of asparagus crown on fresh mass, dry mass, total dry weight and root to shoot ratio over a 2 month growth period.

	Fresh mass[z] (g)			Dry mass (g)			Total dry wt (g)	Root: shoot ratio
	Shoot		Root	Shoot		Root		
	Initial	Subsequent		Initial	Subsequent			
Control	6.3b	34.9a	51.3a	1.5b	8.0a	8.5a	18.0a	0.89a
CPPU	9.3a	17.8b	33.2b	2.1a	3.9b	5.2b	11.2b	0.87a

[z]All values are the mean of seven plants per treatment. Means within columns sharing the same letter are not significantly different by *t* test at $P \leq 0.05$.

4.3.3 Experiment 3

Control and foliar CPPU-treated plants had similar fern numbers throughout the growing period (Table 4.8), except at two weeks when the second fern emerged slightly faster in the control. Soaking crowns in 10 mg L^{-1} CPPU significantly decreased fern numbers per plant from 2 weeks after CPPU treatment (Table 4.8).

Table 4.8 Effect of CPPU foliar spray, foliar dip and crown soak on fern numbers of 'Jersey Giant' plants.

Treatments	Initial fern (no.)	Fern numbers per plant[z] Days after CPPU treatment									
		7	14	21	28	35	42	49	56	63	70
Control	1.0	1.0	2.0a	2.5a	3.0a	3.4a	3.9a	4.4a	4.9a	5.3a	5.8ab
Foliar spray	1.0	1.0	1.4b	2.2a	2.5a	3.3a	4.0a	4.3a	5.1a	5.5a	6.3a
Foliar dip	1.0	1.0	1.4b	2.1a	2.5a	2.9a	3.6a	4.0a	4.8a	5.2ab	6.0ab
Crown soak	1.0	1.0	1.3b	1.6b	1.6b	2.1b	2.5b	2.9b	3.6b	4.0b	4.9b

[z]All values are mean of sixteen plants. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

CPPU as a foliar spray and foliar dip significantly improved the growth of initial ferns 2 weeks after CPPU treatment (Table 4.9). From week 3 onwards, fern height in the foliar dip treatment significantly increased compared to other treatments. However, soaking crowns in 10 mg L^{-1} CPPU significantly decreased height of initial ferns compared to other treatments, for the first four weeks.

Table 4.9 Effect of CPPU foliar spray, foliar dip and crown soak on the height of initial ferns of 'Jersey Giant' plants.

Treatments	Initial fern height (cm)	Fern height per plant[z] Days after CPPU treatment			
		7	14	21	28
Control	15.6	17.9a	22.4b	22.9c	22.9c
Foliar spray	15.5	18.0a	24.8a	25.8b	25.8b
Foliar dip	16.0	18.0a	24.2ab	27.9a	27.9a
Crown soak	15.3	16.5b	18.0c	18.6d	18.6d

[z]All values are mean of sixteen plants. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

For the second to third week after CPPU treatment, all CPPU treatments significantly decreased the height of the subsequent ferns compared to untreated controls (Table 4.10) but this effect was lost after five weeks for the foliar treatments. Soaking crowns in 10 mg L^{-1} CPPU significantly decreased the height of subsequent ferns for the duration of the experiment.

Table 4.10 Effect of CPPU foliar spray, foliar dip and crown soak on the height of the subsequent ferns of 'Jersey Giant' plants.

Treatments	Height of the tallest fern per plant[z]									
	Days after CPPU treatment									
	7	14	21	28	35	42	49	56	63	70
Control	0	9.2a	24.1a	28.9a	31.0a	32.5a	33.3a	34.5a	37.4ab	40.0bc
Foliar spray	0	1.8b	13.5b	25.6ab	29.8a	32.6a	33.8a	35.8a	42.2a	45.0a
Foliar dip	0	3.7b	12.2b	20.3b	25.4a	30.5a	30.3a	35.9a	39.9ab	42.8ab
Crown soak	0	0.6c	4.5c	8.8c	12.0c	17.6b	17.6b	27.4b	34.6b	38.2c

[z]All values are mean of sixteen plants. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

CPPU as a foliar spray, foliar dip and crown soaking stimulated both lower and upper axillary bud burst. All CPPU treatments had a similar effect on axillary shoot numbers except the first week after CPPU treatment (Table 4.11). The axillary shoots of the control plants remained dormant for the duration of the experiment as illustrated in Table 4.11. Even though the three different CPPU treatments were not significantly different, soaking crown treatment may have produced slightly more axillary shoot numbers than the other treatments 3–5 weeks after treatment.

Table 4.11 Effect of CPPU foliar spray, foliar dip and crown soak on axillary shoot numbers of the initial fern.

Treatments	Axillary shoots per plant[z]				
	Days after CPPU treatments				
	7	14	21	28	35
Control	0.0b	0.0b	0.0b	0.0b	0.0b
Foliar spray	0.3ab	5.1a	6.4a	7.4a	7.4a
Foliar dip	0.6a	5.0a	8.2a	8.2a	8.2a
Crown soak	0.0b	3.3a	8.3a	10.2a	10.2a

[z]All values are mean of sixteen plants. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Only small differences in axillary shoot growth between CPPU treatments were observed (Table 4.12) although bud break in the soaking treatment was delayed.

Table 4.12 Effect of CPPU foliar spray, foliar dip and crown soak on axillary shoot length of the initial fern.

Treatments	Axillary shoot length per plant (cm)[z]				
	Days after CPPU treatments				
	7	14	21	28	35
Control	0	0.0b	0.0c	0.0b	0.0b
Foliar spray	0	0.06a	2.7b	6.6a	8.9a
Foliar dip	0	0.04ab	3.6a	6.0a	8.0a
Crown soak	0	0.0b	2.0b	5.7a	7.3a

[z]All values are mean of sixteen plants. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Cladophyll length and diameter in both foliar CPPU treatments were significantly greater than untreated plants but effects in the soaking treatment were small (Table 4.13). Cladode area, specific leaf weight and cladophyll fresh and dry weights of foliar treated plants were significantly greater than that of the crown soaked or control plants.

Table 4.13 Effect of three CPPU treatments on length, diameter, leaf area, fresh and dry weight of cladodes, and specific leaf weight (SLW mg cm^{-2}) of initial fern. Measurements were determined eight weeks after the three CPPU applications.

Treatments	Length[z] (mm)	Diameter (μm)	Cladode area (cm^2) x 10^{-3}	Fresh wt (mg)	Dry wt (mg)	SLW (mg cm^{-2})
Control	21.6b	274.4c	18.8b	1.0d	0.34b	18.5b
Foliar spray	36.0a	381.3a	43.3a	3.4b	1.10a	25.3a
Foliar dip	36.3a	396.9a	45.4a	3.9a	1.26a	27.4a
Crown soak	19.8b	302.5b	18.8b	1.3c	0.41b	22.5ab

[z] Each value is the means of twenty cladodes per treatment. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Stem diameters after 70 days in the foliar spray and dip treatments were significantly higher than those of untreated controls and crown soaking. Primary branch numbers were decreased by the crown soaking treatment and primary branch length was stimulated by the foliar spray (Table 4.14). Crown soaking decreased crown bud numbers, total bud numbers (fern+buds+spears) and root numbers, while foliar spraying increased cluster numbers but not total bud numbers.

Table 4.14 Effect of three CPPU treatments on stem diameter, number of primary branches of subsequent, and crown buds, total buds, clusters and storage roots of the crown 70 days after treatment.

Treatments	Stem dia[z] (mm)	Primary branches		Crown buds (no.)	Total buds (Fern+buds +spears)	Bud clusters (no.)	Root (no.)
		Number	Length				
Control	1.6b	28.1a	20.7bc	26.6a	37.2a	3.6b	52.3a
Foliar spray	1.9a	29.6a	23.6a	27.3a	37.5a	4.5a	56.0a
Foliar dip	1.9a	29.4a	22.3ab	26.3a	36.4a	4.3a	50.4a
Crown soak	1.6b	24.6b	20.0c	17.8b	27.8b	3.2c	37.0b

[z]Each value is the means of twelve plants per treatment. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

CPPU as a foliar spray and foliar dip significantly increased the fern fresh and dry weights, particularly foliar dip (Table 4.15). In contrast, soaking crown severely inhibited the production of ferns. Soaking the crowns also decreased rhizome and root fresh weight. Shoot to root ratio significantly increased in the crown soaking treatment, indicating that crown soaking treatment inhibited roots more than shoots. Shoot to root ratio of CPPU foliar spray and foliar dip were also higher than untreated controls but total dry weight was similar.

Table 4.15 Effect of CPPU foliar spray, dip and treatment of crowns on fresh weight, dry weight, total dry weight, and shoot to root ratio of 'Jersey Giant' plants 70 days after treatment..

Treatments	Fresh wt[z] (g)			Dry wt (g)			Total dry wt (g)	Root: shoot ratio
	Fern	Rhizome	Root	Fern	Rhizome	Root		
Control	17.4c	2.6a	69.7a	4.9b	0.55a	18.9a	24.5a	3.79a
Foliar spray	22.1b	2.9a	77.3a	6.4a	0.62a	20.0a	27.0a	3.11b
Foliar dip	24.6a	2.7a	76.0a	7.0a	0.58a	20.5a	28.1a	2.93bc
Crown soak	13.6d	1.9b	38.2b	3.8c	0.41b	9.8b	14.0b	2.58c

[z] Each value is the means of sixteen plants per treatment. Means within columns sharing the same letter are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

4.4 Discussion

Applying CPPU (10 or 20 mg L^{-1}) to foliage reduced the number of ferns from one to eight weeks (Table 4.1). However, drenching asparagus crowns with CPPU at 10 mg L^{-1} promoted fern emergence from weeks 3 to 7 but then the difference in fern number decreased (Table 4.1). Soil drenching with CPPU promoted fern emergence, suggesting that CPPU may be taken up slowly over a long period of time. Root applied CPPU at 10 mg L^{-1} had no significant difference until 5 weeks and from 5 weeks onwards CPPU-treated plants reduced fern numbers (Table 4.4). Crown soak in 10 mg L^{-1} CPPU significantly decreased fern numbers from two weeks after CPPU treatment but the effect started to be lost by 70 days (Table 4.8). Direct exposure of asparagus crowns to CPPU solution may have resulted in high level of uptake resulting in supra-optimal concentrations of CPPU in the crown. Therefore, inhibition of fern number by CPPU crown soak may be consistent with supra-optimal cytokinin concentration.

CPPU as a foliar spray and foliar dip significantly improved the growth of initial ferns (Figure 4.1; Table 4.9). However, for the second to third week after treatment, foliar applied CPPU significantly decreased the height of the subsequent ferns compared to untreated controls (Table 4.10), but this effect disappeared after five weeks. An increase in fern height could be due to increased cell elongation or increased cell number due to increased meristem activity. CPPU may have increased height of the initial fern by stimulating cell elongation. For subsequent fern inhibition of apical meristem activity would lead to shorter fern and also stimulation of axillary shoot growth by loss of apical dominance.

CPPU sprays increased stem diameter measurements initially, but stem diameter of subsequent ferns was reduced (Tables 4.2 and 4.14). This result agrees with research by Cruz-Castillo (1998) who observed that stem diameter of the CPPU-treated 'Red Caturra' coffee plants was higher than those of the control. However, root applied CPPU had no effect on stem diameter both initial and subsequent ferns (Table 4.6).

All CPPU treatments stimulated axillary shoot numbers and length in asparagus (Figures 4.3, 4.4 and 4.5; Tables 4.11 and 4.12) as has been found for BA and CPPU in rose (*Rosa hybrida* L. cvs. 'Madelon' and 'Motrea') (Kapchina-Toteva et al., 2000).

Although CPPU stimulated the release of axillary buds shoot growth ceased after 35 days (Table 4.12). That is apical dominance was reduced by CPPU but growth of the axillary shoot eventually became inhibited.

It appears that if CPPU stimulated the release of axillary buds but not continued growth. That is apical dominance was reduced by CPPU but continual growth of the axillary shoot did not occur. CPPU is generally transported slowly or is immobile within plants (Woolley et al., 1992; Biasi et al., 1993). If CPPU was poorly transported from root to axillary shoot buds, then the stimulation of axillary buds must be indirect. Indirect effects could be due to a mobile metabolite of CPPU, or stimulation of cytokinin production or other hormones by the roots (Bangerth, 1994; Koukourikou-Petridou and Bangerth, 1997; Walch-Liu et al., 2000).

When CPPU was applied to asparagus foliage, physiological features such as bud break were enhanced. The lower eight axillary buds (about 25 cm at the ground level), that normally do not grow were stimulated by foliar application of CPPU even though extension growth of the fern was stimulated (Figure 4.4). In this case, CPPU application directly to the bud was releasing the bud from apical dominance. CPPU as a spray thus has potential in releasing axillary buds of asparagus seedlings.

Cladophyll length and diameter, and SLW in both foliar CPPU treatments were significantly greater than untreated plants but effects for the soaking treatment were small (Table 4.13). CPPU applied as a foliar spray or foliar dip was effective in producing longer and thicker cladodes. Cladophyll diameter and specific leaf mass may correlate with photosynthetic rate, subsequent influencing yield performance in the harvest season (Bai and Kelly, 1999; Faville et al., 1999; Guo et al., 2002). CPPU-treated plants increased cladophyll length and diameter, and specific leaf weight, suggesting these may increase photosynthesis and asparagus yield.

Crown application of 10 mg L^{-1} CPPU significantly decreased the number of crown buds, roots and total buds (ferns + buds + spears) for 1 month old seedlings but not three month old seedlings (Table 4.14). It is clear that CPPU-treated plants threshold for bud production varies with plant age. CPPU applied to young seedlings (1 month old seedlings) significantly decreased bud numbers, but older seedlings were not

affected. CPPU applied as a crown soak at 10 mg L^{-1}, especially to young seedlings was inhibitory to bud production. Care would be required in the use of CPPU for asparagus production as bud number may be an important factor limiting yield (Woolley et al., 2006). However, crown bud number, root number, and root dry weight were not influenced by foliar application of CPPU (Tables 4.14 and 4.15). The ratio of root dry mass:crown bud numbers were 0.71, 0.73, 0.77, 0.55 for control, foliar spray, foliar dip or root applied respectively (Section 4.3.3). CPPU foliar treatments did not influence the ratio of root dry mass:crown bud number. Root to shoot ratio of plants subjected to foliar applications of CPPU were also lower than untreated controls but total dry weight was similar (Tables 4.14 and 4.15). Thus fern growth was increased without a corresponding increase in root dry weight. More work is required on the hormonal regulation of root: shoot and root: bud ratios. It is clear that these ratios can be affected by CPPU but to increase yields root:fern ratios and bud: root ratios need to be increased rather than decreased as the size of the soluble carbohydrate pool stored in the roots (Wilson et al., 1999b) and the ratio of bud numbers to root dry mass (Woolley et al., 2006) are major determinants of yield.

4.5 Summary

CPPU as a foliar spray ($\geq$ 10 mg L^{-1}) and crown soak decreased fern numbers, but drenching asparagus crowns with CPPU at 10 mg L^{-1} was effective in stimulating fern numbers of asparagus seedlings. CPPU as a foliar spray and foliar dip stimulated the height of initial ferns, but decreased the height of subsequent ferns. All CPPU-treated plants stimulated axillary bud break and shoot length. CPPU was effective in promoting cladophyll diameter and length, features often associated with increased photosynthetic rates and yields. CPPU has the possibility that this may increase photosynthetic rate and thus yield. In particular, it would appear that the effect of CPPU was transient and repeated applications would be required to maintain on effect longer.

CHAPTER 5

EFFECT OF CPPU TREATMENT ON FERN NUMBER, BUD NUMBER, AND PHOTOSYNTHETIC RATE

Abstract

The effects of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) application to foliage and root on fern numbers, bud numbers, cladophyll morphology and photosynthetic rates were observed. Fern numbers and total buds showed a maximum response to CPPU as a foliar spray at 5 mg L^{-1}. Combined root and foliar applied CPPU severely inhibited the production of buds. CPPU as a foliar spray at 5 and 10 mg L^{-1} was effective in producing longer and thicker cladodes, features often associated with increased photosynthetic rates and yields. However, photosynthetic rates were unaffected by CPPU foliar spray. 'Jersey Giant' had a higher photosynthetic rate than 'UC 157'.

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Effect of CPPU Treatment on Fern Number, Bud Number, and Photosynthetic Rate.

Yang Gyu. Ku, D. J. Woolley and M. A. Nichols

5.1 Introduction

Bud break and subsequent spear growth are strongly controlled by apical dominance (Kretschmer and Hartmann, 1979). Apical dominance is defined as being inhibition of lateral buds by the apical region (Cline, 1997) or young shoot tissues (Hosokawa et al., 1990). The growing spear on the crown inhibited the sprouting of adjacent buds until the spear was removed (Tiedjens, 1926). Kretschmer and Hartmann (1979) referred to this as apical dominance but it has been defined as correlative inhibition by Nichols and Woolley (1985). In asparagus, unlike other plants, the spear is repressing the adjacent buds above (Daningsih, 2005). Therefore, in asparagus, correlative inhibition is a more relevant term than apical dominance.

Correlative inhibition can be released by using plant growth regulators. Foliar sprays of benzyladenine (BA) ranging from 100 to 400 mg L^{-1} in 100 mg L^{-1} increments promoted shoot emergence in three asparagus cultivars, Mary Washington, UC 157 F_1 and UC 157 F_2 (Mahotiere et al., 1993). Uesugi et al. (1995) similarly reported that a single BA foliar spray in the autumn promoted additional spear production in cv 'Welcome'. Tiburcio (1961) found that gibberellic acid (GA_3) drenches at 10 mg L^{-1} improved the number, diameter, weight, and length of new ferns. Thus it may be possible to enhance bud break during the harvest season by using plant growth regulators.

Asparagus is a C_3 plant, and photosynthesis occurs mainly in the cladodes, which constitutes the bulk of foliar portion of the fern (Downton and Torokfalvy, 1975; Faville et al., 1999; Guo et al., 2002). Generally, the assimilate produced in the cladodes is translocated into the storage root during fern growth and is subsequently remobilized during spear production and vegetative growth. The objectives of this experiment were to determine the effect of CPPU application to foliage and root on fern numbers, bud numbers, cladophyll morphology and photosynthetic rates.

5.2 Materials and methods

5.2.1 Experiment 1

Asparagus seeds 'Jersey Giant' were germinated and grown in plastic cell trays containing a sterile medium composed of vermiculite. Thirty-six seedlings of uniform size with one fern were selected. The roots of 18 of the seedlings were soaked for two hours in an aqueous solution of CPPU (Sitofex EC 2.5%; SKW, Trostberg, Germany) at a concentration of 10 mg L^{-1}, and the other 18 treated with tap water as controls. All five-week-old seedlings were then transferred into plastic bags (12 cm diameter x 22 cm high) filled with bark-peat-pumice mix (1:1:1) mixed media with agricultural lime (100g / 100L), dolomite (300g / 100L), and osmocote (16 N-3.5 P-10.8 K) (200g of long term and 100g of short term: 300g / 100L). Three days after the CPPU crown treatment, the fern of 6 seedlings in each root application treatment were sprayed to runoff with an aqueous solution of CPPU at either 0, 5, or 10 mg L^{-1} ($\approx$ 10 ml per each plant). The CPPU treatments were thus as follows:

1. Control + 0 mg L^{-1} foliar spray
2. Control + 5 mg L^{-1} foliar spray
3. Control + 10 mg L^{-1} foliar spray
4. Root application at 10 mg L^{-1} + 0 mg L^{-1} foliar spray
5. Root application at 10 mg L^{-1} + 5 mg L^{-1} foliar spray
6. Root application at 10 mg L^{-1} + 10 mg L^{-1} foliar spray

Thus the experiment was a completely randomized design (CRD) with a factorial arrangement of three foliar sprays and two root applied CPPU treatments, with six plants per treatment. The glasshouse was ventilated at 25°C during the day and heated to 17°C at night. The seedlings received natural radiation and were irrigated as needed.

Two weeks after CPPU treatment, the two largest cladodes per plant were selected. Cladophyll length (L) was determined, and the diameter (D) of the middle part of the cladophyll was measured with a calibrated eye-piece micrometer in a compound microscope (OLYMPUS, Tokyo). Fern numbers were recorded weekly after CPPU

treatment. Crowns were thoroughly washed with mild water pressure and crown bud numbers were counted.

5.2.2 Experiment 2

Five-week-old seedlings 'Jersey Giant' and 'UC 157' were transplanted into plastic bags (13 cm diameter x 27 cm high). The growing media was as described in Section 5.2.1. Four-month-old seedlings of two cultivars were sprayed to runoff with CPPU 10 mg L^{-1}. Control plants were sprayed with tap water. Twenty-four days after the CPPU foliar spray, photosynthetic rates (Pn, μmol g^{-1} s^{-1}) were measured using a portable photosynthesis system (CIRAS-2, PP Systems, USA). Measurements were made on fully expanded lateral branches of mature ferns between 12:00 and 14:00 hours local time. The Pn rate was expressed per unit of cladodes dry weight because of the difficulty of measuring cladode surface area (Woolley et al., 1999).

5.3 Results

5.3.1 Experiment 1

Foliar applied CPPU at five mg L^{-1} stimulated fern production ($P \leq 0.001$). Root applied CPPU severely restricted fern production, particularly if CPPU had also been applied as a foliar spray (Figure 5.1).

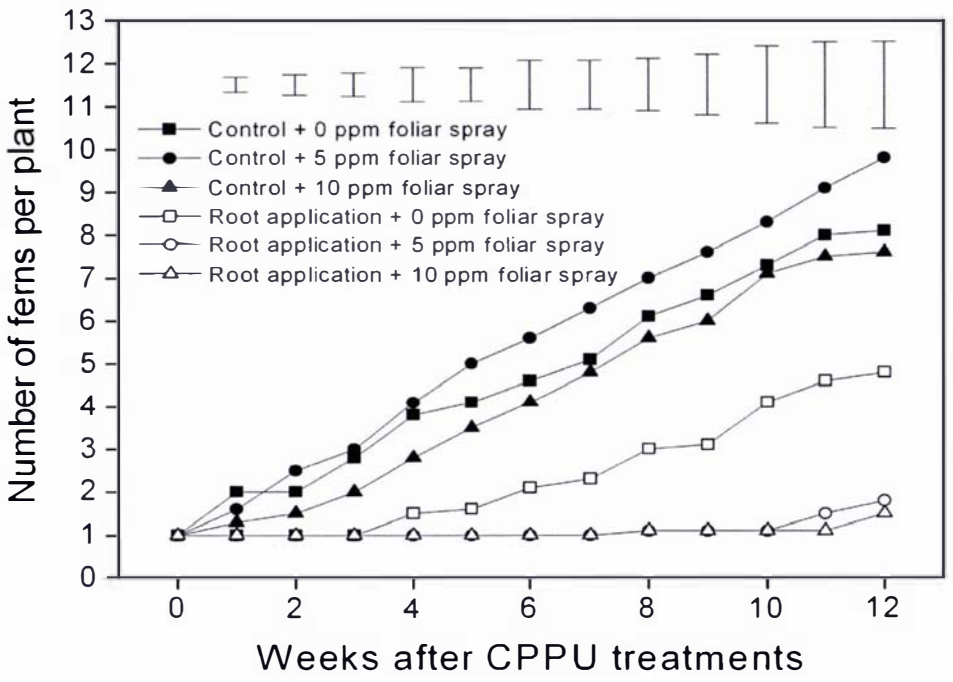

Figure 5.1 Effect of foliar applied and root applied CPPU at 10 mg L^{-1} on fern numbers over 12 weeks. Each point is means of six plants. Vertical bars represent the LSD at $P \leq 0.05$.

CPPU as a foliar spray at 5, and 10 mg L^{-1} alone significantly increased length, diameter, fresh and dry weight of cladodes compared to control plants (Table 5.1) but root applied CPPU had no significant effect.

Table 5.1 Effect of foliar applied and root applied CPPU at 10 mg L^{-1} on length, diameter, fresh weight, and dry weight of cladodes 2 weeks after CPPU treatment.

Treatment		Cladodes[z]			
Root soak ($mg\ L^{-1}$)	Foliar spray ($mg\ L^{-1}$)	Length (mm)	Diameter (μm)	Fresh weight (mg)	Dry weight (mg)
0	0	20.58b	277.5b	0.9b	0.30b
0	5	34.33a	365.8a	2.9a	0.82a
0	10	34.25a	388.3a	3.3a	0.89a
10	0	23.00b	275.0b	1.1b	0.35b
10	5	23.50b	249.1b	0.8b	0.26b
10	10	22.75b	257.5b	0.8b	0.26b

[z] Each value is the mean of twelve cladodes per treatment.
Means sharing the same letter in a column are not significantly different by Duncan's multiple range test at $P \leq 0.05$.

Total buds (ferns + spears + crown buds on the crown) were significantly increased by CPPU as a foliar spray at 5 mg L^{-1} without root applied CPPU (Figure 5.2). Buds on the crown of the 5 mg L^{-1} CPPU-treated plants without root applied CPPU were significantly higher than those of other treatments (Figure 5.2). Control plants and 10 mg L^{-1} CPPU foliar spray, without root-applied CPPU, did not significantly affect total bud numbers and buds on the crown. However, root applied CPPU alone or combined root and foliar applied CPPU significantly decreased the initiation and production of buds on the crown.

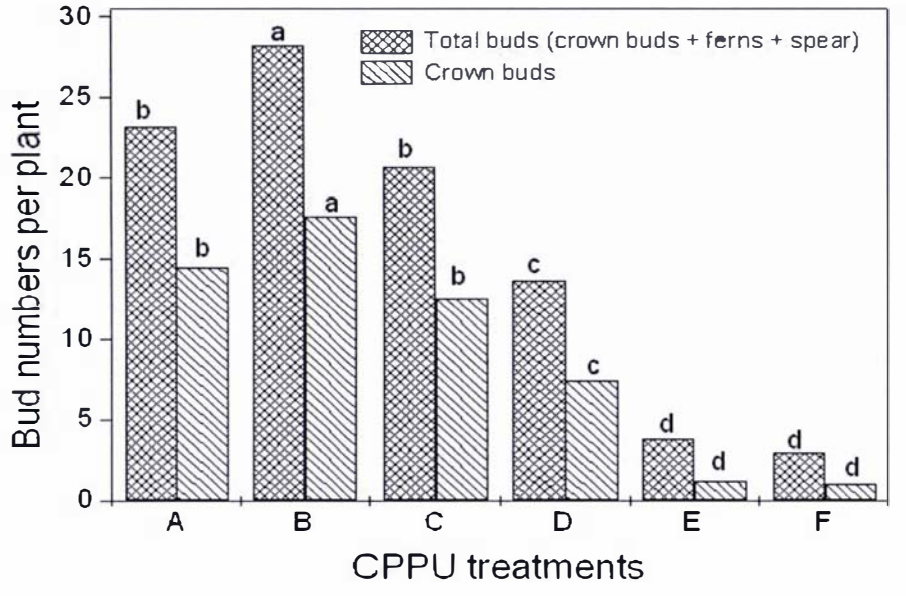

Figure 5.2 Effect of foliar applied and root applied CPPU at 10 mg L^{-1} on total buds (ferns + spear +crown buds) and buds on the crown 12 weeks after CPPU treatments (Treatments: A control + 0 mg L^{-1} foliar spray, B control + 5 mg L^{-1} foliar spray, C control + 10 mg L^{-1} foliar spray, D root application + 0 mg L^{-1} foliar spray, E root application + 5 mg L^{-1} foliar spray, F root application + 10 mg L^{-1} foliar spray). Bars with different letter are significant different by Duncan's multiple range test at $P \leq 0.05$.

5.3.2 Experiment 2

For both the cultivars CPPU-treated plants produced longer and thicker cladodes, and an increase in cladode dry weight per cladode. However, photosynthetic rates were unaffected by CPPU treatment (Table 5.2). 'Jersey Giant' cultivar had higher photosynthetic rates than 'UC 157'.

Table 5.2 Effect of CPPU foliar spray on photosynthetic rate, cladode dry weight and root dry weight of two cultivars twenty four days after treatment.

Treatment		Rate of photosynthesis[z]	Cladode dry weight
Cultivar	Foliar spray ($mg\ L^{-1}$)	($\mu mol\ g^{-1}s^{-1}$)	per cladode ($g \times 10^{-4}$)
Jersey Giant	0	149.86	2.83
Jersey Giant	10	164.27	3.61
UC-157	0	125.81	2.50
UC-157	10	106.52	3.74
Significance[y]			
Cultivar (C)		*	ns
Foliar spray (FS)		ns	*
C x FS		ns	ns

[z]Each value is the mean of five measurements per treatment.
[y]Treatment effects were not significant (ns) or significant at $P \leq 0.05$(*).

5.4 Discussion

CPPU as a foliar spray at 5, and 10 mg L^{-1} alone was effective in producing longer and thicker cladodes (Table 5.1). However, root applied CPPU at 10 mg L^{-1} plus foliar sprays did not increase cladophyll length and diameter suggesting supra-optimal levels of CPPU. However, this seems unlikely as concentrations as high as 100 mg L^{-1} CPPU applied as a foliar spray to developing fern can promote cladode size (Woolley D J, unpublished data). Although foliar spray of CPPU increases cladode dry weight per cladode, photosynthetic rates were unaffected by CPPU foliar spray (Table 5.2).

'Jersey Giant' had a higher photosynthetic rate than 'UC 157'. Daningsih (2005) reported a higher amount of chlorophyll and higher root dry weight in 'Jersey Giant' than 'UC 157'. It is suggested that 'Jersey Giant' has a potentially more active photosynthetic system and was more effective in storing assimilates in the roots. Genotypic variability in photosynthetic rate has been reported in asparagus plants. For example, the higher yield genotype, 'Franklim' and 'Hart-3', had higher photosynthetic rate (27.8 μmol m^{-2} s^{-1} and 24.1 μmol m^{-2} s^{-1}, respectively) and the lowest yield genotype, '86Sam3', had the lowest photosynthetic rate (15.7 μmol m^{-2} s^{-1}) (Bai and Kelly, 1999). Such variation in photosynthesis among genotypes is related to physiological differences in fern respiration, carboxylation efficiency and stomatal limitation (Woolley et al., 1999) or is related to genotypic differences in cladophyll diameter, content of photosynthetic biochemical constituents, and mesophyll resistance to CO_2 diffusion (Faville et al., 1999).

CPPU stimulates and retards asparagus fern numbers depending on whether CPPU was applied as a foliar spray, or root application. Root applied CPPU at 10 mg L^{-1} significantly reduced the number of ferns (Figure 5.1). These results suggest that CPPU or a metabolite was being transported to the crown and stimulating fern production, but that root applied CPPU at 10 mg L^{-1} with foliar spray inhibited fern production, possibly due to a high level of uptake resulting in supra-optimal concentrations of CPPU. An alternative explanation is that CPPU is affecting other hormones such as gibberellins, as has been found for thidiazuron

As bud numbers may limit yield (Woolley et al., 2006) an interesting effect of the cytokinin-like compound, CPPU, was that foliar sprays at 5 mg L^{-1} produced a small but significant increase in bud numbers. Five parts per million may well not be the optimum concentration, as 10 mg L^{-1} was somewhat inhibitory. Root application of 10 mg L^{-1} CPPU significantly decreased the number of total buds (Figure 5.2). The application of CPPU at 5 mg L^{-1} to the foliage was effective in increasing bud initiation and development whereas CPPU root application particularly as a crown soak at 10 mg L^{-1} was highly inhibiting. For bud production, it would appear that crown buds were very sensitive to CPPU and that CPPU itself, or some signal from foliar supplied CPPU was transmitted to the crown.

The ratio of root mass: total bud number of seedling is a possible limiting factor for yield performance of established asparagus in the field. Wilson and Sinton (2004) reported that the larger crowns had twice as many buds and roots as the small ones, but did not report the bud to root mass ratio. Daningsih (2005) and Woolley et al. (2006) found a lower number of buds per unit root mass in large crowns compared to small and medium crowns. As root mass increase the number of large buds which form high quality, spears do not increase in proportion. The present experiment indicated that foliar application at 0, 5 mg L^{-1} or 10 mg L^{-1} CPPU alone changed the ratio of root dry mass: crown bud number to 0.46, 0.47, or 0.44. CPPU as a foliar spray did not affect the ratio of root dry mass: crown bud number. Therefore, foliar application at 5 mg L^{-1} CPPU alone increased total bud numbers and crown buds on the crown, suggesting that CPPU could improve yield performance in the subsequent harvest season.

5.5 Summary

CPPU as a foliar spray at 5 mg L^{-1} alone produced highest fern numbers and total buds. Together root and foliar applied CPPU severely inhibited the production of buds. CPPU was effective in producing longer and thicker cladodes, but photosynthetic rates were unaffected by CPPU foliar spray. 'Jersey Giant' had a higher photosynthetic rate than 'UC 157'.

CHAPTER 6

EFFECTS OF REPEATED CPPU APPLICATION ON PLANT GROWTH AND DEVELOPMENT

Abstract

The effect of repeated application of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) to foliage on cladophyll morphology using growth analysis was investigated. Seven-week-old asparagus seedlings cvs. 'Jersey Giant' and 'UC 157' were sprayed to run off with CPPU at 10 mg L^{-1}. Untreated plants were sprayed with tap water. The application of CPPU or tap water (control) were repeated after 4, 8, and 12 weeks. Repeated CPPU foliar sprays significantly increased cladophyll area and cladophyll dry weight, but decreased root dry matter and the number of buds. Despite the higher cladophyll area, CPPU did not increase photosynthetic efficiency as measured by relative growth rate (RGR) and net assimilation rate (NAR). 'UC 157' produced more buds than 'Jersey Giant', but root dry weight did not differ between the two cultivars. 'UC 157' and 'Jersey Giant' had similar RGRs and NARs.

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Effects of Repeated CPPU Application on Growth and Development of *Asparagus officinalis* L.

Yang Gyu. Ku, D. J. Woolley, and M. A. Nichols

6.1 Introduction

In asparagus, although photosynthesis occurs in all green tissue photosynthesis occurs mainly in the cladodes (Downton and Torokfalvy, 1975; Faville et al., 1999; Guo et al., 2002). Asparagus, unlike most vegetable crops, is a perennial plant and photosynthesis does not directly contribute to spear yield until the subsequent growing season (Guo et al., 2002), when assimilate produced in cladodes by photosynthesis are translocated into the underground storage roots and then utilized for spear growth and vegetative growth during the following year (Haynes, 1987; Pressman et al., 1993).

High-yielding genotypes of asparagus primarily depend on photosynthetic capacity, which was found to correlate with cladophyll diameter (Faville et al., 1999). The high-yielding cultivar 'ASP-69' had a significantly greater cladophyll diameter compared to low-yielding cultivar 'ASP-03' (Guo et al., 2002). The previous experiment (Chapter 4) indicated that the cytokinin-active compound, *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) as a foliar spray was effective in producing longer and thicker cladodes. It was also observed that the cladodes of new ferns were of normal size, and therefore it was considered that repeat foliar sprays of CPPU would be required to increase the diameter and length of cladodes of subsequent ferns. The objective of this research was to determine the effect of repeated CPPU application on the plant growth of two asparagus cultivars.

6.2 Materials and methods

6.2.1 Plant materials and experimental conditions

Seeds of two asparagus cultivars 'Jersey Giant' and 'UC 157' were germinated and grown in plastic cell trays (45 ml volume/cell) containing a sterile medium (vermiculite). After four-weeks, the seedlings were transferred into plastic bags (12 cm diameter x 22 cm high). The growing media was as described in Section 5.2.1. After seven weeks, three hundred and twenty uniform size seedlings of each cultivar were selected. One hundred and sixty seedlings of each cultivar were sprayed to runoff with CPPU (Sitofex EC 2.5%; SKW, Trostberg, Germany) at a concentration of 10 mg L^{-1},

and the other 160 treated similarly with tap water (control). The CPPU application and the tap water (control) were repeated after 4, 8, and 12 weeks.

The treatments were thus as follows:

1. 0 mg L^{-1} foliar spray on Jersey Giant
2. 0 mg L^{-1} foliar spray on UC 157
3. 10 mg L^{-1} foliar spray on Jersey Giant
4. 10 mg L^{-1} foliar spray on UC 157

The pots were arranged in a randomized complete block design with each block containing 4 plots with 40 plants per treatment. The glasshouse was ventilated at 25°C during the day and heated to 17°C at night. The seedlings received natural radiation and were irrigated as needed.

6.2.2 Measurements of plant growth

Five plants per treatment from each block were harvested destructively at fortnightly intervals. Adhering medium on each crown was removed manually and the crown of each seedling was thoroughly washed with mild water pressure. The number of buds, ferns, and storage roots was counted. Fern was dissected into stalk and cladodes. All cladodes and fern stalk were separated and collected from five plants per plot. To obtain total cladode area, they were carefully arranged to avoid overlap on the belt of a leaf area meter (model Li 3100; Li-Cor, Lincoln, Neb, USA). The fresh weight of shoots, rhizome, and roots of five plants per plot was weighed and then oven dried at 60°C to a constant weight.

6.2.3 Relative growth rate (RGR), Leaf area ratio (LAR), Net assimilation rate (NAR), Specific leaf area (SLA), and Leaf weight ratio (LWR).

Cladode area, cladode dry weight, and total dry weight were individually transformed to natural logarithms, and these components were fitted using quadratic equations against harvest time. The parameter values obtained from these functions were used to

derive the growth analysis terms, RGR, LAR, NAR, SLA, and LWR for each treatment at two week intervals.

A quadratic function was fitted to:
Log_c total dry weight against time
Log_c cladode area against time
Log_c cladode dry weight against time

These three functions were used to derive the growth analysis parameters:

(1) Relative growth rate (RGR) $= \frac{1}{W}\frac{dW}{dt}$

(2) Leaf area ratio (LAR) $= \frac{A}{W}$

(3) Net assimilation rate (NAR) $= \frac{1}{A}\frac{dW}{dt}$

(4) Specific leaf area (SLA) $= \frac{A}{WL}$

(5) Leaf weight ratio (LWR) $= \frac{WL}{W}$

When W= total plant dry weight, WL =cladode weight, and A= 'cladode area'.

6.2.4 The root-total dry weight allometric ratio

The root to total plant dry weight ratio was calculated using an allometric relationship between root and total plant dry weight. This allometric relationship overcomes the effect of harvest date by obtaining the root to total ratio as a constant number for harvest weeks after CPPU treatment. The allometric relationship between root and total is:

$$\text{Log}_e Y = \text{Log}_e a + b \text{Log}_e X$$

when:

Log_e a is the regression constant the intercept.

b is the allometric constant (the slope)

Log_e Y is natural logarithm of total dry weight

Log_e X is natural logarithm of root dry weight

The allometric constant 'b' is essentially the ratio of the $\log_e$ of the total weight, and the $\log_e$ of the root dry weight. A higher 'b' than 1.0 indicates an increasing partitioning of dry matter to shoots over time while a lower 'b' than 1.0 indicates more partitioning of dry matter to root over time (Hughes et al., 1990).

6.2.5 Data analysis

The experiment was a 2 x 2 factorial experiment with four blocks. Data were analysed using randomized complete block model in the GLM procedure of SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). To stabilise the variances, total dry weight, and fern, root and rhizome dry weights were transformed to natural logs. Means are reported after back transforming. Means separation of variables such as number of ferns, roots, crown buds, and total buds used a protected LSD at $P \leq 0.05$.

6.3. Results

There was no significant interaction between CPPU foliar sprays and cultivars for any measurement. Therefore, the results are presented only for main effects of CPPU foliar spray and cultivar separately. The $\log_e$ total, root, and rhizome dry weights of the CPPU-treated plants were generally lower, while $\log_e$ fern dry weight were generally higher when compared to untreated controls (Figure 6.1). Some ferns of both treatments senesced from 10 weeks after CPPU treatment.

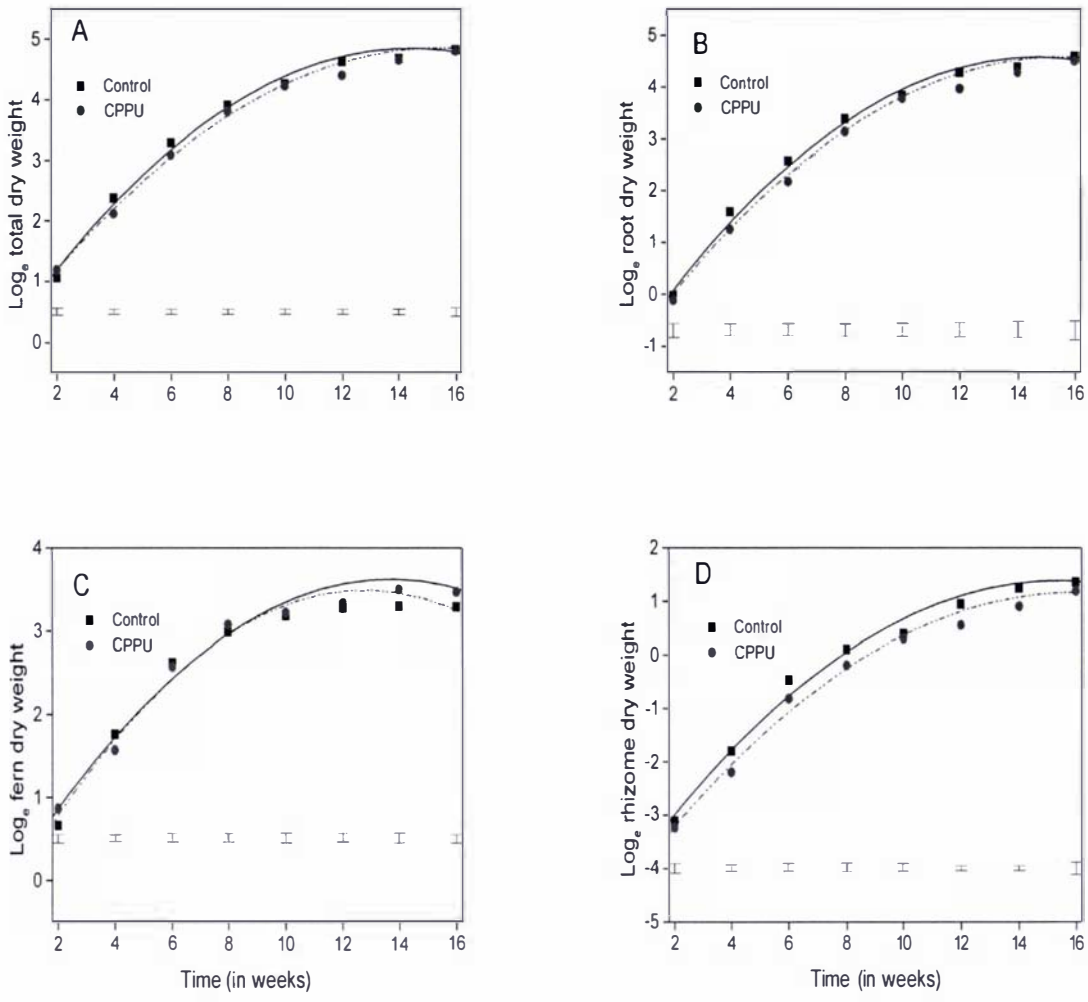

Figure 6.1 Log_c dry weights of total (A), roots (B), ferns (C), and rhizomes (D) of asparagus treated with or without CPPU foliar sprays. CPPU application was repeated after 4, 8, and 12 weeks. Each point is the means of eight plots. Vertical bars represent the LSD at $P \leq 0.05$.

Treatment with CPPU consistently produced plants with fewer ferns, roots and buds, but the differences were not always significant. Even though fern dry weight was generally higher, number of ferns produced in both treatments significantly increased up to 10 weeks after CPPU treatment, but slowed considerably after the 12th week (Figure 6.2A). The number of ferns on CPPU-treated plants was significantly lower than those of the control in all weeks except weeks 2, and 16 (Figure 6.2A). The number of roots, crown buds, and total buds increased linearly but foliar sprays decreased the numbers compared to control plants (Figures 6.2B, 6.2C and 6.2D).

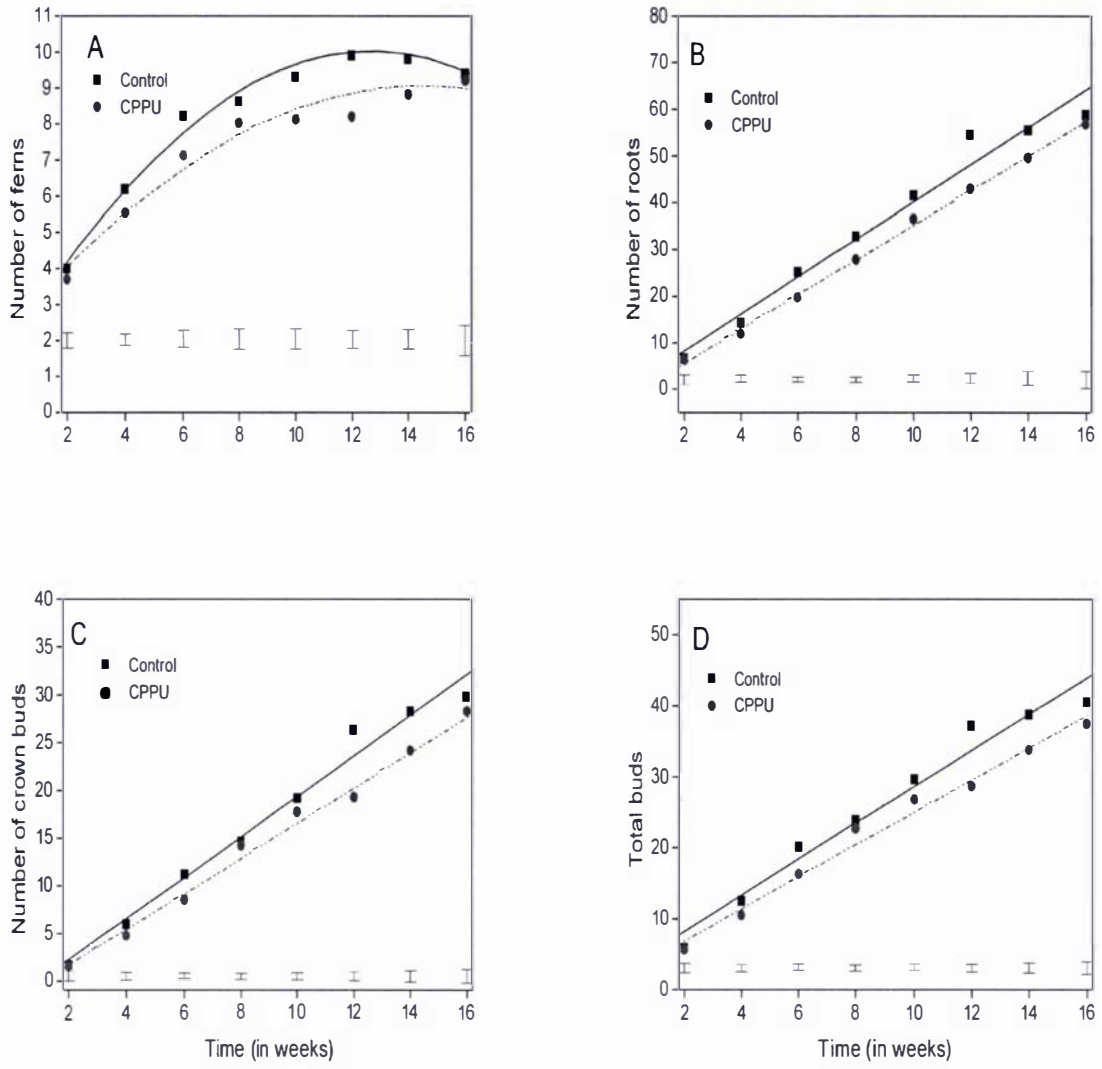

Figure 6.2 Number of ferns (A), roots (B), crown buds (C), and total buds (fern + spear + bud) (D) of asparagus treated with CPPU foliar sprays. CPPU application was repeated after 4, 8, and 12 weeks. Each point is the means of eight plots. Vertical bars represent the LSD at $P \leq 0.05$.

CPPU significantly increased $\log_e$ cladophyll area and dry weight (Figure 6.3).

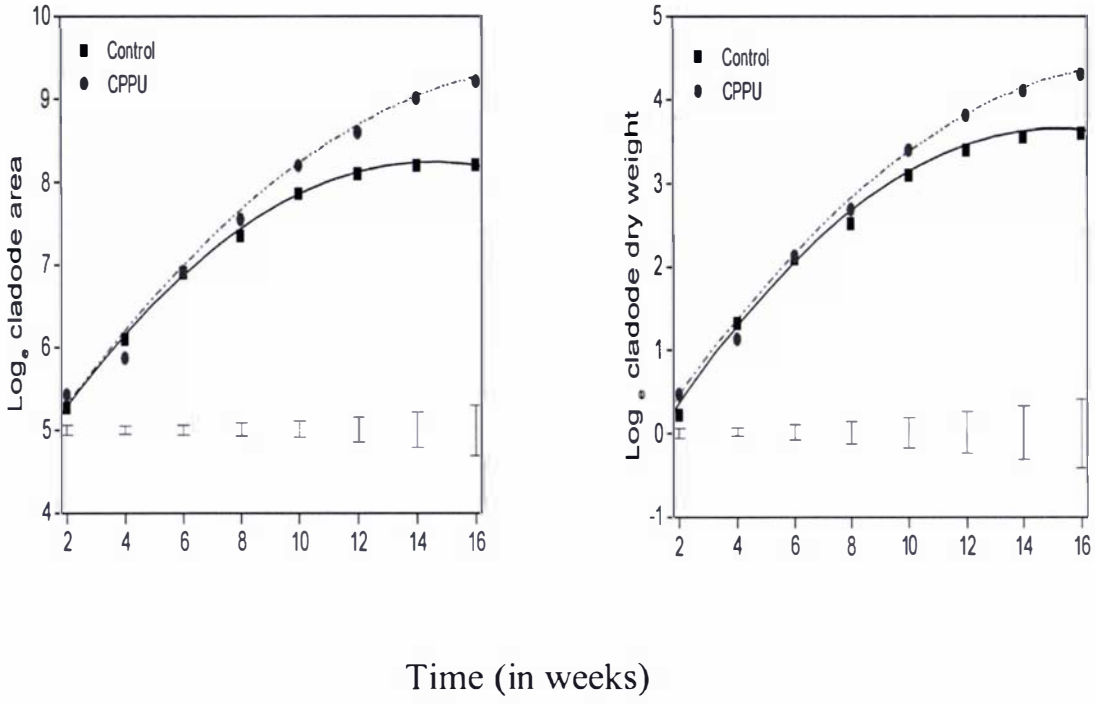

Figure 6.3 Log_e cladode area and cladode dry weight of asparagus with CPPU foliar spray. CPPU application was repeated after 4, 8, and 12 weeks. Each point is the means of eight plots. Vertical bars represent the LSD at $P \leq 0.05$.

RGR (g g^{-1} $week^{-1}$) was not significantly different between cultivars and not affected by CPPU (Figure 6.4).

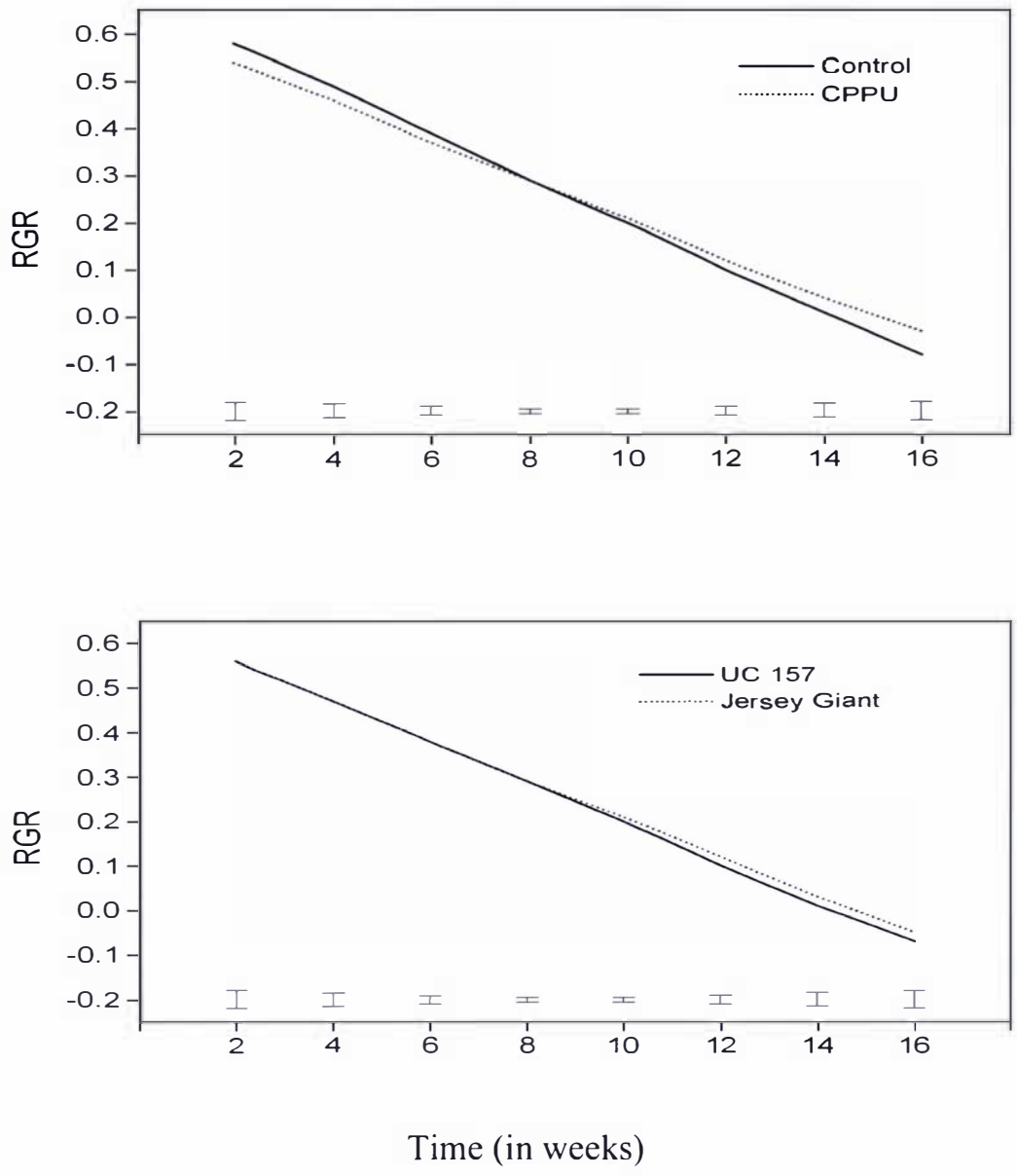

Figure 6.4 Relative growth rate (RGR) of two cultivars of asparagus seedlings treated with foliar sprays of CPPU.

Leaf area ratio (LAR) of CPPU-treated plants was significantly higher than control plants in all except week 2, but there was no significant difference between cultivars (Figure 6.5).

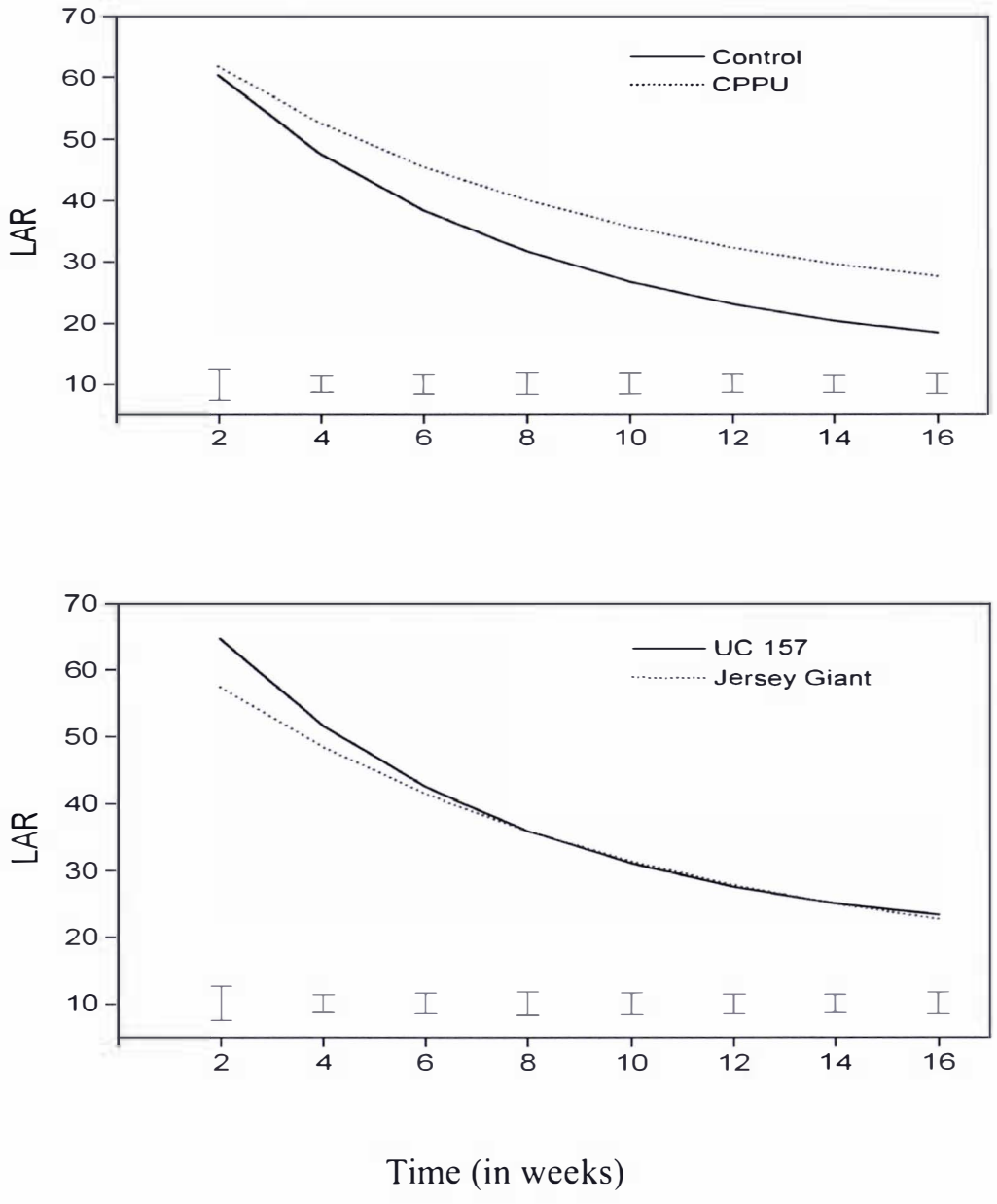

Figure 6.5 Leaf area ratio (LAR) of two cultivars of asparagus seedlings treated with foliar sprays of CPPU. Vertical bars represent the LSD at $P \leq 0.05$.

NAR (g m^{-2} $week^{-1}$), a measure of the efficiency of the plant in gaining carbon per unit leaf area (i.e. total photosynthesis - total respiration), of the CPPU-treated plants was significantly less than that of the untreated plants from weeks 4 to 10 (Figure 6.6). However, there were no significant difference in NAR between 'UC 157' and 'Jersey Giant'.

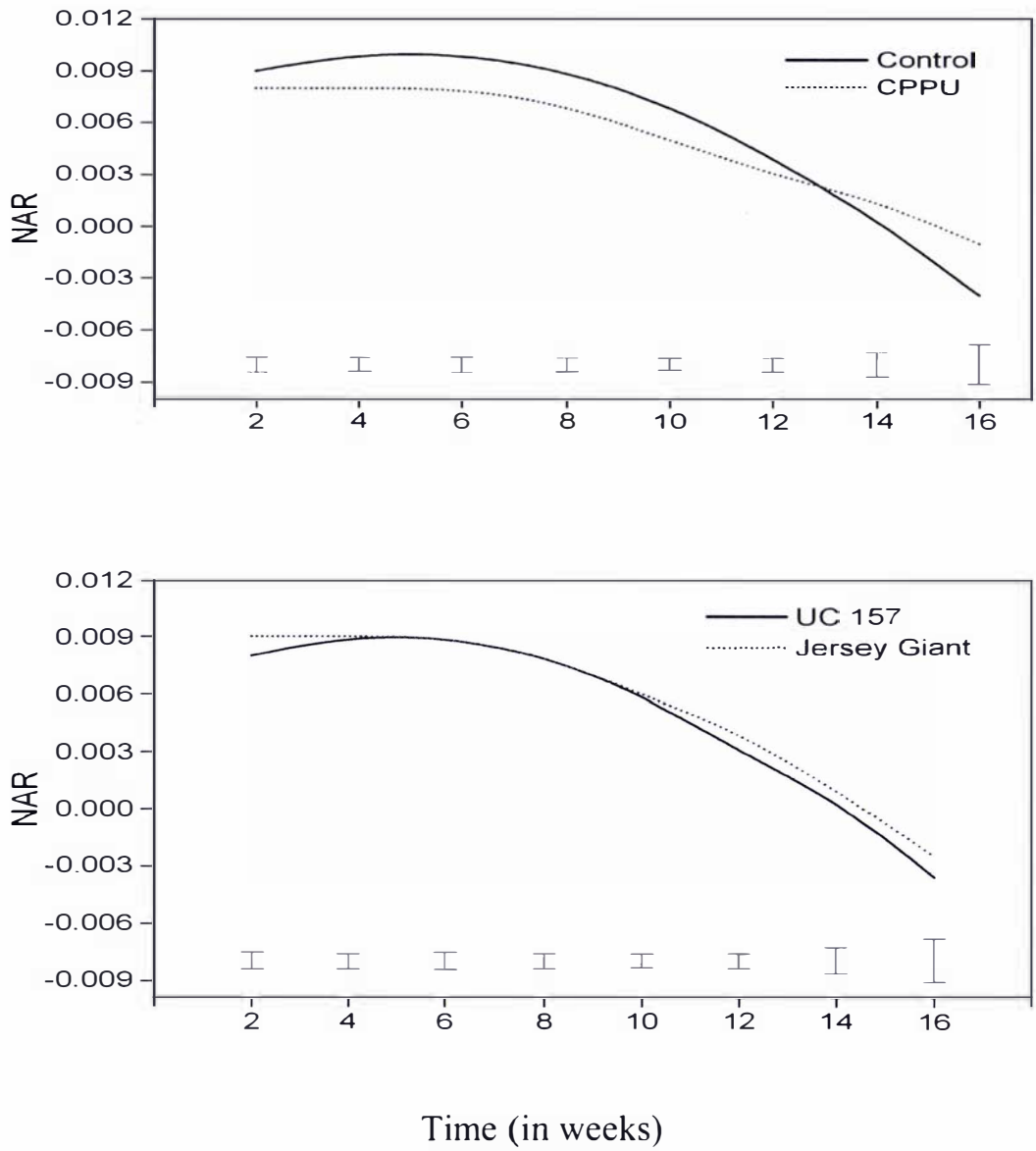

Figure 6.6 Net assimilation rate (NAR) of two cultivars of asparagus seedlings treated with foliar sprays of CPPU. Vertical bars represent the LSD at $P \leq 0.05$.

SLA (m^2 g^{-1}), a measure of leaf density or relative thickness, increased in CPPU-treated plants for weeks 14 and 16, but there were no significant differences between 'UC 157' and 'Jersey Giant' (Figure 6.7).

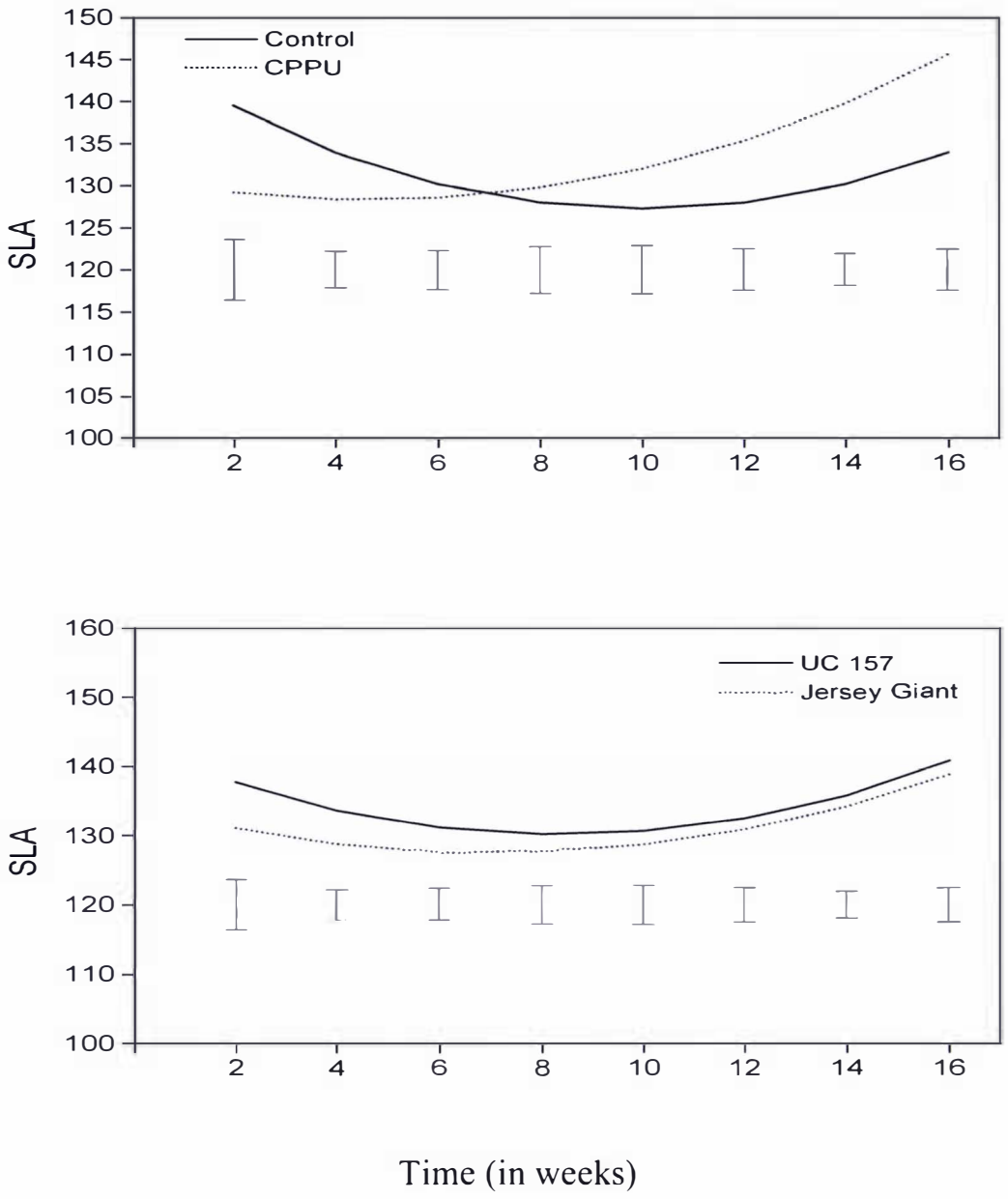

Figure 6.7 Specific leaf area (SLA) of two cultivars of asparagus seedlings treated with foliar sprays of CPPU. Vertical bars represent the LSD at $P \leq 0.05$.

LWR (g g^{-1}), defined as dry weight of leaves per dry weight of whole plant, of the CPPU-treated plants was significantly higher except at week 2 compared to untreated controls (Figure 6.8). However, LWR of 'UC 157' and 'Jersey Giant' was similar.

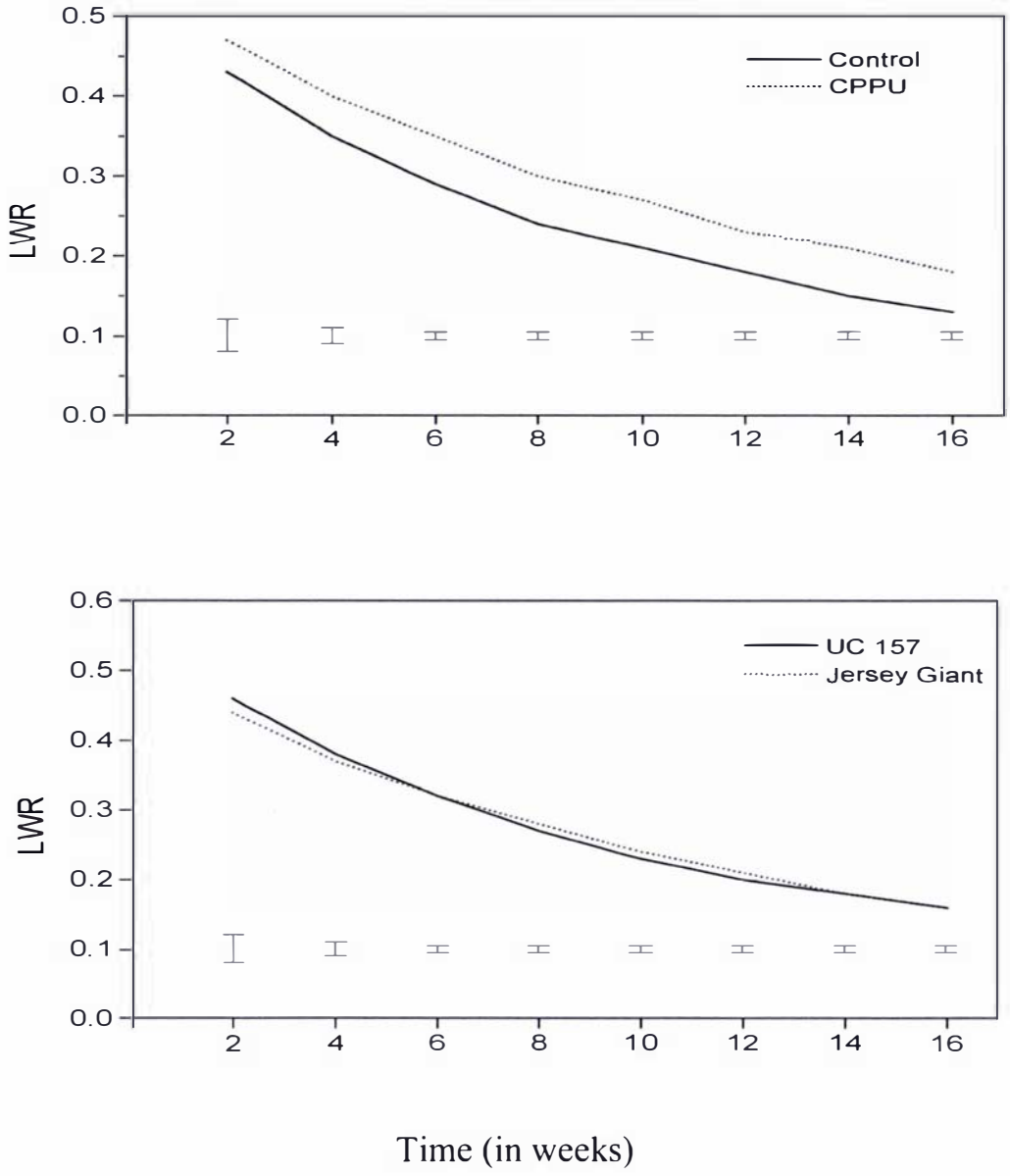

Figure 6.8 Leaf weight ratio (LWR) of two cultivars of asparagus seedlings treated with foliar sprays of CPPU. Vertical bars represent the LSD at $P \leq 0.05$.

There were no significant differences in the intercept and slope of the total dry weight and crown dry weight allometric relation between two treatments (Figure 6.9). The allometric constant numbers for CPPU-treated plants and control plants were 0.78 and 0.82 (Figure 6.9). The allometric constant from both treatments was similar, indicating CPPU-treated plants and control plants showed similar root to total dry weight ratio over the period of the experiment.

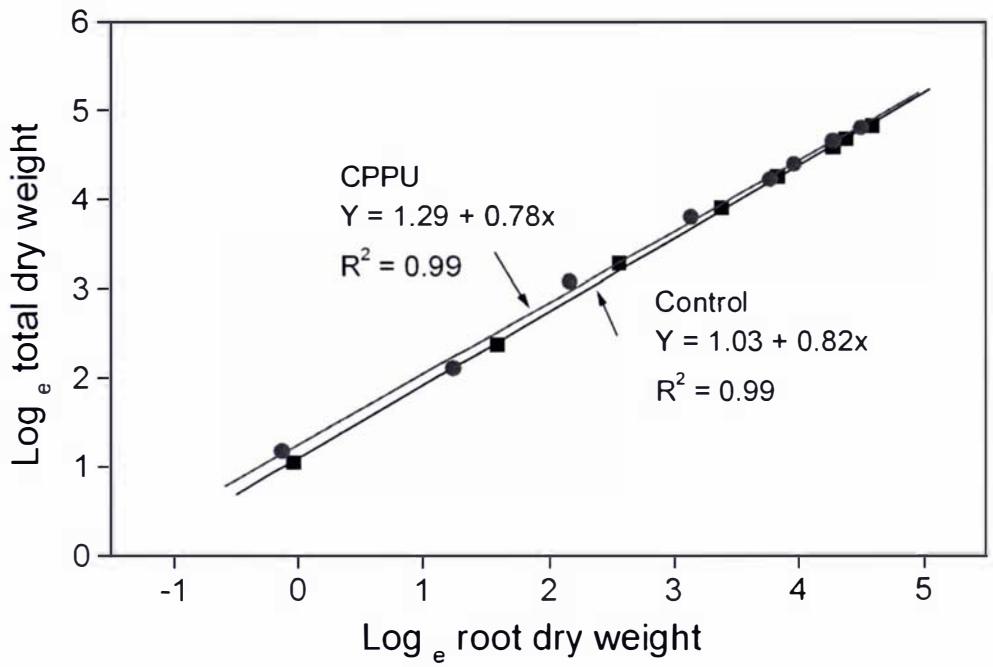

Figure 6.9 Relationship between total and root dry weight for CPPU-treated and control plants. The regression of relationship between $\log_e$ total weight and $\log_e$ root weight for CPPU-treated plants and control plants are Log_e total dry weight = Log_e 1.20 + 0.78 x (Log_e root weight) and Log_e total dry weight = Log_e 1.03 + 0.82 x (Log_e root weight) respectively.

The Log_e total dry weight of 'UC 157' was slightly (but not significantly) higher than that of 'Jersey Giant' (Figure 6.10A). There was no significant difference between cultivars for Log_e root dry weight (Figure 6.10B), however the log_e fern dry weight and rhizome dry weights were significantly greater for 'UC 157' (Figures 6.10C and 6.10D). Although fern dry weight of 'UC 157' showed a higher trend from 8 weeks to the end of the experiment, fern dry weight did not significantly differ.

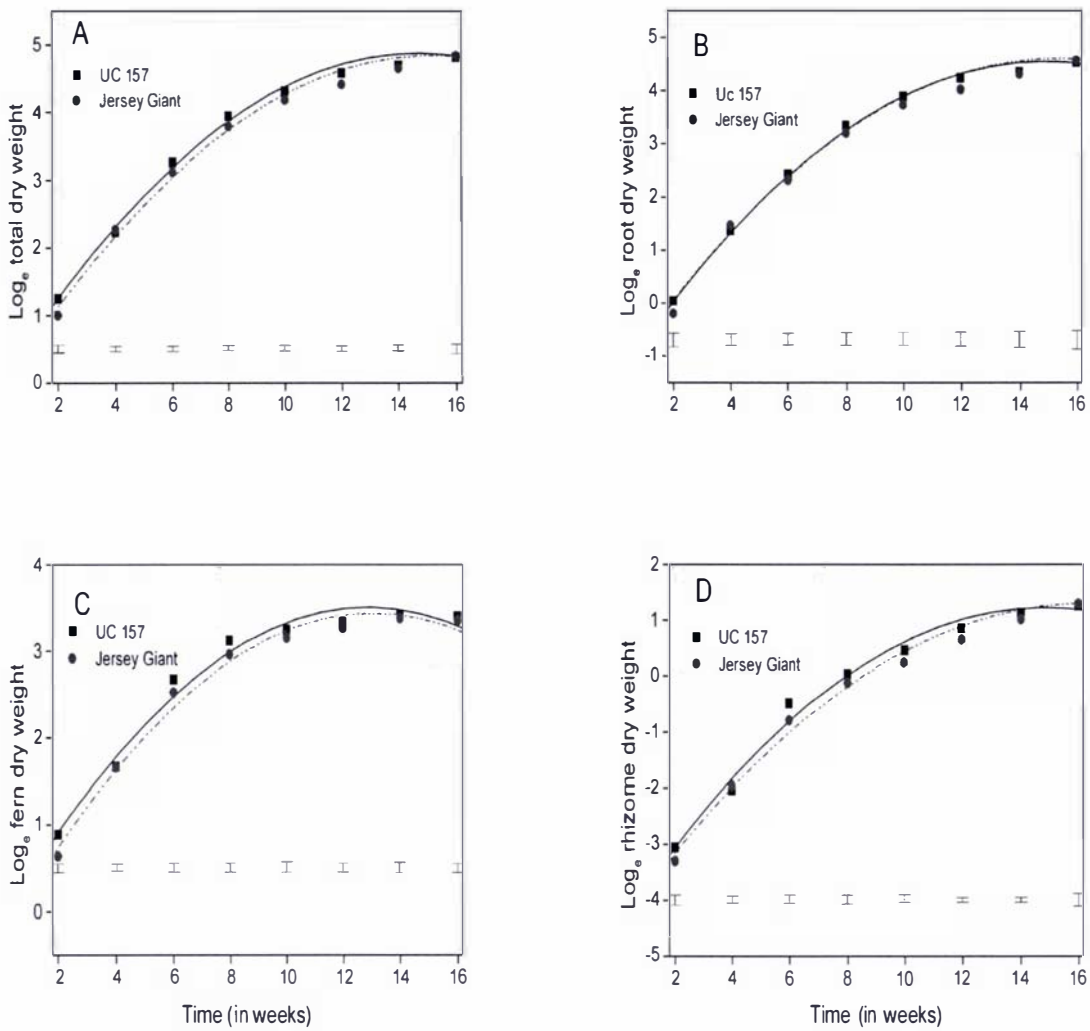

Figure 6.10 Log_e total dry weight (A), root (B), fern (C), and rhizome (D) of 'UC 157' and 'Jersey Giant' asparagus seedlings during 16 weeks growth after CPPU treatment. CPPU application was repeated after 4, 8, and 12 weeks. Each point is the means of eight plots. Vertical bars represent the LSD at $P \leq 0.05$.

The number of ferns per plants did not differ statistically between the two cultivars (Figure 6.11A), but the root numbers of 'UC 157' were significantly higher than for 'Jersey Giant' from week 2 to the end of the experiment (Figure 6.11B) and the number of buds on the crowns per plants and total buds (ferns + spears + crown buds) were consistently lower in 'Jersey Giant' than in the 'UC 157' (Figures 6.11C and 6.11D).

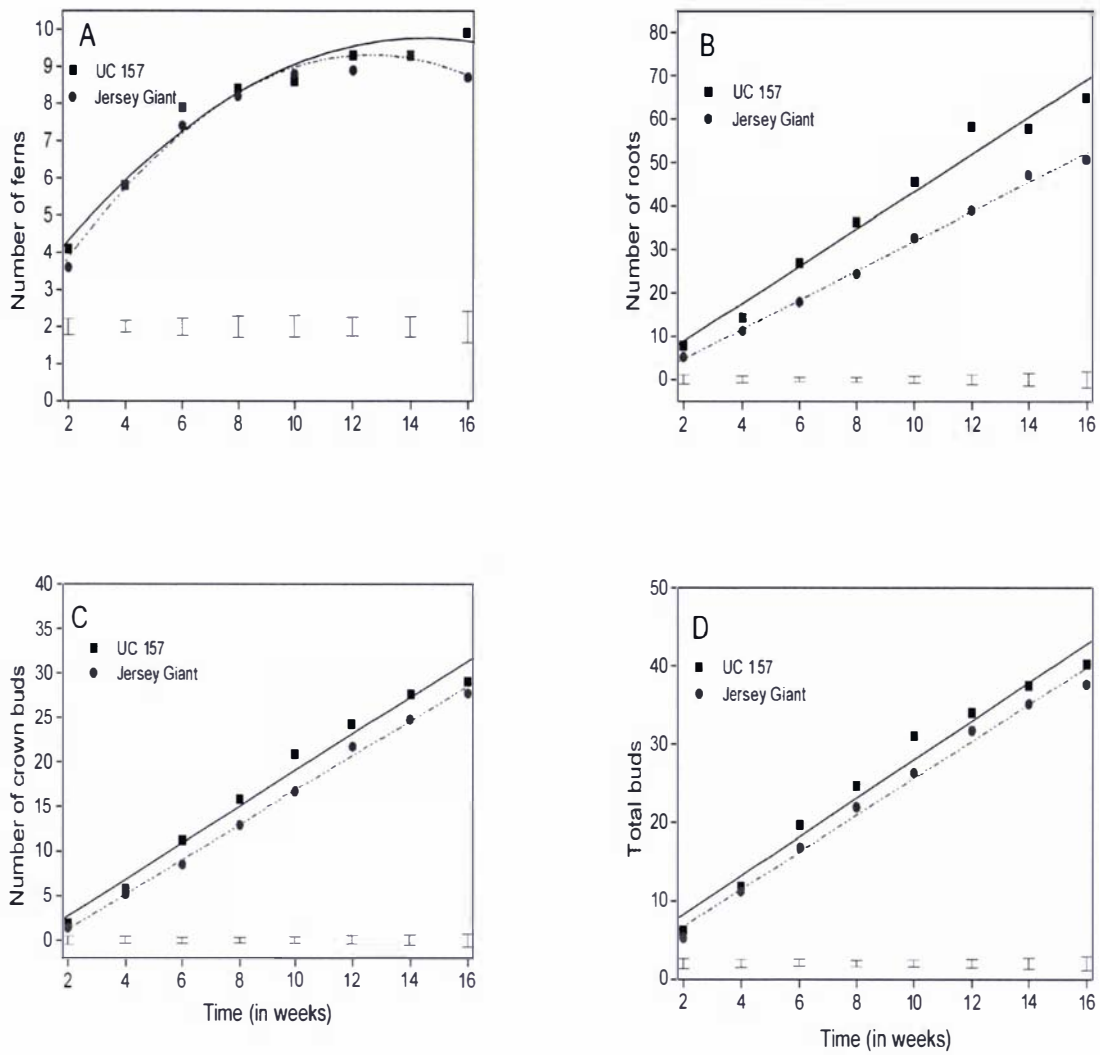

Figure 6.11 Number of ferns (A), roots (B), crown buds (C), and total buds (fern + spear + bud) of 'UC 157' and 'Jersey Giant' asparagus seedlings during 16 weeks growth after CPPU treatment. CPPU application was repeated after 4, 8, and 12 weeks. Each point is the means of eight plots. Vertical bars represent the LSD at $P \leq 0.05$.

The slope of the allometric relationship for 'UC 157' and 'Jersey Giant' were 0.79 and 0.82 respectively. This result indicates that the cultivars had a similar pattern of dry matter distribution over time (Figure 6.12).

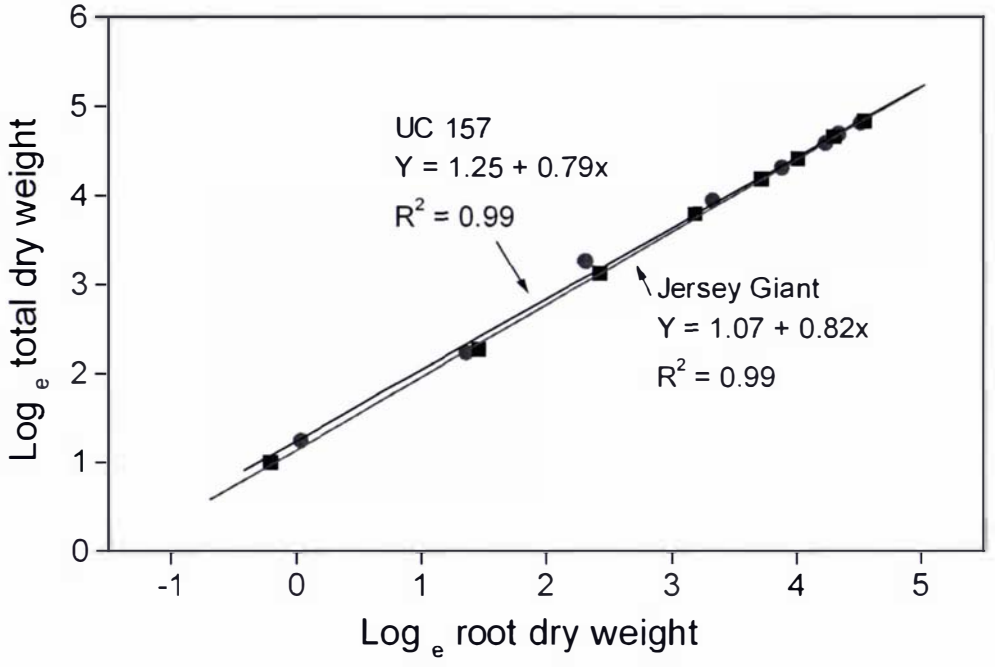

Figure 6.12 Relationship between total and crown dry weight with two cultivars. The regression of relationship between $\log_e$ total dry weight and $\log_e$ root weight for UC 157 and Jersey Giant are Log_e total dry weight = Log_e 1.25 + 0.79 x (Log_e root weight) and Loge total dry weight = Log_e 1.07 + 0.82 x (Log_e root weight) respectively.

6.4 Discussion

Repeated CPPU foliar sprays were effective in increasing cladode area and dry weight, and fern dry weight (Figures 6.1C and 6.3). Longer and thicker cladodes might be expected to produce more assimilates for transport into root storage and to stimulate crown activity. However, root dry weight, root numbers, bud numbers on the crown, and total bud numbers (ferns + spears + buds) were all significantly lower for the CPPU-treated plants than the untreated plants (Figures 6.1B, 6.2B, 6.2C and 6.2D), suggesting that CPPU did not increase photo-assimilation.

Although repeated CPPU foliar sprays increased cladode area and dry weight, CPPU-treated plants had similar RGR's and had a lower LAR compared to control plants (Figures 6.4 and 6.5). Since in growth analysis terms, RGR = NAR x LAR it follows that CPPU-treated plants significantly decreased NAR from 4 to 10 weeks, indicating that CPPU caused a suppression in over-all efficiency of carbon accumulation (Figure 6.6), therefore either the photosynthetic efficiency of CPPU-treated plants was lower than that of untreated plants, or respiration rates were similar. This result demonstrates that the control plants exhibited a strong sink effect due to the greater root mass (Figures 6.1B and 6.2B), but as the root mass was suppressed by the CPPU, then the strong sink effect was absent, and thus photosynthetic rate was significantly reduced by the CPPU treatment. It would appear that the CPPU simply increased the sink strength of developing cladodes compared to the crown.

'UC 157' had a higher bud numbers on the crown and total bud numbers than 'Jersey Giant' (Figures 6.10C and 6.10D). Daningsih (2005) has similarly reported that 'UC 157' cultivar should have a higher yield potential in terms of bud number, total bud numbers, and root numbers, than 'Jersey Giant'. However, 'Jersey Giant' is well known to be higher yielding cultivar in most conditions (Nichols and Fisher, 1999; Bussell, 2000). 'UC 157', 'Jersey Giant', and 'Karopiro' were defined as low, medium, and high yield, respectively, by Faville et al. (1999). Total dry weight were significantly greater for the 'UC 157' at weeks 2, 6, and 8 weeks after treatment, resulting in increasing fern dry weight 2, and 6 weeks after treatment (Figure 6.10A). However, root dry weight did not differ between cultivars (Figure 6.10B). The similar root mass in terms of dry weight, but fewer root number in 'Jersey Giant' implied the average weight of

individual roots was higher in 'Jersey Giant' than in 'UC 157'. Both cultivars showed no significant difference in RGR, LAR, NAR, LWR and SLA (Figures 6.4, 6.5, 6.6, 6.7 and 6.8).

These results indicated that although CPPU-treated plants had increased fern growth, net assimilation rates were significantly reduced by the CPPU treatment. Further work is clearly required on the anatomy of CPPU-treated cladophylls in relation to cladode dimensions and photosynthetic rates in CPPU-treated plants, and on possible effects of CPPU on changing relative sink strengths of the shoots compared to the roots.

6.5 Summary

Repeat foliar sprays of CPPU increased cladophyll area and cladophyll dry weight, but decreased root dry matter and the number of buds. Repeated CPPU foliar sprays did not increase photosynthetic efficiency as measured by RGR and NAR. Root masses of repeated CPPU foliar sprays were decreased and then the strong sink effect was absent, and thus photosynthetic rate was significantly reduced by the CPPU treatment. Although 'UC 157' cultivar increased numbers of root and bud, root mass did not differ between 'UC 157' and 'Jersey Giant'. RGR and NAR were not influenced by cultivars.

CHAPTER 7

EFFECT OF CPPU TREATMENT OF *CAPSICUM ANNUUM* L. cv. 'Ancho St Luis' ROOTS ON PLANT GROWTH CHARACTERISTICS

Abstract

This study explored the influence of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) treatment of the roots on shoot growth, root growth and photosynthetic rate, and investigated the effects of a gibberellic acid (GA_3) foliar spray on reversing the growth suppression of chilli 'Ancho St Luis' shoots due to CPPU treatment of the root system. In the first experiment, six-week-old chilli seedling roots were soaked in an aqueous solution of CPPU (10 mg L^{-1}), for two hours, transplanted into pots and grown in a glasshouse. In the second experiment, the roots of six-week-old seedlings were soaked in 3 concentrations (1, 5, or 10 mg L^{-1}) of CPPU for two hours, transplanted into pots, and grown in a glasshouse. Four weeks after CPPU treatment, the plants were sprayed with GA_3 at 100 mg L^{-1} as a single foliar spray. Results showed that root applied CPPU at 10 mg L^{-1} significantly decreased plant height, probably by inhibiting the activity of the sup-apical region of the shoot, reduced internode number and length at 2 and 4 weeks after treatment, and suppressed root growth. CPPU-treated plants also resulted in delayed flowering. Application of GA_3 to shoots did not reverse CPPU effect. Therefore, CPPU applied to roots may not only block GA_3 biosynthesis but also block GA_3 action.

7.1 Introduction

Applying CPPU (10 or 20 mg L^{-1}) to asparagus foliage significantly stimulated elongation of initial ferns (Figure 4.1; Table 4.9), however, CPPU treatment of crowns significantly suppressed fern elongation, resulting in decreased asparagus fern height, fresh weight and dry weight (Tables 4.5, 4.9 and 4.10). Thus, CPPU stimulated or retarded growth depending on whether it was applied as a foliar spray or as a root soak.

Further, root applied CPPU at 10 mg L^{-1} in combination with foliar sprays inhibited fern height and production, possibly due to a high level of uptake resulting in supra-optimal concentrations of CPPU (Chapter 5). Possibly CPPU was affecting the production of other hormones such as gibberellins. However, CPPU is poorly transported within the plant as demonstrated by Woolley et al. (1992) and Biasi et al. (1993) who found low CPPU translocation within kiwifruit following a dip application and that little CPPU reached the fruit from the leaves. It is generally accepted that cytokinins are synthesized in root apical meristems and transported to the shoot through the xylem via the transpiration stream (Morris, 1981; Morris et al., 1986; Letham, 1994; Emery and Atkins, 2002; Kuroha et al., 2002; Aloni et al., 2005). Gibberellins are also synthesized in the apical part of the root, transported into shoot parts (Davies and Rappaport, 1975; Yim et al., 1997) and influence shoot internode elongation (Heidmann, 1982; Anderson et al., 1988). For asparagus, CPPU as a soak decreased fern height and root growth, caused in part by a decreased supply of growth substances from the roots. In asparagus plants, it was difficult to collect xylem sap and so further experiments were undertaken with pepper plants. The objective of these experiments was to determine the effect of CPPU-treated roots on plant growth and development of pepper plants and to assess the similarity the responses to those of asparagus plants.

7.2 Materials and Methods

7.2.1 Experiment 1

7.2.1.1 Experimental conditions and treatments

The experiment was carried out at the Plant Growth Unit, Massey University, Palmerston North (Latitude 40.2°S, Longitude 175.4°E), New Zealand. Pepper seeds (*Capsicum annuum* L. cv. 'Ancho St Luis') were germinated and grown singly in plastic cell trays (45 ml volume) containing vermiculite. Ten seedlings were chosen for uniformity of plant height and leaf number. The roots of five chilli seedlings were submerged for two hours in an aqueous solution of CPPU (Sitofex EC 2.5%; SKW, Trostberg, Germany) at 10 mg L^{-1}, and the other five treated with tap water as controls. All seedlings were then transplanted into polythene bags (15 cm diameter x 31 cm high) containing a bark-peat-pumice mix (1:1:1) mixed media with agricultural lime (100g / 100L), dolomite (300g / 100L), and osmocote (16N-3.5P-10.8K) (200g of long term and 100g of short term, 300g / 100L) and grown in a glasshouse. The glasshouse was ventilated at 28°C during the day and heated to 17°C at night. The seedlings received natural radiation and were irrigated daily.

7.2.1.2 Measurements of plant height, internode number and length, flower number and dry weights

Plant height was measured weekly from top of the potting mix to the tip of the growing point. Internode number was measured from first true leaf prior to branching at 2, 4, and 12 weeks after CPPU treatment. Internode length was calculated by dividing plant height (from first true leaves prior to branching) by internode number. Days to first flower were recorded. At final harvest, plants were divided into main stem, leaves, roots and the fresh weights were obtained before oven drying at 60°C to a constant weight.

7.2.1.3 Measurements of stomatal conductance (gs) and photosynthesis

To determine how CPPU treatments affected plant growth, measurements of photosynthetic rate (Pn, μmol m^{-2} s^{-1}) and leaf stomatal conductance (gs, mol m^{-2} s^{-1}) were taken using a portable photosynthesis system (Li-Cor 6200, Li-Cor Inc., Neb., USA). Measurements were made on two middle leaves per plant between 12:00 and 14:00 hours five weeks after CPPU treatment.

7.2.1.4 Data analysis

The experiment was a completely randomised design with five single plant replicates per treatment. Data were analysed using the GLM procedure of SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). Treatment means were separated by the LSD test at $P \leq 0.05$.

7.2.2 Experiment 2

7.2.2.1 CPPU concentration and GA_3 treatments

Six week old pepper seedlings were removed from the potting mix, and the seedling roots were soaked in an aqueous solution of either 0, 1, 5, or 10 mg L^{-1} of CPPU for two hours and then transplanted. Control plants were soaked with tap water for two hours. Four weeks after applying the CPPU soaking treatments, five plants in each CPPU soaking treatment were sprayed on the foliage with GA_3 (Sigma, G-7645) at two concentrations (0 or 100 mg L^{-1}) to run-off, using a hand-held sprayer. Controls were sprayed with water. The growing medium was covered with paper during GA_3 spray applications to protect it from drips (Cox, 1991). Plant material and experimental conditions were as described in Section 7.2.1.1. Measurements of plant height and flower number were as described in Section 7.2.1.2.

7.2.2.2 Data analysis

The experiment was a completely randomized design with a 4 x 2 factorial arrangement of treatments with five single-plant replicates. Data were collected and analyzed using the GLM procedure of SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). The LSD at $P \leq 0.05$ was used to separate means in Experiment 1 (Section 7.3.1). Mean separations were done by orthogonal polynomial contrasts to test whether there was a significant linear relationship between CPPU concentrations and GA_3 foliar spray (Section 7.3.2).

7.3 Results

7.3.1 Experiment 1

CPPU significantly retarded plant height when applied to roots (Figure 7.1). One week after CPPU treatment, the height of the CPPU-treated plants was significantly lower than the untreated controls. CPPU-treated plants were half the height of untreated plants between 4-6 weeks after treatment (Figures 7.1 and 7.2). Untreated plants flowered at 4 weeks and plant height did not increase between 7 and 12 weeks. In contrast, CPPU-treated plants flowered at 6 weeks and height slowly increased so that by the end of experiment plant height was significantly different from the control (Figure 7.1).

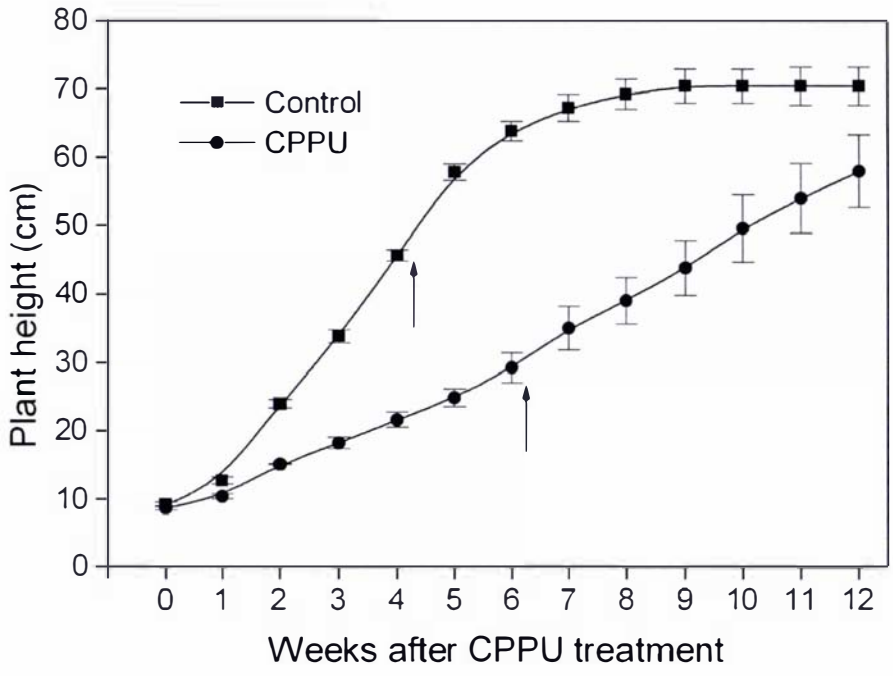

Figure 7.1 Effect of 10 mg L^{-1} CPPU treatment of roots on chilli (*Capsicum annuum* L. cv. 'Ancho St Luis') plant height. Each point is the mean of five plants. Vertical bars represent the standard error of the mean. Upward arrows indicate the week after CPPU treatment when the first flower opened.

Figure 7.2 Pepper plants (*Capsicum annuum* L. cv. 'Ancho St Luis') treated with CPPU at 0 (control, left) and 10 mg L^{-1} (right) 5 weeks after CPPU treatment. Note the shorter internodes and increased lateral shoot number of the treated plants.

The reduction of shoot length in CPPU-treated plants was associated with decrease in both internode number and internode length at week 2 and 4 after CPPU treatment. Internode number and length of the CPPU-treated plants decreased by 15% and 34% respectively at week 2, and by 31% and 42% at week 4 (Table 7.1). Shoot elongation ceased in control plants at week 8 after treatment, while CPPU-treated plants were still growing at the end of experiment. Twelve weeks after CPPU treatment, CPPU-treated plants had significantly reduced internode length but a similar internode number on the main shoot to the controls (17 and 15.8 respectively).

Table 7.1 Effect of 10 mg L^{-1} CPPU treatment of roots on plant height, internode number and length at week 2, 4, and 12 weeks after application.

Treatment	Plant height[z] (cm)	Internode	
		Number	Length[y] (cm)
	Two weeks after treatment		
Control	20.1a	7.8a	2.58a
CPPU	11.5b	6.6b	1.74b
	Four weeks after treatment		
Control	37.4a	15.6a	2.43a
CPPU	15.1b	10.8b	1.40b
	Twelve weeks after treatment		
Control	45.0a	15.8a	2.83a
CPPU	30.4b	17.0a	1.80b

All values are means of five plants.
[z] Plant height from true leaves to prior to branching was measured 2, 4, and 12 weeks after treatment.
[y]Internode length was calculated by dividing plant height by internode number.
Means within columns sharing the same letter are not significantly different by LSD test at $P \leq 0.05$.

Photosynthetic rate was unaffected by CPPU treatment (Table 7.2). Stomatal conductance and intercellular CO_2 concentration also were not affected by CPPU treatment.

Table 7.2 Effect of CPPU-treated roots on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and stomatal resistance at week 5 after treatment.

Treatments	Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)	Intercellular CO_2 concentration (ppm)
Control	9.81a	0.43a	308.8a
CPPU	8.83a	0.38a	312.9a

All values are mean of 10 measurements per treatment. Means within columns sharing the same letter are not significantly different by LSD test at $P \leq 0.05$.

Shoot dry weight and root dry weight of the CPPU-treated plants were significantly less than those of control plants. Root growth was severely inhibited by CPPU, resulting in reduction of root fresh and dry mass (Table 7.3; Figure 7.3). Total dry weight of the CPPU-treated plants were decreased by 35% compared to the control. The root to shoot ratio was unaffected by CPPU treatment.

Table 7.3 Effect of root-applied CPPU on dry weight and total dry weight of plants, and root:shoot ratio at week 12.

Treatments	Dry weight (g)			Total dry wt (g)	Root: shoot ratio
	Leaf	Stem	Root		
Control	4.8a	9.2a	5.0a	18.9a	0.35a
CPPU	3.9a	5.7b	2.7b	12.3b	0.27a

All values are the mean of five plants. Means within columns sharing the same letter are not significantly different by LSD test at $P \leq 0.05$.

Figure 7.3 Pepper roots treated with CPPU at 10 mg L^{-1} (left) and 0 (control, right).

7.3.2 Experiment 2

There was no significant interaction between root applied CPPU treatments and GA_3 foliar spray for all measurements. CPPU applied as a soak at 1, 5 or 10 mg L^{-1} reduced plant height (Figure 7.4). Plant height reduced linearly with increasing CPPU concentration. From week 3 onwards, plant height in all CPPU treatments was significantly reduced relative to the untreated plants. At the final measurement, 1, 5, and 10 mg L^{-1} CPPU treatments decreased plant height by 18, 321, and 576% respectively, compared to the untreated controls.

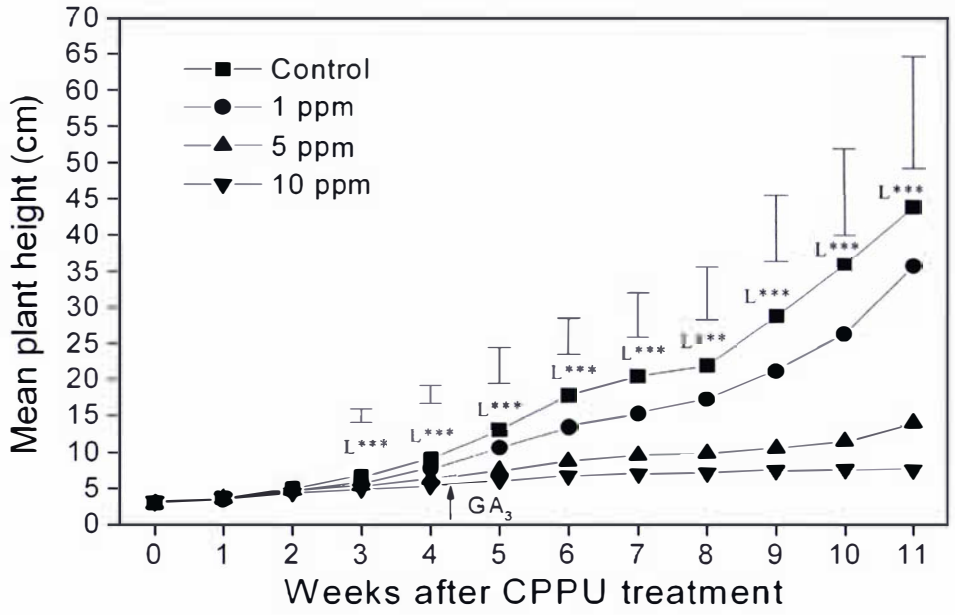

Figure 7.4 Effect of root applied CPPU concentrations on plant height of chilli pepper plants. Each point is the mean of ten plants. Vertical bars represent the LSD at $P \leq 0.05$. L*** represents a significant linear trend to response to CPPU concentration at $P \leq 0.001$.

For the first three weeks after GA_3 foliar spray, height of plants treated with GA_3 was significantly higher than unsprayed controls (Figure 7.5). A single foliar spray of GA_3 at 100 mg L^{-1} did not have a long term stimulating effect on the height of chilli plants.

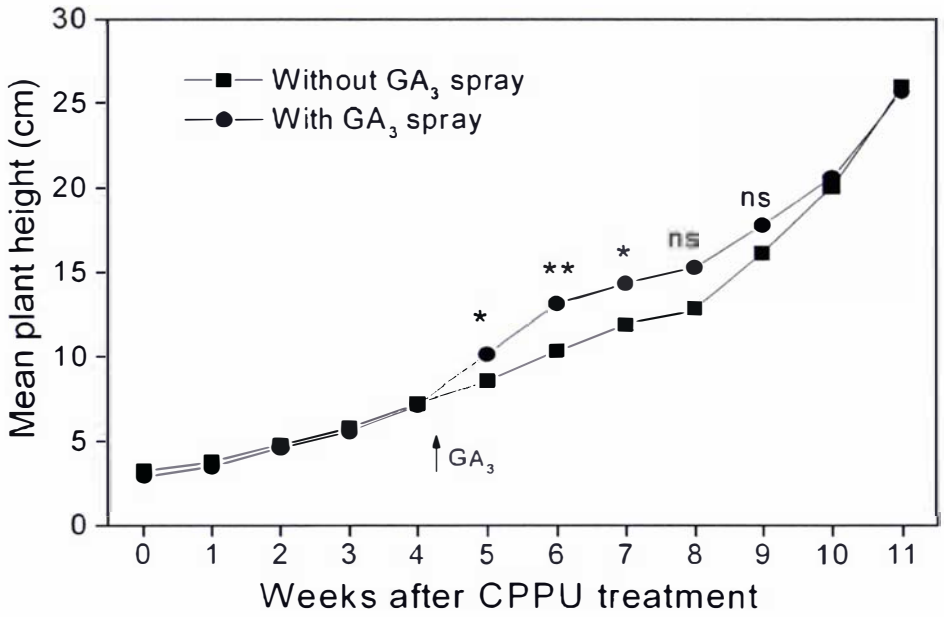

Figure 7.5 Effect of 100 mg L^{-1} GA_3 foliar sprays on plant height of CPPU-treated chilli pepper plants. Each point is the mean of twenty plants. ns, * and ** represents nonsignificant, significant at the $P \leq 0.05$ and significant at $P \leq 0.01$.

Plants treated with GA_3 in all CPPU treatments except 10 mg L^{-1} CPPU were higher than those without GA_3 (Figure 7.6). Plants soaked with 1 mg L^{-1} CPPU-treated with GA_3 were similar to control plants for the first 4 week after GA_3 treatment (Figure 7.6). However, GA_3 applied as foliar spray did not reverse the growth suppression of plant height caused by root applied CPPU at 10 mg L^{-1}.

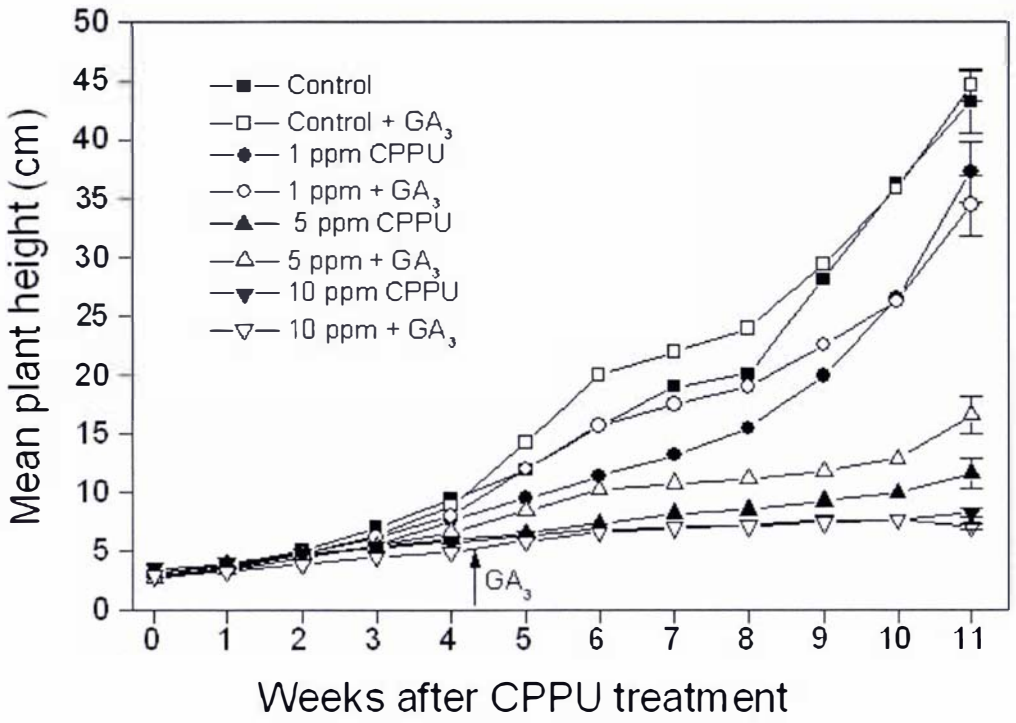

Figure 7.6 Effect of GA_3 foliar sprays following root applied CPPU on plant height of chilli pepper plants. Each point is the mean of five plants. Vertical bars at week 11 represent the standard error of the mean.

There was no significant difference in flower number between controls and 1 mg L^{-1} CPPU without a GA_3 foliar spray. However, GA_3 alone dramatically decreased flower number. Plants with CPPU-treated roots at 5 or 10 mg L^{-1} did not start flowering even after 11 weeks (Figure 7.7) even though controls started after 8 weeks.

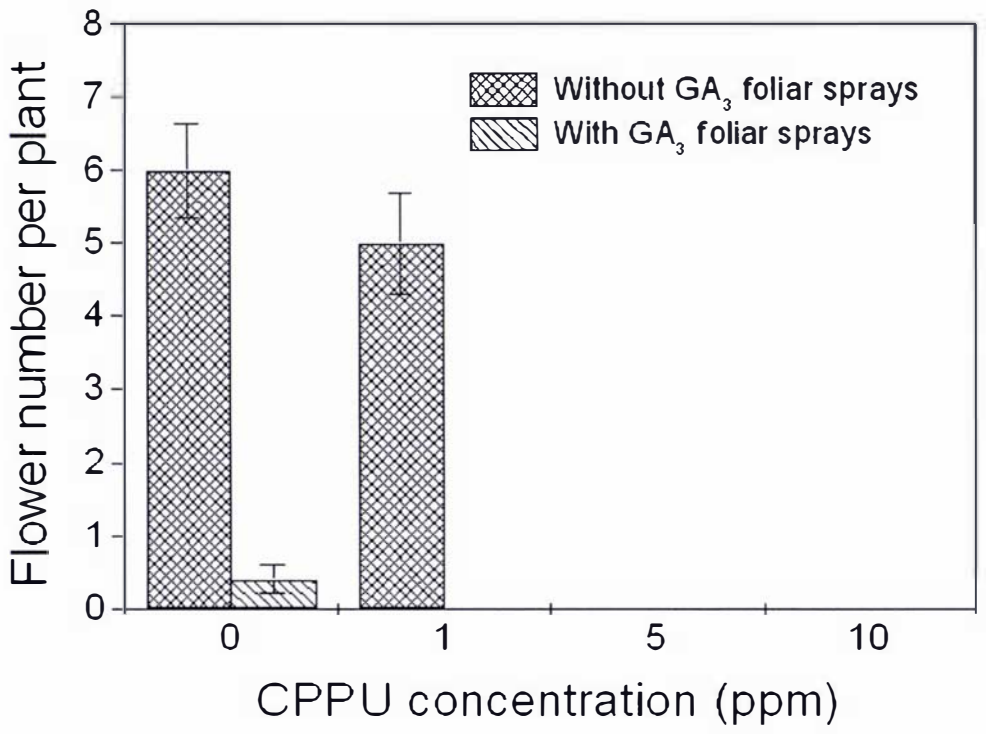

Figure 7.7 Effect of GA_3 foliar spray following CPPU soaked roots on flower number 11 weeks after treatment. Bars are the standard error of the mean.

There was no significant interaction between root applied CPPU treatment and GA_3 foliar spray for any measured variables (Table 7.4). All CPPU-treated plants had a decreased leaf, shoot and root fresh and dry weights compared to untreated controls (no CPPU and no GA_3). Shoot and root dry weights decreased as CPPU concentrations increased. Total dry weights of all CPPU-treated plants were significantly decreased compared to control plants. Root to shoot ratio did not differ among CPPU soaking treatments. However, plants treated with CPPU at 5 mg L^{-1} without a GA_3 foliar spray had a significantly greater root to shoot ratio compared to the other treatments. GA_3 foliar spray had no effect on root, shoot and total dry weights.

Table 7.4 Effect of GA_3 foliar sprays following root applied CPPU on plant characteristics of 'Ancho St Luis' pepper plants after 11 weeks.

CPPU soak (mg L^{-1})	GA_3 sprays	Fresh weight			Dry weight			Total (g)	Root: shoot ratio
		Leaf (g)	Stem (g)	Root (g)	Leaf (g)	Stem (g)	Root (g)		
0	Minus[z]	8.8[x]	14.3	10.6	1.7	2.6	1.9	6.2	0.43 ± 0.03[v]
	Plus[y]	11.0	12.8	11.6	2.3	2.5	1.9	6.7	0.40 ± 0.03
1	Minus	7.8	12.1	8.9	1.5	1.9	1.6	5.1	0.44 ± 0.02
	Plus	5.8	7.7	5.2	1.2	1.3	1.1	4.0	0.42 ± 0.02
5	Minus	1.5	2.1	0.5	0.2	0.3	0.2	0.9	0.56 ± 0.16
	Plus	1.7	2.8	0.6	0.3	0.4	0.2	1.1	0.30 ± 0.03
10	Minus	0.9	1.0	0.2	0.1	0.2	0.1	0.4	0.34 ± 0.06
	Plus	0.5	0.8	0.1	0.1	0.1	0.1	0.2	0.20 ± 0.03
ANOVA[w]									
CPPU		L***	L***	L***	L***	L***	L***	L***	ns
GA_3		ns	ns	ns	ns	ns	ns	ns	*
Interaction		ns	ns	ns	ns	ns	ns	ns	ns

[z] Minus represents no GA_3 application.

[y] Plus indicates GA_3 application at 100 mg L^{-1} four weeks after CPPU soaking treatment. [x] All values are mean of five plants per treatment.

[w] Treatment effects were not significant (ns) or significant at the $P \leq 0.05$ (*), or $P \leq 0.01$ (**), or $P \leq 0.001$ (***) level and were linear (L) response to increasing CPPU concentration..

[v] ± represent the standard error of the mean.

7.4 Discussion

Shoot growth in plants is due to activity of the apical and sub-apical meristems and elongation of cell in the resulting internodes. CPPU, when applied to roots reduced plant height (Figures 7.1 and 7.4). Gibberellin biosynthesis inhibitors such as chlormequat chloride (CCC) and paclobutrazol significantly reduced plant height of pepper (Aloni and Pashkar, 1987; Starman, 1993; Lurie et al., 1995; Deka and Shadeque, 1996). CCC reduced cell division rates in the sub-apical meristem (Wareing and Phillips, 1981). Suzuki et al. (2004) reported that paclobutrazol strongly inhibited etiolated and non-etiolated plant height, although no apparent effect was observed on apical meristem activity. Sachs et al. (1960) reported that although treatment of tall chrysanthemums with CCC caused them to be dwarfed, their apical meristem remained unaffected. In contrast, GA_1 promotes shoot elongation by stimulating the activity of the sub-apical meristem (Sachs, 1965; Hansen et al., 1999; Sommer et al., 1999; Little and MacDonald, 2003), leading to greater numbers of cells in the internode, but also stimulating the elongation of internode cells (Wareing and Phillips, 1981; Daykin et al., 1997).

In this experiment, CPPU decreased both internode number and internode length markedly decreasing shoot length at week 2 and 4 after CPPU treatment (Table 7.1). Gad et al. (1997) reported that *Fuchsia magellanica* L cv. 'Beacon' treated with paclobutrazol decreased internode length by 52.2% and 60.9% compared to controls when applied as a foliar spray or soil drench, respectively. Paclobutrazol reduced shoot elongation due to reductions in both length and number of internodes (Gad, 2003). Umezaki et al. (1991) reported that uniconazole, another gibberellin biosynthesis inhibitor, retarded internode elongation of soyabeans. Reduction of internode elongation can result from fewer cells in the internode due to low sub-apical meristem activity (Brown and Sommer, 1992), less cell elongation (Hays et al., 2002) or less cell division (Wang and Dunlap, 1994).

Root applied CPPU severely decreased root growth (Table 7.3; Figure 7.3). Tanimoto (1991) reported that ancymidol inhibited root elongation of lettuce (*Lactuca sativa* cv. 'Grand Rapids'). He demonstrated that ancymidol not only inhibited root elongation but also caused thickening of roots. The present study has shown that CPPU significantly

suppressed root elongation of pepper seedlings and parts of the root became thicker (Figure 7.3), suggesting that CPPU-treated plants possibly have less gibberellin biosynthesis.

If CPPU blocks GA_3 biosynthesis, applying exogenous GA_3 should reverse growth suppression caused by at root applied CPPU. However, if CPPU blocks gibberellin action GA_3 application may not overcome the inhibition caused by CPPU. It is well documented that gibberellic acid reverses the growth suppression caused by growth retardants such as paclobutrazol and uniconazole in several crops including apple (Steffens et al., 1985), cucumber (Cho et al., 2002), marigold (Moore and Schekel, 1985), chrysanthemum (Holcomb et al., 1991), peach (Casper and Taylor, 1989; Tagliavini and Looney, 1991), grapevine (Reynolds and Wardle, 1990), and geranium (Cox, 1991). Cox (1991) reported that gibberellic acid reversed plant growth suppression by paclobutrazol whether paclobutrazol was applied as a drench or as a foliar spray. Ancymidol effects on the elongation growth and the morphological changes in root cells were completely overcome by exogenously applied GA_3 (Tanimoto, 1994). Root-applied gibberellins are transported upwardly into shoot portions (Davies and Rappaport, 1975) and stimulate shoot growth of both herbaceous (Anderson et al., 1988) and woody perennial plants (Heidmann, 1982). Although in the present experiment there was no significant interaction between CPPU and GA_3, Figure 7.6 does appear to indicate that at 1 mg L^{-1} CPPU, GA_3 treatment resulted in plants similar to the controls (i.e. a reversal of the inhibition that was caused by 1 mg L^{-1} CPPU). However it should be noted that the response of 1 mg L^{-1} CPPU-treated plants to GA_3 was not significantly different from control plants. It is therefore not clear that GA_3 resulted in any real reversal of CPPU-induced inhibition. In contrast it is clear that at 10 mg L^{-1} CPPU, GA_3 had no measurable effect.

The emergence of flowers on pepper plants with CPPU-treated roots was significantly delayed (Figures 7.1 and 7.5). This observation contrasts with reports of stimulated flowering in plants treated with GA biosynthesis inhibitors. For example, Cox (1991) reported that 'Mustang' geranium treated with paclobutrazol flowered about ten days earlier than untreated controls. Similarly, exogenous applications of GA_3 delayed flowering of geranium (Armitage, 1986).

7.5 Summary

Root applied CPPU at 10 mg L^{-1} decreased plant height by reducing internode number and length. CPPU as a root soak at 10 mg L^{-1} significantly decreased plant height. A single foliar spray of GA_3 stimulated plant height (Figure 7.6). However it is not clear the extent which GA_3 may have partially reversed the CPPU inhibition effect. Possibly CPPU may block gibberellin synthesis at low concentration and both synthesis and action at higher concentrations. A preliminary study on the effect of CPPU on gibberellin levels in root exudates is presented in the next chapter.

CHAPTER 8

PLANT GROWTH, CHLOROPHYLL CONTENT, LEAF WATER POTENTIAL, PHOTOSYNTHETIC RATES AND GIBBERELLIN ASSAY OF ROOT EXUDATE OF *CAPSICUM* PLANTS TREATED WITH CPPU.

Abstract

The roots of six week old capsicum seedlings were soaked in 2 concentrations (1 or 10 mg L^{-1}) of *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU) for two hours. Root applied CPPU at 10 mg L^{-1} treated plants significantly decreased plant height and leaf expansion, and by three weeks after CPPU treatment, chlorophyll content of 10 mg L^{-1} CPPU was significantly lower than untreated controls. Photosynthetic rates were not affected by CPPU treatment. Leaf water potential of the 10 mg L^{-1} CPPU-treated plants was lower than untreated controls, suggesting the smaller root systems of CPPU-treated plants may limit water uptake. Xylem sap collected from the roots of CPPU-treated *Capsicum* plants had less detectable gibberellin, possibly associated with blocking of gibberellin biosynthesis.

8.1 Introduction

Preliminary experiments indicated that root applied CPPU at 10 mg L^{-1} caused large reductions in plant height through decreased internode number and length (Figures 7.1 and 7.2; Table 7.1). The inhibiting effect of root applied CPPU may involve the activity of gibberellins. The inhibitory effect of 1 mg L^{-1} CPPU appeared to be temporarily overcome by adding gibberellic acid (GA_3) (Figure 7.6) but the effect of 10 mg L^{-1} CPPU was not. Root growth was severely inhibited and some roots became thicker when CPPU was applied to roots (Figure 7.3). Tanimoto (1991) similarly reported that ancymidol, an inhibitor of gibberellins, not only inhibited root elongation but also caused thickening of roots. Both responses could have a negative impact on water

uptake. The objective of the research presented in this chapter was to determine the effect of CPPU-treated roots on plant growth, leaf chlorophyll content, leaf water potential, photosynthetic rates, and biological activity of xylem exudate as determined by a lettuce hypocotyl bioassay, with a view to, at least partially, explaining the effect of root applied CPPU.

8.2 Materials and methods

8.2.1 Experimental conditions and treatments

Bell pepper (*Capsicum annuum* L cv. 'California Wonder') seeds were germinated and grown in plastic cell trays (45 ml volume) containing vermiculite. Four-week-old seedlings were transplanted into 1-litre plastic containers containing a bark-peat-pumice mix (1:1:1), agricultural lime (100g / 100L), dolomite (300g / 100L) and short term osmocote (16N-3.5P-10.8K) (150g / 100L) and grown for two weeks in a glasshouse. Six-week-old seedlings including pots were immersed in an aqueous solution, of either 1 or 10 mg L^{-1} of CPPU for one hour. Control plants were soaked with tap water for one hour. All seedlings were repotted into large plastic bags (15 cm length x 31 cm high) containing a bark-peat-pumice mix (1:1:1), agricultural lime (100g / 100L), dolomite (300g / 100L) and osmocote (16N-3.5P-10.8K) (200g of long term and 100g of short term, 300g / 100L). The seedlings received natural radiation and were watered daily with a drip irrigation system. The experiment was a completely randomized design and each treatment comprised of six plants.

8.2.2 Measurement of plant height, leaf area and leaf length & width

At each harvest, plants were destructively harvested at one, three, six and nine weeks after CPPU treatment. Plant height was measured from top of the potting mix to the tip of the growing point. Leaf mid-rib length and width of the three largest leaves on each plant were measured at each harvest. Leaf area per plant was measured using a leaf area meter (Li 3100; Li-Cor, Lincoln, Neb, USA).

8.2.3 Chlorophyll content

Leaf samples (1.5g for harvests at one and three weeks and four grams for six and nine weeks) were extracted in 100 ml of 80% acetone. The samples were gently shaken in dark room at 2°C for 5 days and the optical density of the chlorophyll extracts recorded using a spectrophotometer (Hitachi Model type U-2000), set at 663 (chlorophyll a) and 645 nm (chlorophyll b). The concentrations of chlorophyll a and chlorophyll b was calculated as milligrams of chlorophyll per gram of leaf calculated according to the equation of (Wellburn, 1994).

8.2.4 Measurement of leaf water potential and photosynthesis

Leaf water potential was measured by using a pressure bomb (Soil moisture Equipment Corp, Santa, Barbara, CA, USA) between 12:00 and 15:00 local hours before each harvest. Two fully expanded leaves from the middle position of the plant were sampled and measured within 30 seconds of leaf removal.

Leaf stomatal conductance (gs, mol m^{-2} s^{-1}) and photosynthetic rate (Pn, μmol m^{-2} s^{-1}) were measured using a portable photosynthesis system (Li-Cor 6200, Li-Cor Inc., Neb., USA). Measurements were made on two middle leaves per plant between 12:00 and 14:00 hours local time five weeks after CPPU treatment.

8.2.5 Xylem sap collection and lettuce bioassay method

Pepper plants were cut 4.0 cm above the ground. Plants were laid horizontally and xylem sap allowed to drip directly into test tubes for 13 hours and then frozen until assayed. To quantify gibberellin activity in xylem sap, the method of Frankland and Wareing (1960) was used. Lettuce seeds cv. 'Marksman' were germinated in petri dishes in a growth cabinet. Gibberellic acid crystals were weighed and dissolved in 50% ammonium hydroxide. The GA_3 solution was placed in a water bath (30°C) and ammonia removed by rotary evaporation. The GA_3 solution was adjusted pH 6.1 and diluted with distilled water to the desired concentration of 100 mg $litre^{-1}$. The 100 mg L^{-1} GA_3 stock solution was serially (x 10) diluted to obtain the following concentrations:

100 mg L^{-1}, 10 mg L^{-1}, 1 mg L^{-1}, 0.1 mg L^{-1} and 0.01 mg L^{-1}. Two mls of each GA_3 standard was pipetted into 5 cm petri dishes lined with filter paper. Fourteen seed with radicles of 2 mm length were transferred into each test solutions and grown at 21°C under fluorescent light for three days. Lettuce seedlings were put on graph paper and hypocotyl length measured. The two maximum and two minimum lengths of the hypocotyl among the fourteen seedlings were eliminated to reduce variation in the sample.

8.2.6 Data analysis

Six single seedling replicates per treatment per harvest were arranged in a completely randomised design in the glasshouse. Leaf mid length and width, three leaves were measured per plant as subsamples for analysis. Analysis of variance (ANOVA) was employed to analyze data using SAS package version 8.2 (SAS Institute, Cary, North Carolina, USA). Data were analyzed at each harvest separately. Treatment means were separated by the LSD test at $P \leq 0.05$.

8.3 Results

Untreated controls and plants treated with 1 mg L^{-1} CPPU were of similar height but treatment with 10 mg L^{-1} CPPU reduced plant height (Figure 8.1). Growth inhibition was first noticeable 3 weeks after the 10 mg L^{-1} CPPU treatment; the amount of inhibition increased from week three to week six.

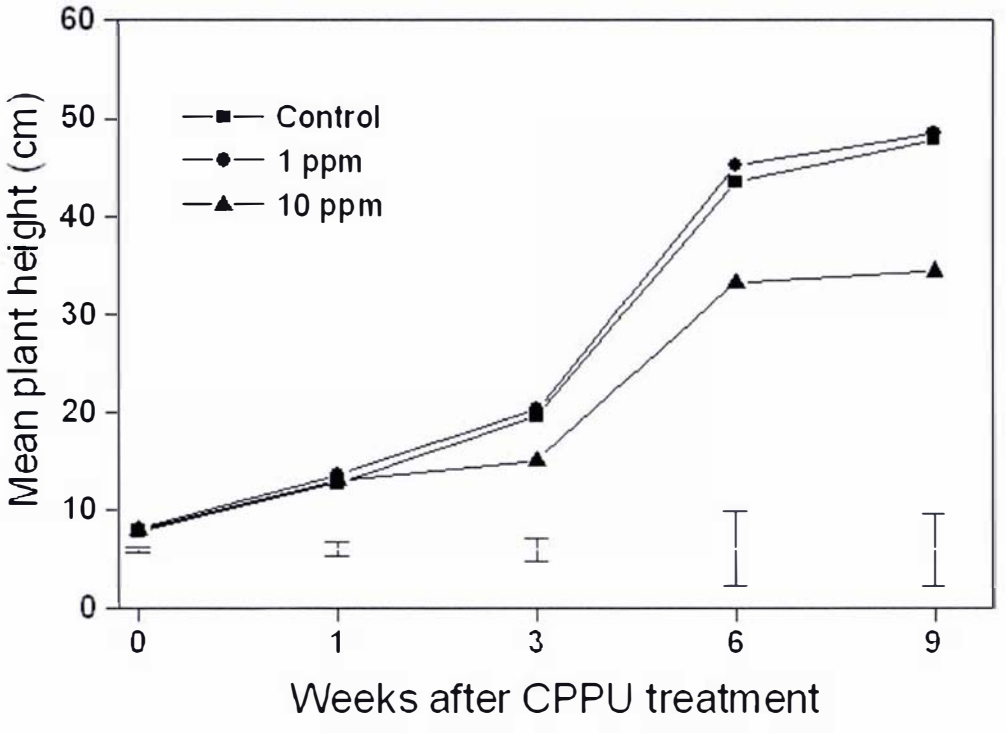

Figure 8.1 Effect of CPPU-treated roots on plant height of 'California Wonder' pepper. Each point is the mean of six plants per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

Root applied CPPU at 10 mg L^{-1} reduced leaf width and length (Figure 8.2), with this reduction first noticeable 6 weeks after treatment.

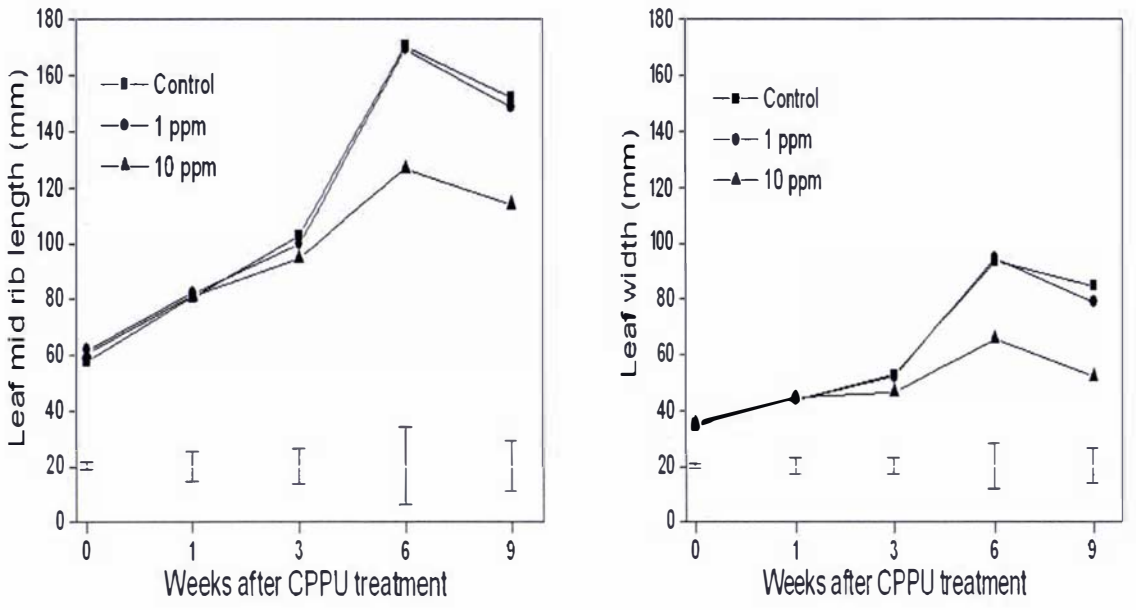

Figure 8.2 Effect of CPPU-treated roots on leaf mid rib length and maximum width of 'California Wonder' pepper. Each point is the mean of eighteen leaves per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

The 10 mg L^{-1} CPPU treatment significantly suppressed the production of leaf area from week three to week nine compared to controls and 1 mg L^{-1} CPPU (Figure 8.3).

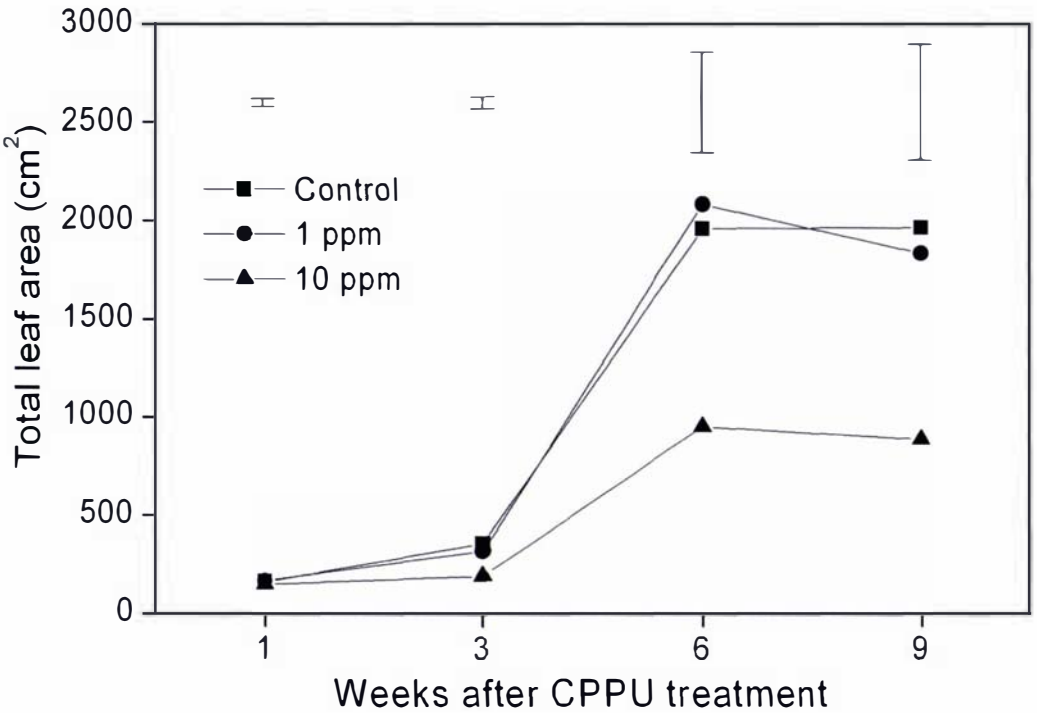

Figure 8.3 Effect of CPPU-treated roots on total leaf area of 'California Wonder' pepper. Each point is the mean of six plants per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

Average leaf area of the 10 mg L^{-1} CPPU-treated plants was significantly lower than untreated control and 1 mg L^{-1} CPPU-treated plants from week six to week nine (Figure 8.4 left). Mean leaf number of the 10 mg L^{-1} CPPU-treated plants from week three to week six was significantly less compared to untreated control and 1 mg L^{-1} CPPU-treated plants (Figure 8.4 right).

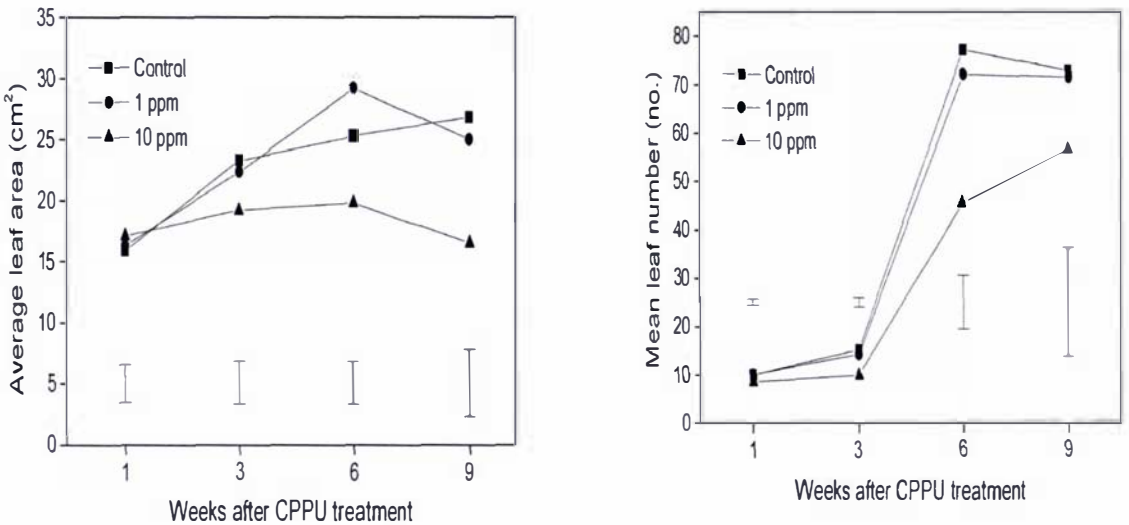

Figure 8.4 Effect of CPPU-treated roots on average leaf area and mean leaf number of 'California Wonder' pepper. Each point is the mean of six plants per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

Chlorophyll a and total chlorophyll content were similar in all treatments after one week. In contrast, chlorophyll b content of leaves from roots treated with 10 mg L^{-1} CPPU was significantly higher than those of the untreated plants (Table 8.1). After 3 weeks, chlorophyll a, b and total chlorophyll content in both CPPU treatments had significantly decreased compared to control plants. However, this effect was lost by 6 weeks after treatment. CPPU at 1 mg L^{-1} had significantly increased chlorophyll a, b and total chlorophyll content in comparison to 10 mg L^{-1} CPPU nine weeks after treatment.

Table 8.1 Effect of CPPU-treated roots on chlorophyll content (mg/g fresh weight) of 'California Wonder' leaves at 1, 3, 6, or 9 weeks after CPPU treatment.

Week	CPPU (mg L^{-1})	Chlorophyll-a	Chlorophyll-b	Total chlorophyll
1	0	0.819a	0.248b	1.068a
1	1	0.875a	0.258ab	1.133a
1	10	0.884a	0.271a	1.155a
3	0	0.962a	0.432a	1.395a
3	1	0.832b	0.387b	1.219b
3	10	0.741b	0.343c	1.085b
6	0	0.858a	0.378a	1.236a
6	1	0.906a	0.410a	1.316a
6	10	0.904a	0.412a	1.317a
9	0	0.758ab	0.276ab	1.034ab
9	1	0.892a	0.327a	1.219a
9	10	0.665b	0.258b	0.924b

Means for the same time sharing the same letter within columns are not significantly different by LSD test at $P \leq 0.05$.

Ten mg L^{-1} CPPU resulted in significantly lower leaf water potentials than control plants and those treated with 1 mg L^{-1} CPPU at 1, 3, and 9 weeks after treatment (Figure 8.5).

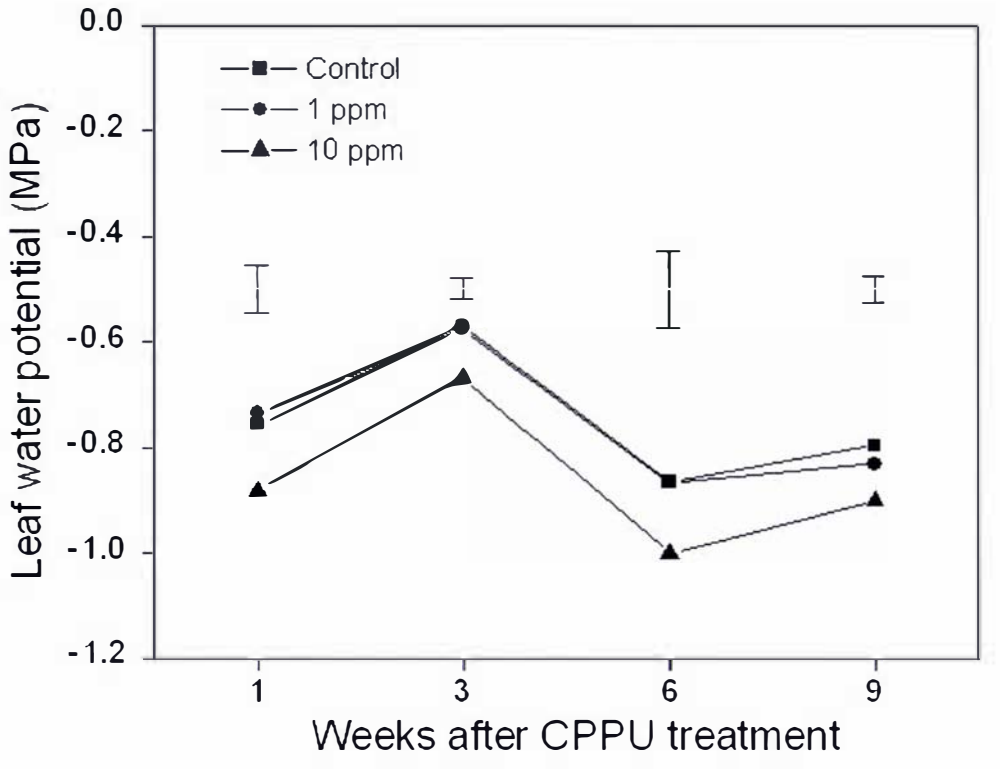

Figure 8.5 Effect of CPPU-treated roots on leaf water potential of 'California Wonder' pepper. Each point is the mean of twelve leaves per treatment. Vertical bars represent the LSD at $P \leq 0.05$.

Photosynthetic rates were not significantly different between any of the treatment over the period of the experiment (Table 8.2). Stomatal conductance, and stomatal resistance were not affected by CPPU treatment. The intercellular CO_2 concentration of the 10 mg L^{-1} CPPU-treated plants was lower than those of control plants, however, six weeks after CPPU treatment, intercellular CO_2 concentration of the 10 mg L^{-1} CPPU plants was higher.

Table 8.2 Effect of CPPU-treated roots on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and stomatal resistance by five weeks after treatment.

Week	CPPU (mg L^{-1})	Photosynthetic rate (μmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Intercellular CO_2 concentration (ppm)	Stomatal resistance (s cm^{-1})
1	0	12.24a	1.24a	323.2a	0.32a
1	1	11.82a	1.08a	317.6ab	0.38a
1	10	11.81a	1.01a	313.9b	0.40a
6	0	9.66a	0.45a	320.2b	2.52a
6	1	7.82a	0.50a	321.1ab	2.32a
6	10	10.28a	0.36a	328.3a	2.97a
9	0	7.56a	0.85ab	334.9a	0.52a
9	1	7.40a	0.72b	333.4a	0.55a
9	10	8.74a	1.14a	331.5a	0.36a

All values are mean of 12 measurements per treatment. Means for the same time sharing the same letter within columns are not significantly different by LSD test at $P \leq 0.05$.

One week after CPPU treatment, controls and 10 mg L^{-1} CPPU plants were similar except for shoot fresh weight and root dry weight. However, leaf, shoot, and root fresh and dry weights of the 10 mg L^{-1} CPPU-treated plants were significantly less at week three, six, and nine weeks compared to control plants (Table 8.3). Shoot dry weight was severely reduced by 10 mg L^{-1} CPPU by week six, however, root growth was severely suppressed by 10 mg L^{-1} CPPU by week three. All CPPU-treated plants had significantly higher root to shoot ratios than control plants and 1 mg L^{-1} CPPU-treated plants at week three. By nine weeks after treatment shoot growth was severely affected resulting in the root to shoot ratio being similar between control and 10 mg L^{-1} CPPU-treated plants. Total dry weight of the 10 mg L^{-1} CPPU-treated plants was significantly less than the control or 1 mg L^{-1} CPPU-treated plants.

Table 8.3 Effect of CPPU-treated roots on plant characteristics of 'California Wonder' at each harvest, 1, 3, 6, and 9 weeks after application.

		Plant characteristics							
Week	CPPU (mg L^{-1})	Fresh weight			Dry weight			Total dry wt (g)	Root: shoot ratio
		Leaf (g)	Shoot (g)	Root (g)	Leaf (g)	Shoot (g)	Root (g)		
1	0	4.38a	2.14b	2.66a	0.52a	0.22b	0.35b	1.09a	0.48a
1	1	4.26a	2.41ab	2.76a	0.58a	0.29a	0.46a	1.34a	0.54a
1	10	4.31a	3.04a	2.29a	0.58a	0.34a	0.45a	1.38a	0.49a
3	0	10.15a	6.67a	10.42a	1.56a	0.89a	0.90ab	3.35a	0.36b
3	1	8.66a	6.24a	11.97a	1.30a	0.89a	1.08a	3.27ab	0.50a
3	10	6.33b	5.75a	6.79b	0.83b	0.85a	0.83b	2.52b	0.49a
6	0	59.04a	73.30a	71.23a	9.48a	10.26a	7.01a	26.76a	0.35a
6	1	64.16a	73.61a	80.40a	10.15a	10.19a	7.67a	28.02a	0.38a
6	10	31.90b	36.83b	36.25b	6.37b	5.17b	3.87b	13.54b	0.42a
9	0	59.18a	65.29a	85.73a	9.99a	11.98a	10.34a	32.32a	0.47ab
9	1	54.00a	61.63a	72.21a	9.14a	10.88b	9.14a	29.17a	0.45b
9	10	26.56b	30.13b	30.01b	4.93b	4.93b	3.98b	13.98b	0.57a

All values are mean of six plants per treatment. Means for the same time sharing the same letter within columns are not significantly different by LSD test at $P \leq 0.05$.

Root exudates were collected in order to investigate gibberellin supply from the roots to the shoots. Root exudation from the 10mg L^{-1} CPPU-treated roots was low compared to the control plants or 1 mg L^{-1} CPPU for the first three weeks (Table 8.4) but not significantly different among the treatments by six weeks.

Table 8.4 Effect of CPPU-treated roots on root exudate (g $hour^{-1}$) of 'California Wonder' at 1, 3, 6, or 9 week after CPPU treatment.

CPPU concentration (mg L^{-1})	Time after treatment (weeks)			
	1	3	6	9
	Root exudation rate			
0	--	0.06[z] ± 0.01	0.60 ± 0.11	0.52 ± 0.05
1	--	0.06 ± 0.02	0.47 ± 0.14	0.52 ± 0.04
10	--	0.02 ± 0.00	0.44 ± 0.14	0.47 ± 0.03

[z] All values are mean of six measurements per treatment. ± denotes standard error of the mean.

Lettuce cv. 'Marksman' gave a significant response to GA_3 at 0.01 mg L^{-1}. The response was maximum at 10 mg L^{-1} while 100 mg L^{-1} was supra-optimum but still significantly greater than 1 mg L^{-1} (Figure 8.6, left). The lettuce hypocotyl bioassay indicated that CPPU had significantly decreased gibberellin levels in the xylem exudates at 3 and 9 weeks after treatment. At 6 weeks after treatment only 10 mg L^{-1} CPPU had significantly lowered gibberellin levels (Figure 8.6, right).

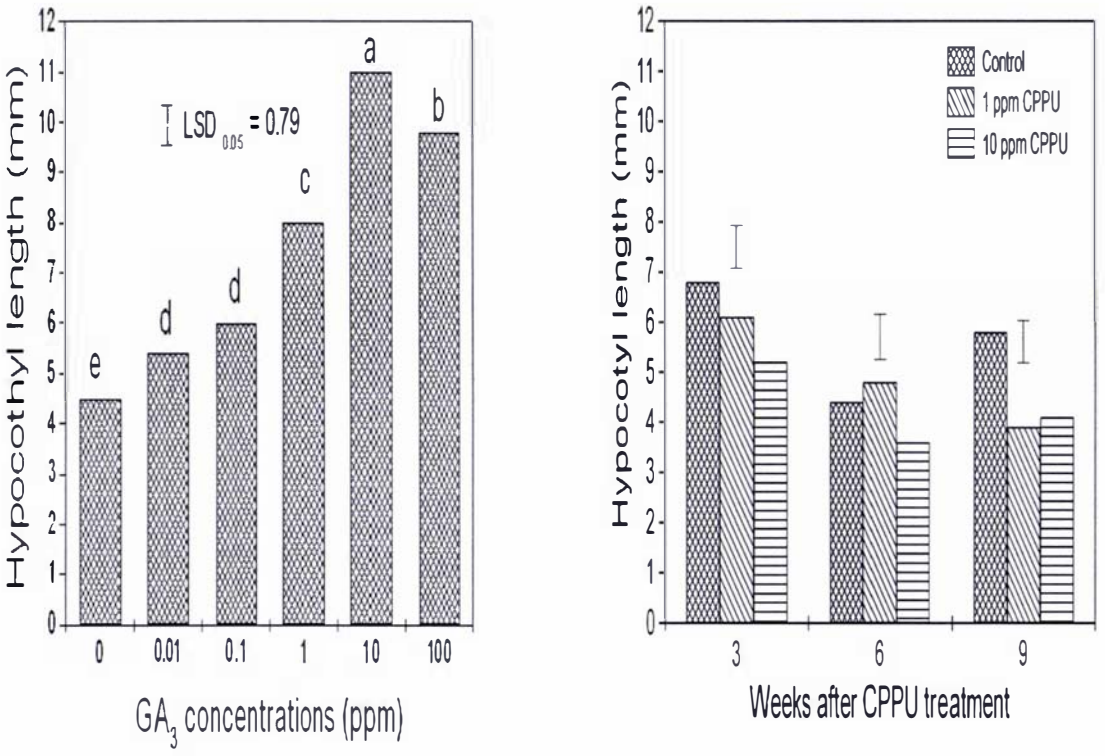

Figure 8.6 Lettuce hypocotyl assay of root exudates of CPPU plants (right graph) and corresponding standard curve for GA_3 response (left graph). Values followed by different letters are significantly different by LSD test at $P \leq 0.05$ (left graph). Vertical bars represent the LSD at $P \leq 0.05$ (right graph).

8.4 Discussion

Root applied CPPU at 10 mg L^{-1} exerted a strong inhibitory effect on plant growth (Figure 8.1). One mg L^{-1} CPPU had no effect on plant height (Figure 8.1) but significantly reduced plant height in Experiment 2, Chapter 7 (Figure 7.4) compared to untreated plants. A possible reason for this could be that pepper seedling roots were soaked in CPPU solution at 1 mg L^{-1} for two hours in the previous experiment (Figure 7.4), while seedlings including the pots were submerged at 1 mg L^{-1} for one hour in the present study. The longer CPPU treatment may, presumably, result in greater CPPU uptake, but CPPU would saturate the soil so uptake would be over a long period. In addition, cultivars were 'Ancho St Luis' (Section 7.2.2) and 'California Wonder' in Section 8.2.

CPPU applied as a foliar spray at 30 mg L^{-1} promoted the growth of leaves of 'Red Caturra' coffee plants (Cruz-Castillo, 1998). However, in the current experiment, root applied 10 mg L^{-1} CPPU reduced both the size of the leaves and leaf number when applied to roots (Figures 8.2 and 8.3). Thus, CPPU may stimulate or retard leaf growth depending on whether it is applied to the plant leaves or roots, respectively. CPPU applied to roots may possibly damage the roots, affecting water uptake and cytokinin production both of which are involved in leaf growth and expansion. Tanimoto (1987) reported that plant growth retardants reduced leaf expansion.

Leaf chlorophyll content of tissue cultured microshoots treated with CPPU was higher than in shoots on normal medium (Singh and Syamal, 2001). Leaf chlorophyll content of growth retardant CCC (chlormequat) treated plants was higher than the control (Ojeda and Trione, 1994). These results disagree with the present study that chlorophyll a, b, and total chlorophyll content in all CPPU-treated plants were significantly reduced compared to control plants at week 3 (Table 8.1). However, photosynthetic rate was not significantly different among treatments. This result is consistent with previous experiment on CPPU (Table 7.2). Wieland and Wample (1985b) reported that paclobutrazol had no effect on photosynthetic rate when applied as a soil drench or as stem application to one-year-old 'Topred Delicious' apples, as was also found here for CPPU-treated pepper plant.

Leaf water potentials in all 10 mg L^{-1} CPPU-treated plants were significantly lower than in the controls (Figure 8.5). In contrast, Wieland and Wample (1985a) reported that soil and stem applied paclobutrazol caused higher leaf water potential in apple. They explained that paclobutrazol treated plants had increased stomatal conductance and leaf water potentials, probably due to the reduced water requirements for these dwarfed plants. The root to leaf area ratios were increased in all paclobutrazol treated plants compared to control plants (Wieland and Wample, 1985a). The present study indicated that leaf water potential was significantly lower in 10 mg L^{-1} CPPU-treated plants, suggesting that smaller root system of CPPU-treated plants may have limited H_2O uptake. However, there was no effect on photosynthesis, and little or no effect on stomatal conductance (Table 8.2). Also the difference in leaf water potential due to CPPU was small.

Root growth was severely reduced by application of 10 mg L^{-1} CPPU (Tables 7.3 and 8.3). Roots of 10 mg L^{-1} CPPU-treated plants were less branched and shorter than controls (Figure 7.3). Thus, root applied CPPU at 10 mg L^{-1} caused large reductions in root fresh and dry weights (Table 8.3). Shoot growth was also severely reduced resulting in similar root: shoot ratios by week nine (Table 8.3). It is generally believed that some gibberellins are synthesized in root meristems, transported upwardly into shoots (Davies and Rappaport, 1975) and influence shoot growth (Heidmann, 1982; Anderson et al., 1988). In the previous experiment it was shown that applying GA_3 to the foliage did not reverse growth suppression caused by root applied CPPU (Figure 7.6) i.e. the growth stimulation caused by GA_3 was similar in control and CPPU-treated plants. However, the root exudate of CPPU-treated plants produced a reduced response in the lettuce hypocotyl bioassay compared to the control plants and 1 mg L^{-1} CPPU at week 3, 6, and 9 (Figure 8.6). These results indicate that root applied CPPU may be associated with blocking biosynthesis of gibberellins that affect root, shoot or leaf growth. Thus CPPU appears to be acting similarly to a growth retardant that blocks gibberellin biosynthesis, but the results are not entirely consistent. That is it would be expected that GA_3 application would be reverse the effect of CPPU inhibition. There are several ways in which CPPU gives a different response to most growth retardants. GA_3 as a foliar spray at 100 mg L^{-1} did not reverse the CPPU inhibition (Figure 7.4). CPPU-treated plants had decreased chlorophyll levels (Table 8.1). Leaf water potential of the CPPU-treated plants was significantly lower than in the control (Figure 8.5). Thus it is

not clear that CPPU is acting as an anti-gibberellin growth retardant, nor the extent to which CPPU may block gibberellin action as well as synthesis.

8.5 Summary

Leaf water potential was lower in CPPU-treated plants than control plants. This result may indicate that CPPU-treated plants have limited water uptake. However although chlorophyll content of the CPPU-treated plants decreased, photosynthetic rate was unaffected. The root exudate of CPPU-treated plants significantly reduced hypocotyl length in the lettuce gibberellin bioassay, suggesting that CPPU blocks gibberellin biosynthesis in roots. However GA_3 application did not appear to reverse the effect of CPPU.

CHAPTER 9

GENERAL DISCUSSION

9.1 Physiological factors affecting productivity in temperate climates

In New Zealand the length of the asparagus harvest season is limited by spring temperatures and the need to establish a fern canopy in early summer in order to replenish carbohydrate reserves to be used for spear growth the following season. The amount of fern produced and its photosynthetic rate will determine the amount of carbohydrate stored, provided a sufficiently large storage root system can be established. That is, in asparagus, the balance between source strength (supply of carbohydrates) and sink strength (accumulation of carbohydrates) is critical to productivity. Most evidence indicates that high yielding cultivars partition a large proportion carbohydrates to the root system resulting in a high root to shoot ratio (Benson and Takatori, 1980; Haynes, 1987; Drost, 1997; Sinton and Wilson, 1999). Nevertheless an adequate fern canopy must also be established to fully charge the root system with fructans.

Provided the amount of reserve carbohydrates (fructans) is adequate yield will then depend on the number of buds available and how quickly spears are produced and grow during the time-limited harvest season. In this discussion some attention will be paid to establishing fern numbers, photosynthetic rates and root to shoot ratios (Chapters 4, 5 and 6), but more emphasis will be placed on spear growth rates and bud numbers and bud burst (Chapters 2 and 3). An attempt was also made to determine the influence of mineral nutrition on fern, bud and root production, using a hydroponics system, but results were extremely variable and not reported in this thesis. A "mind map" illustrating the relationship between the physiological factors and yield discussed in this thesis is given below (Figure 9.1).

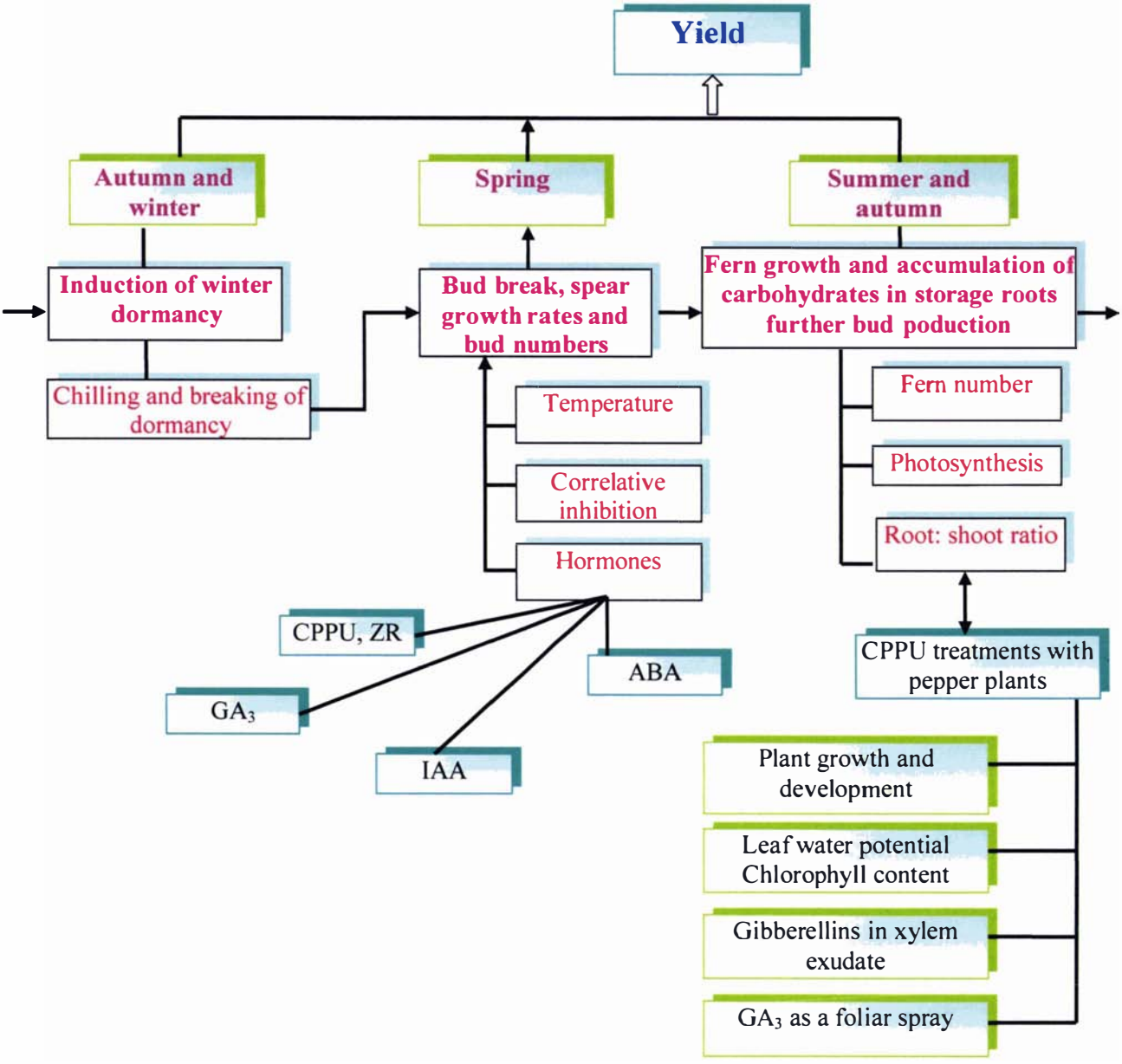

Figure 9.1 Relationship between main physiological factors affecting asparagus yield as discussed in this thesis.

9.2 Bud break, spear growth rates and bud numbers

In temperate climates yield is strongly correlated with spring and early summer temperatures. Growing temperatures are important in relation to bud break, spear growth rates, spear growth pattern (linear or exponential) and bud numbers. Hughes (1992) provided some evidence that chilling temperature prior to bud break might influence the dynamics of bud break. She found that the optimum chilling temperature was difficult to determine, but appeared to be closer to 5°C than to 10°C or 2°C for 'Rutgers Beacon' plants grown at 12.5°C.

9.2.1 Bud break

The minimum temperatures for bud break have been reported as 4.4°C for 'Mary Washington' (Bouwkamp and McCully, 1975), 5.8 ° C for 'Martha Washington' (Culpepper and Moon, 1939a), below 7.2°C for 'Raritan' (Blumenfield et al., 1961), 10 °C for 'Glenn Smith Mary Washington' (Dean, 1999), and around 10°C for 'UC 157', around 12.5°C for 'Rutgers Beacon' and 'Jersey Giant' (Hughes, 1992). The present result indicated that the minimum temperature for asparagus bud break was near to 10° C for 'Apollo' (Figure 3.5). This agrees closely with the minimum temperatures found by Dean (1999) and Hughes (1992) for 'UC 157'. However, the minimum temperature of 10°C for bud break was higher than the air temperatures for bud break given by Culpepper and Moon (1939a), Blumenfield et al. (1961) and Bouwkamp and McCully (1975), and lower than Hughes (1992) for 'Rutgers Beacon' and 'Jersey Giant'. These differences in minimum temperatures may reflect differences between early, medium and late cultivars; for rapid bud burst of large numbers of buds, suitable for the start of commercial harvest, spring temperatures need to be above 8°C for cv. 'JWC1' and above 13 ° C for 'Pacific 2000' (Falloon, personal communication and see Section 1.3.1.1.2). From the results presented in this thesis late cultivars that require a high spring temperature, such as 'Pacific 2000', may benefit from additional winter chilling. However, for the cultivars investigated here the number of chill units required appear to be quite low.

In the present work the temperatures at which bud burst occurred depended on prior chilling. Prior chilling at 5°C promoted bud break at 10°C and 15°C, with 6 weeks chilling being significantly more effective than 3 weeks chilling (Figure 3.5). These chilling periods corresponded with accumulated chill units of 504 or 1008 respectively with a chill unit calculated as one hour at 5°C. This is somewhat lower than that required for bud break of 'Excel' in South Korea, e.g, 1000 to 1250 chill units (Seong et al., 2002) but it is possible that more than six weeks chilling should have been given. However, Hughes (1992) reported that asparagus plants appeared to have a relatively low chilling requirement of about 500 chill units for low-chill asparagus cultivar such as 'Rutgers Beacon'. Thus there appears to be substantial differences in chilling requirements amongst cultivars and if insufficient winter chilling were received bud break could be delayed at normal spring temperatures. Therefore, in some climates production may be reduced if insufficient chilling is received or an inappropriate cultivar used.

9.2.2 Spear elongation

Growing temperatures not only influenced mean number of days to bud break for 'Dariana' (Figure 3.3) and for 'Apollo' plants (Figure 3.5) but also the elongation rate of spears increased dramatically with increasing temperature from 10°C to 30°C (Figures 3.4 and 3.6). Prior chilling had no effect on relative spear growth rate (RSGR) of 'Dariana' from 20°C to 30°C and 'Apollo' from 10°C to 30°C (Figures 3.4 and 3.6). However, at 15°C the growth of spears for chilled-plants cv. 'Apollo' was faster than those from non-chilled plants (Figure 3.2). Thus, in some cultivars if a warm winter is followed by a cool spring growth rates may be slow, due to both low growing temperatures and insufficient winter chilling.

Spear elongation can be linear, exponential, two phases of exponential, or mixed (not clearly linear or exponential) (Figure 1.4). Spears grew exponentially with time from 20°C to 30°C for 'Apollo' and 'Desto' (Table 3.1) and from 15°C to 30°C for 'Apollo' (Table 3.2). However, at 10°C, only 20% of the spears showed exponential growth, 20.7% linear growth and the remaining 59.3% exhibited an unclear growth pattern. At 35°C, 46.4% of the spears showed linear growth and 36% exponential growth. These

results confirm that at 10°C and 35°C, elongation rates of spears tended to become linear while between 15°C and 30°C spears mainly grew exponentially. Thus, growing temperature is a major factor affecting spear growth if plants are not limited by other resources, such as carbohydrates in the crown and correlative inhibition (Nichols and Woolley, 1985; Daningsih, 2005). Fastest growth rates normally follow an exponential growth pattern with adverse conditions leading to slower exponential growth or linear growth. A slow growth rate not only delays the harvest of that spear, but also delays the release of the next bud in the cluster due to correlative inhibition and thus reduces total yield within a fixed harvest period.

9.2.2.1 Correlative inhibition

Daningsih (2005) defined correlative inhibition between spears of asparagus as the inhibition period between the growth of one spear in a cluster and the establishment of rapid growth of the next spear (Figure 9.2). Thus correlative inhibition was manifest in two ways, a lag period when the second spear did not grow and a period when the spear grew slowly, generally until the previous spear was harvested. If correlative inhibition does not exist, spears elongate simultaneously (i.e. little or no lag period), and at similar rates. Nichols and Woolley (1985) had previously found that when several spears from different bud clusters on a crown grew simultaneously, they grew at a similar rate, but the growth of other spears on a rhizome was severely inhibited. Thus correlative inhibition normally occurs between buds in the same cluster but may occur between clusters on connected rhizomes. If a bud starts growing but the spear is still subjected to some correlative inhibition then growth tends not to be exponential, or the rate constant is very low. A consequence of this correlative inhibition is that growth of the subsequent spear will be substantially delayed due to slow growth of the previous spear. Correlative inhibition occurred most strongly for the first five spears on a rhizome and gradually reduced thereafter (Daningsih, 2005).

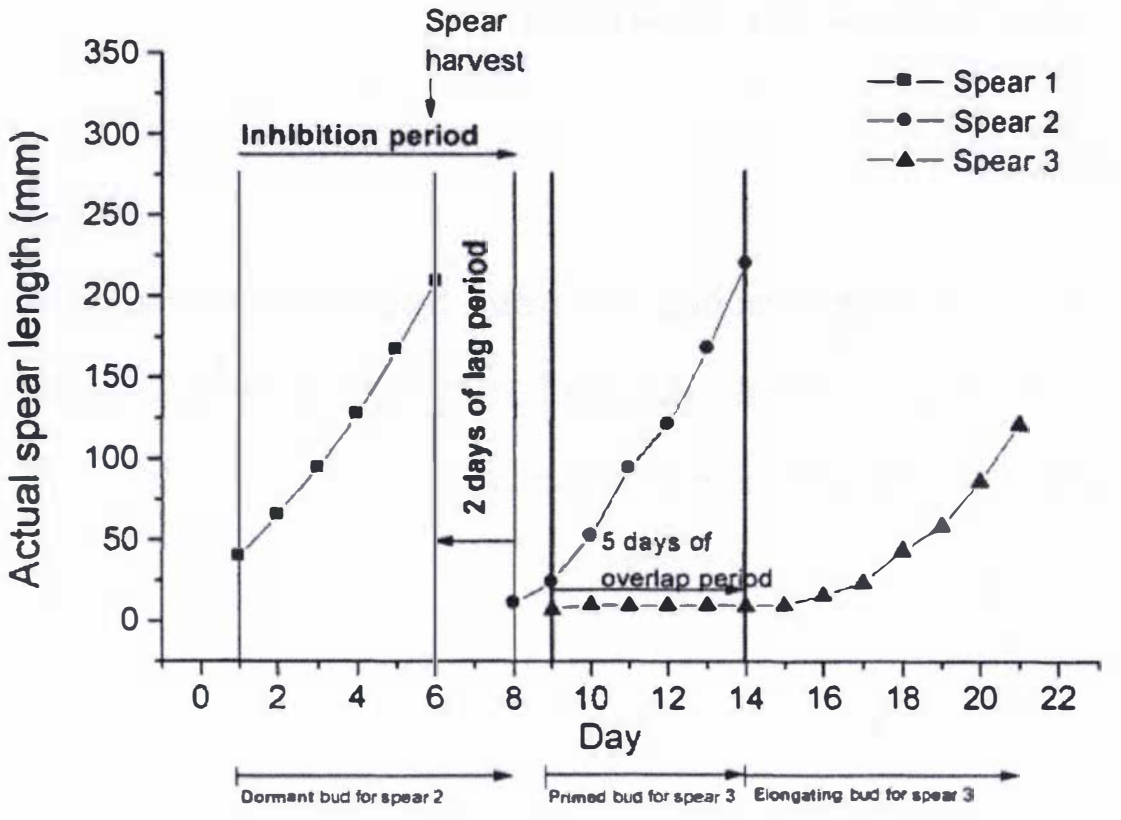

Figure 9.2 Three phases of correlative inhibition in asparagus bud clusters. 1. Inhibition period – time between growth of one spear and rapid growth of the subsequent spear. 2. Lag period – time between harvest of the previous spear and growth of the following spear. 3. Overlap period – period when both spears are growing simultaneously but the growth rate of one spear is reduced by the other (from Daningsih, 2005).

RSGRs were approximately 0.10 mm mm^{-1} day^{-1} at 10°C and 0.24 mm mm^{-1} day^{-1} at 15°C. Days taken for spears to grow between 1 and 20 cm were approximately 28 days at 10°C and 16 day at 15 °C, respectively (Table 9.1). If it is assumed that all the bud clusters produced spears equally (41 clusters for 10°C and 32 clusters for 15°C) and that there was no residual effect of correlative inhibition once a spear had been harvested then the expected total spears would be 205 spears at 10°C and 288 spears at 15°C within the same harvest period (Table 9.1). The difference between actual number of spears produced and the expected number of spears produced was 92 spears at 10°C and 128 spears at 15°C and represents delayed growth (correlative inhibition) after the previous spear had been harvested. The percentage decrease at 10°C and 15°C was

therefore 45% for both temperatures. That is these temperatures appeared to have no effect on correlative inhibition.

Table 9.1 Difference between actual and estimated spear numbers grown at 10 and 15°C.

Temeprature (°C)	Clusters (no.)	Spear[y] (1-20 cm) (days)	Harvest cycle[x]	Harvest period (days)	Estimated spear (no)	Actual spear (no.)	Difference (no.)
10	41	28	5	144	205	113	92
15	32	16	9	144	288	160	128

[y] Days taken for spears to grow between 1 and 20 cm. [x] Each spear was harvested after a mean period of 28 days at 10°C and 16 days at 15°C. At 10°C and 15°C estimated spear numbers were calculated as cluster number x harvest cycles within each harvest period.

Correlative inhibition and growth rate in relation to temperature may be influenced by plant age and plant cultivar. Nichols and Woolley (1985) found that RSGRs were 0.33 mm mm^{-1} day^{-1} at 10°C and 0.36 mm mm^{-1} day^{-1} at 15 °C for 'UC 157'. Days taken for spears to grow between 1 and 20 cm were 9 days at 10°C and 8 days at 15°C. The present result for 'Apollo' showed lower spear growth rates than for 'UC 157', suggesting that the growing temperature threshold for bud break and spear growth varies with age and/or cultivar. Robb (1984) stated that 1 and 2-year-old plants produce earlier than old plants, and some cultivars are earlier than others. It is suggested from the above comparison that young asparagus plants cv. 'UC 157' would produce spears earlier in spring than five-year-old plants of cv. 'Apollo'. Hughes (1992) reported that asparagus plants cv. 'UC 157' appeared to have a relatively weak correlative inhibition compared to cvs. 'Rutgers Beacon' and 'Jersey Giant', while in the present work 'Apollo' plants appeared to have relatively strong correlation inhibition. It is interesting to note that 'UC 157' tends to be grown in the South Island of New Zealand where spring temperatures are low, compared to 'Apollo', which tends to be grown in the North Island (Woolley, personal communication).

9.2.3 New bud numbers (buds produced during the harvest period)

During the spear harvest season new buds were produced, but the number of new buds per cluster were not influenced by temperatures between 15°C to 30°C, but at 10°C new bud production decreased (Table 3.3). New bud formation during spear harvest was three to four additional buds per cluster at temperatures between 15°C to 30°C (Table 3.3; Figure 3.7). In a commercial field planting, the number of new buds produced during the spear harvest averaged 51 buds per m^2 (Table 3.4). These results agree with those of Woolley et al. (2004) and Daningsih (2005) that new buds formed between 20 and 30 percent of the total buds produced during the spear harvest period in a commercial field planting near Bulls (New Zealand). Daningsih (2005) reported that 16% of new buds produced spears in the current harvest. The present result from this research showed that some of the new buds formed during the spear harvest grew and contributed to new ferns (Figure 3.8). Thus new buds formed during the spear harvest contribute to the carbohydrate supply of the next season's yield. These buds therefore need to produce vigorous, healthy fern and new methods of managing the crop need to be considered. For example, asparagus growers normally provide fertiliser in early spring before spear harvest and/or after spear harvest before fern growth in summer. Three application times of nitrogen fertiliser application; namely; prior to fern growth, prior to harvest, and early harvest, prior to the main period of spear production increased spear production by 12% in the first year over the nil nitrogen treatment but there was no significant effect of time of nitrogen application (Ledgard et al., 1992). Split applications of nitrogen (75% before and 25% after harvest, or 50% before and 50% after harvest) had no significant effect on cumulative 3-year yields (Krarup, 1991). Most uptake after application of nitrogen fertiliser occurred prior to the period of fern growth or during the first 8 weeks of foliage growth in summer (Ledgard et al., 1992; Ledgard et al., 1994). Thus it would appear that earlier application of at least some of the nitrogen, i.e. at the time of bud development during harvest, would be beneficial if uptake at this time could be improved. Lack of uptake may reflect low root activity due to low temperatures.

9.2.4 Yield

In temperate climates such as those experienced in New Zealand, the mean growing temperature during spear harvest from September to November is normally between 10°C - 15°C (Tabe 9.2). As the season progresses from early spring to the end of spring, environmental factors, such as growing temperatures increase and bud break and spear growth rates increase (Hughes, 1992; Chapter 3). Differences in the effect of growing temperatures at 10°C and 15°C clearly demonstrated that bud break at 15°C occurred earlier than at 10°C (Figure 3.5) however the effect of chilling on spear growth rate was unclear. This lack of clarity was because in the main experiment (Section 3.2.4) the plants received a small amount of chilling due to lack of temperature control during cold nights (Figures 3.2 and 3.6). At 15°C spears grew exponentially (56.8%) and 160 spears (harvest period-144 days) were produced, while at 10°C only 113 spears (harvest period-144 days) were produced of which only 20% grew exponentially (Table 9.1). Clearly both growth rate and number of spears that can grow at any one time due to correlative inhibition, are going to affect yield. Earlier bud break, a higher percentage of spears exhibiting exponential growth and increasing new bud numbers at 15°C are important factors influencing spear yield under temperate climatic conditions.

Gradual increases of temperature from 10°C to 15°C were not investigated here but may be important in relation to asparagus yield. It is clear that temperature change during spring and early summer is a major environmental determinant of asparagus yield, and more attention should be given to investigating the physiological and genetic differences between cultivars in response to temperatures between 8°C and 20°C. For example from our results on spear growth rates and Table 9.2 it is clear that some cultivars would be better suited to the Auckland regions compared to Dunedin.

Table 9.2 Average monthly mean, maximum and minimum air temperatures (1971-2000 year) at National Climate Centre (http://www.niwascience.co.nz/ncc).

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	Mean	19.3	19.8	18.5	16.2	13.7	11.6	10.8	11.3	12.6	14.1	15.8	17.8
A	Max	23.3	23.7	22.4	20.0	17.4	15.2	14.5	15.0	16.2	17.8	19.6	21.6
	Min	15.3	15.8	14.6	12.3	10.0	8.0	7.1	7.6	8.9	10.5	12.1	13.9
	Mean	17.9	18.2	16.6	14.1	11.3	9.2	8.6	9.3	11.0	12.7	14.3	16.3
P	Max	22.4	22.9	21.1	18.4	15.3	13.1	12.5	13.3	14.9	16.7	18.5	20.7
	Min	13.4	13.5	12.1	9.8	7.4	5.4	4.7	5.4	7.2	8.7	10.2	12.0
	Mean	17.4	17.1	15.5	12.8	9.6	6.9	6.6	7.7	10.0	12.3	14.0	16.0
C	Max	22.5	22.2	20.4	17.8	14.6	11.7	11.3	12.4	14.9	17.4	19.2	21.2
	Min	12.2	12.1	10.6	7.7	4.5	2.1	1.9	2.9	5.1	7.2	8.9	10.9
	Mean	15.2	15.1	13.7	11.9	9.2	7.0	6.5	7.5	9.3	10.9	12.4	13.9
D	Max	18.9	18.7	17.2	15.5	12.6	10.2	9.8	11.0	12.8	14.6	16.1	17.4
	Min	11.5	11.4	10.2	8.3	5.7	3.8	3.2	4.1	5.7	7.2	8.6	10.4

Station details. A: Auckland-Owaikraka (Mt Albert Research Centre), P: Palmerston North (AgResearch, Airport), C: Christchurch (Airport, Botanical Gardens), D: Dunedin (Musselburgh).

9.3 Bud break and spear growth rates in relation to hormones

Release of winter dormancy by prior chilling could be either a paradormancy or endodormancy phenomena. It could be endodormancy if the bud meristem required chilling before bud break occurred, and paradormancy if the bud scales were involved. The response of the bud to removal of bud scales would help to clarify this situation but has not been reported in the literature. Robb (1984) stated that high levels of abscisic acid (ABA) in the crown buds of asparagus appeared to control dormancy in winter. Matsubara (1980) reported that the resting buds accumulated high levels of ABA levels compared to the buds of developing stages, such as sprouting buds and shoots 1 or 1.5 cm in length. Evidence that gibberellins may be involved was indicated many years ago when Tiburcio (1961) found that maximum concentration in asparagus buds coincided with release of winter dormancy. Thus changes in hormone levels with dormancy appeared to follow patterns typical of bud dormancy in general.

ABA and IAA inhibited bud break in asparagus plants (Tiburcio, 1961; Matsubara, 1980). However, although high levels of ABA are associated with dormancy it is interesting that relatively high levels of ABA and IAA in the spear tip were present during the periods of active growth in spring (Kojima et al., 1993; Kojima and Sakurai, 1994). The present result showed that ABA slightly inhibited spear elongation rates while IAA and GA_3 slightly promoted spear elongation (Figure 2.1). However, CPPU greatly stimulated the growth of the first and second segment (20-60 mm) below the spear tip (Figures 2.2 and 2.3). The naturally occurring cytokinin, zeatin riboside (ZR) was also effective in stimulating spear length (Figure 2.5). These results not only indicated that CPPU and ZR may stimulate the zone of cell elongation along spear but may also extend the elongation region. Kojima et al. (1993) found that the zone of elongation was 15-35 mm below spear tip but results presented in Chapter 2 suggested that the region of elongation may be much longer. Unfortunately cell numbers, length and width were not measured here, but cell activity, especially in the zone of spear elongation, would appear to warrant further study, especially with regard to the relationship between spear growth rate and yield.

The present work showed that spear leaf scale removal significantly reduced spear elongation irrespective of CPPU treatment (Table 2.2). CPPU-treated spears with intact spear leaf scales stimulated spear growth but without spear leaf scales CPPU was ineffective. That is, CPPU did not reverse the growth suppression caused by removing spear leaf scales. These results indicate that other plant growth hormones may interact with cytokinins in promoting elongation, and that cytokinins may block the inhibitory effect of ABA (Robertson and Berrie, 1977; Lu et al., 1992; Dewar et al., 1998; Hansen and Dorffling, 2003). Spear growth would appear to be influenced by hormonal interaction effects, but further research is required to explain exponential growth and the general kinetics of spear growth.

9.4 Yield and partitioning of dry matter in relation to CPPU treatments.

The number of fern that should be produced per plant will depend on plant density and the amount of light that should be intercepted on a bright sunny day. Increasing plant densities from 21,550 to 43,100 plants/ha increased the cumulative yield from 64% to 80% for three hybrid lines ('UC 157', 'WSU 1', and 'WSU 2') (Sanders et al., 1998). At the higher densities, a lower number of ferns per plants were produced and the total fern numbers resulting from high density was similar to a normal planting system.

As ferns grow and develop, the fern canopy progressively intercepts more solar radiation. Light interception is mainly determined by leaf area index (LAI) and leaf angle, but leaf angle of asparagus is random. This allows good penetration of radiation to the lower leaves. In asparagus plants, an increase in fern numbers has the potential for a larger photosynthetically active plant surface area, however, this must be balanced by the danger of an excessively high leaf area index, or the development of fern late in the season using assimilates for fern production which return less assimilates than they use. A favourable root:shoot ratio may assist in the development of a large root system and thus a good fern canopy in subsequent seasons (Dufault and Greig, 1983). The maximum fern stalk area and dry weight depend on root:shoot partitioning of carbon in a cultivar, and affect its ability to produce spear yield (Wilson et al., 2002b).

The number of stalks and stalk diameter has been correlated with total harvested yield (Ellison and Scheer, 1959; Moon, 1976). Large (> 15 mm) and medium stalks (10 to 15 mm) were highly correlated with total yield (Wolyn, 1993). On the other hand Dufault and Greig (1983) reported that the growth of fern rather than the number of ferns produced was a better indicator of large crown growth, since crown fresh weight vs. fern fresh weight correlated highly and crown fresh weight vs. number of ferns correlated poorly.

9.4.1 Fern numbers

Fern numbers were stimulated by foliar applied CPPU at 5 mg L^{-1} (Figure 5.1) and drenching asparagus crowns with CPPU at 10 mg L^{-1} (Table 4.1). These results suggest that CPPU applied as a foliar spray or by soil drenching was being transported to the crown buds and stimulating fern production, which may increase canopy size and potential photosynthetic surface area. However, applying CPPU to foliage (10 mg L^{-1}) or roots at 10 mg L^{-1} reduced the emergence of ferns (Tables 4.4 and 4.8) and the repeated application of CPPU to foliage significantly reduced fern numbers (Figure 6.2). This reduction in fern numbers with CPPU-treated plants may be due to a high level of uptake resulting in supra-optimal concentrations of CPPU. Thus the response appears to be very sensitive to CPPU concentration and much further work is required if growth regulators are to be used to stimulate fern production in a commercial situation.

9.4.2 Root: shoot ratio

The repeated CPPU treatments had no effect on root: shoot dry weight ratio compared to control plants. Root to shoot ratio of plants treated once with CPPU-treated plants ($\geq$ 10 mg L^{-1} foliar sprays) was lower than controls, but total dry weight was similar (Tables 4.3 and 4.15). This increase in fern growth might be expected to stimulate crown activity such as root dry weight, crown bud number and total bud numbers (crown buds + ferns + spear). In fact, there was no corresponding increase in root dry weight and in crown activity (Chapter 4). Special care would be required in the use of CPPU ($\geq$ 10 mg L^{-1}), whether as a foliar spray or crown soak, as crown activity in terms of root dry matter and bud numbers are important factors limiting yield (Woolley et al., 2006).

9.4.3 Photosynthesis

Cladophylls are the main sites of photosynthesis in asparagus plants (Downton and Torokfalvy, 1975) and there appears to be a correlation between high cladophyll length and diameter and high photosynthetic rates. Once the optimum cladode density has been ascertained the highest possible root: shoot ratio needs to be obtained. Carbohydrate production could be increased by modification of cladode characteristics.

Cladophyll properties such as cladophyll length and diameter appear to be important in explaining difference in photosynthesis between cultivars (Faville et al., 1999; Guo et al., 2002). The close relationship between cladophyll thickness and photosynthetic capacity are associated with increased root growth and asparagus yields should increase. CPPU stimulated the formation of longer and thicker cladodes (Tables 4.13 and 5.2). However, photosynthetic rate was unaffected by 10 mg L^{-1} CPPU treatment (Table 5.2). Plants sprayed at intervals with CPPU had a lower net assimilation rate (NAR) compared to control plants (Figure 6.6). Thus CPPU-treated plants had suppressed photosynthetic rates although fern growth increased, possibly due to a reduction in sink strength, and thus net assimilation rates were significantly reduced by the CPPU treatment. Further work is clearly required on the anatomy of CPPU-treated cladophylls in order to ascertain the reason for this lack of correlation between cladode dimensions and photosynthetic rates in CPPU-treated plants.

9.4.4 Bud numbers

The number of buds developed affect the potential number of spears and may be a yield limiting factor (Daningsih, 2005), as well as carbohydrates availability in the roots (Wilson et al., 1999b). Foliar application of CPPU at 5 mg L^{-1} stimulated bud numbers whereas higher concentrations, or root-applied CPPU, inhibited bud development. It is suggested that CPPU concentrations less than 5 mg L^{-1} CPPU should be investigated in relation to both cladophyll morphology and bud production.

9.5 Effect of CPPU treatments on pepper plant growth and development

A crown soak in 10 mg L^{-1} CPPU decreased asparagus fern height, root dry weight and crown buds compared to control plants (Sections 4.3.2 and 4.3.3). It is postulated that CPPU applied as a crown soak may affect plant hormones in roots such as gibberellins. The collection of xylem sap from asparagus plants was needed, but it was not possible to collect xylem sap in useable quantities from young asparagus plants. Therefore further experiments were undertaken on the response of pepper plants to CPPU.

Root applied CPPU at 10 mg L^{-1} significantly decreased plant height and root growth of pepper plants (Table 7.1; Figure 7.2) as has been found in asparagus plants (Tables 4.5, 4.7, 4.9, 4.10 and 4.15). Root applied CPPU at 10 mg L^{-1} significantly decreased leaf size, leaf area and leaf water potential of pepper plants compared to controls (Figures 8.3 and 8.4), suggesting that CPPU-treated plants had a smaller root systems and possibly limited water uptake. In pepper plants, xylem exudates from CPPU-treated plants significantly reduced hypocotyl length in a GA_3 lettuce hypocotyl bioassay (Figure 8.6), which suggests that CPPU could be blocking GA_3 biosynthesis, resulting in inhibition of plant growth. However, GA_3 as a foliar spray at 100 mg L^{-1} did not reverse the effect of CPPU inhibition (Figure 7.4) suggesting that CPPU may also block gibberellin action (Cruz-Castillo et al., 1999; Merino et al., 2002).

CPPU applied as a crown soak at 10 mg L^{-1} may act similarly to growth retardants that blocks gibberellin synthesis. Plant growth retardants reduced internode elongation because of fewer cells in the internode due to low sub-apical meristem activity (Brown and Sommer, 1992), less cell elongation (Hays et al., 2002) or less cell division in the internode (Wang and Dunlap, 1994). However GA_3 did not completely reverse growth inhibition caused by CPPU, unlike growth suppression caused by growth retardants (Cox, 1991). In addition CPPU increased chlorophyll levels, unlike growth retardants which normally reduce chlorophyll. Thus the mode of action of CPPU in this system is unclear.

9.6 Suggestions for further investigations

1. CPPU and ZR increased elongation rate of spears. However, CPPU did not reverse growth suppression caused by removing spear leaf scales suggesting that other factors in addition to cytokinins may be involved in stimulating spear length. Further experiments on hormonal interactions, especially CPPU or ZR and gibberellins combinations, will be required to provide further clues to the mechanisms that control spear growth.

2. High growing temperatures may stimulate cell elongation and/or cell division in the spear tip and elongation zone. Further work is required to investigate cell elongation and/ or cell division in spears in relation to growing temperatures and plant hormones. Since low spring temperature can be severely limit yield in New Zealand the commercial use of protective covers such as polythene tunnels, should be investigated.

3. Chilling is an important factors for releasing bud dormancy and sometimes influencing spear growth rates. For 'Apollo' prior chilling had a positive effect on bud break for some cultivars at 10°C and 15°C but the effect of prior chilling on RSGR was unclear. Further work is required on the effect of prior chilling on spear growth, bud break patterns, bud break time of each bud within a cluster, between cluster or between plants, in relation to factors such as temperature, chilling, cultivar and plant sex.

4. Application of CPPU as a foliar spray increased cladophyll length and diameter, but did not increase photosynthetic rate and decreased NAR. Further work is needed to investigate the anatomy of CPPU-treated cladophylls, and its relationship to photosynthesis.

5. CPPU applied as a foliar spray or crown soak ($\geq$ 10 mg L^{-1}) decreased fern production but 5 mg L^{-1} CPPU treatment stimulated fern and crown bud production. CPPU concentrations less than 5 mg L^{-1} CPPU should be investigated in relation to fern and bud production and also the extent to which gibberellic acid may overcome some of the deleterious effects of higher concentrations of CPPU.

6. Formation of the new buds during the spear harvest season indicates the need to reassess crop management practices such as time of application of nitrogen fertilisers and, if they are cost effective, plant growth regulators. Timing of fertiliser nitrogen application could have a significant impact on the physiology of asparagus plants. Prior to spear harvest or during the harvest period, lack of nitrogen uptake may reflect low root activity due to low temperatures; much of the nitrogen may then be lost by leaching. It is suggested that split fertiliser application or slow-release nitrogen may increase fertiliser nitrogen uptake during the period when new buds develop.

7. Reciprocal relationship between ABA and gibberellins are important for induction of winter dormancy, release of bud dormancy and possibly spear growth rates. The relationship between ABA and GA_3 should be elucidated for asparagus in relation to bud dormancy and spear growth.

8. The transition from exponential to linear spear growth, which may be due to low levels of stored carbohydrates in the roots, may be a useful method of assessing exhaustion of carbohydrate. This method may be easier to apply to the Aspire New Zealand system (Wilson et al., 2000; 2002a) then the present method of measuring refractive index of expressed root sap.

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