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**GROWTH AND PHYSIOLOGICAL RESPONSES OF ASPARAGUS
(*Asparagus officinalis* L.) AT HIGH TEMPERATURES**

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fulfilment of the requirements for the degree
of Doctor of Philosophy in
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Abstract

Asparagus is now planted in tropical climates, hence a series of experiments were carried out to examine the physiological responses of asparagus to high temperature. These included analysis and modelling of growth, and the measurement of heat tolerance of four asparagus cultivars at high temperatures.

Asparagus seeds of four cultivars were sown and grown in controlled climate rooms. These results showed that growth of young asparagus plants was exponential, and thus the parameters RLGR (relative leaf area growth rate), RFGR (relative fern dry weight growth rate), RCGR (relative crown dry weight growth rate), RPGR (relative total plant dry weight) were constant for any specific temperature regime or cultivar. The growth rate could be classified according to the parameters NAR (net assimilation rate), LAR (leaf area ratio) and RGR (relative growth rate), and could be grouped into high : 'D25/N25°C and D30/N30°C', normal : 'D20/N20°C, D30/N20°C, D35/N15°C, and D35/N25°C', and poor growth rates : 'D35/N35°C, D40/N20°C, and D40/N30°C'. The effects of these temperature regimes on growth were greater than the differences among cultivars, although there were different responses at high temperature among cultivars.

Generally, NARs decreased with increasing age, while LARs increased with age. Both NARs and LARs varied with temperature regime, plant age and cultivar. The effects of high temperature on NAR or LAR were greater than the differences between cultivars.

The leaf production rate was the largest contributor to total plant relative growth rate, followed by the root, the stem, and the rhizome production rate. The stem and the rhizome production rates declined with age, the leaf production rate increased, and the root production rate was maintained nearly constant.

The allometric coefficients of root in relation to fern for cultivars and for the various temperature regimes were essentially the same. On the contrary, the allometric intercepts between plants at various temperatures or between cultivars were significantly different, with Tainan No.1 having the highest and Larac the lowest root/ shoot ratio except at supra-optimal temperatures. The lower temperature regimes had the higher root : shoot ratios. The root : shoot ratio was higher with a 10°C day/night temperature differential compared to the equivalent constant temperature regimes.

Day or night temperatures around 26.5°C were optimal for RLGR, RFGR and RPGR, but a night temperature of 23.8°C was optimal for RCGR.

The experiment on spear yield and fern development showed that not only did high temperature depress spear yield and quality, but it also depressed total fern weight and individual fern height. The plant characteristics such as the first branch height and fern height were also depressed at high temperature. Brocks and UC157 maintained better fern characteristics than the others at high temperatures. From the parameters of Richard's equation on fern, and of the RSGRs on spear, the ability of adaptation to high temperature was in the order: Tainan No.1 > Brocks > UC157 > Larac.

In a high temperature study with germinated asparagus seedlings, the higher the temperature was the more stunted the growth. High concentrations of ABA application also markedly depressed seedling growth. There was an additive effect of heat stress and application of high ABA concentration on seedling growth, while there was an ameliorative effect with the application of ABA at a low concentration (0.1 - 1 μ M) on heat stressed seedlings. At high temperature the sensitivity difference to ABA between cultivars was clearly expressed and thus the difference in heat tolerance of asparagus cultivars may be determined by ABA insensitivity.

The studies of the effect of high temperature on endogenous ABA levels showed that the endogenous ABA levels decreased with temperature and then increased to a peak around 38°C for Larac and Tainan No.1, but peaked at around 36°C or lower for Brocks and UC157 for both roots and shoots. The spears of Tainan No.1 had an extremely high ABA content at 28°C and 33°C and fell to similar levels as the other cultivars at 36°C. It is concluded that the peak of endogenous ABA occurred at supra-optimal temperature and then decreased to low levels at extreme high temperatures.

The assay of membrane thermostability (T_m) is a potentially valuable means of determining heat tolerance of asparagus. T_m varied with genotype, age, and heat acclimation. Heat acclimation may increase the membrane thermostability of young tissues. UC157 may be expected to be best adapted to tropical climate on the basis of membrane thermostability, because UC157 had the highest T_m of spears grown at high temperature. Tainan No.1, Larac and Brocks grown at high temperature also had increased heat tolerance, presumably due to heat acclimation.

The study on the differences between cultivars in heat shock protein production showed that changes in protein synthesis occurred when asparagus was heat shocked at 34°C or 37°C for 2 or 6 hours. Specific heat shock proteins were produced and the levels of normal proteins changed. Most of the HSPs were of low molecular weight (about 24 kD to 13 kD). A small number of the HSP's appeared to be cultivar specific. A number of ABA induced proteins might be HSPs, but ABA also depressed the production of some HSPs. However most HSPs were induced at high temperature even in the presence of ABA.

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List of Abbreviations

<u>Abbreviation</u>	<u>Meaning</u>
ABA	Abscisic acid
D20/N20°C	D : day; N : night; Day 20°C/night 20°C
GA	Gibberellic acid
HMW	High molecular weight
HSPs	Heat shock proteins
IEF/SDS-PAGE	Isoelectric focus/sodium dodecyl sulfate polyacrylamide gel electrophoresis
J_{leaf}	Leaf production rate
J_{rhizome}	Rhizome production rate
J_{root}	Root production rate
J_{stem}	Stem production rate
kD	Kilo-Dalton
LAR	Leaf area ratio
LMW	Low molecular weight
MAGR	Mean absolute growth rate
NAR	Net assimilation rate
RCGR	Relative crown growth rate
RFGR	Relative fern growth rate
RGR	Relative growth rate
RLGR	Relative leaf area growth rate
RPGR	Relative plant growth rate
RSGR ₁₀₀	Relative spear growth rate of spear from 10 to 100 mm.
RSGR ₂₀₀	Relative spear growth rate of spear from 10 to 200 mm.
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
WMRGR	Weighted mean relative growth rate

Introduction

Asparagus officinalis L. var. *altilis*) is a long-lived monocotyledonous, herbaceous perennial, which is grown for its edible spears. The carbohydrate source for spear growth is in the storage roots, therefore this pool of carbohydrate is of major concern to both asparagus grower and researcher. The environment, especially temperature, strongly influences total plant growth or the growth of the individual components. In particular the response of root growth to temperature may be different from that of other plant components. Traditionally asparagus has been grown in temperate climates, but in the past few years, commercial production of asparagus has begun in tropical climates. Thus asparagus appears to have a fairly wide environmental adaptability. To date the growth and physiological responses and genetic adaptability of asparagus to high temperature has not been well researched.

A series of experiments were carried out to examine the physiological responses of asparagus to high temperature. These included the analysis and modelling of plant growth at high temperature, and the measurement of heat tolerance.

The experiment on growth analysis and modelling was carried out in controlled climate rooms to examine the effect of high temperatures on plant growth, when the growth parameters of different cultivars at various temperature regimes were compared. Various growth parameters were fitted against day/night temperatures to establish a response surface able to predict plant growth at various temperatures, or to predict the optimal temperature and the optimal growth of a specific plant component or an individual genotype.

The potential for spear flush and fern development is determined by the carbohydrate pool in the roots, but temperature has a major effect on spear yield, quality and growth, and later, on fern development.

ABA is considered a stress hormone, hence the possibility of ABA being involved in heat tolerance and in enhancing growth at high temperature were examined. These studies included the effects of ABA application on seedling growth at high temperature, the effect of high temperature on endogenous ABA levels, and the changed pattern of ABA induced protein synthesis.

The measurement of thermotolerance of asparagus in my studies included the measurement of membrane thermostability and the changed pattern of heat shock induced protein synthesis. The membrane thermostability of asparagus spears was measured to determine the differences in heat tolerance between cultivars, and the effect of heat acclimation on enhancing heat tolerance.

Heat shock protein patterns were analyzed to examine the genetic diversity of heat tolerance between cultivars. In addition, the heat shock induced protein patterns were compared to the ABA induced protein patterns to reveal the possibility of ABA involvement in thermotolerance at the molecular level. These results were then linked to the critical thermostability of asparagus at high temperature.

Sound recommendations on asparagus production and the appropriate genotype for use in tropic environments are essential for profitable commercial production. The objects of my researches was to determine physiological responses and genetic adaptability of asparagus at high temperature. Subsequently it is hoped that an appropriate technology to produce high quality asparagus in the tropics may be developed.

CHAPTER ONE

Review of Literature

1.1. Asparagus Growth and Environmental Requirement

1.1.1. Classification

Asparagus (*Asparagus officinalis* L. var. *altilis*, a member of the Liliaceae family) is a long-lived dioecious monocotyledonous, herbaceous perennial. Although authorities have argued as to the exact place of origin of asparagus, all agree that the eastern Mediterranean region is the most likely. The plant is found growing wild, and is grown commercially in temperate and tropical climates throughout the world. Although there are at least 150 species of asparagus, only *A. officinalis* is cultivated for food (Nonnecke and Reinhold 1989).

The plant consists of above ground stems (referred to as the fern) bearing cladophylls (which perform as leaves but are actually modified stems) and scale leaves (together referred to as the fern), underground stems (rhizomes), and fleshy and fibrous roots. The fleshy roots serve as storage organs while the fibrous roots are absorption organs. The plant is grown for its succulent fleshy spears, which in temperate climates appear after a winter rest period. If the spear is not harvested, then it becomes a fern. The true leaves are the scale-like structures that form at the tip of the spear and down the stem. As the plant grows, the cladophylls come out of the axis of scale, and in turn give rise to the flowers (Peirce 1987; Nonnecke and Reinhold 1989). *Asparagus* produces male and female florets on separate staminate and pistillate plants. As a rule, male plants produce more spears and are consequently higher yielding than female plants, however the individual spears from female plants are generally heavier. Male plants are more vigorous than female plants, and have a lower mortality in the field (Lloyd and Webb 1977), but Bouwkamp and McCully (1972) concluded that the higher mortality rate of

the female plant could be not attributed to competition from adjacent male plants. Since males appear to be more vigorous, earlier and higher yielding (Yeager and Scott 1938; Ellison *et al.* 1960; Ellison and Schermerhorn 1958), breeders have tended to develop all male cultivars that will produce higher, more stable yields of spears (Peirce and Currence 1962; Falloon 1982).

1.1.2. Genotypes / Cultivars

In the early 1900s, J. B. Norton in the United States was commissioned to develop asparagus cultivars free from rust (*Puccinia asparagi*). Two notable selection were released as a result of this work: Mary Washington and Martha Washington, which until recently were the two main North American types (Peirce 1987). A number of strains have since been selected from Mary Washington, including Paragon, California 500, Roberts Strain, Viking and Tainan No.2. Because of the inherent variability within most asparagus cultivars, these selections are substantially different from the original Mary Washington in adaptation and yield (Peirce 1987). For example, Tainan No.2 was derived from Mary Washington but was selected for a subtropical/tropical climate in order to produce good quality spears in Taiwan.

In California newer cultivars such as UC72 and more recently UC157 are replacing the Mary Washington types and introducing some tolerance to *Fusarium oxysporum*. From New Jersey a whole new group of all-male cultivars (such as Jersey Giant) have been developed, as has a series of Lucullus cultivars from Germany. From France has come a number of new cultivars, e.g. Larac, and from the Netherlands varieties such as Libras 10 and 22, which are also adapted to culture under glass or plastic for forcing purposes (Nonnecke and Reinhold 1989). All the above cultivars (except Tainan No.2) were developed in (and for) temperate climates, and their potential production in subtropical and tropical climates is almost unknown.

1.1.3. Geographic / Climatic Requirements and Production Regions

As a perennial herbaceous species, asparagus has specific environmental requirements. Asparagus has a wide geographic compatibility spectrum in very wide-ranging environments including sea-shore and desert. Thus the asparagus plant has considerable tolerance to heat, drought and salinity stresses. Although these diverse environmental conditions indicate its wide adaptability, asparagus is still considered to be a typical cool season crop with 24 to 29°C day and 13 to 19°C night temperatures favouring productivity and longevity (*Peirce* 1987; *Nonnecke* and *Reinhold* 1989).

1.1.4. Potential Production Regions in Future and Present Studies



In recent years there has been a growing interest in developed and affluent countries to have fresh asparagus available year round, therefore off-season asparagus for temperate countries in the northern hemisphere must be sourced from the tropics or southern hemisphere. Also from an economic objective these tropical countries are interested in producing asparagus. For example, asparagus was the most important processing vegetable in Taiwan 20 years ago, when Taiwan created a unique production system, called the 'mother fern' method, to overcome the problem in the tropics of not having a dormant period, and in order to maintain a balance between carbohydrate production and removal (*Lin* and *Hung* 1978; *Huang* 1979). Although Taiwan has developed these unique production systems along with local strains (selected from California cultivars), there still exist many problems of production in the tropics. The major problem is the poorer quality of the asparagus compared with that produced in temperate climates. Until now there has been very little reliable information available on how best to grow asparagus in the tropics. Therefore, an appropriate technology to produce high quality asparagus in the tropics has still to be developed. It is clear however that high temperature stress is a major factor which can influence productivity and quality (*Nichols* 1990). The present studies were undertaken to

characterize the physiological responses of asparagus to high temperature stress. In particular, the growth and partitioning of dry matter in four different cultivars grown in a range of different temperature environments was studied together with the ability of the plant to produce heat shock proteins in the presence or absence of applied abscisic acid (ABA).

1.2. Previous Studies of Asparagus Growth and Physiology

Most asparagus cultivars are evaluated on the basis of yield. Such evaluations usually take place over several years, and thus require a lot of labour to collect data, and to effectively manage the trial plots. Perennial plants usually have a large variation within treatments due to environmental variation and due to the plant itself. For example, the works of *Hanna* (1934) and *Moon* (1976) showed that there exists a wide variation in yield, average weight of spear, cross section and shape, earliness, compactness of head and colour of spear among individual plants within a cultivar. *Knaflowski* (1985) evaluated asparagus cultivars over 10 years and found that only 40 to 60% of the plants of most cultivars and only about 1 % plants of California 500W (because of the severe infection by fusarium) survived. Thus a short term controlled environmental study may be more preferable than a long term field trial. For these reasons, growth analysis carried out in the early stages of plant growth in controlled environments, may be of more value.

A number of workers have shown that the spear yield of plants correlates well with early yield, fern numbers and fern diameter (*Young* 1937; *Ellison* and *Scheer* 1959; *Ellison et al.* 1960), so spear yield can be evaluated with these characteristics within 3 - 5 years from planting, but this is still too long. *Benson* (1980) suggested that it might be possible to explain difference in yield between cultivars on the basis of differences in their early growth stages. Furthermore, he showed that in UC72 and UC157 this variation in plant growth was due to differences in the partitioning of dry matter in seedlings and that this also related to the yielding ability of the mature plants. In comparing

UC157 and UC72, the root biomass was greater in the UC157 throughout the experiment, and a very high positive correlations were found between root and fern number, fern height and root length, and leaf area and cladophyll weight. Asparagus yield depends firstly upon the ability of the plant to accumulate assimilate in the storage roots, and secondly on the plants ability to remobilize these reserves to produce spears in the harvest season (*Nichols and Woolley* 1985). They suggested that because there was very little difference between the RGR (relative growth rate) of UC157 and UC72 in *Benson's* study, that the main difference between the two cultivars was in the distribution of dry matter, 46% of UC157's total dry weight being fern compared to 55% for UC72, so that the photosynthetic efficiency of UC157 must be higher than that of UC72. It was suggested that this may be due to the strong sink effect of the greater root mass of UC157.

Hughes et al. (1990) examined the effect of temperature on the growth of asparagus seedlings. They found that dry matter accumulation peaked at about a constant 25°C, but that a day/night difference of 10°C resulted in increased growth at mean temperatures of 15 and 20°C, but reduced growth at a mean temperature of 25°C, when compared with growing the plant at a constant temperature. This suggests that night respiration is more important than photo-respiration at the two lower temperatures, and that therefore the higher night time temperature at the constant temperature led to greater dry matter losses, but that photo-respiration at 30°C and night respiration at 20°C is greater than the sum of both types of respiration at 25°C. However, there was no significant difference between the growth rates of the four cultivars used in the study. Furthermore, *Sawada et al.* (1962) found that photoassimilation at 18°C is more efficient than at 13 or 28°C, and that 28°C is the least efficient of these 3 temperatures. Thus the growth difference between constant 25°C and D30/N20°C (day/night) may be due to the photosynthetic efficiency at 25°C being greater than at 30°C during day. The ratio of photosynthesis to respiration in asparagus fern (2 - 5:1), is, however, a great deal lower than the 10 - 20:1 commonly found in leaves of woody

perennials (*Downton and Törökfalvy 1975*). It appears therefore that respiration may have a very large effect on dry matter accumulation, and therefore that high temperature may lead to a high respiration, low rate of net assimilation and thus reduced growth.

Spear production depends strongly on temperature, but there are only a few reports involving its quantification. In general, temperature has a major influence on the growth rate of spears. *Nichols and Woolley (1985)* have shown a linear relationship between log spear length and time over the temperature range, 10 to 30°C for spear heights from 1 to 20 cm. i.e. the taller the spear the greater its growth rate. Furthermore, spears grow more during the day than during the night, and these growth difference were attributed mainly to the temperature difference between day and night in the field (*Blumenfield et al. 1961*). The growth of fern appeared to follow a sigmoidal shape, in which the growth rate was slow at first, increased rapidly to about 65 cm (when it was at maximum), and then slowly decreased as the fern became taller (*Culpepper and Moon (1939a,b)*). *Lampert et al. (1980)* described the region of maximal growth rate of spear as being a short distance behind the tip and being very sensitive to temperature.

The work of *Bouwkamp and McCully (1975)* and *Hartmann (1985)* suggested that temperature, (especially air temperature), can have a strong influence on the number of spears, so temperature may be a major factor influencing total yield. However as spear numbers increase spear diameter decreases. In fact, there are many factors (e.g. bud number, carbohydrate pool) affecting spear yield other than temperature; bud numbers (and therefore spear number produced) also depend on genotype (*Tiedjens 1924*). The size of carbohydrate pool is positively related to plant vigour, spear number, spear size, spear vigour and fern growth (*Robb 1984; Haynes 1987*), while *Shelton and Lacy (1980)* have shown that the longer the harvest duration the previous season the greater the yield reduction, because the more extended the harvest duration, the greater will be the depletion of storage carbohydrates and the

longer the fern growth will be delayed. Consequently, the period in which carbohydrates are translocated down to the rhizomes will be shortened and so too will be the period in which new buds and root can develop (*Haynes 1987*). Moreover, the accumulated amount of root dry matter will depend not only on the period of accumulation, but also on the rate of accumulation. Therefore temperature plays a major factor in dry matter accumulation in the roots, because the net assimilate rate and assimilate partitioning strongly depend on temperature, which therefore has an important role in determining spear yield, quality and fern development (see section 1.5.2 and 1.5.3). Unfortunately, little is known about the causal mechanism by which high temperatures affect fern growth.

Elongation of the spear is directly correlated with temperature and *Dedolph et al.* (1963) showed that the growth rate of decapitated spears is decreased, but can be stimulated by the application of naphthaleneacetic acid (NAA). This suggests that endogenous growth regulators in the tip are probably auxin or auxin-like substances. The auxin content in the spear increases with spear growth, which may explain why, in the presence of an intact apex and associated primordia, only one basal bud in a cluster is usually able to develop at a time (*Nichols and Woolley 1985*). High auxin level may promote apical dominance (*Knox and Wareing 1984*), by indirectly inhibiting other buds from developing in the cluster (*Nichols and Woolley 1985*). Therefore when spears longer than 3 - 5 cm are cut, a flush of new spears may be triggered (*Bouwkamp and McCully 1975*). In addition, *Matsubara* (1980) found that ABA (abscisic acid) levels in spears was very high and increased with spear development. *Makus and Guinn* (1992) found that green spears contained higher levels of ABA and IAA than white spears. The higher IAA levels in green spears were consistent with their greater elongation than white spears (*Makus and Gonzalez 1991*), but the higher ABA levels were not. The balance of growth-promoting and growth-inhibiting hormones on spear development may be more important than the concentration of either one alone. Cytokinins and gibberellins may also influence growth. This agrees with the work of

Matsubara (1980), who showed that GA levels in spears were high. *Koda* and *Okazawa* (1980) reported that cytokinins are not only produced at the root tip, but are also produced at the shoot apex of asparagus, although the root tip is hypothesized as the major site of cytokinin production in higher plants.

Atkin et al. (1973) showed that optimal-warm temperature may also lead to rapid growth, while sub- or supra-optimal temperatures lead to slow growth, which may be due to a low activity of auxin in sub or supra-optimal temperatures. Likewise high temperatures also reduce the amount of cytokinins and gibberellins produced. Thus high temperature may cause changes in hormonal levels and lead to a modification in spear and fern growth. Since, the endogenous cytokinins to GA ratio may regulate sexual expression of plants (*Ombrello and Garrison* 1987), and temperature can cause changes in cytokinins and GA concentrations, climate may modify the sexual expression of asparagus (*Peirce and Currence* 1962). *Iwamura* (1990) showed that the s-triazine and carbamate compounds (anticytokinins) had a flower-inducing activity in asparagus seedlings.

Plant hormones influence not only fern development, but also spear production. For example, it has been suggested that the positional dominance in asparagus may be overcome by GA application, as crowns drenched with GA increase the number, diameter, height and weight of asparagus fern (*Mahotiere et al.* 1988 citing the work of *Wittwer and Bukovac* 1958; *Tiburcio* 1961). However *Mahotiere* (1976) showed that the potassium salt of GA₃ alone did not affect the time of spear emergence and was not effective in increasing the number of shoots per crown. GA₃ actually negated the effect of ethephon when the chemicals were applied together, because dipping asparagus crowns in ethephon significantly increased the number and fresh weight of both ferns and roots. The variation of these results suggest that GA may interact with other plant hormones or environmental factors to produce differing effects.

1.3. Influences of High Temperature on Agricultural Ecology

Global warming can be expected to increase photosynthetic rates, but vegetative production depends not only on temperature, but is also limited by moisture, radiation, fertilizer, CO₂ concentration and other factors (*Budyko* 1982). In agriculture, with no change in precipitation and radiation, a 1 °C increase might decrease average yields about 5%, and a 2°C might reduce average yields about 10%, because higher temperatures are usually associated with higher evapotranspiration rates and therefore greater moisture stress during critical stage of growth. This effect is likely to be important in regions where inadequate soil moisture is already a problem. The higher temperatures can be expected to stimulate photosynthesis slightly but accelerate development, and senescence and shorten the duration of plant growth to the detriment of yields. This phenomenon may have a considerable effect on asparagus growth in tropical climates, because accelerated development of the ferns may shorten the duration of fern growth and thus decrease fern size. In addition, the accelerated senescence of the ferns will shorten the assimilation period thereby decreasing dry matter accumulation and the carbohydrate pool. It may be possible to prevent a decline in crop yields due to climate change by developing a new suite of cultivars (*Warrick et al.* 1986), leading to improvements in crop response and final yield. It is therefore important to determine asparagus responses to warm temperatures to take full advantage of future developments in the tropics.

1.4. Physiological Responses to High Temperature

1.4.1. Metabolism at High Temperature

The ability of biological entities to grow at high temperature has been known for almost 200 years, with a considerable body of the literature being concerned with thermal stability. Many of these studies have addressed the question of how organisms live at high temperatures which would normally

destroy or inactivate the cellular components of most forms of life. Two explanations have been offered : the first (and most obvious) is that the essential cell components of thermophilic forms are relatively more heat stable than those of their mesophyllic counterparts, the second is that the cells are capable of rapid resynthesis of the destroyed or inactivated components (*Campbell and Pace 1968*).

From a physiological viewpoint, a decrease in photosynthesis and an increase in the degradation of protein and carbohydrates will lead to a decrease in dry matter accumulation. The work of *Al-khatib and Paulsen (1984)* showed that high temperatures accelerated the normal decline in viable leaf blade area and photosynthetic activities per unit area, electron transport declined earlier and faster than other photosynthetic processes at the suitable temperature, and protease activity during senescence was markedly accentuated. They suggested that a major effect of high temperature was the acceleration of the deterioration of photosynthetic activities, and the degradation of proteinaceous constituents.

It has been suggested that the primary effect of high temperature is the disruption of cell metabolism, possibly by protein denaturation, or by the production of toxic substances or by membrane damage (*Fitter and Hay 1981*). Some toxins produced in heated leaves, can be translocated and then injure the unheated leaves (*Yarwood 1961*).

C₃ plants (such as asparagus; *Downton and Törökfalvy 1975*) exhibit a decline in photosynthetic rate at high temperatures. There is a large scope for modification of the temperature effects via modification of RuDP carboxylase properties (*Ehleringer and Björkman 1977; Berry and Björkman 1980*). As temperatures increase, the specific enzymatic activities of the photosynthetic apparatus are lost and specific function of the photosynthetic membranes are altered (*Berry and Björkman*). Thus the changes in the thylakoid membrane may play a role in temperature regulation of the overall photosynthetic process.

Heat inactivation of biomembranes may be prevented by the synthesis or accumulation of protective compounds such as heat shock proteins surrounding the membrane or by biochemical and /or ultrastructural changes within the membrane (*Santarius* 1973; *Krause* and *Santarius* 1975; *Thebud* and *Santarius* 1982). However in connection with the stabilizing effect of soluble non-sugar stroma compounds, *Santarius* and *Müller* (1979) proposed that changes in the ultrastructure of thylakoids are responsible for acclimation of the photosynthetic apparatus to high temperature conditions.

Gent and *Enoch* (1983) citing previous work showed that at a constant temperature plant growth rate was linearly related to the photosynthetic rate, but the effect of temperature on growth and photosynthesis was not the same, because the photosynthetic rate increased with temperature in an asymptotic manner to a plateau above 15°C, but the growth rate increased exponentially from 5°C to 20°C and fell rapidly at temperatures above 25°C. The divergence occurred because only a part of the assimilates was used to promote growth and the rest is used to maintain the plant in the current state, that is respiration could be divided into maintenance respiration and growth respiration (*Thomley* 1977; *Barnes* and *Hole* 1978). Although growth respiration and maintenance respiration increased exponentially up to 20°C, maintenance respiration was promoted by higher temperature, while growth respiration was not (*McCree* 1974). Therefore, thermoperiodism (warm days and cool nights) may result in faster growth than constant temperature due to the high levels of nonstructural carbohydrate used for growth and smaller requirement for maintenance during cool nights (*Szaniawski* 1983). At high temperature growth is limited by the supply of nonstructural carbohydrate due to maintenance having priority for nonstructural carbohydrate. The optimal temperature for growth maintains both a high rate of supply of carbohydrate and energy to convert to structural material (*Gent* and *Enoch* 1983). However often growth is at least as fast at constant temperatures (*Robson* 1972, 1973; *Warrington et al.* 1977; *McCree* and *Amthor* 1982), perhaps because low night temperatures may lead to a shortage of respiratory energy for specific species. In addition, *Rajan* and

Blackman (1975) concluded that a difference between the night and day temperature has been found to have a positive effect only when a sub-optimal day temperature is combined with a supra-optimal night temperature.

Gerik and Eastin (1985) with sorghum showed that growth respiration is less dependent on temperature, and maintenance respiration is strongly dependent on temperature. In fact, maintenance respiration is not only strongly modified by temperature, but also depends on growth rate, and varies with plant organs (e.g. root > top) (*McCree* 1974; *Hansen and Jensen* 1977; *McCree and Silsbury* 1978; *McCree* 1982). *Heichel* (1971) showed that the higher respiration levels occurred in varieties having lower photosynthetic capacity and slower dry matter accumulation. Thus reduction of dark respiration may improve the carbohydrate balance and ultimately the yield (*Volence et al.* 1984; *Gerik and Eastin*. 1985), so genetic variation in maintenance respiration at high temperature may be useful to screen for high yield or high temperature tolerant lines.

1.4.2. Thermotolerance of Plants

Usually the term 'stress' may be defined as the exposure of plants to extraordinarily unfavourable conditions (*Larcher* 1980). These responses are often slight and difficult to detect in supra-/sub-optimal environments. In the case of temperature, active plant growth is generally confined to a temperature range from 10 to 40°C, and temperature extremes above and below this impose stress on the plant's metabolic activities leading to varied symptoms such as leaf chlorosis, fleck, and scorch; needle blights; stem lesions and cankers; fruit scald; and finally complete breakdown (*Treshow* 1970).

Heat injury is more complex than low temperature injury, since all the reactions in the plant are already taking place rapidly, and a further rise in high temperature might easily disturb the balance. Heat injuries may be divided into direct injury (e.g. disorganization of membrane), indirect injury (e.g. growth

inhibition, starvation, toxicity, biochemical lesions, protein breakdown) and coincident drought stress injury (*Yarwood 1961; Langride 1963; Daniell et al. 1969; Itai and Benzioni 1973; Larcher 1980; Levitt 1980; Wehner and Watschke 1984; Harding et al. 1989a,b; Lin and Markhart 1989*).

The plant with heat tolerance is able to prevent, decrease or repair heat injury, leading to an increased capacity for survival at extreme temperature (*Crisan 1973; Larcher 1980*). Thus genotypes having heat tolerance maintain growth under a greater temperature range and show better growth and greater yield at high temperatures (*Saadalla et al. 1990a*). The mechanisms of heat tolerance may involve many adaptations such as, increased protein thermostability leading to prevention of protein denaturation, increased resynthesis of protein to repair heat damage (*BrodI 1989; Kimpel et al. 1990*), or alteration of membrane lipid composition leading to improved thermostability (*Pearcy 1978; Santarius and Müller 1979; Hugly et al. 1988; Hunst et al. 1989*).

Thermotolerance depends on plant status. Resting (dormant) organisms, such as dry seeds, being able to survive as high as 120°C while in contrast highly hydrated tissues are killed by temperatures as low as 50 - 60°C (*Levitt 1980*). The work of *Martin and Wehner (1987)* showed that annual bluegrass under frequent watering was heat sensitive, whereas under infrequent irrigation differences appeared. In addition, plants under a low nitrogen application were more heat tolerant than those under a high nitrogen application. Annual bluegrass was less tolerant than Kentucky bluegrass at low nitrogen levels but there was no difference at high levels. So the heat tolerance of plants may depend on the surrounding environment and the genotype. Presumably, these elevated heat tolerances were induced by drought stress or nitrogen deficiency, which may be moderated by abscisic acid (ABA), because these stresses were found to elevate ABA levels (*Daie et al. 1979; Bray 1988*), leading to cross-adaptation (*Boussiba et al. 1975*).

1.4.3. Thermotolerance Adaptation

Adaptation is important to prevent injury by a stress which injures the unadapted organism. Plant response to heat stress by rapid adaptation can take place within hours, so that resistance is higher in the afternoon than in the morning. The enhanced heat adaptation disappears within a few days. The molecular mechanism of adaptation to heat is probably based chiefly on changes in the conformation of protein compounds and stabilization of the structure of macromolecular and biomembranes (*Larcher* 1980). For instance, plants grown at low temperature (4°C) reach optimum protein synthesis at 27.5°C, whereas plants kept at 36°C have the highest rate of protein synthesis at 35°C (*Weidner and Zeimens* 1975). Therefore the plant response to heat tolerance depends on heat acclimation (*Saadalla et al.* 1990a). Elevated ABA levels during heat acclimation may also be involved in heat tolerance (*Itai et al.* 1978; *O'Connor et al.* 1991).

1.5. Growth at High Temperatures

Increase in the yield of crop plants can come from many directions, such as better adaptation to environmental conditions, improved agronomic practices, or increased genetic potential. With asparagus we are concerned with the physiological basis of increased genetic yield potential, and in particular with the control and improvement in distribution and storage of assimilates. Thus a marked increase in the size of carbohydrate pool (roots) of asparagus is required because this is a major determinant of high spear yield.

In order to achieve high yield it would be best to increase photosynthetic rate and RGR (relative growth rate, see section 1.5.1), and direct partitioning of assimilates to the desired organ (sink) (*Gifford and Evans* 1981). A shift in temperature is often marked by changes in the pattern of development of plants, much of which is presumably caused by direct alteration in the growth rate of developing organs. The work of *Al-hamdani and Todd* (1990) with

alfalfa showed that the fleshy roots were the major sink, and photosynthate export from source to sinks was increased by increased temperatures (up to mildly high temperature). *Seddigh and Jolliff (1984)* showed that cool nights (10°C) restricted soybean seed growth rate and favoured partitioning of photosynthates to vegetative organs and pod wall .

At high temperature, carbon fixation and export decreased, because the total carbon pool in the leaves is reduced, thereby limiting the total carbon transport from the source leaf. It is speculated that the rate of transport under limiting conditions is controlled by the rate of reserve remobilization, (mainly by starch), so it appears that starch regulation and metabolism could play an important role in the supply of available sucrose for export when carbon fixation is limiting (*Dinar et al. 1983*). *Dinar and Rudich (1985a,b)* reported that high temperatures modified carbon metabolism in tomato, so that with increasing temperature, sucrose level in the source organ increased while starch levels decreased. These processes may be the result of heat stress on invertase activity, the heat-tolerant cultivar being able to hydrolyse sucrose more efficiently than the heat-sensitive cultivar. In addition, grapevines exposed to heat stress significantly increased the concentration of sucrose found in most of the plant fractions. Specifically, relatively higher concentrations of sucrose were found in leaf tissue rather than in woody tissue. The reasons for the sucrose accumulation may be a lack of utilization in leaves, or transport to other plant parts, or that sucrose is not being hydrolysed due to inhibition of the invertase enzyme system at high temperature. Generally, heat stress increased glucose and fructose accumulation in trunks and roots, but had the opposite effect on all other above ground fractions (*Sepúlveda et al. 1986; Sepúlveda and Kliwer 1986*).

When the growth rate at high temperature is limited by lack of assimilates the growth of roots is reduced more than other organs (*Pardales et al. 1991*), due to shoot growth having priority for the limited carbohydrate supply. Total root respiration (growth and maintenance respiration) is reduced relative to the

shoot (*Szaniawski* 1983). *Wardlaw* (1968) showed that temperature affects the distribution of assimilates in a plant largely through its effect on the organ with the greater demand for assimilates; this results a marked change in the pattern of development of growth. *Terry* (1968) showed that the storage root growth and sugar accumulation in sugar-beets was controlled more by internal mechanism determined by genotype than by temperature.

Fukai and Silsbury (1976) showed that although at high temperature the clover plant had a high rate of leaf appearance, high death rates rapidly reduced total leaf number, thus high rates of leaf appearance were not clearly associated with high dry matter yield. In fact, the leaf area duration was closely associated with dry matter yield because leaf longevity reflects the time interval during which a leaf was able to contribute to the photosynthetic gain of the whole community. In asparagus more research is needed to determine the interactions between temperature and genotype on root / shoot ratios.

1.5.1. Analysis and Modelling of Plant Growth

The adaptation of plant to environment can take many morphological and physiological forms. The rate at which new plant material is accumulated is an integrated measure of plant response to environment and is a useful character when comparing either genetic differences between populations or effects of different environmental conditions on populations (*Nicholls and Calder* 1973). Several approaches have been made to the study of quantitative plant growth. There have been two approaches to this type of the work: either to rely strictly on the harvest data sets, with quantities like the mean relative growth rate (RGR) being calculated between a pair of harvests; or to try to use a fitted equation. The main problem experienced in the first case is that the result of the calculation of growth rates is very susceptible to slight errors in either data set. The use of a regression equation allows the trend in RGR to be much better defined and a clearer perception of ontogenetic drift to be determined (*Elias and Causton* 1976; *Poorter and Lewis* 1986; *Poorter* 1989).

Paradoxically the fitted function can be of more value to the experimenter than the data from which it was derived (*Hunt 1979*).

The plant growth functions have been used for many years, usually to provide a mathematical summary of time-course data on the growth of an organism or part of an organism. Thus a general growth function connecting plant size (e.g. dry weight W) to age (t) is

$$W=f(t)$$

where f denotes some function.

The major problem of using growth functions is the choice of a suitable function to fit data (*Elias and Causton 1976*). Usually the choice of growth function is largely empirical, thus the function f will sometimes be chosen by simply looking at the data and making an informed guess; however, it is preferable to select or construct a function to simulate curves of limited growth. There are various advantages:

1. The function fitted provides a convenient summary of the data.
2. A series of estimates of the growth attribute may be calculated at as many times as desired, and these estimates are less disturbed by biological variability.
3. The logarithmic form of the function may be differentiated to produce a relative growth rate function.
4. If the function employed is based on some biologically meaningful model, then the parameter of the function may provide useful information, either by themselves, or in various combinations (*Richards 1959; Causton et al. 1978; France and Thomley 1984*).

1.5.1.1. Relative Growth Rate (RGR) Derived from A Simple Exponential Growth Equation

In the commonly encountered exponential growth equations, it is assumed that growth rate is proportional to size of plant or plant organ, therefore the RGR is constant and does not change over time. This equation is often found empirically to fit the early phase of growth (*Erickson 1976; Causton 1977*), even though at high temperature the RGR still appears constant but low (*Duncan and Hesketh 1968; Hardacre and Turnbull 1986; Tollenaar 1989; Tollenaar et al. 1991*). In fact higher plant growth rarely shows true exponential growth (*Causton 1977*). However asparagus growth during the young stage is expected to be approximately exponential with constant RGR, then departs from the simple exponential relationship to be curvilinear with ontogeny. The differential exponential growth equation is

$$\frac{dW}{dt} = R * W$$

$$R = \frac{1}{W} * \frac{dW}{dt}$$

If differential exponential equation is integrated, then the exponential equation is

$$W = W_i * e^{Rt}$$

$$\log_e W = \log_e W_i + Rt$$

- W size of plant or of plant organ (i.e. plant weight, leaf weight, leaf area, root weight, reproductive organ weight, ..., etc.).
- W_i initial size of plant or of plant organ
- R relative growth rate (RGR)
- t plant age. (*France and Thornley 1984; Hunt 1990*).

1.5.1.2. Derived Growth Parameters

An important requisite for analysing plant growth is the availability of accurate data regarding the dimensions of the assimilatory apparatus-leaf area and assimilation rates (*Sivakumar and Shaw 1978*). These attributes of growth of individual plants most commonly studied in growth analysis can be derived from RGRs of plant organs and leaf area, as follows:

$$RGR = LAR * NAR$$

where

$$LAR = \frac{L_A}{W}$$

and

LAR = leaf area ratio

L_A = leaf area

W = total plant dry weight

and

$$NAR = \frac{1}{L_A} * \frac{dW}{dt}$$

where

NAR = net assimilation rate

(*Hughes and Freeman 1967; Hunt 1990*)

1.5.1.3. Root-Shoot Allometric Relationship

This is index of the balance of growth between root and shoot components of the plant integrated over a period of time. The allometric relationship is

$$W_R=b*W_s^K$$

$$\log_e W_R=\log_e b+K*\log_e W_S$$

also

$$K=\frac{RGR_R}{RGR_S}$$

where

K	allometric coefficient
b	allometric intercept
W_R	root weight
W_S	shoot weight
RGR_R	RGR of roots
RGR_S	RGR of shoot

1.5.1.4. Component Production Rate (CPR)

An index of the current commitment of the whole plant to the production of one of its components, such as roots, stems or leaves. Therefore the whole plant's relative growth rate (RGR) can be subdivided into an expression which includes the relative growth rate of the individual organs of the plant.

$$RGR=RGR_1\frac{W_1}{W}+RGR_2\frac{W_2}{W}+...+RGR_n\frac{W_n}{W}$$

where

- $W_1, W_2, , W_n$
the size of the individual parts of plant.
- $RGR_1, RGR_2, , RGR_n$
the relative growth rate of $W_1, W_2, , W_n$.

then

$$RGR = J_1 + J_2 + \dots + J_n$$

where

$$J_1, J_2, \dots, J_n$$

the component product rate of $W_1, W_2, \dots, W_n$.

(Hunt and Bazzaz 1980; Hunt 1990).

1.5.1.5. Growth Models Derived with Richards Growth Equation

If the growth of a plant or a plant organ is followed over a long period it departs from the simple exponential relationship. Several equations have been proposed to account for such curves e.g. polynomial functions; Richards equation; logistic equation.

The polynomial equations are especially useful as they can provide arbitrary functions for these purposes since the equation can be evaluated by fairly simple numerical procedures, and the statistics of polynomials is well understood (Nicholls and Causton 1973; Elias and Causton 1976; Erickson 1976).

The Richards function is empirical, general and flexible. For particular values of the parameter N , it encompasses three growth equations: the monomolecular ($N = -1$), the logistic ($N = 1$), and the Gompertz ($N \rightarrow 0$) (Richards 1959; Causton et al. 1978). The differential equation for the Richards equation may be written

$$\frac{dW}{dt} = \frac{k * W * (W_i^N - W^N)}{N * W_i^N}$$

where

k, N and W_i are constants

and k and W_f have positive values
 then $-\infty \geq N \geq -1$ but not equal to 0

With $N = -1$, the differential Richard equation becomes

$$\frac{dW}{dt} = k*(W_f - W)$$

W size of plant or plant organ.

W_f final size of plant or plant organ.

This is identical with the monomolecular growth equation, in which it is assumed that the quantity of growth machinery is constant and independent of size, and growth is also modified by substrate availability.

With $N = 1$, the differential Richards equation becomes

$$\frac{dW}{dt} = kW(1 - \frac{W}{W_f})$$

This is identical with the logistic growth equation, in which it is assumed that the quantity of growth machinery is proportional to size, and growth is modified by substrate availability (*Caloin and Yu 1982; France and Thornley 1984*).

With $N \rightarrow 0$, the differential Richards equation becomes

$$\frac{dW}{dt} = k*W*\log_e(\frac{W_f}{W})$$

This is identical with the Gompertz growth equation, in which it is assumed that the quantity of growth machinery is proportional to size, and the effectiveness of the growth machinery decays with time. This decay may be due to degradation (possibly of enzymes), or senescence, or development and differentiation.

When the differential Richards equation is integrated, it can be written

$$W = \frac{(W_f * W_i)}{[W_i^N + (W_f^N - W_i^N) * e^{-k * t}]^{\frac{1}{N}}}$$

where

W_i initial size of plant or of plant organ

Equating the Richards equation to zero at age $t=t^*$ (the inflexion point), therefore

$$\frac{dW}{dt} = 0$$

$$W(t=t^*) = W_f \left(\frac{1}{N+1} \right)^{\frac{1}{N}}$$

$$t^* = \frac{1}{N} * \log_e \left(\frac{W_f^N - W_i^N}{N * W_i^N} \right)$$

Therefore N defines the shape of the growth curve, which the rate constant, k , has no significance by itself, it has to be viewed in combination with N . In the formulae $k/(N+1)$ and $W_f * k/(2 * (N+2))$ the former is a weighted mean relative growth rate over the whole period of growth, the latter is the corresponding mean absolute growth rate. The weighting is proportional to the absolute growth. Therefore the Richards function may be used to describe quantitatively growth and growth characteristics (Causton *et al.* 1978).

1.5.1.6. Plant Growth Prediction with Response Surface Technique

Research on the influences of the environment on plant growth usually deal with single factor, but in the field changes in one factor may also result in changes by other factors. In trying to elucidate environmental conditions which produce a given response, experiments with a factorial combination of treatments usually result in only one or two combinations which yield the desired response. A full complement of factorial combinations to test 3 factors at p levels results in p^3 treatments which may be too costly or too time consuming, especially if each treatment is lengthy (*Armitage et al.* 1981). Therefore modelling plant growth as a function of the total environment is extremely complex due to multiple factors such as radiation, photoperiod, humidity, CO_2 , etc. (*Hammer and Langhans* 1976 citing the work of *Krizek et al.* 1970). Response surface techniques (*Box* 1954) minimize the number of experimental treatments required to cover a range of factors. This often incorporates a composite design (*Armitage et al.* 1981). Using the response surface technique (*Hammer and Langhans* 1976) partially overcame the difficulty of not being able to visually present the results of five factors (root temperature, day temperature, night temperature, day length, light intensity) on sunflower growth. Their work showed that solving the equation of the response surface for the stationary point and reducing the surface to the canonical form which gave some sense of the shape of response surface, which enabled the maximum growth of sunflower, and the sunflower plant growth at any specific combination of the five factors to be predicted. The work of *Armitage et al.* 1981; *Armitage and Carlson* 1981; *Armitage et al.* 1981; *Erwin et al.* 1986; *Karlsson and Heins* 1986; *Hopper and Hammer* 1991; *Lieth et al.* 1991 showed that using the second order model of the response surface successfully predicted plant growth and flowering at a combination of various environmental factors. Previous studies have shown that the effect of either day or night temperature on plant growth is especially critical, but the effect of combinations of day / night temperature is still not clear. It is therefore suggested that the response surface model can be used to predict asparagus

growth at desired combinations of day / night temperature.

1.5.2. Dry Matter Accumulation at High Temperatures

The production of dry matter by vegetation can be separated into two processes: (1) the interception of solar energy by foliage, and (2) the storage of this energy as dry matter. The fraction of available energy intercepted during the life of a crop depends on the rate of expansion of leaves and on their longevity, the efficiency of conversion depends on the balance between photosynthesis and respiration. Many workers have shown that temperature has a strong influence on factors that determine interception, for example, increasing temperature accelerates germination and leaf expansion and shortens the time from germination to maturity. However there is insufficient experimental evidence to show whether an increase in interception caused by the more rapid formation of a canopy can compensate for the decrease caused by earlier maturity (*Ong 1983a; Squire and Ong 1983*). Even less is known about the temperature response of solar energy conversion for a crop in the field. The correlation between dry matter production and temperature is difficult to demonstrate as the growth rates of complete canopies are influenced predominantly by irradiance and are much less sensitive to temperature. In the laboratory, both photosynthesis and respiration show well defined responses to temperature, but are not immediately relevant to the long term carbon balance of a crop stand (*Squire et al. 1984*).

The work of *Squire et al. (1984)* suggested that the optimal temperature for growth rate of the canopy did not correspond with the optimal temperature for total dry weight, because growth rate was maximal at 25 - 27°C and total dry weight at 20 to 22°C. Consequently the increased rate of canopy formation did not compensate for the shorter life of leaves forming the active part of the canopy.

The work of *Hunter et al.* (1974) on the effect of photoperiod and temperature on vegetative and reproductive growth of maize showed that both photoperiod and temperature affected plant dry matter yield, with the longer photoperiod and cooler temperature producing the highest final dry weight. Final yield (the integral of rate and duration of dry matter accumulation) was largely attributed to leaf area. However, grain yield depended on the capacity of grain growth during the grain filling period. Photosynthesis affected grain yield by increasing post anthesis assimilate production at the longer photoperiod, and temperature affected grain yield resulting in a greater proportion of the post anthesis assimilate being allocated to the grain at the lower temperature. Temperature effects were more important than photoperiod.

The additional loss of carbon associated with increased respiration does not appear to support the theory that respiration responses would be an important factor limiting dry matter accumulation at high temperature during kernel development of sorghum, because the respiration rate of the kernels developing at high temperature may not greatly exceed that of a grain developed at a lower temperature. In fact the movement of assimilate was enhanced at higher temperatures in wheat (*Wardlaw and Moncur 1976; Wardlaw et al. 1980*). The rate of dry matter accumulation by the kernels of wheat, sorghum and rice was not significantly changed, and any increase in assimilate movement was possibly balanced by an increase in respiratory loss, but these grain developments were reduced at high temperature, e.g. wheat or low temperature, e.g. rice (*Chowdhury and Wardlaw 1978*). *Wardlaw et al.* (1980) explained that at high temperatures the grain dry weight was reduced, due to a shortened duration of grain development.

Fussell et al. (1980) reported that grain growth has little connection with carbohydrate production and the export of current photosynthate from source leaves. The work of *Jones et al.* (1981) and *Nicolas et al.* (1984) also showed that the assimilate availability does not play a limiting role in kernel growth at high temperature. Moreover *Egli and Wardlaw* (1980) showed that the

reduction in size of grain of soybean is mainly due to internal factors within the grain itself, and is not an effect on the availability of assimilate. Temperature affects the rate and duration of grain filling, but the reduced duration of grain filling at high temperature did not result in lower yield for these field grown crops, as higher daily incident solar radiation can compensate for the shorter grain-filling period (*Muchow* 1990). Thus the fill of the carbohydrate pool of asparagus may be greatly associated with duration. Furthermore the final size of carbohydrate pool may be the product of interplay between genetic potential and environment, especially temperature.

1.5.3. Assimilate Partitioning at High Temperatures

Tollenaar (1989a,b) showed that total dry weight of maize gave a curvilinear response to temperature with an optimum between 15 and 23°C. There was a high positive correlation between SPC (shoot partition coefficient, increase in shoot dry weight per unit increase in total dry weight) and RGR, but the association between SPC and LPC (leaf area partition coefficient, increase in leaf area per unit increase in total dry weight) showed opposite trends. The close association between shoot partition coefficient and RGR may be, in part, the result of the similar response of shoot partition coefficient and photosynthetic rate per unit leaf area to temperature. Shoot partition increased linearly and leaf photosynthetic rate showed a curvilinear response in the 11 to 31°C range with an optimum temperature of 27°C.

Most of the research that has been reported on dry matter distribution during the vegetative development of plants has focused on the root : shoot ratio (*Tollenaar* 1989a). The allometric equation, can be used to represent the balance of growth between root and shoot components of the plant integrated over a period of time, and thus effectively the allometric coefficient is the ratio between root and shoot RGR (*Hunt* 1990). The work of *Stanhill* (1977a,b) working with carrot showed that the allometric equation enabled more than 95% of the variation in the weight of tap root, to be accounted for by reference

to the weight of leaves. He suggested that environmental factors, plant development and cultivar could affect the allometric coefficient. The allometric intercept might be neglected, but the two parameters were not independent, as they had an inverse linear relationship. Moreover he concluded that the large difference between genotypes in their final root weight was not due entirely to differences in absolute growth rates but was, at least in part, due to differences in allometry. He suggested that relative growth of root and shoot must be related to their morphogenesis and therefore that any marked difference in root shape will be accompanied by changes in the allometric coefficient. However *Currah and Barnes* (1979a) showed that age influenced the allometric intercept of carrot more than the allometric coefficient. They cited previous work and showed that nutrient (but not light and water) also shifts the allometric intercept. *Terry* (1968) showed that light intensity had no effect on assimilate partitioning in sugar-beet, but temperature, which may alter physiological age did. *Nichols and Woolley* (1985) found a clear effect of temperature on the root : shoot ratio in asparagus. Moreover they showed that, after adjustment to the new environment, the allometric coefficient was constant between cultivars and between temperatures, and that the major differences were the allometric intercepts.

The analysis of CPR (component production rate) represents the current commitment of the whole plant to its components (e.g. leaf, shoot, root). The CPRs sum up to the whole-plant RGR, thus the analysis also reveals the distribution of growth activity throughout the plant at various times (*Hunt and Bazzaz* 1980; *Hunt* 1990). *Hunt and Bazzaz* (1980) analyzed the growth of *Ambrosia trifid* L. to fertilizer response at component levels, and showed that there was a heavy early commitment to root production by the whole plant. This was most marked in unfertilized plants. Stem production was moderately high throughout the whole growth period for both fertilized and unfertilized plants. Leaf production was clearly enhanced by the more favourable nutrient regimes.

There is a assumption that high NAR should correlate well with growth, because high NAR indicates a greater photosynthetic rate. The work of *Al-khatib* and *Paulsen* (1990) showed that the temperature response of NAR in young maize canopies is similar to the temperature response of leaf photosynthetic rate, but the closeness of the relationship between net assimilation and leaf photosynthetic rate varied with phase of development and temperature. For example, there was a close relationship between the reduction in photosynthetic ratio and decreased biomass of seedlings ($r=0.943$) and decreased grain yield of maturing plants ($r=0.807$) of the 10 genotypes of wheat from the moderate to high temperature.

Potter and *Jones* (1977) (citing previous work) showed that plant growth is not well correlated with NAR, but good correlations existed between growth and rates of leaf area expansion. Their work on maize growth analysis also confirmed that RGR was not well correlated with NAR or LWP (*leaf weight partition* = $(dL/dt)/(dW/dt)$ where L = leaf weight, W = total plant weight, t = time), but, RGR had a good correlation with LAP (*leaf area partition* = $(dA/dt)/(dW/dt)$ where A = leaf area), thus the partitioning of assimilates into new leaf area is an important component of growth. *Tollenaar* (1989a) showed that the LPC (leaf partition coefficient, increase in leaf dry weight per unit increase in total dry weight) increases with rising temperature in the range 11 to 31°C, because a change in dry matter partition is related to the relative activity of the plant components involved. Thus when the leaf partition coefficient increases with rising temperature, the shoot activity and root activity decline with rising temperature. Furthermore it was found that the NAR increase within the temperature range 15 to 27°C corresponded with the increase of the leaf area partition coefficient in maize.

1.5.4. Growth Parameters in Relation to Temperature

The effect of temperature on NAR is not consistent, as some investigations showed a positive correlation with mean temperature, others no significant

correlation, or even a negative correlation. These contrasting results may represent real differences between species, climates, varieties and age (Watson 1947a; Rajan *et al.* 1971; 1973). The relationship of NAR to temperature varied not only in magnitude, but also in sign (positive or negative), as NAR had a positive relationship with day temperature, due to NAR rising with temperature up to a maximum, and falling with a further increase in temperature. There was a negative relationship with night temperature as a high night temperature increased respiration and so reduced NAR (Watson 1947a; Wilson 1966). The work of Tollenaar (1989b) in maize showed that the response of the NAR of corn to temperature was curvilinear with an optimum at D27/N27°C. The response of LAR to temperature was also curvilinear with a minimum at D19/N7°C. NAR contributed the major variation in RGR. RGR at constant temperature was higher than at the equivalent alternating day/night temperature because NAR and, to a lesser extent, LAR were higher at the constant temperature, except that the RGR at the alternating day/night regime was higher than at the constant temperature of 15°C. NAR and LAR showed opposite trends, with a lower LAR and higher NAR at the alternating vs. constant temperature regime. RGR also declined during the period from the 4th to the 12th leaf stage, the decline in RGR was the result of a decline in both NAR and LAR in the D23/N11°C to D31/N31°C range, but the NAR did not decline in the range D15/N3°C to D19/N19°C.

Generally, NAR has little variation from moderate to warm temperatures, thus in a natural climate NAR has little effect on RGR, nevertheless RGR is very temperature dependent, because LAR increases with increasing temperature around the optimal temperature (Wilson 1966). In addition, the effect of temperature on NAR was related to specific species, for example in the field the NAR of rape had a greater dependence on temperature than maize. The work of Watson (1947a,b) showed that at any time the mean NAR was found to be almost constant, this was because high NAR was correlated with a small leaf area, but NAR decreased as leaf area increased. Therefore the variation in dry matter accumulation between years, species and varieties is determined

by differences in the size and duration of the assimilating system, such as leaf area, and variation in NAR are of minor importance. The differences between varieties and years in yield of dry matter mainly reflected the differences in leaf area, and showed no close relation to difference in NAR. Thus in spite of the wide variation in NAR between species the reduction in RGR with time was associated with increases in plant size which were partly reflected by reduction in the LAR (Davidson and Milthorpe 1965). Hanson (1971) showed that high NAR was not necessarily reflected in increased RGR, because high NAR was frequently accompanied by a low LAR, thus variation in NAR did not play a major role in determining differential RGR.

Eagles (1967) showed that the marked changes in RGR of *Dactylis glomerata* populations at different temperatures were positively correlated with changes in both NAR and LAR, whereas the differences between the populations in RGR were the result of differences in NAR, and were negatively correlated with differences in LAR. It may be concluded from these very different results that although $RGR = NAR * LAR$ the magnitude of RGR contributed by NAR or LAR varied with crop, temperature, or genotype. In my studies the RGR, NAR and LAR of asparagus plants at various temperature will be presented.

1.5.5. Seed Germination at High Temperatures

In the case of millet seed (Garcia-Huidobro *et al.* 1982a,b), the rate of emergence increased linearly with temperature from a base of 10 - 12°C to a sharply defined optimum at 33 - 34°C and declined to zero at about 45 - 47°C. When seeds were exposed to pairs of alternating temperatures, the rate of emergence and the germination percentage were close to values predicted from measurements at a constant temperature, provided the maximum temperature did not exceed 42°C during imbibition. Exposure to high temperature during imbibition slowed germination and reduced the number of seeds which germinated. No seeds germinated when the maximum temperature of the cycle exceeded 47°C. The germination of pearl millet also

increased linearly with temperature from a base temperature to a sharply defined optimum temperature beyond which the rate decreased linearly with temperature, reaching zero at the maximum temperature.

In the tropics, the temperature a few centimetres below the surface of bare soil is frequently in the range 40 to 50°C during the day leading to a poor seed germination (*Ong and Monteith 1985*). Pre-sowing heat stressed seed was suggested as a simple, effective and practical method to overcome the poor germination of seed at high temperatures. For instance, *Khan et al. (1973)* reported that if cotton seed was treated with supra-optimal temperature stress before sowing, not only faster seedling emergence and establishment may result, but there may also be induced resistance in the established plant to drought and higher temperature. The work of *Onwueme and Adegroye (1975)* explained that the reduced emergence could be accounted for by a reduction in the rate of hypocotyl elongation. In the case of corn, the shoot and radicle elongation rates in germinating seedlings showed a minimum temperature of 9°C, an optimum of 30°C and a maximum of 40°C (a lethal temperature for both root and shoot growth) (*Blacklow 1972; Warrington and Kanemasu 1983a*).

1.5.6. Leaf Growth at High Temperatures

In seedlings, the rate at which leaf and root primordia appear, extend and subdivide depend strongly on the temperature of the appropriate meristematic tissue. After seedling emergence, both temperature and light influence yield since dry matter production is almost proportional to intercepted radiation during vegetative growth. *Ong (1983a)* and *Ong and Monteith (1985)* showed that the temperature of the soil surface determined the rate of leaf initiation, so that more leaves were produced at high temperature, and the rate of leaf expansion also increased almost linearly with temperature, but declined rapidly between 35 and 40°C. *Watts (1972a)* concluded that at root temperatures from 5 to 35°C and shoot temperatures from 5 to 30°C, water stress was not a

major factor restricting the extension of corn leaves, but between 30 to 35°C, in an unsaturated atmosphere, restrictions of leaf extension were associated with low water potential, probably because the evaporative demand at 35°C greatly exceeded water uptake. The above results are an agreement with the work of *Arnold* (1969) who showed that there was no effect on the total number of leaves of corn in the period from planting to the 4th leaf stage when plants were exposed to warm or cool temperature, but from the 4th to the 9th leaf stage the warm temperature treated plants had 3 more leaves than the cool temperature treated plants. Exposing the plants to warm temperature suppressed the elongation of the lower internodes. In general, the leaf number of maize, sorghum and hungarian millet was profoundly effected by temperature and photoperiod (*Hesketh et al.* 1969).

Fukai and Silsbury (1976) reported that the rate of leaf appearance and the rate of leaf death of subterranean clover were constant at each temperature, with high temperatures favouring a high rate of leaf appearance. Dry matter accumulation increased with high temperature during the early stages, but as high temperature also favoured a high death rate in the canopy, green leaves did not accumulate faster. High dry matter yield was not associated with a high rate of leaf appearance, because leaf longevity reflects the time interval during which a leaf can contribute to the photosynthetic gain of the whole community. *Duff and Beard* (1974) showed that the weekly yields of *Agrostis palustris* were significantly reduced at the supra-optimal temperature regimes of D35/N25°C and D40/N30°C, and that the chlorophyll content was lowest at D40/N30°C. Similar observation on alfalfa indicated that growth declined during the hot summer weather due to the temperature stress. When repeated high temperatures occurred at intervals in the field during hot weather, heat induced growth suppression might prevail throughout the summer (*Bula and Massengale* 1972). *Pulgar and Laude* (1974) showed that the period of depressed growth was extended as intensity of stress was increased either by elevating temperature or length of exposure.

Thiagarajah and *Hunt* (1982) reported that the specific leaf weight (lamina weight / lamina area) increased throughout ontogeny, but there was a general decrease in specific leaf weight with increase in temperature. These results are in agreement with many other results which postulate that at low temperature assimilates are incorporated into existing leaves, rather than used for the expansion of new leaf tissue. Hence growth in a cool regime of D25/N20°C is restricted. *Wilson* and *Ludlow* (1968) showed that the leaf area expansion of *Phaseolus* had an optimum rate of 30 > 25 > 35 > 20°C. A small change in geography and topography can result in large differences in night temperature which can strongly affect crop productivity; for example *Seddigh* and *Jolliff* (1984) showed for soybean that higher night temperature enhanced vegetative growth. Warmer night temperature considerably shortened the time period between plant emergence and appearance of first flower at the node immediately below the uppermost node, but the plant height, number of nodes, and the number of auxiliary branches were not significantly different among the treatments at maturity since the growth of these components also terminated earlier for plants receiving the higher night temperature.

Erwin et al. (1989) examined *Lilium longiflorum* growth at a number of day and night temperatures from 14 to 30°C, and concluded that there was an interaction between day and night temperature. Stem elongation and leaf orientation in lily were influenced more by the difference between day and night temperature than the absolute day or night temperatures. The influence of the difference between day and night temperature on stem elongation suggested that thermomorphogenesis was not a function of total plant carbohydrate translocation, but a function of endogenous GA content or the response of plant tissue to GA. However *Onwueme* and *Laude* (1972) showed that heat induced growth retardation of barley and wheat coleoptiles could not be prevented by exogenous application of 0.5 - 100 ppm IAA or 0.5 - 5 ppm GA nor by dilute aqueous solutions of calcium chloride, cobalt nitrate or zinc sulphate. Thus growth retardation at high temperature may be caused by a change in hormone balance rather than just a change in gibberellins (*Dedolph*

et al. 1963; Matsubara 1980; Makus and Guinn 1992).

Warrington and Kanemasu (1983b,c) using corn reported that the leaf initiation rate and leaf appearance rate increased linearly as mean temperatures were increased from 15 to 28°C; maximum rates occurred at 30 to 34°C, beyond which the rate declined. Moreover, Warrington *et al.* (1977) showed that the response of corn leaf number to temperature was strongly curvilinear; leaf number was highest at both the cooler and warmer temperatures and minimum at a mean temperature near 18°C. The decline in leaf number at temperatures above 30°C, indicated a possible response to high temperature stress, as plants growing in the 38/33°C temperature were partially stunted with considerable senescence of lower leaves at the time of anthesis of the corn. Leaf growth of asparagus in tropical climates has not been documented.

1.5.7. Root Growth at High Temperatures

Studies with roots are particularly difficult, because soil temperatures at different depths are correlated, and the development of different organs and different root numbers is also correlated. These multiple correlations make definite statements about which environmental factor controls individual processes very difficult. The rapid development of main and lateral roots could result from either, a direct effect of local temperature or a direct effect of shoot meristem temperature or, an indirect effect of the shoot meristem, or temperature affecting shoot growth and therefore the supply of assimilates and hormones to the roots (Gregory 1983). In addition, the work of Watts (1972b) has drawn attention to the significance of the shoot meristem, but the soil temperature was also recognized as an important determinant of early shoot growth, because the temperature of the root system can affect plant growth by altering the rate of absorption and movement of water and nutrients, and the production and translocation of plant regulating hormones. Air temperature is also important as high leaf temperature can lower rates of net photosynthesis thus lowering the supply of carbohydrates which leads to a greater decline in

root growth than shoot growth and hence high shoot : root ratios.

Lahav and Trochoulis (1982) demonstrated that at low temperature avocado plants had better root growth and more dry matter accumulation compared with high temperature, as at high temperature the pattern of distribution of assimilates had less going to the roots and no change in the movement of carbohydrates out of the leaves. Furthermore, more roots appeared to have died during the high temperature treatment. The optimal temperature for root growth was D21/N14°C for cv. 'Hass' (heat sensitive) and D25/N18°C for cv. 'Fuerte' (heat insensitive). Thus high temperature was more advantageous in all parameters measured except for root dry matter production, and the heat insensitive genotype had a higher optimal temperature compared to the heat sensitive one. However, the shoot/root balance was changed in favour of shoots by high and supra-optimal temperatures. A similar situation may exist in asparagus, where growth in tropical climates may favour the shoot, but heat insensitive genotypes may maintain a lower shoot/root ratio at high temperatures.

Hurewitz and Janes (1983) showed that tomato plants grown with a cool root zone temperature (18°C and below) had thicker darker green leaves with increasing purplish coloration on the underside of their leaves. Their roots were short and thick as opposed to plants grown at warmer root zone temperatures (26°C and above) whose roots were long, thin and profusely branched. It appears, therefore that an environment which provides for satisfactory root growth would also benefit the shoot growth of the plant. Cool root zone temperatures are capable of inhibiting and warm temperature are capable of stimulating plant growth when air temperatures are not limiting growth, because vital temperature dependent processes (e.g. sink activity of roots) which occur in the root exert control over shoot as well as root activities.

The examination of rape by *Cumby and Nye (1982)* also indicated similar results, in that root dry weight (and root length) were clearly temperature

dependent; increasing from 10 to 30°C, and decreasing at 35°C. Root branching increased with higher temperatures as did the intensity of brown pigmentation. In apple trees, supra-optimal root temperatures showed no serious damage to roots up to 35°C, except for their reduced size. At 40°C, different degrees of dieback appeared, and the quantity of dead roots increased. Leaf senescence was noticeable at 40°C. At 35°C similar symptoms occurred much later and less severely. It was suggested that the reduction of leaf chlorophyll level at supra-optimal root temperature was due to a reduction in the supply of cytokinins from less active roots (*Gur et al.* 1976).

Hence it is likely that the control of the root growth of asparagus in tropical climates will be complex, because root growth is associated with assimilation and assimilate partitioning, which is strongly influenced by temperature. A thermotolerant line may have a greater ability to maintain normal growth. High rates of root growth at high temperature would be a favourable characteristic in asparagus, because the carbohydrate pool for spear growth is the roots.

1.6. Effects of Absciscic acid on Growth at High Temperatures

1.6.1. Plant Hormones and High Temperatures

Environmental stress is known to cause hormonal changes. Such changes are evident when plants are exposed to heat, water, salinity or flooding, and may be part of the mechanism enabling plants to survive environmental stress. When the stress is relieved, the reverse process begins in which metabolic processes tend to normal conditions (*Itai et al.*, 1978). *Yakushkina* and *Tarasov* (1982) reported that the high growth rate of maize seedling at 25°C was accompanied by high activity of auxins, GA, and cytokinins and low activity of ABA; the reverse occurred at 46°C. Application of GA at 100 mg/l increased growth and removed the inhibitory effect of high temperature. This result was contrary to that of *Onwueme* and *Laude* (1972), who found that 0.5 - 5 mg/l GA did not prevent growth retardation at high temperature. Perhaps

the GA concentration was too low. *Matsui et al* (1986) also found that when grapevines were exposed to temperatures exceeding 40°C during the growing season, the content of GA in the berries declined thus indicating that fruit cells appeared to lose their ability to synthesize or import the growth promoting hormone and assimilate from leaves and buds. The growth inhibition resulting from decreased GA level was partially reversible by application of GA₃ or with promalin.

1.6.2. Changes in Endogenous ABA at High Temperatures

Plants exposed to stress, such as high temperature or low moisture, react by producing ABA, which functions to slow/stop growth and induce resistance, which in turn may hasten the senescence process (*Hellali and Kester* 1979). For example the ABA concentrations was significantly higher in young leaves of N-deficient (stressed) plants of tomato (*Daie et al.* 1979). *Durley et al.* (1983) examined the ABA and IAA concentrations in leaves of sorghum under drought stress, and found that there was considerable genotypic variation, and that the mean leaf ABA concentration in drought-stressed plants was positively related to a reduction in grain yield. They concluded that it is possible to evaluate genotype drought resistance to a given stress treatment in sorghum by examination of ABA levels.

It is suggested that ABA is a stress hormone, which regulates stress responses common to many environmental stresses (*Boussiba et al* 1975). *Itai et al.* (1978) reported that the ABA content was not consistent with this assumption. *Rikin et al.* (1975) showed that ABA and GA are involved in an interrelationship between morphogenesis and cold resistance in seedlings of two alfalfa cultivars (Ranger and Hairy Peruvian), while *Waldman et al.* (1975) found that ABA-like activity was hardly affected by thermophotoperiod (alterating day / night temperature regime). Thus they suggested that the modification of the ABA/GA balance through the decrease of the GA level, monitors the capacity of the two alfalfa cultivars to become cold acclimated

when exposed to low temperature. *Atkin et al.* (1973) measured the inhibitor content in xylem exudate of maize at various temperatures, and found that there was a lower inhibitor activity of xylem exudate at 33°C than at both 28°C and 23°C, and that at 33°C the temperature was optimal for shoot extension growth. *Itai et al.* (1973) treated the root system of tobacco and bean for 2 minutes at 46-47°C (extremely high temperature), and found that heat treatment reduced cytokinin levels and increased ABA levels in the xylem exudate of maize.

Daie and Campbell (1981) proposed that ABA may be a common mediator for all stressed plants, and may also be involved in a stress syndrome response, because stressful temperatures, whether constant or diurnal, caused an increase in ABA levels of tomato plants (*Daie and Campbell* 1981). However when *Eze et al.* (1983) examined the effects of temperature and moisture stress on the accumulation of ABA in bean, they suggested that the rapid accumulation of ABA during temperature stress is a function of induced moisture stress deficits and does not result from high or low temperature *per se*, in contrast with those responses reported for tomato by *Daie and Campbell* (1981).

Radin and Hendrix (1986) found that the accumulation of ABA in leaf discs of corn incubated in an osmotic stress solution (polyethylene glycol 8000) from 20 - 35°C depended strongly upon temperature, being maximum at 20°C, while at 35°C the amount of ABA accumulated was 45-80% less than at 20°C. In fact, the production of ABA in cotton leaf at 35°C is higher than at 20°C, but due to the higher turnover rate at 35°C, the ABA accumulation was less at 35°C than at 20°C. *Daie et al.* (1981) exposed the seedlings of cool season crops (beet, lettuce, radish, pea) and warm season crops (bean, corn, eggplant, okra) to supra- and sub-optimal temperatures (40, 25, 10°C), and the results supported the hypothesis that ABA is produced under temperature stress. For cool-season crops 40°C was a marginally stressful temperature, and at temperatures higher than 40°C, these plants produced high levels of ABA. In

contrast, all warm-season crops showed as much as a 5-fold increase in free ABA at 10°C compared to the concentration at 25°C. Thus the ABA levels or turnover rate appears to reflect the extent of stress, the effect depending on species.

1.6.3. Exogenous ABA on Growth at high temperatures

The retardation of plant growth under high temperature has been described earlier, but the way in which high temperature retards growth is not clearly understood although, as discussed earlier, one cause may be the elevated ABA content in both roots and shoots at high temperature (*Biddington and Dearman* 1982), since elevated ABA depressed cell elongation and cell division in roots (*Torre et al.* 1972). However, *Gaither et al.* (1975) reported that although ABA probably retains its physiological role as a growth inhibitor in roots of many species, it may also play a promotive role in the hormonal regulation of pea root growth. For example, the application of 1 μM ABA increased root growth about 25 - 30%, while 0.01 μM ABA enhanced and 1 μM - 100 μM ABA inhibited the elongation of maize root segments (*Pilet and Rebeaud* 1983). Consequently it is suggested that low concentrations of ABA may stimulate the growth and development of roots and shoots (*Yamaguchi and Street* 1977; *Abou-Mandour and Hartung* 1980). Thus ABA can act as a growth promoter and this action probably involves an interaction with other endogenous growth regulators (*Yamaguchi and Street* 1977).

The work of *Biddington and Dearmen* (1982) showed that the addition of ABA to the nutrient solution increased the root to shoot ratio of hydroponically grown cauliflower by reducing the dry weight of the shoot and increasing that of the root. Similarly, *Watts et al.* (1981) reported that elevating endogenous ABA by water stress or by the application of exogenous ABA had similar effects on the growth of roots and shoots and ultimately the root to shoot dry weight ratio. Generally, low concentration ABA applications and mild water stress stimulated root growth, but higher levels of ABA application and water

stress reduced root growth. Overall the effect of stress was to increase the ratio of roots to shoots. However, *Creelman et al.* (1990) reported that exogenous ABA did not induce the changes in sugar accumulation, polysome status and mRNA populations that were observed after soybean seedlings were water stressed.

1.6.4. Possible Role of ABA in Plant Growth at High Temperatures

The possibility that ABA is involved in a common stress regulation system had been proposed by *Boussida et al.* (1975), who showed that tobacco plants pre-exposed to drought, mineral deprivation or salinity, exhibited elevated levels of endogenous ABA and demonstrated resistance to a secondary stress of sub-zero temperature. Furthermore, it has been reported that application of ABA to cultured tobacco cells accelerated the rate of adaptation of cells to NaCl stress and cold (*Keith and McKersie* 1986; *Orr et al.* 1986; *Chen and Gusta* 1983; *Singh et al.* 1987). The work of *Bonham-Smith et al.* (1987) also showed that maize seedlings can acquire thermotolerance by pretreating seedlings through the roots, with either a heavy metal stress or a water stress. *Bonham-Smith et al.* (1988a,b) also reported that maize seedlings pretreated with an exogenous application of ABA or Triadimefon, (a fungicide capable of blocking GA synthesis), enhanced the plant's ability to withstand the effects of a 3 hour sub-lethal (40°C) or lethal (45°C) heat shock. The level of protection provided by these agents was dependent upon the length of time that the plant was exposed, as prolonged exposure reduced tolerance to subsequent stress.

1.6.5. Endogenous Hormone Levels and Application of Plant Hormones on Growth at High Temperatures

Roots are known to produce hormonal factors that are required for sustained shoot growth and metabolism. *Atkin et al.* (1973) found that the export of GA from corn root declined markedly due to root heating. The heat stress not only affected root growth, but also affected the tops; thus temperature had a

diverse and complicated impact on plant growth. *Menzel* (1980; 1981; 1983a,b; 1985) found that a mildly high root temperature increased the activity of GA, increased stem growth and inhibited tuberization of potato. Moreover when *Hiller et al.* (1979) measured the relationship between seedstalk height and GA content of the vernalized seedstalk of carrots, they found that supra-optimal temperatures severely reduced ultimate seedstalk height and endogenous GA activity. In addition *Matsui et al.* (1986) found that Napa Gamay grape berries from heat stressed vines were smaller than from non-stressed vines. The effect of heat stress was overcome by application of GA, but not BA. Thus high temperature stress may cause a decline in the GA level, but in contrast, slightly lower temperatures may increase GA levels. Furthermore, *Rikin* (1975) showed that ABA and GA are involved in the interrelationship between morphogenesis and cold-resistance in the seedlings of alfalfa.

Chen et al. (1986) found that GA applied to mungbean during the heat-shock period not only increased the subsequent thermotolerance of seedlings treated at 45°C but also allowed survival at a higher temperature (48°C). Some of this effect appeared to be due to the inhibition of RNA and protein synthesis at high temperature. They suggested that GA treatment may enhance the thermotolerance of mungbean seedlings through stimulation of induction and accumulation of heat shock proteins in the seedling during the heat shock period. *Lin et al.* (1985) reported that heat shock proteins function to prevent leakage of various substance from soybean cells. This suggests that GA may promote the heat shock process by protecting membrane integrity. In addition, *Lafuente et al.* (1991) showed that although heat shock elevated ABA levels, endogenous ABA levels were not directly related to the rate of electrolyte leakage.

1.6.6. Other Hormones in Relation to High Temperatures

Cytokinins are synthesized in the roots and appear to play an important role in the processes of plant development. *Atkin et al.* (1973) found that a high temperature (33°C) caused a decline in cytokinin activity. This result is the reverse of that of *Skene and Kerridge* (1967), who showed that a mildly high temperature increased cytokinin activity, but *Atkin et al.* (1973) explained his high temperature result as being due to a reduction in exudate flow from the roots.

Itai et al. (1973) exposed the root system of tobacco and bean to a temperature of 46-47°C for two minutes, and found a lower cytokinin activity and a higher ABA content. *Mauk and Langille* (1978) also showed that zeatin riboside levels in potato plants grown at D28/N13°C with 10 hour photoperiod were significantly higher than those in plants grown at D30/N28°C with 18 hours photoperiod. *Skogovist* (1974) speculated that if a certain content of cytokinins in roots is required for keeping the cells alive, heat treatment could have suppressed or inhibited the cytokinin synthesis to such an extent that the root meristem died. Application of kinetin was found to maintain endogenous cytokinin above critical levels, possibly by preventing the formation of hydrolytic enzymes such as proteinase, peptidase, RNase and B-1,3-glycan hydrolase. *Kuroyanagi and Pausen* (1988) also showed that high root temperature increased the activities of protease and RNase, and caused a loss of chlorophyll, protein and RNA in shoots, whereas low root temperature had the opposite effect. High root temperature directly induced shoot senescence, probably by the disruption of root processes (i.e. the export of cytokinins).

Optimal or mildly high temperatures may maintain or increase the level of auxins. In contrast, at supra-optimal temperatures, the levels of auxins are significantly decreased (*Lee et al.* 1979). *Hadid et al.* (1986) showed that high and low temperatures caused a depression of the concentration of auxins, but high temperature caused an increase of ABA on tomato flowers. *Kuo and Tsai*

(1984) also reported that high temperature decreased the level of auxin, and caused a problem with fruit set of tomato.

A number of environmental factors such as temperature, drought, salinity, flooding, light, CO₂ and O₂ have been shown to influence ethylene production in plant tissue. High temperature may cause impairment of ethylene production by perturbing cellular membranes, resulting in the inhibition of membrane associated oxidation of ACC to ethylene. Moreover the conversion of ACC to ethylene in auxin treated mungbean hypocotyl was inhibited only at temperatures at and above 42.5°C. High temperature inhibited auxin induction of ACC synthase (Yang and Hoffman 1984; Biggs *et al.* 1988). Yu *et al.* (1980) also concluded that the conversion of ACC to C₂H₄ is highly vulnerable to high temperature inhibition. Also Chan (1986a,b) demonstrated that heat can decrease the activity of EFE (ethylene forming enzyme system). Horiuchi and Imaseki (1986) also reported that the conversion of ACC to ethylene was greatly inhibited at high temperature.

1.7. Cell Membrane Thermostability and Heat Tolerance

1.7.1. Electrolyte Leakage from Heat Injured Cell Membranes

The identification of heat tolerant genotypes is often desirable, but field evaluations are frequently inefficient, so a laboratory procedure for measuring heat tolerance, using controlled environment conditions, is preferable. The conventional method used for the estimation of heat tolerance is by exposing the entire plant to heated air or immersing the shoot or root in heated water for a few hours, the plant then being returned to the normal growing condition. The degree of damage by the heat exposure is evaluated one or two weeks later. Although carried out in a controlled environment, this still has two major disadvantages: (1) at least some of the plants being tested must be killed or severely damaged during the test, and (2) the entire test lasts a long time, requiring about 2 weeks before the results can be known (Onwueme 1979).

Therefore, reliable measurements of physiological characteristics, e.g. photosynthesis or enzymes are desirable. Membrane permeability for evaluating heat tolerance is preferable in the laboratory, because cell membranes are thought to have a primary involvement in injury caused by extreme temperature.

1.7.2. Membrane Thermostability

Basically, when plant tissue is injured by exposure to high temperatures, the cell membrane permeability is increased, and electrolytes diffuse out of the cell, thus the amount of electrolyte leakage is a function of the degree of injury by the elevated temperature. The works of *Martineau et al. (1979)*, *Bousslama and Schapaugh (1984)*, *Marsh et al. (1985)*, *Saadalla et al. (1990a)* and *Shanahan et al. (1990)* proved that using the membrane thermostability test is suitable for ascertaining heat tolerance of soybean, winter wheat and spring wheat. Furthermore, *Saadalla et al. (1990a,b)* determined the relationship between membrane thermostability and other agronomic traits of winter wheat, and reported that heat tolerant strains of winter wheat had more yield, greater grain volume weight and kernel weight, so they suggested that the membrane thermostability test would be a suitable procedure for selecting heat tolerant winter wheat genotypes in a breeding program.

Chen et al. (1982) showed that membrane thermostability tests can be used to measure heat tolerance in bean, potato, soybean and tomato. Moreover they suggested that when breeding for high yield under high temperature conditions, one should select those genotypes with higher heat adaptability rather than those with higher pre-acclimation levels of heat tolerance. This characteristic cannot be distinguished among genotypes unless plants are first subjected to temperature above 30°C for about 12 to 24 hours, and then the heat acclimation detected by measuring the ion leakage immediately after a stress at high temperature, e.g. 50°C, when heat tolerance increases dramatically and differences between tolerant and susceptible genotypes

became clear. However, when the temperature was increased above a certain point, the hardening effect declined. This may indicate that, in nature, when the environmental temperature reaches a high level (about 35 - 37°C), the hardening mechanism is triggered, resulting in an increase in heat tolerance. This enables plants to endure more severe heat stress. However, the acclimation mechanism may only work within a narrow temperature range, becoming less efficient at temperatures above this range.

Lester (1985) reported that aging is a major factor in the ability to acquire heat tolerance, and for *Cucumis melo*, the heat tolerance of plants 10 days old or older was not affected significantly by cultivar or growing temperature differences. *Lin et al.* (1985) postulated that 15 - 18 kD (kilo-dalton of molecular weight) heat shock proteins (see section 1.8) play a role in preventing leakage of the cell membrane, although a direct association of 15 kD HSP with plasma membrane, which protects the cell from solute leakage, has not yet been found.

1.7.3. Establishment of a Criterion for Assessing Heat Tolerance - a Model

In order to evaluate heat resistance, it is necessary to select a criterion by which it is possible to judge the degree of cell injury after a specific level of heat stress. Most researchers estimate heat tolerance by exposing plants or tissue to various temperatures for a fixed time, and determining the temperature at which 50% tissue injury or death occurs. Thus a heat killing temperature is obtained. On the other hand, the duration of stress at a fixed temperature has also been used for determining heat tolerance. The plant tissue is exposed to a selected temperature for a certain period and the relative heat tolerance among genotypes is compared, based on the percentage of tissue injury. *Chen et al.* (1982) found that heat killing time provided a much wider range of difference between heat tolerance and susceptible genotypes. In fact, heat killing temperature and heat killing time

were highly correlated, thus heat killing time and killing temperature are useful measurements of heat tolerance for both experimental use and genotype selection.

Because the electrolyte leakage against exposure time shows a sigmoidal response (*Martineau et al.* 1979), a mathematic model has been developed by *Ingram* (1985) to fit the sigmoidal response. The inflection point of the response curve is the critical high temperature, causing irreversible cell damage or death, for a given exposure duration. Moreover, *Ingram* (1986) demonstrated that the critical temperature for roots decreased linearly as exposure time increased exponentially. Thus the critical high temperature can be derived and used as a predictor of heat tolerance of cultivars or suitable growing temperatures (*Ingram and Buchanan* 1981, 1984; *Ingram* 1985, 1986; *Ahrens et al.*, 1988; *Inaba and Crandall* 1988).

1.8. Heat Shock Induced and Absciscic Acid Induced Proteins

1.8.1. Heat Shock Induced Proteins

A transient increase in temperature (generally 5 - 10°C above the normal growth temperature for a period of from 15 minutes to a few hours) induces the synthesis of a new set of proteins, the so-called "heat shock proteins" (HSPs), which are either not present or are present at a low level in unstressed cells. The proteins that the cells have been synthesizing prior to heat shock (referred to as normal cellular proteins in this discussion), or at least a subset of them, are no longer synthesized (*BrodI* 1989). *Kimpel* and *Key* (1985a) describe the heat shock protein response of plants as follows:

1. Immediately following an abrupt shift of 8 - 10°C above the normal growing temperature, the synthesis of a new group of proteins, ("HSPs") is induced and, at the same time, there is a decline in synthesis of the normal complement of cellular proteins.

2. The synthesis of these HSPs is due to the transcription of a new set of heat shock genes. Many of these genes have now been sequenced and a consensus promoter or enhancer-like sequence has been identified in the 5' flanking regions. Evidence is accumulating that this core 15 bp (base pair) sequence is critical to the heat-inducability of these genes.
3. When tissue are returned to their normal growing temperature, synthesis of HSPs ceases, synthesis of other proteins recovers gradually, and the HS mRNAs decay with a half-life of less than 2 hours.
4. Heat treatments which induce HSP synthesis can also lead to the development of thermal tolerance.

Presumably, such a shift in protein synthesis would allow an organism to direct its energy toward coping with heat stress. Although the precise role of HSPs is not clear, their synthesis has been correlated with the acquisition of thermotolerance (*BrodI* 1989).

There are 3 different size groups of HSPs present in higher plants, including soybean, tobacco, tomato, maize, cotton, gladiolus, and wheat (*Key et al.* 1981; *Baszczynski et al.* 1983; *Cooper and Ho* 1983; *Lin et al.* 1984; *Kanabus et al.* 1984; *Burke et al.* 1985; *Ginzbury and Salomon* 1986; *Ougham and Stoddart* 1986; *Krishnan et al.* 1989). The composition of groups of HSPs are dependant on the plant species and cultivars, shock temperatures and time. These HSPs are described as follows:

1. The large HSP group ranging in size from 68 to 104 kD. This group is ubiquitous among all the organisms including bacteria, animals and plants.
2. The intermediate size group between 20 and 33 kd.
3. The small HSP group about 15 - 18 kd in size. This group is unique to higher plants.

Kimpel and *Key* (1985a) divided the HSPs into two groups: the high-molecular-weight (HMW) and the low-molecular-weight (LMW) HSPs. The LMW proteins were resolved into more than 30 polypeptides, the exact profile and complexity varying among plant species, but with a relatively high amount of this group of proteins being observed in all plant species. The HMW proteins of plants seemed less complex than the LMW proteins. For a given plant species the HMW proteins resolved into less than 10 polypeptides. *Marmioli et al.* (1986) compared two genotypes of barley tissues and showed that 'Onice' and 'Georgie' barley had differences in cold shock-induced proteins and cold-repressed protein pattern, and the two genotypes of barley also had different thermotolerances. *Oughum* and *Stoddart* (1986) suggested that the lack of HSP synthesis in germinating sorghum at high temperature may be partly responsible for the particular heat-sensitivity of this stage of development.

1.8.2. Heat Shock Protein Difference between Genotypes

Genetic differences in the high-temperature susceptibility of crop plants may be correlated with variation in the temporal development of the capacity to synthesise HSPs and acquire thermotolerance. This may lead to the development of laboratory techniques for the assessment of high temperature susceptibility in crop varieties. *Krishnan et al.* (1989) suggested that significant quantitative difference between the wheat cultivars 'Mustang' and 'Sturdy' were observed in the HSPs exhibiting molecular weights of 16, 17, 22, 26 and 33 kD. Their work revealed unique proteins (16, 17 and 26 kD) in the thermal tolerant variety 'Mustang' that were absent in the more thermal sensitive variety 'Sturdy'. These results provide a correlation between the synthesis of specific low molecular weight HSPs and the degree of thermal tolerance expressed following exposure to elevated temperatures. In the examination of the heat shock response of three genotypes (two parents of *Lycopersicon esculentum*, *L. pennellii* and their F_1 progenies) of tomato (*Fender* and *O'Connell* 1990), the response of F_1 lines to heat shock protein synthesis were

intermediate to the parental responses for duration of, and recovery from, heat shock. Only half the HSPs of *L. esculentum* were found in the heat shocked F_1 lines but all the HSPs of *L. pennellii* were present.

In carrot cell cultured lines, the HSPs among cell lines appeared to have both quantitative and qualitative differences (Hwang and Zimmerman 1988). Wheat also exhibited similar differences, so the genetic diversity of HSPs within a species opens up several new areas of investigation of the heat shock response, such as the analysis of specific, unique HSPs with respect to thermal tolerance. Current research is focused on studying the correlation between heat shock protein synthesis and cellular thermal tolerance. More sensitive DNA probes could then be used to identify HSPs that play a direct role in thermal tolerance.

The synthesis of particular HSPs also depends on the shock temperature treatments and time courses. For instance, HSP 80 kD has maximum synthesis at 34°C, HSP 75 kD and 16 kD around 37°C, HSP 16 kD around 42°C. The normal temperature proteins 44, 42 and 38 kD were decreased by heat shock, and the protein was more rapidly degraded in the sudden heat shock treatment compared with a gradual temperature increase (Altschuler and Mascarenhas 1985). Whether or not the different kinds of HSPs associated with different temperatures provide a different level of protection, is a question for the future.

1.8.3. Linking ABA Induced Proteins and Heat Shock Proteins

The plant hormone ABA (see section 1.5) appears to moderate the responses of plants under adverse conditions, so an elevated endogenous ABA level under stress conditions or exogenous application of ABA may cause enhanced tolerance to stress environments. ABA induced proteins and mRNA are part of a general response by the plant to high temperature, however only a little work has been carried out comparing ABA induced and heat induced proteins.

The metabolites of ABA may relate to the appearance of HSPs (*Radin and Hendrix 1986*). Other stresses, i.e. cold, salinity, drought, also induce some HSPs and enhance heat tolerance. Recently it was proposed that there are inducers linking HSP genes and stresses. These inducers may be the denaturation products of proteins and/or abnormal proteins produced at high temperature which activate HSP genes (*Rees et al. 1988*). The work of *Lafuente (1991)* indicated that increased tolerance to chilling injury induced in cucumber cotyledons by temperature conditioning was more likely related to the appearance of HSPs, which would protect plants against further stress temperatures such as chilling, than to the induction of higher endogenous levels of ABA.

Bray (1991) showed that the response of plants to heat is different to many other environmental stresses. Because heat induced alterations in gene expression of tomato were the same in the wild type and in the ABA deficient mutant, heat tolerance of the mutant was only marginally less than for the wild type. In contrast, ABA applications significantly reduced the heat tolerance of the leaves of the wild type. She concluded that elevated levels of endogenous ABA are not involved in the tomato heat shock protein response. *Heikkila (1984)* reported that heat shock induced specific HSPs and the intensification of synthesis of two proteins with a molecular weight of approximately 70 kD. Water stress and ABA also stimulated synthesis of these 70 kD proteins and other unique proteins distinct from those induced by heat shock. Moreover he showed that heat shock, water stress and ABA caused the accumulation of mRNA specific to a cloned genomic probe of the 5'end of the 70 kD *Drosophila* heat shock protein gene. The HSP 70 gene was activated in corn by a variety of diverse stresses, which may explain why other stresses may induce partial HSPs and induce heat tolerance.

Recently, *Jacobsen and Shaw (1989)* also showed that ABA induced the synthesis of at least 25 polypeptides in mature barley aleurone cells, and that most of these ABA induced polypeptides were heat stable and had higher

glutamic acid / glutamine and glycine levels and lower levels of neutral amino acid than normal. The possibilities that the accumulation of the heat stable polypeptides during grain development was controlled by ABA and that the function of these polypeptides was related to their abundance and extraordinary heat stability were considered. In addition, the work of *Ried* and *Walker-Simmons* (1989) demonstrated that ABA inhibits the embryonic germination and synthesis of heat stable proteins of wheat, but they could not demonstrate that those heat stable proteins are the cause for limiting germination. Whether these heat stable proteins are HSPs is still not clear. Generally ABA may act on some HSP genes to synthesise part HSPs and lead to some heat tolerance.

1.8.4. Role of Heat Shock Proteins in Thermostability

In plant cells the two main sites of HSP accumulation are : (1) the nucleus, which undergoes heat shock-specific changes of its ultrastructure in connection with the interruption of ribosome biosynthesis and (2) new cytoplasmic structures (heat-shock granules). In addition, the majority of HSPs from leaves of sorghum and barley were associated with soluble proteins. Several low molecular mass (17 - 24 kD) HSPs were clearly identified in the membrane fraction (*Clarke and Critchley* 1990). Distinct HSPs were also found in organelles, thus chloroplasts contain a membrane-bound HSP (22 kD), which was synthesized by cytoplasmic polysome, whereas the prominent HSPs of mitochondria (60 kD) are formed within the organelle itself (*Sinibaldi and Turpen* 1985). *Lin et al.* (1984) found that in soybean HSPs 15 - 18 kD and 69 - 70 kD are associated with nuclei, mitochondria, and ribosomes, HSPs 22 - 24 kD with mitochondrial fractions and HSPs 84 and 92 kD with the post-ribosomal supernatant.

The precursor of HSPs 22, 28 and 27 kD can be taken up by isolated chloroplasts of soybean, pea, and maize in vitro (*Sachs and Ho* 1986). *Neumann et al* (1987) observed heat shock dependent recompartmentation of

HSP 70 kD and HSP 17 kD. Moreover, *Cooper and Ho* (1987) provided evidence that HSP 25 kD and HSP 72 kD were in the fractions containing closely associated Golgi bodies and ER (endoplasmic reticulum), while HSP 18, 29, and 72 kD were in fractions containing overlapping plasma membrane, mitochondria, and glyoxysome.

It has been shown that the induction of HSPs has been correlated with the ability of the cell to establish thermotolerance in many organisms, *Lin et al* (1984) showed that briefly subjecting soybean seedlings to 45°C following incubation at 28°C results in the induction of HSP and a concomitant establishment of thermotolerance. *Key et al.* (1981) and *Cooper and Ho* (1983) postulated that proteolytic activity induced by high temperature in wheat bears some resemblance to heat shock proteins in soybean and maize, because both occur in these species at similar temperatures and within similar time frames.

In *E. coli*, the synthesis of the HSPs is positively regulated by the *htpR(hin)* gene product. Mutants of *E. coli* lacking this gene product also fail to acquire thermal tolerance during heat shock at a permissive temperature (*Kimpel and Key*, 1985a). So the conservation of the heat shock response across all evolutionary lines implies that it is of central importance for the survival of the cell. It is therefore critical to understand the regulation of its expression. In general, it appears that the mechanism by which cells switch over from a program of normal cellular protein, to a program of HSP gene expression is not ubiquitous. Furthermore a study of normal cellular protein synthesis during heat stress in other organisms could significantly broaden our understanding of potential mechanism for regulating gene expression. Such an understanding will become immeasurably useful as genetic engineering in higher plants expands the boundaries of traditional plant breeding in the future.

1.8.5. Linking Heat Shock Proteins and Response of Plants at High Temperatures

Recently the linking of HSPs with the response of plants to high temperatures at particular developmental stages have been demonstrated by many workers. *Dupuis and Dumas (1990)* observed that the fertilization of maize was reduced when spikelets were exposed to temperatures over 36°C. When pollen and spikelets were exposed separately to temperature stress, the female tissues appeared resistant to 4 hours of heat stress (40°C) and HSPs were induced. In contrast mature pollen was sensitive to heat stress, unable to synthesis HSPs, and was responsible for the failure of fertilization at high temperature. It appears that the female tissues are able to synthesize heat shock proteins leading to thermotolerance, but the inability of the pollen to synthesize heat shock proteins leads to sensitivity to heat stress. This may be because the mature pollen is a specialized haploid tissue, which contains only a store of presynthesised mRNA used for pollen germination and tube growth. Because the synthesis of HSPs is primarily regulated by a transcriptional control, this allows the rapid induction of mRNA and a preferential translation during heat shock. This explanation is in agreement with the speculation that preheat treatment induces the production of a heat shock messenger in addition to the normal complement that is already in production. These messengers are exported as ribonucleoproteins to the cytoplasm where they attach to polysomes for translation or enter into an inactive pool (*Mitchell et al. 1979*)

In developing seeds HSPs probably have some function in addition to protecting the embryo from heat induced damage during development. As they are synthesized in normal regimes, two broad possibilities are that HSPs help protect the embryo from desiccation induced damage, or that they have developmental functions unrelated to protection from stress induced damage. Thus HSPs may have multiple functions. Such functions could include action in developmental processes which have nothing to do with stress tolerance, or the protection from desiccation stress during embryo maturation, and

enhanced stress tolerance during imbibition (*Helm and Abernethy* 1990). This may explain why arsenite, ABA and water stress also induced HSP synthesis. In addition, a wide variety of agents may cause the accumulation of mRNA encoding HSPs, e.g. methomyl, an insecticide and nematicide (*Rees et al.* 1989).

In the absence of HSPs, heat shock caused the degradation of ER lamellae and α -amylase mRNA, so that thermotolerance may in part be the resultant synergistic effect of HSPs (*Brodí et al.* 1990). HSP 70 kD may be involved in the disassembly of protein aggregate formed in the cytoplasm during heat shock, a variation of their unfolding role at the ER and mitochondria. HSP 15 to 18 kD and possibly 70 kD provide protection of mitochondrial oxidative phosphorylation coupled to O₂ uptake at high temperature (42.5°C). But the HSPs of 24 and 22 kD, specifically found in association with mitochondria seem not to be required for maintaining coupled phosphorylation (*Chirico et al.* 1988; *Deshaies et al.* 1988; *Chou et al.* 1989).

It has been suggested that a greater diversity of HSPs is induced in sorghum compared with barley, because sorghum is a subtropical C₄ species having adaptive ability to high temperature, while barley is a temperate C₃ species with less adaptation to high temperature (*Clarke and Critchley* 1990). All genotypes of sorghum have two major HSPs, 65 kD and 62 kD, but pearl millet had more HSPs and these ranged from 30 - 70 kD (*Sivaramakrishnan et al.* 1990). *Conner et al.* (1990) also suggested that HSPs 30 - 90 kD are involved in a general mechanism of regulation, controlling a variety of cellular functions, during the heat shock response. This is postulated to be the modulation of eIF-2 α phosphorylation and subsequent regulation of translation of cellular mRNA.

HSPs are products of mRNA translation, so the transcription activity of HS genes and pool size or half-life of HSP mRNA, are important factors. In wheat (*Helm and Abernethy* 1990), found there were no detectable differences in the

mRNAs found in mature embryos from field grown, from 25°C growth chamber cultivated, or from plants given 38°C heat stresses at different stages of seed development. The mRNA encoding for developmentally dependent HSPs was among those found in the dry embryos. The mRNAs encoding the low molecular weight HSPs decayed rapidly upon imbibition and the mRNAs for developmentally dependent HSPs persisted longer and were detectable following 16 hours of imbibition. After 1½ hours of imbibition, the mRNA for the developmentally dependent HSPs did not accumulate in response to heat shock, even though the synthesis of the proteins was enhanced. Thus, an applied heat shock appeared to lead to the preferential translation of preexisting developmentally dependent mRNA. It would appear that induced preferential translation of these RNAs reflects not a response to heat *per se*, but a recognition of stress protein mRNA by the protein synthetic machinery of heat shocked embryos.

Kimpel et al. (1990) demonstrated that the active transcription of heat shock genes occurs only during the first few hours at 40°C, nonetheless, mRNA for these genes are present in relatively high abundance even after 9 hours of exposure to 40°C. Because HS mRNAs have a fairly short half life (less than 3 hours at 28°C), these results indicate that HS mRNAs are inherently more stable at 40°C. Thus this suggests that the regulatory controls for the transcription of the HS genes must involve more than a simple sensing of ambient temperature. Plant tissues not only monitor the changes in temperature but, through an unknown sensing mechanism they also quantify the severity of the change and measure both the magnitude before and during the stress. A level of response is then initiated that is commensurate with the degree of the imposed stress. HSP 70 kD in soybean may have a role in regulating the HS response. Alternatively, it is also possible that one or more of the abundant low molecular mass HSPs in soybean has a regulatory role. Thermoinducability of mRNA transcription is mediated through the HSF (heat shock transcription factor) which bind to the HSE (heat shock consensus element). So the HSF exists in normal cells in a nonbinding form, and is

converted upon heat shock to a high affinity, HSE-specific form. The conversion seems to play a crucial role in transcriptional activation of heat shock genes (*Czamecka et al.* 1990). In addition, the level of transcription homologous to this cDNA is low in 2 week old *Arabidopsis* plants but is rapidly enhanced by elevated temperature (*Conner et al.* 1990).

Asparagus is regarded as a classical temperate crop, but is now being planted in tropical climates. Thus asparagus is fairly thermotolerant, and different growth responses to high temperature exist between genotypes. In this thesis a study on the possible association between ABA, HSPs and thermotolerance in asparagus will be presented.

CHAPTER TWO

An Investigation on the Growth of Asparagus Plants at High Temperature

2.1. Introduction

Asparagus is usually evaluated on the basis of yield and quality, but because asparagus is a perennial crop, evaluations usually proceed for a long period in the field with considerable expense of effort work and time. *Benson* (1980) suggested that yield differences might be analyzed on the basis of differences in early growth. *Nichols* and *Woolley* (1985) have also shown that although at this early stage any growth differences among cultivars may be very small, the distribution of dry matter can be very different. Thus growth analysis at the early growth stage in controlled environments may be a favourable method to assay cultivar differences. In this experiment seeds of asparagus were sown and grown in controlled climate rooms to measure the growth of young plants, the distribution of dry matter and the adaptability of asparagus to high temperature. The differences among cultivars at high temperature was compared. These results might be expected to be of application to asparagus production in tropical climates.

The growth of seedling asparagus can be represented by an exponential function, thus when the logarithm of plant size against chronological time is fitted to a linear regression, the slope is the relative growth rate (RGR). In these experiments the relative growth rates of leaf area, fern weight, crown weight and total plant dry weight (RLGR, RFGR, RCGR and RPGR) were determined in order to analyze the adaptability of four asparagus cultivars to high temperature. In addition, NAR and LAR were determined to provide some explanation of the differences in RGRs at high temperature (*Hughes* and *Freeman* 1967; *France* and *Thomley* 1984; *Hunt* 1990).

The partitioning of assimilates is partly governed by temperature, therefore the allometry of crown in relation to fern, the component production rate and their interrelationships were analyzed to determine the change of leaf, fern, rhizome and root growth activity and their dynamic changes at high temperature.

The growth experiments were only carried out in a limited number of temperature regimes, and the growth parameters were then fitted to a response surface to predict the plant growth at various temperature regimes.

2.2. Materials and Methods

2.2.1. Seeds

Four cultivars of asparagus were used in these experiments, namely:

Brocks Imperial

ex California Asparagus Seed and Transplants Inc., Davis, California,
U.S.A.

Larac

ex INRA, Versailles, France

Tainan No.1

ex Asparagus Research Centre, Tainan, Taiwan, R.O.C.

UC157

ex California Asparagus Seed and Transplants Inc., Davis, California,
U.S.A.

2.2.2. Sowing and Planting

Seeds were sown about 1 cm deep in 4.5 l pots in a growing medium of sieved peat : pumice (50/50 v/v), which were immediately placed in growth rooms at the experimental temperature regimes. After emergence seedlings were thinned according to a pre-determined plan to fit the space and harvesting of plants (Appendix 1A, 1B).

2.2.3. Treatment Temperatures and Experimental Design

This experiment involved 9 different temperature treatments, and it was carried out in 9 growth rooms and run between November 1989 and October 1990 in the Controlled Climate Facility of Fruit and Trees Division, DSIR, Palmerston North (Appendix 2). These treatments are shown in Table 2.1.

Each growth room was divided into 4 blocks. Each block consisted of 28 pots (7 pots each of the 4 cultivars), placed at random. One pot was sampled at

each harvest (7 days interval) from each block for each cultivar.

2.2.4. Environmental Conditions of Growth Rooms and Nutrients

The environmental conditions used in the growth rooms are shown in Appendix 3A, 3B, 3C.

A modified half-strength Hoagland's nutrient (Appendix 4) was supplied via an automatic tube system at rates considered adequate to minimize moisture stress (Appendix 5).

2.2.5. Harvesting and Plant Component Measurements

At each harvest the plants were separated into leaves, stems, rhizomes and roots. The roots were carefully washed out of the medium. Because the "leaves" are cladophylls, the fern was separated at the cladophyll attachment position to become two parts namely "stem" and "leaves". The area of "leaves"

Table 2.1. The treatment temperatures, sowing date, harvesting on growth analysis.

Day / night temperature	Date of sowing	Age of 1st harvesting from sowing	Age of 7th harvesting from sowing
D20/N20°C	25/07/1990	24 days	66 days
D25/N25°C	27/11/1989	17 days	59 days
D30/N20°C	19/03/1990	24 days	73 days
D30/N30°C	28/11/1989	17 days	59 days
D35/N15°C	18/06/1990	24 days	66 days
D35/N25°C	20/03/1990	24 days	66 days
D35/N35°C	29/11/1989	24 days	66 days
D40/N20°C	19/06/1990	31 days	73 days
D40/N30°C	21/03/1990	24 days	66 days

was measured with a Li-cor area meter before the "leaves" were dried. Although a Li-cor does not accurately measure cladophyll area, it can provide measurements to compare leaf area differences between temperature regimes or cultivars. All the plant components were dried in a vacuum oven for 48 hours at 40°C, and then weighed in a low humidity balance room (temperature 25°C, humidity < 40%).

2.2.6. Growth Analysis

Due to the different temperature treatments, germination was inconsistent and therefore the plants from the different treatments were not all harvested on the same days after sowing. The growth data were therefore processed using dynamic (fitted curve) growth analysis methods, then the growth indices of relative growth rates, leaf area ratios, net assimilation rates, allometry and component production rates were derived. These indices were compared to distinguish differences between temperatures and cultivars (*Hunt 1990*), as follows,

2.2.6.1. Relative Total Plant Dry Weight Growth Rates (RPGR)

Relative growth rate expresses growth in terms of a rate of increase in size per unit of size.

Definition :

The rate of increase of total dry weight per plant, W , expressed per unit of W .

Formula :

Instantaneously,

$$RPGR = \frac{1}{W} \cdot \frac{dW}{dt}$$

Methods of calculation :

Instantaneously derived from functions fitted to $\log_e W$ versus t ;

if

$$\log_e W = fw(t)$$

then

$$RPGR = fw'(t)$$

also

$$W_F = W_I e^{Rt}$$

$$\log_e W_F = \log_e W_I + Rt$$

When

W_F : Final plant weight.

W_I : Initial plant weight.

RPGR : Relative total plant dry weight growth rate.

R : RGR (relative growth rate).

t : Harvesting age of plant

2.2.6.2. Relative Fern Weight Growth Rates (RFGR)

2.2.6.3. Relative Crown Weight Growth Rates (RCGR)

2.2.6.4. Relative Leaf Area Growth Rates (RLGR)

The above three relative growth rates have a similar definition, formula and calculation as the relative growth rate of the whole plant, except that the total plant dry weight is replaced by fern dry weight, crown dry weight (rhizomes and roots weight) and leaf area, respectively.

2.2.6.5. Leaf Area Ratios (LAR)

A morphological index of the leafiness of the plant

Definition :

The ratio between total leaf area per plant, L_A , and total dry weight per plant, W .

Formula :

Instantaneously,

$$LAR = \frac{L_A}{W}$$

Methods of calculation :

Instantaneously derived from functions fitted to $\log_e L_A$ and to $\log_e W$ versus t ;
if

$$\log_e L_A = fL_A(t)$$

and

$$\log_e W = fW(t)$$

then

$$LAR = \frac{L_A}{W} = \exp(fL_A(t) - fW(t))$$

2.2.6.6. Net Assimilation Rates (NAR)

An index of the productive efficiency of plants calculated, not in relation to total dry weight, but in relation to total leaf area.

Definition :

The rate of dry weight (W) production of a plant, expressed per unit of total leaf area L_A .

Formulae :

Instantaneously,

$$NAR = \frac{1}{L_A} \frac{dW}{dt}$$

Methods of calculation:

Instantaneously obtained from functions fitted to $\log_e W$ and to $\log_e L_A$ versus t ;
if

$$\log_e W = fW(t)$$

and

$$\log_e L_A = fL_A(t)$$

then

$$NAR = \frac{1}{L_A} \frac{dW}{dt} = \frac{fW'(t)}{\exp(fW(t) - fL_A(t))}$$

or because

RGR = NAR x LAR then

NAR can also be obtained by dividing RGR by LAR

2.2.6.7. Allometry of Crowns in Relation to Ferns

An index of the balance of growth between crowns in relation to ferns, integrated over a period of time.

Definition :

The allometric coefficient, K , is the ratio between the mean RGRs of RCGR and RFGR.

$$K = \frac{RCGR}{RFGR}$$

Formulae :

$$W_R = bW_F^K$$

there

b is a constant (the natural logarithm crown weight when the fern weight is 1 gram)

K is the allometric coefficient

Methods and calculation :

Derived from a series of paired measurements of W_R and W_F . The function

$$\log_{10} W_R = f(\log_{10} W_F)$$

is fitted, then a linear regression of the form

$$\log_{10} W_R = \log_{10} b + K \log_{10} W_F$$

may be calculated to determine both the K coefficient and the constant b . K is also (of course) the ratio of RCGR/RFGR.

2.2.6.8. Component Production Rates

A mathematical identity which constitute the interrelating equation of relative growth rate of plant components (*Hunt and Bazzaz 1980; Hunt 1990*).

Definition :

The whole plant's relative growth rate in weight, R_w , can be subdivided into an expression which includes the relative growth rate of the individual organs of the plant.

Formulae :

$$RPGR = \frac{1}{W} \frac{dW}{dt}$$

$$RPGR = \frac{1}{W_1} \frac{dW_1}{dt} \frac{W_1}{W} + \frac{1}{W_2} \frac{dW_2}{dt} \frac{W_2}{W} + \dots + \frac{1}{W_n} \frac{dW_n}{dt} \frac{W_n}{W}$$

there $W_1, W_2, \dots, W_n$ are the dry weight of the individual parts of plant.

Methods of calculation :

$$RPGR = RGR_1 \frac{W_1}{W} + RGR_2 \frac{W_2}{W} + \dots + RGR_n \frac{W_n}{W}$$

$$RPGR = J_1 + J_2 + \dots + J_n$$

there $RGR_1, RGR_2, \dots, RGR_n$ are the relative growth rate of $W_1, W_2, \dots, W_n$ in the experiment

$$RPGR = R_R \frac{W_R}{W} + R_Z \frac{W_Z}{W} + R_S \frac{W_S}{W} + R_L \frac{W_L}{W}$$

$$RPGR = J_{root} + J_{rhizome} + J_{stem} + J_{leaf}$$

R_R : relative root weight growth rate

R_Z : relative rhizome weight growth rate

R_S : relative stem weight growth rate

R_L : relative leaf weight growth rate

J_{root} : root production rate

$J_{rhizome}$: rhizome production rate

J_{stem} : stem production rate

J_{leaf} : leaf production rate

2.2.7. Growth Predictions

A surface response function was applied to predict relative growth rates. The response function is defined as follows (Box 1954):

$$Y=f(D,N)$$

Y : RGR

D : day temperature

N : night temperature

The relative growth rates were fitted to a secondary degree equation for the two variables D and N using the equation:

$$Y=bB_0+b_1D+b_2N+b_{11}D^2+b_{22}N^2+b_{12}DN$$

When coefficients

b_1 and b_2 are linear effects

b_{11} and b_{22} are quadratic effects

b_{12} is interaction effect

The PROC RESEG of SAS software released 6.04 was applied to estimate the coefficients of b , b_1 , b_2 , b_{11} , b_{22} , b_{12} . Then the PROC CONTOUR was applied to produce a contour diagram to predict growth.

2.3. Results

2.3.1. Plant Growth

There was considerable variation in seed emergence time among cultivars, and temperature regimes. Seeds sown at more optimal temperatures (about 25 - 30°C) germinated faster than those at higher and lower temperatures ($\geq 35^\circ\text{C}$ and $< 25^\circ\text{C}$), so the plant sizes at any particular time were the combination of emergence time and growth rate. Because the inconsistent germination may have masked the real growth rate, the plant sizes were not used directly to determine growth difference among temperature regimes and cultivars. Growth indices which were derived from the growth data were compared to determine growth differences among temperature regimes and cultivars, because the growth indices exclude the influence of the time of germination of the seeds.

Linear regressions were fitted to the natural logarithm of plant size (leaf area, fern weight, crown weight and total plant dry weight) against chronological time, and very high correlation parameters were obtained ($0.986 \geq r^2 \geq 0.861$, $0.978 \geq r^2 \geq 0.893$, $0.982 \geq r^2 \geq 0.899$, $0.976 \geq r^2 \geq 0.898$ for leaf area, fern weight, crown weight, total plant dry weight, respectively), except that the plants grown at the extreme high temperature regimes (D35/N35°C, D40/N20°C, D40/N30°C) had poorer correlation parameters ($0.870 \geq r^2 \geq 0.567$, $0.882 \geq r^2 \geq 0.747$, $0.899 \geq r^2 \geq 0.662$, $0.891 \geq r^2 \geq 0.758$ for leaf area, fern weight, crown weight, total plant dry weight, respectively). These plants had very poor growth and a large variation of plant sizes within treatments, which resulted in lower r^2 values though still significant at $p < 0.001$.

Seeds sown at high and low temperature regimes had lower and slower germination than seeds sown at suitable temperature regimes. Plants grown at high temperature regimes showed poor and abnormal growth, with the tips of leaves and leaf yellowing occurring earlier than in plants grown at suitable

or low temperature regimes. At supra-optimal temperature ferns were also thinner and weaker, roots were smaller, shorter, brown in colour and had less secondary roots, while the rhizomes were also smaller, brown and had fewer buds. Although the seeds sown at low temperature regimes (about 20°C) were slow in germinating, the plants usually had normal growth, thick cladophylls, strong ferns and large, white, fleshy roots, even though the growth rates were slower than those grown at more suitable temperature regimes (Plates 2.1, 2.2, 2.3). The seeds sown at suitable temperature regimes not only emerged early but also had good germination. In addition, these plants grew rapidly, and the original bud cluster could develop up to 2 - 4 bud clusters and even a few flowers appeared within 59 days. The cladophylls were also dark green in colour, thick and still alive when harvested at the end of the experiment.

At suitable and low temperatures the fern produced a high first branch, and the spears were compact, thick and fleshy. At high temperatures the spears had open tips, and appeared fibrous. In addition, when the seedlings were grown at the high temperature regimes ($\geq 35^\circ\text{C}$), the fern was short, had fewer cladophylls and often had flowers on the extreme top of the ferns. Generally, the plants at extreme high temperatures had very poor growth, sometimes even becoming too weak to stand.

2.3.2. Relative Growth Rates

Because of the highly significant correlation between the natural logarithm of plant growth (leaf area, fern weight, crown weight, total plant dry weight) and chronological time (Fig. 2.1), it was clear that these plant growth parameters could be defined by the slope of the regressions.

2.3.2.1. Analysis of Variance of the Influences of Temperature and Cultivar on Plant, Fern, Crown and Leaf Relative Growth Rates

The experiment showed that plant growth was strongly influenced by

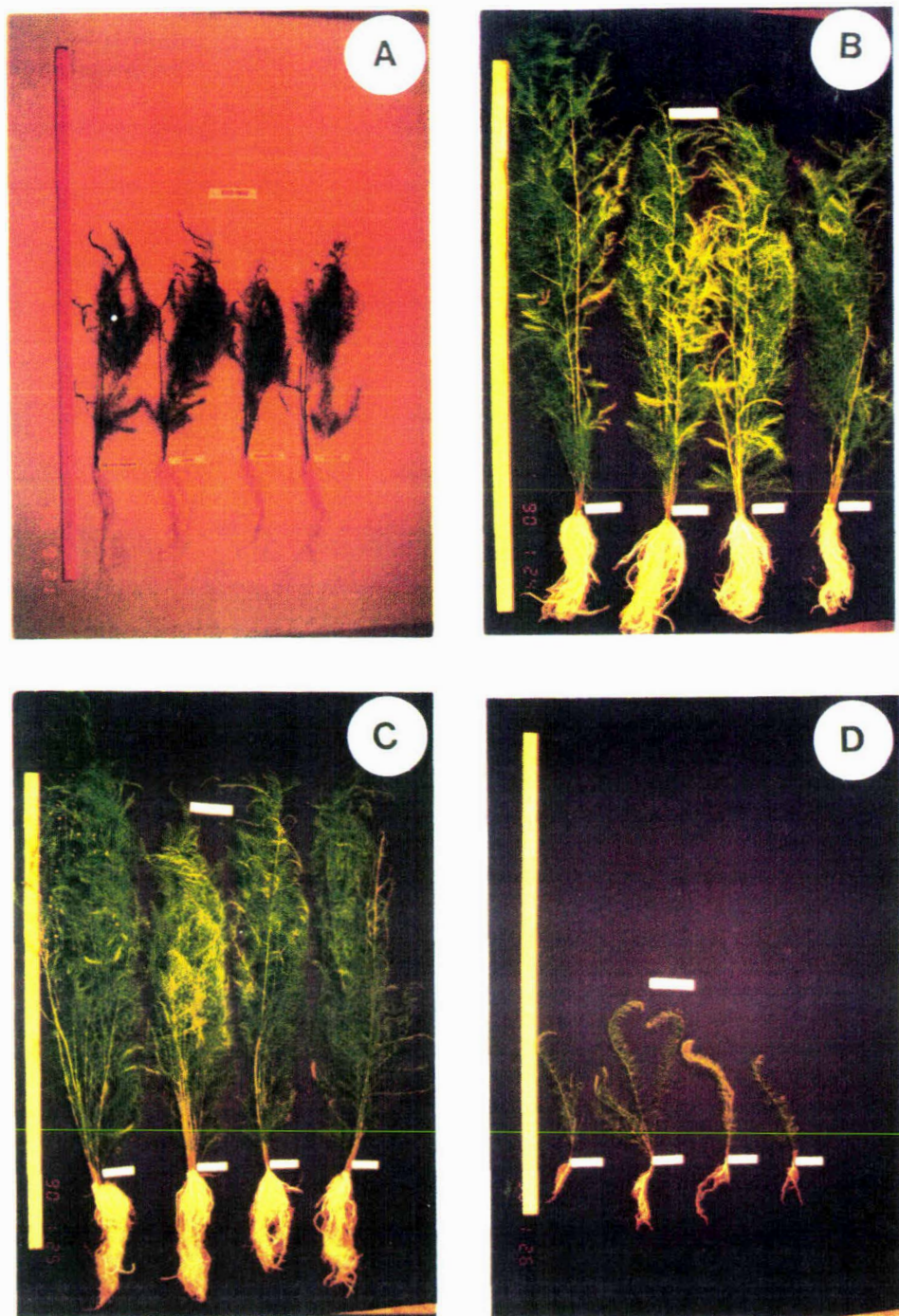


Plate 2.1. 59 days old plants at (A) D20/N20°C. (B) D25/N25°C. (C) D30/N30°C. (D) D35/N35°C.

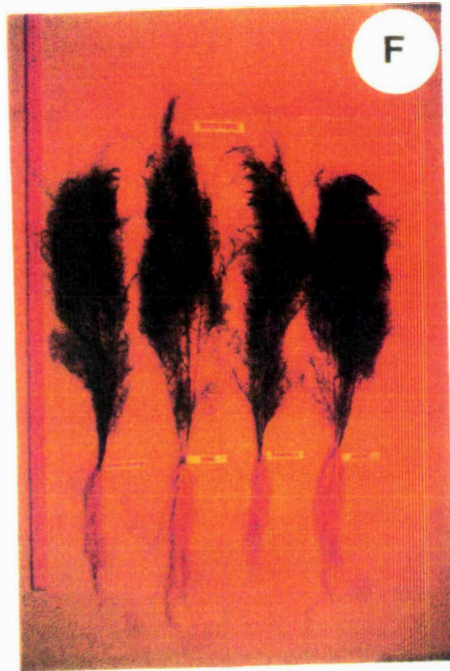


Plate 2.2. 59 days old plants at (E) D30/N20°C. (F) D35/N25°C. (G) D40/N30°C.

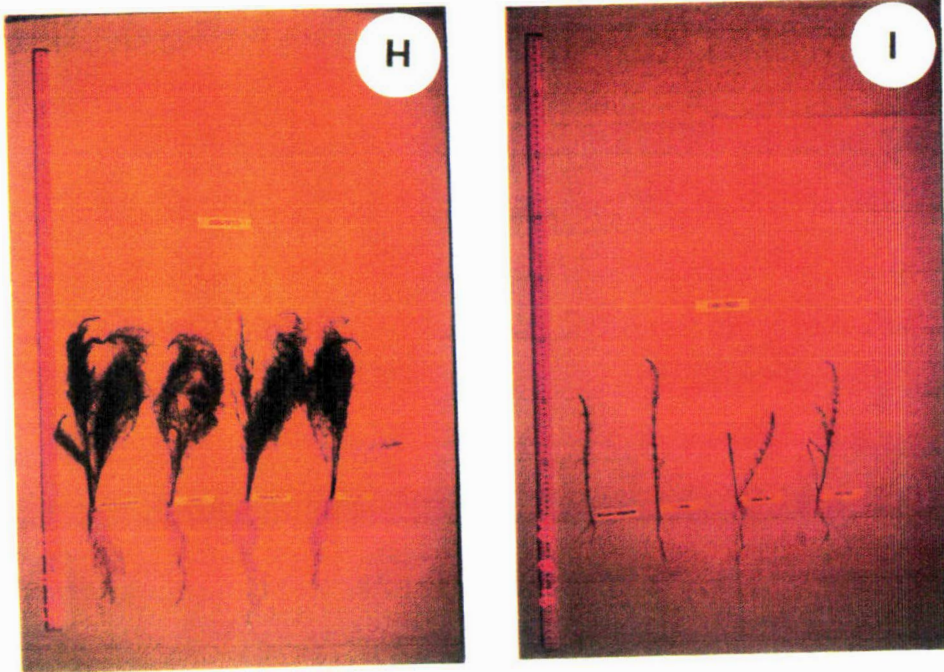


Plate 2.3. 59 days old plants at (H) D35/N15°C. (I) D40/N20°C.

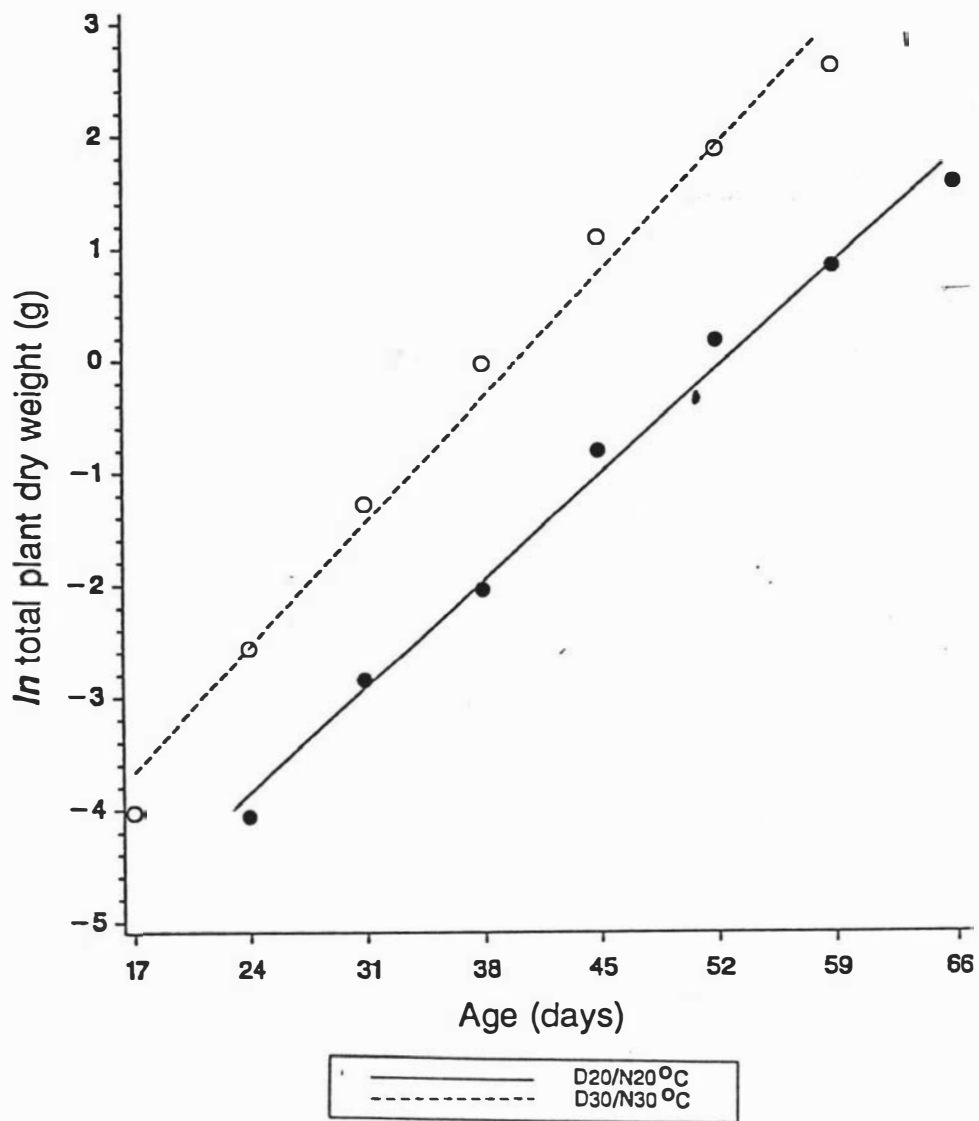


Fig. 2.1. The plot of natural logarithm total plant weight against age fitted to a linear regression

temperature, and to a lesser extent by cultivar. The analyses of the influence of temperature and cultivar on RLGR, RFGR, RCGR and RPGR are presented in Appendix 6A, 6B, 6C, 6D. The effect of cultivar on RFGR was significant at the 5% level, but none of the effects of cultivar on RLGR, RCGR and RPGR were significant. The effects of day temperature and of night temperature on RLGR, RFGR, RCGR and RPGR were significant, and the interaction between day and night temperature, and the interaction between cultivar and night temperature were significant for RLGR, RFGR and RPGR, but not for RCGR. Conversely, none of interactions between cultivar and day temperature, and the interactions between cultivar, day and night temperature on RLGR, RFGR, RCGR and RPGR were significant.

The influences of day temperature, night temperature and the interaction between day and night temperature on RLGR, RFGR, RCGR and RPGR of each cultivar are represented in Appendix 7A, 7B, 7C, 7D. Generally, the effects of day temperature, night temperature and the interaction between day and night temperature on RLGR, RFGR, RCGR and RPGR of all cultivars were significant except that the interaction between day and night temperature on RCGRs of Brocks, Tainan No.1 and UC157 and the interaction on RPGR of Brocks were not significant. The effect of night temperature was not significant on RPGR, RFGR and RCGR of Tainan No.1. It appeared that Tainan No.1 was fairly insensitive to night temperatures except for leaf growth.

These results showed that a number of factors significantly influenced RLGR, RFGR, RCGR and RPGR. However the effects of temperature, cultivar and their interactions varied with plant components; for example, the effects of these factors on RLGR, RFGR and RPGR were similar, but on RCGR was different. In detail, day temperature significantly influenced all plant components of each cultivar, but the night temperature and the interaction between day and night temperature did not significantly influence any plant components of any of the cultivars.

2.3.2.2. Comparison of Relative Growth Rates among Plant Components

Temperature had a greater effect on plant growth than either cultivar or plant component, hence the RGRs showed greater variation among temperatures, and a much smaller variation among cultivars and components (Fig. 2.2, 2.3, 2.4, 2.5). The highest RLGR, RFGR, RCGR and RPGR (means of cultivars) were 0.181, 0.162, 0.155 and 0.159 per day, respectively, and the lowest were 0.073, 0.070, 0.080 and 0.073 per day, respectively. Thus plant components above the ground had faster growth rates than those under the ground. In addition, the highest RLGR and RFGR occurred at D30/N30°C, but the highest RCGR and RPGR occurred at D25/N25°C, although at D25/N25°C the RLGR, RFGR, RCGR and RPGR were not significantly different from at D30/N30°C.

The relationship of RGRs to temperatures were curvilinear, with a peak between 25 - 30°C. RPGR, RFGR and RLGR at a constant temperature were higher than at different day/night temperatures at the same mean temperature. All RGRs declined when the temperature was outside the optimum temperature, but the decline was not symmetrical. A 5°C increase from the optimum temperature led to poor growth and a dramatic decline in RGRs. Conversely, a 5°C reduction from the optimum temperature maintained near normal growth and only a small decline in RGR.

At a day temperature of 40°C (D40/N20°C and D40/N30°C) and at a constant temperature of 35°C plant growth was very poor, but when the high day temperature was combined with a suitable or low night temperature the plants had better growth. At a day temperature of 35°C and a night temperature of 25°C plants had normal growth, but at a day temperature 35°C and night temperature of 15°C growth was poorer than at D35/N25°C. Thus the growth at a high day temperature of 35°C could be improved by suitable night temperature. However, at a supra-optimal day temperature of 40°C growth was only slightly improved by a suitable night temperature (D40/N20°C and

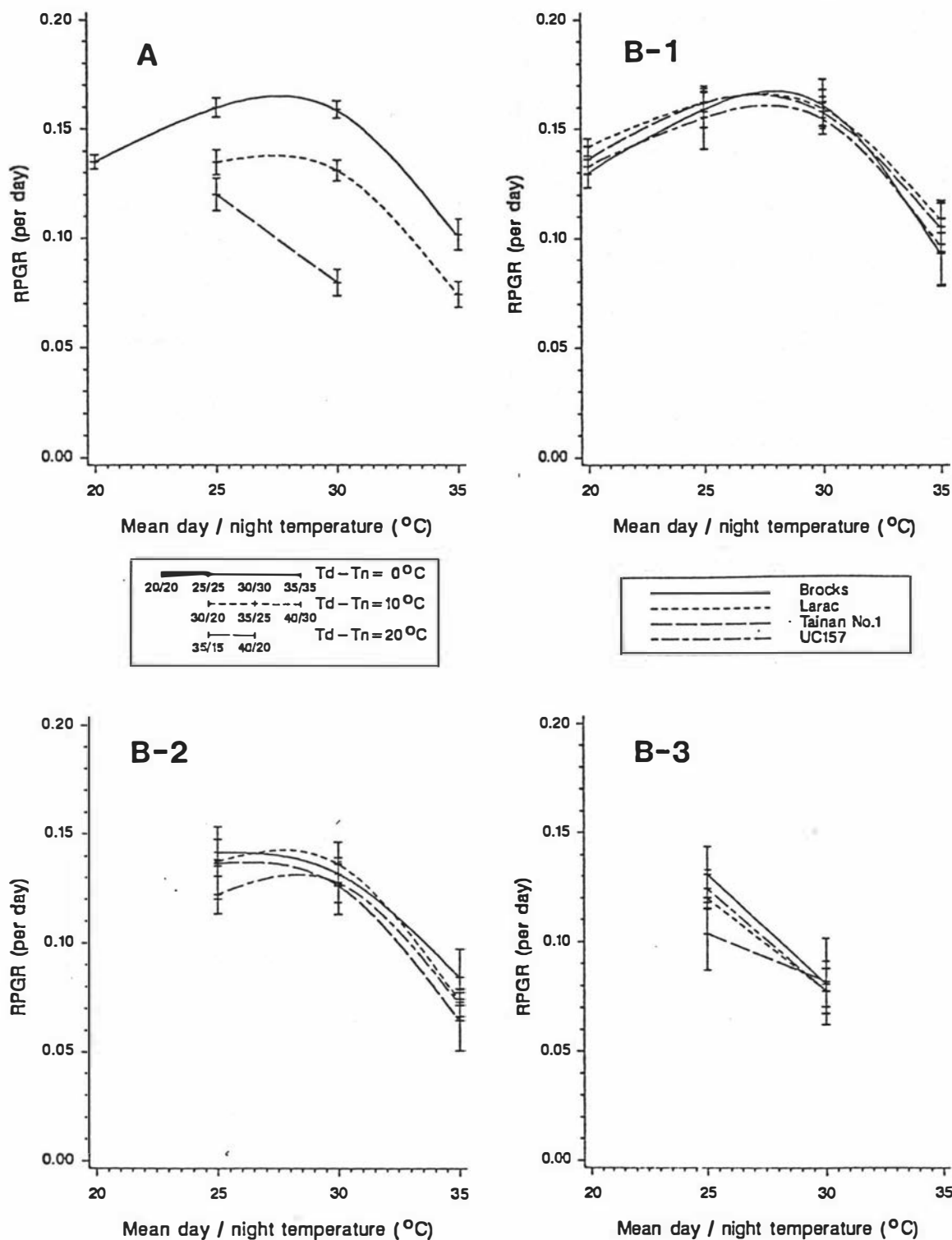


Fig. 2.2. The comparison of the RPGRs between plants

A : at various temperature regimes (means of four cultivars).

B-1 : of cultivars at the constant day/night temperatures.

B-2 : of cultivars at the 10°C day/night differential.

B-3 : of cultivars at the 20°C day/night differential.

(Vertical lines indicate standard errors of the means)

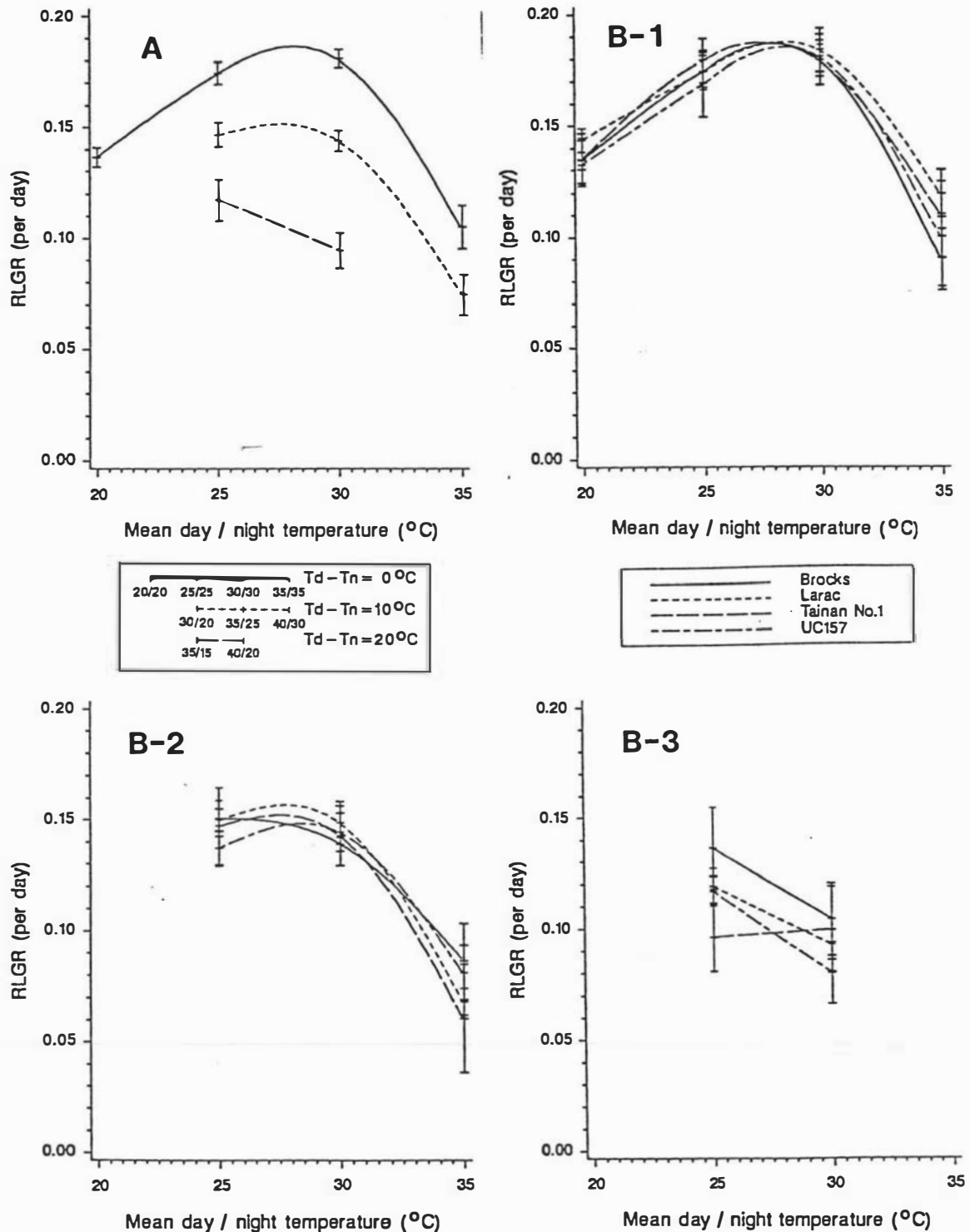


Fig. 2.3. The comparison of the RLGRs between plants

A : at various temperature regimes (means of four cultivars).

B-1 : of cultivars at the constant day/night temperatures.

B-2 : of cultivars at the 10°C day/night differential.

B-3 : of cultivars at the 20°C day/night differential.

(Vertical lines indicate standard errors of the means)

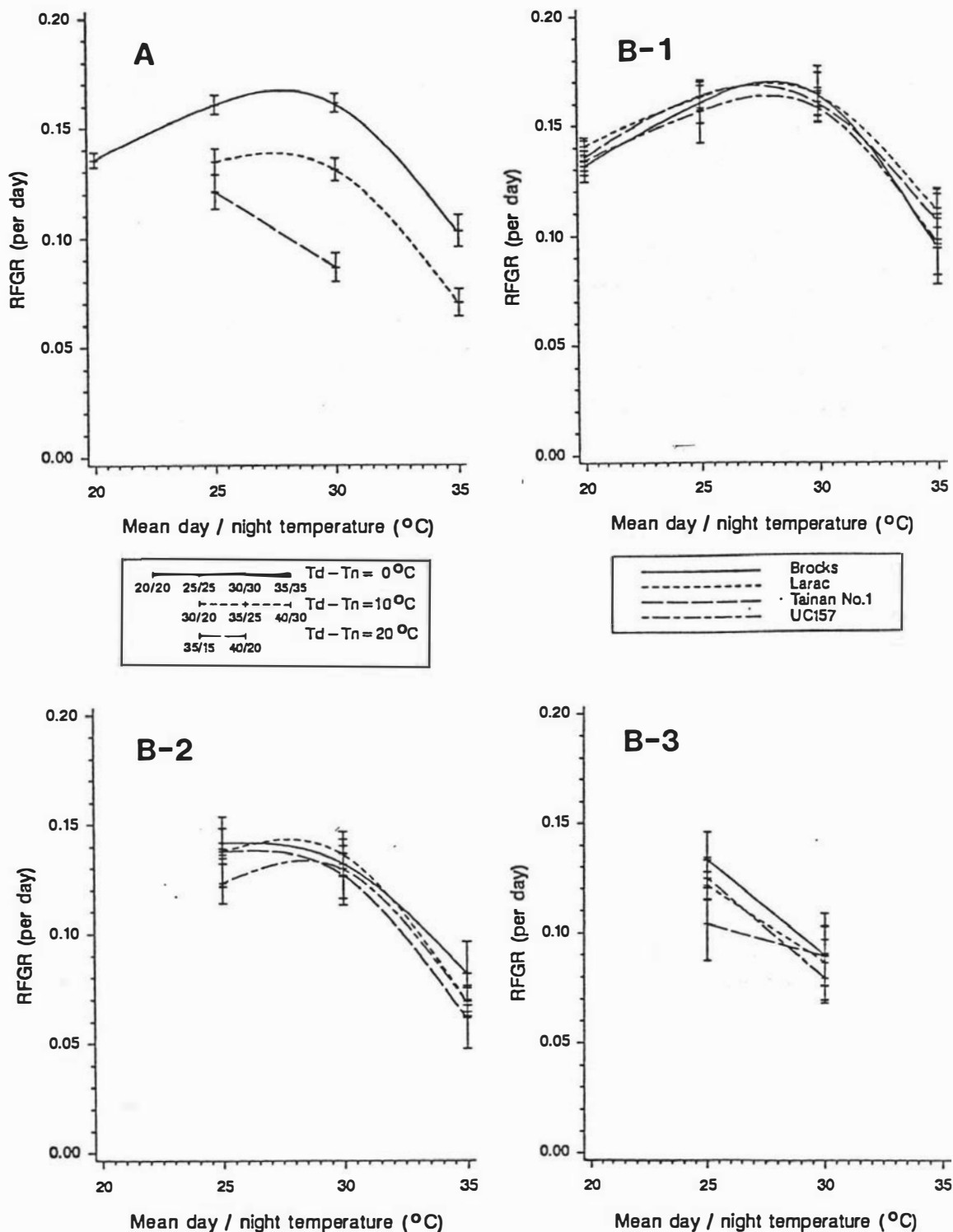


Fig. 2.4. The comparison of the RFGRs between plants

- A : at various temperature regimes (means of four cultivars).
- B-1 : of cultivars at the constant day/night temperatures.
- B-2 : of cultivars at the 10°C day/night differential.
- B-3 : of cultivars at the 20°C day/night differential.

(Vertical lines indicate standard errors of the means)

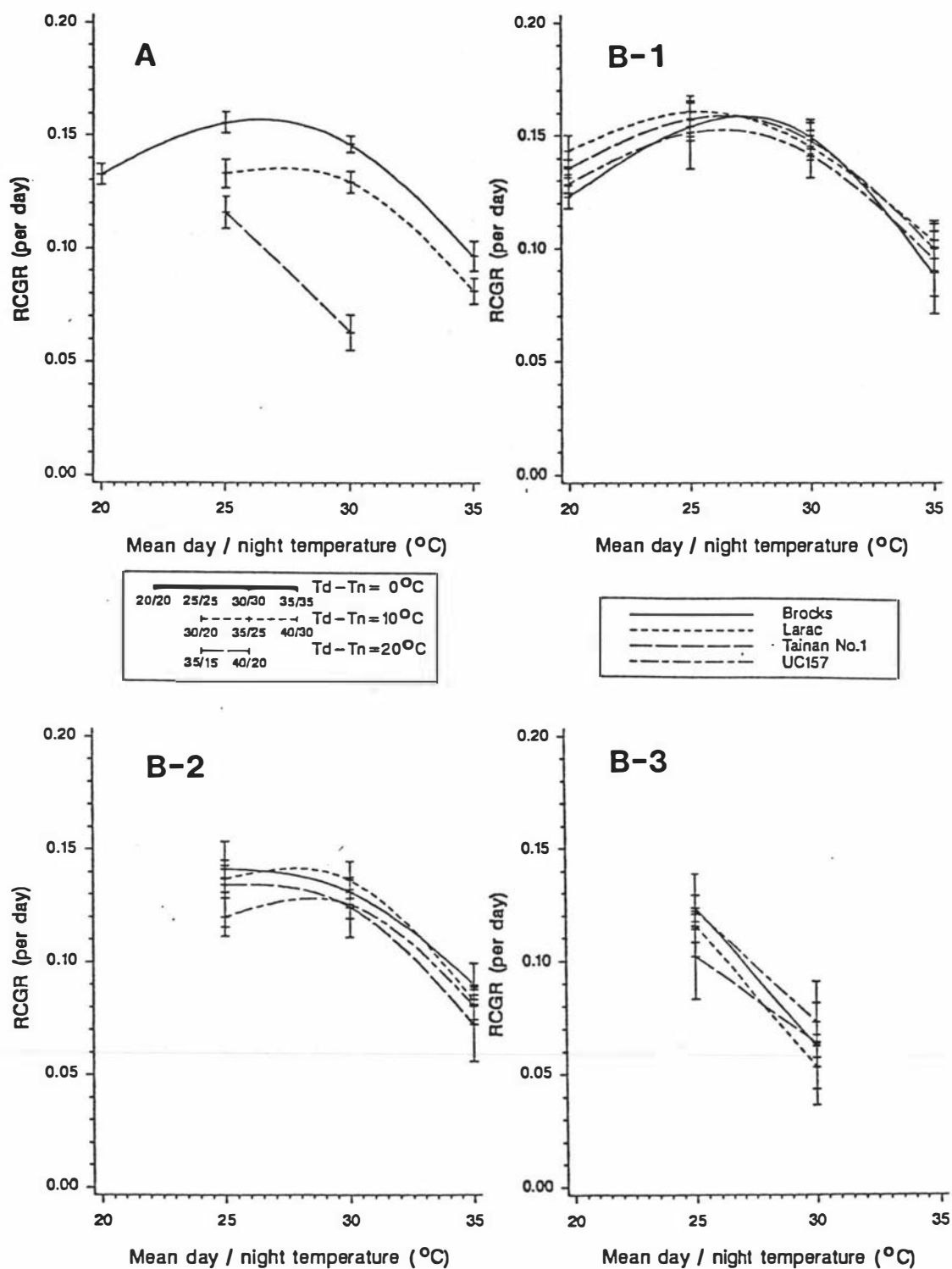


Fig. 2.5. The comparison of the RCGRs between plants

- A : at various temperature regimes (means of four cultivars).
- B-1 : of cultivars at the constant day/night temperatures.
- B-2 : of cultivars at the 10°C day/night differential.
- B-3 : of cultivars at the 20°C day/night differential.

(Vertical lines indicate standard errors of the means)

D40/N30°C). At D40/N30°C the plants had better growth than at D40/N20°C and D35/N25°C was better than D35/N15°C.

2.3.2.3. Comparison of Relative Total Plant Growth Rates

The total plant dry weight is the sum of all the plant components, hence RPGR represents the response of the whole plant to temperature (Fig. 2.2A). RPGR had a peak at between 25°C and 30°C constant temperature. Generally there were no significant differences between RPGRs among cultivars except that at D20/N20°C Larac was significantly higher than Brocks and UC157. At D30/N20°C UC157 was significantly lower than Brocks, and at D35/N15°C Tainan No.1 was significantly lower than both Brocks and UC157 (Fig. 2.5B-1, 2.5B-2, 2.5B-3). Brocks had a smaller variation in RPGR at D35/N35°C, D40/N20°C and D40/N30°C than the other cultivars.

2.3.2.4. Comparison of Relative Leaf Growth Rates

The response of RLGR (overall and individual cultivars) to a number of temperature regimes is represented in Fig. 2.3. This shows that D30/N30°C and D25/N25°C were optimum temperatures, that D20/N20°C, D30/N20°C, D35/N15°C and D35/N25°C were less suitable temperature, and that at D35/N35°C, D40/N20°C and D40/N30°C the RLGRs were very low; while at D35/N15°C Brocks had a near normal RLGR. Low night temperature had a positive effect on RLGR, but too low a night temperature was counter productive.

At optimum temperatures the RLGR differences among cultivars were small. e.g. at D20/N20°C, D25/N25°C, D30/N30°C, D30/N20°C and D35/N25°C the RLGRs for cultivars were similar. At D35/N35°C, D40/N30°C, D35/N15°C and D40/N20°C, however, the differences in RLGRs among cultivars were larger.

2.3.2.5. Comparison of Relative Fern Growth Rates

The effects of temperature on RFGR were similar to RLGR and RPGR. D30/N30°C and D25/N25°C were optimum temperatures, D20/N20°C, D30/N20°C, D35/N15°C and D35/N25°C were less suitable temperatures, and at D35/N35°C, D40/N20°C and D40/N30°C the RFGRs declined dramatically (Fig. 2.4A). Generally, at D20/N20°C, D25/N25°C, D30/N30°C, D30/N20°C and D35/N25°C the RFGRs were not significantly different among cultivars (Fig. 2.4B-1, 2.4B-2). At D35/N15°C Tainan No.1 RFGR was significantly lower than that for Brocks (Fig. 2.3B-3). At the supra-optimal regimes (35/35°C, 40/20°C and 40/30°C) RFGR was not different among cultivars except that at D40/N30°C RFGR for Tainan No.1 was significantly lower than for Brocks.

2.3.2.6. Comparison of Relative Crown Growth Rates

The optimum temperature for RCGR was slightly lower than for RLGR, RFGR and RPGR (Fig. 2.5A). At D25/N25°C the RCGR was higher than at D30/N30°C. In addition, at D20/N20°C, D30/N20°C and D35/N25°C the RCGRs were not significantly different, but lower compared to rates at D25/N25°C and D30/N30°C. At D35/N25°C (high day temperature and suitable night temperature) the RCGR was higher than at D35/N35°C (high constant regime), D40/N20°C and D40/N30°C (high day temperature). Thus temperatures above 35°C were too warm and below 15°C was too cool for good crown growth.

At D20/N20°C the RCGRs showed a marked variation among cultivars, e.g. Larac (0.143 per day) was higher than Brocks (0.125 per day) and UC157 (0.129 per day) (Fig. 2.5B-1, 2.5B-2, 2.5B-3). Moreover, at D30/N30°C the RCGRs of Brocks, Tainan No.1 and UC157 were not significantly different from at D25/N25°C, but Larac was significantly lower. At D20/N20°C, D30/N20°C and D35/N25°C there was no significant difference between RCGRs of individual cultivar except that at D20/N20°C they were significantly lower than at D30/N20°C for Brocks. At D35/N15°C, D35/N25°C and D35/N35°C the

RCGR of Tainan No.1 were not significantly different, but Brocks, Larac and UC157 were significantly different. Generally at D35/N25°C the RCGR was always higher than that at D35/N15°C and D35/N35°C.

2.3.3. Comparison of Net Assimilation Rates

The NARs varied with temperature, plant age and cultivar (Fig. 2.6, 2.7). The effect of temperature on NAR appeared more extensive than the effect of cultivar. Generally, NARs decreased markedly with increasing age except that at D20/N20°C, D35/N35°C, D35/N15°C and D40/N30°C the NARs changed little throughout the experimental period. At D30/N30°C and at D25/N25°C NAR was the highest at the first harvest and then declined with increasing age to become the lowest at the final harvest. Generally at optimum temperatures (D25/N25°C, D30/N20°C, D30/N30°C, D35/N25°C) the NARs were high at the early stages and declined with increasing age. At D35/N35°C the NAR was high at the early stage and declined slowly, and hence had the highest NAR at the later stage. At D20/N20°C and D35/N15°C the plants had low NARs at the early stage and maintained stable NARs with increasing age, and therefore had high NARs at the later stage.

The NARs among cultivars were similar except at the stress temperature regimes (D35/N15°C, D35/N35°C, D40/N20°C and D40/N30°C) (Fig. 2.7). At D35/N35°C Brocks had very low NAR at the early stage which increased with increasing age while the NARs for the other cultivars fell with increasing age. At D40/N20°C Brocks had a very high NAR at the early stage but this dramatically declined with increasing age. NAR for Tainan No.1 and Larac also fell with age. At D40/N30°C the NAR of Larac increased with increasing age, while the NARs for the other cultivars decline.

2.3.4. Analysis of Leaf Area Ratios

The LARs also varied with temperature, cultivar and age. The effect of

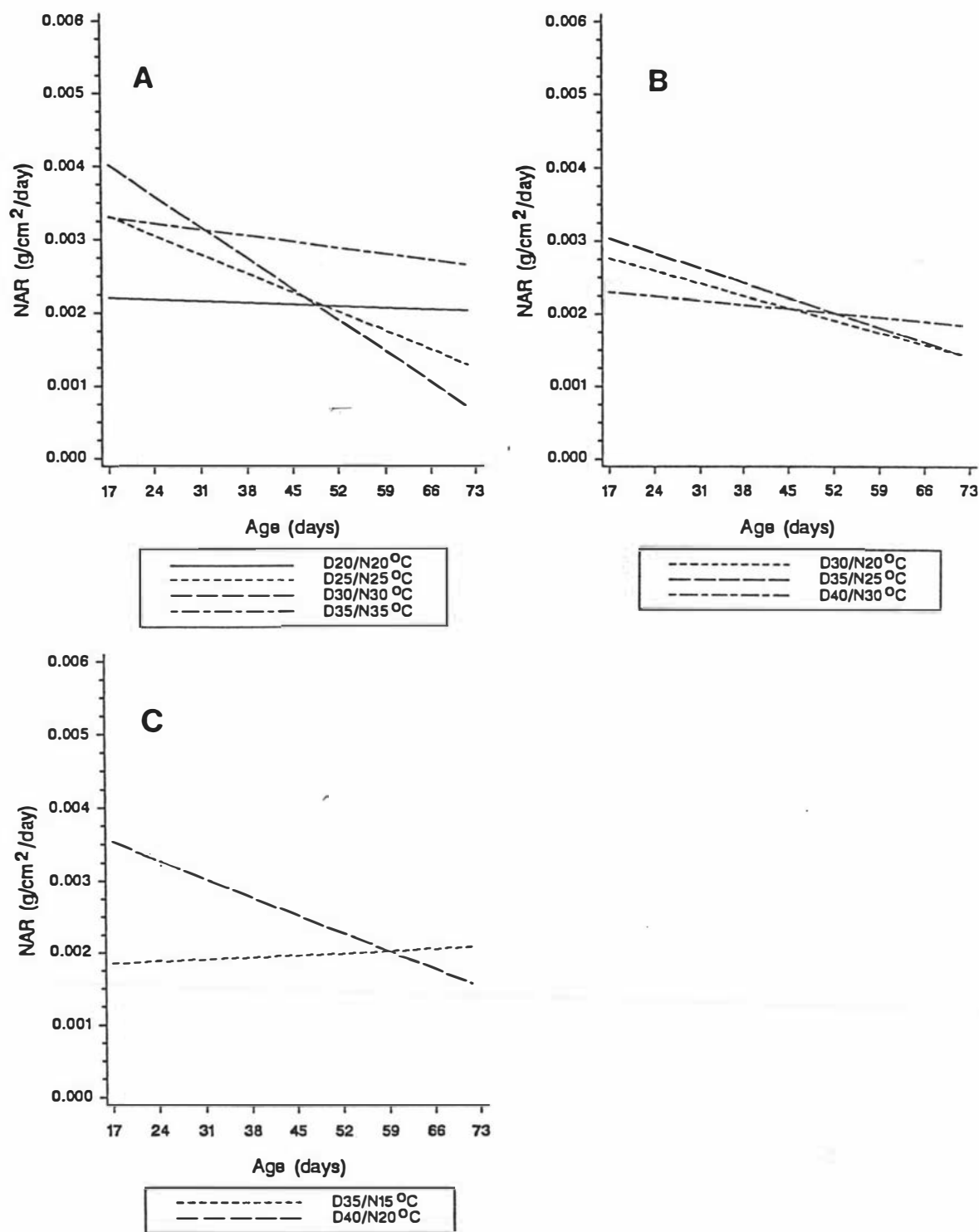


Fig. 2.6. The comparison of the NARs (means of four cultivars)
A : at the constant day/night temperatures.
B : at the 10°C day/night differential.
C : at the 20°C day/night differential.

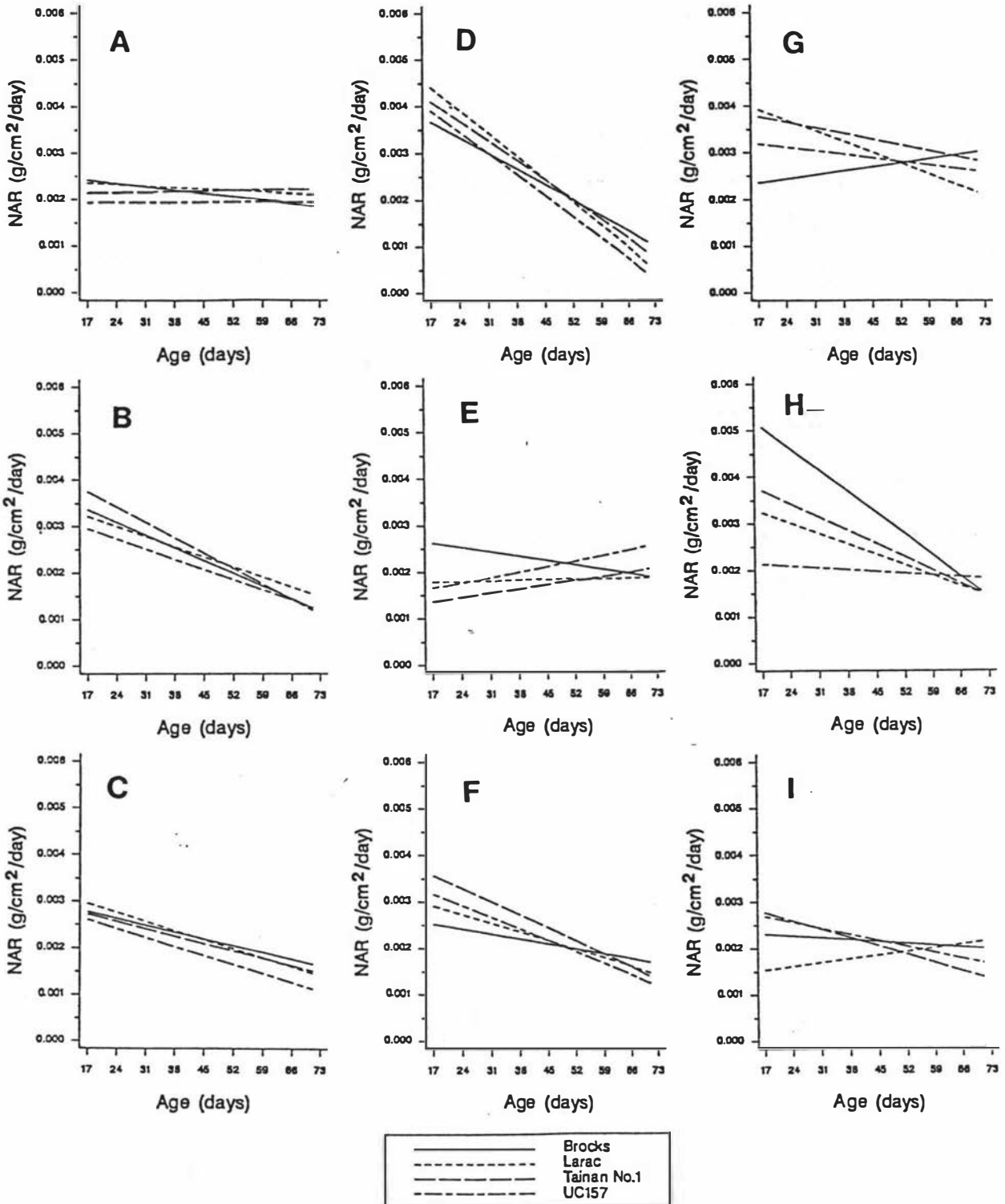


Fig. 2.7. The comparison of the NARs between cultivars at

A : D20/N20°C.	D : D30/N30°C.	G : D35/N35°C.
B : D25/N25°C.	E : D35/N15°C.	H : D40/N20°C.
C : D30/N20°C.	F : D35/N25°C.	I : D40/N30°C.

temperature was more extensive than for cultivar. Generally, LARs could be classed into 4 groups (Figs. 2.8). The first group of D30/N30°C and D25/N25°C the plants had the highest mean LAR, increasing LARs, and the best growth; the second group of D30/N20°C and D35/N25°C the plants had the second highest mean LAR, increasing LARs, and good growth; the third group of D20/N20°C and D35/N15°C the plants had nearly stable LAR ($50 - 70 \text{ cm}^2\text{g}^{-1}$), normal growth; the last group of D35/N35°C, D40/N20°C and D40/N30°C the plants had low mean LAR, very poor growth, except that at D40/N20°C the LAR increased with age from a very low initial level. In general, high LAR plants had high RGRs, while plants with low LARs had low RGRs.

LARs showed considerable variation among temperatures and a much smaller variation among cultivars (Fig. 2.9). The LARs among cultivars at each temperature were similar except at a few temperature regimes, for example, at D30/N30°C UC157 had the highest LAR at the later stages. At any specific individual temperature regime the difference in LAR between cultivars did not related to RGR's differences.

2.3.5. Analysis of Allometry of Crowns in Relation to Ferns

The relationship between crown weight and fern weight can be analyzed using the linear allometric function (*Hunt* 1990). This showed that the natural logarithm of crown weight against the natural logarithm of fern weight of plants at various temperatures was highly correlated ($0.99 > r^2 > 0.95$), except at D40/N20°C ($r^2=0.88$) (Fig. 2.10). At a very early growth stage (fern dry weight $< 0.01 \text{ g}$) the crown weight lagged behind fern weight, but after this stage the relationship between crown weight and fern weight appeared to follow a linear function (Fig. 2.10H). The slope of the linear allometric function is the allometric coefficient. The allometric intercept represents the crown weight when the fern weight is 1 gram.

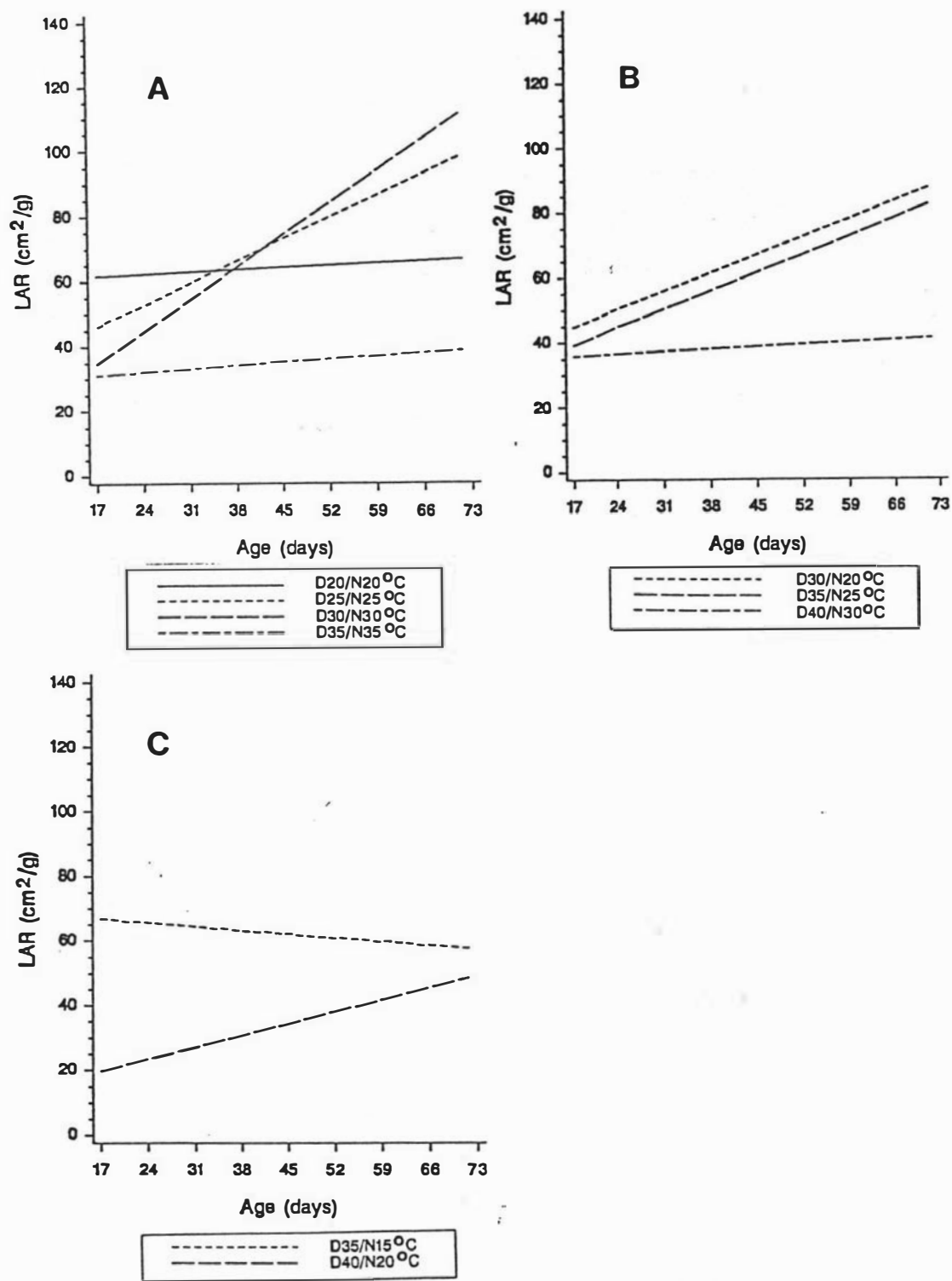


Fig. 2.8. The comparison of the LARs (means of four cultivars)

A : at the constant day/night temperatures.

B : at the 10°C day/night differential.

C : at the 20°C day/night differential.

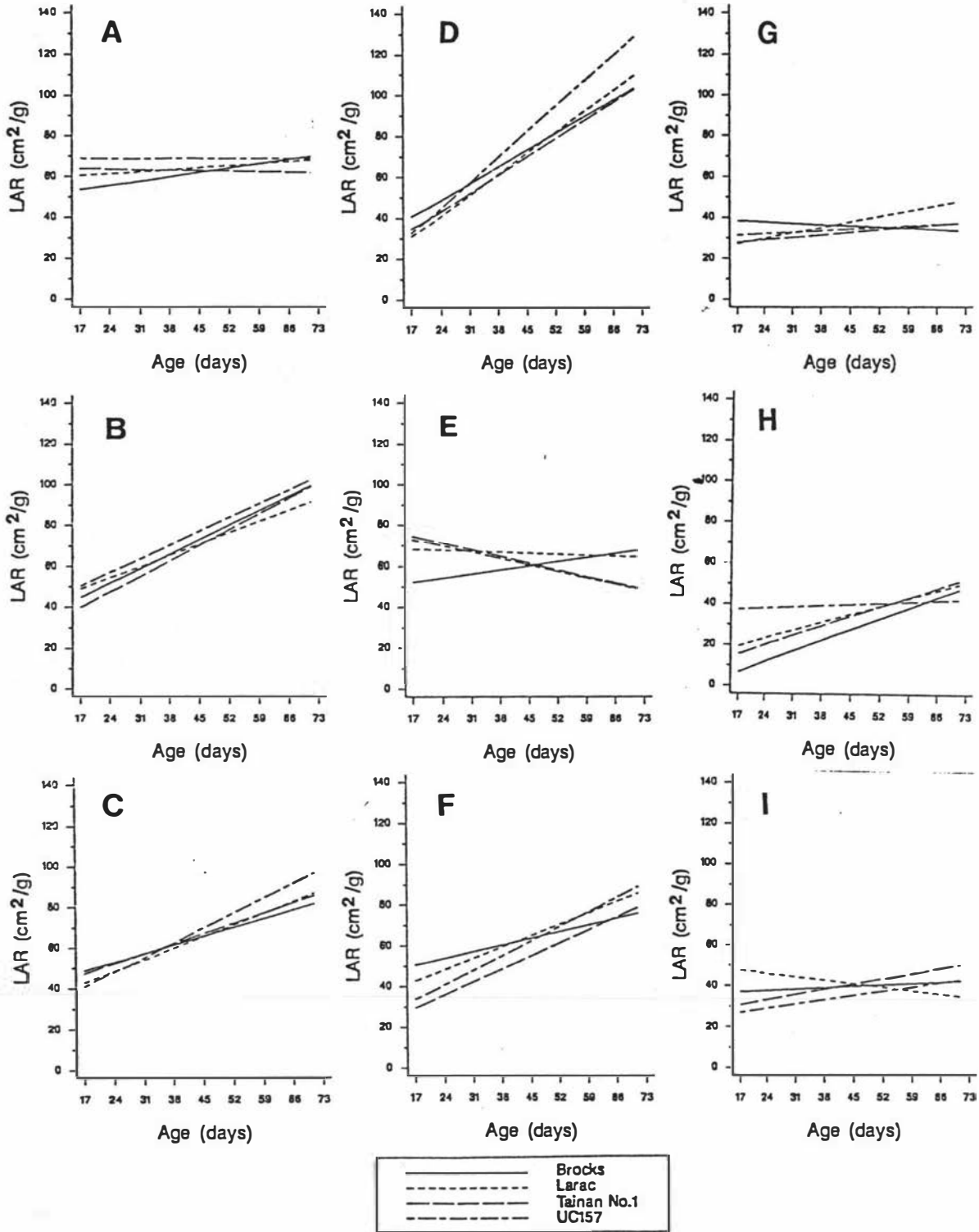


Fig. 2.9. The comparison of the LARs among cultivars at

A : D20/N20°C.	D : D30/N30°C.	G : D35/N35°C.
B : D25/N25°C.	E : D35/N15°C.	H : D40/N20°C.
C : D30/N20°C.	F : D35/N25°C.	I : D40/N30°C.

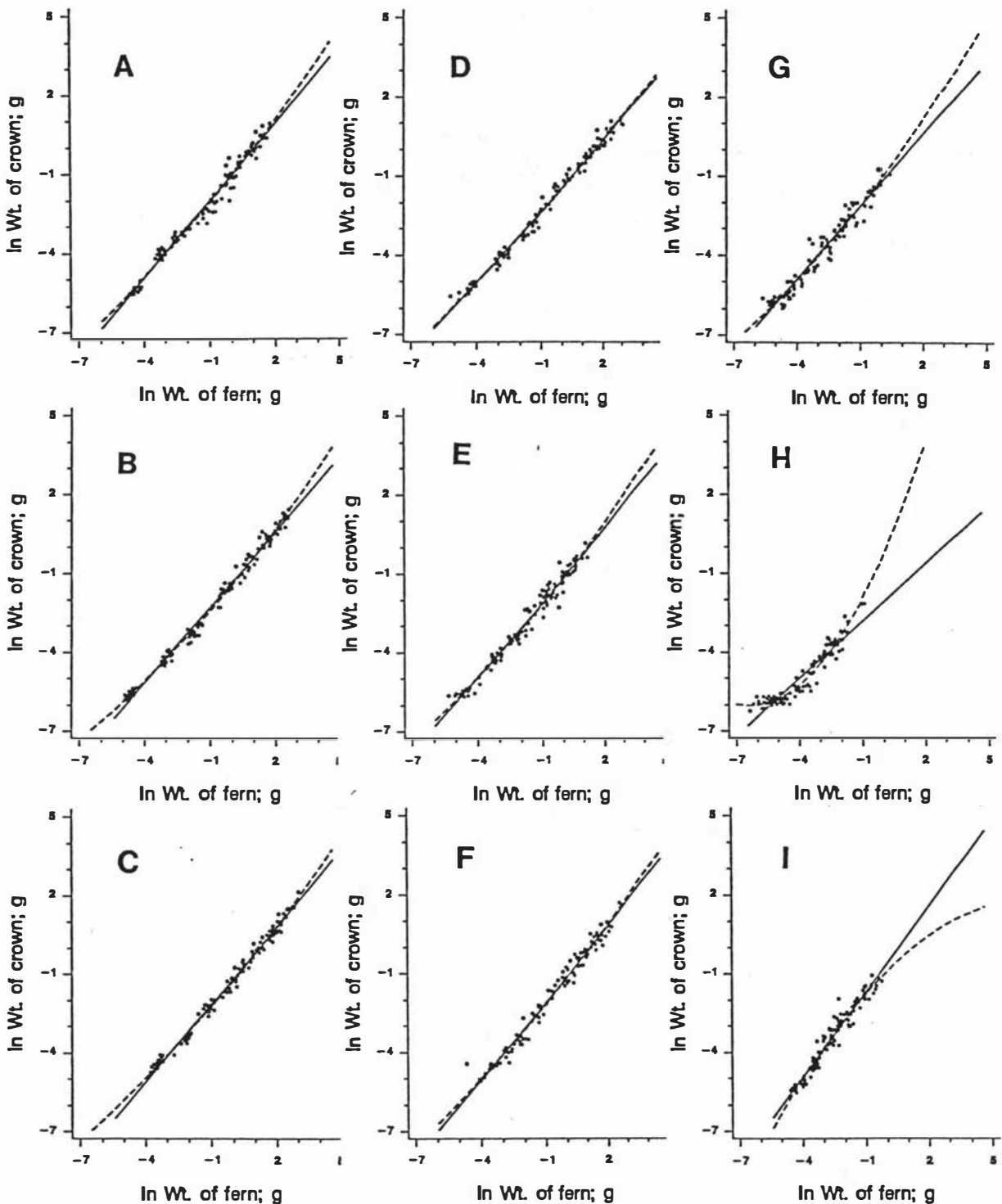


Fig. 2.10. The plot of natural logarithm crown weight against natural logarithm fern weight fitted to linear regression (solid line) and secondary order polynomial function (dotted line) at (mean of four cultivars):

A : D20/N20°C.	D : D30/N30°C.	G : D35/N35°C.
B : D25/N25°C.	E : D35/N15°C.	H : D40/N20°C.
C : D30/N20°C.	F : D35/N25°C.	I : D40/N30°C.

2.3.5.1. Comparison of Allometric Coefficients between Temperatures and Cultivars

The allometric coefficient is an index of development of the organism. When the allometric coefficient of the crown in relation to the fem is higher than 1 the plant is "rooty"-has a high proportion of root, and less than 1 is "ferny"-has a high proportion of fern (Hunt 1990). Therefore, the growth of asparagus appeared "ferny" at most temperature regimes due to the allometric coefficient being less than 1 (Table 2.2). Generally at D20/N20°C, D25/N25°C, D30/N20°C, D35/N15°C and D35/N25°C the plants had an allometric coefficient close to unity, while at D30/N30°C the plants produced the lowest allometric

Table 2.2. Comparing the effects of temperature on allometric coefficients of asparagus.

Temperature	Brocks	Larac	Tainan No.1	UC157	Mean
D20/N20°C ¹	0.930 ^a	1.004 ^a	0.992 ^a	0.950 ^a	0.969 ^{ab}
D25/N25°C	0.954 ^a	0.975 ^a	0.954 ^a	0.959 ^a	0.960 ^{ab}
D30/N20°C	0.988 ^a	0.990 ^a	0.970 ^a	0.971 ^a	0.980 ^a
D30/N30°C	0.908 ^a	0.882 ^c	0.919 ^a	0.893 ^b	0.900 ^c
D35/N15°C	0.902 ^a	0.945 ^{ab}	0.974 ^a	0.965 ^a	0.946 ^{ab}
D35/N25°C	0.986 ^a	0.990 ^a	0.971 ^a	0.964 ^a	0.978 ^a
D35/N35°C	0.918 ^a	0.906 ^{bc}	0.927 ^a	0.987 ^a	0.934 ^{bc}
D40/N20°C ²	0.672	0.882	0.705	0.875	0.723
D40/N20°C³	0.812	0.904	1.183	1.055	1.044
D40/N30°C ²	1.065	1.020	1.096	1.139	1.100
Mean ⁴	0.941 ^a	0.956 ^a	0.958 ^a	0.956 ^a	

¹ Within a temperature treatment, numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

² The allometric coefficients for D40/N20°C and D40/N30°C are excluded from the analysis of variance.

³ The allometric coefficients (in bold) indicate that fern weight less than 0.018 g was excluded from the allometric analysis.

⁴ The mean of allometric coefficients over all temperature regimes excluding both D40/N20°C and D40/N30°C. Within cultivars (row), numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

coefficient (0.9). At D40/N30°C and D40/N20°C the plants had unusual allometric coefficients (the former had the highest coefficient (1.1) and the latter had the lowest coefficient (0.7)). Therefore these coefficients were excluded when the allometric coefficients were compared.

The effects of temperature (except D40/N20°C and D40/N30°C) on the allometric coefficient for each cultivar were almost constant except that at D30/N30°C (Larac and UC157), and D35/N35°C (Larac). If it is assumed that a small establishment period is required before the allometric coefficient becomes stable, then the coefficient for D40/N20°C in excess of log weight of fern -4.0, becomes similar to the other coefficients. The mean of allometric coefficients at all temperature regimes among cultivars were not significantly different.

2.3.5.2. Comparison of Allometric Intercepts between Temperatures and Cultivars

The analysis of variance on the allometric intercept was different from that found for the allometric coefficient, as some allometric intercepts were significantly different among cultivars and among various temperature regimes. At D40/N20°C the plants produced the lowest allometric intercept and allometric coefficient, while at D40/N30°C the plant produced the highest coefficient and intercept (Table 2.2, 2.3). If the allometric analysis excludes fern weights less than 0.018 g for the D40/N20°C treatment, the adjusted allometric coefficient is similar to D40/N30°C, and the allometric intercept is normal in magnitude. At D25/N25°C and D30/N30°C all cultivars had small allometric intercepts while at D20/N20°C cultivars had large allometric intercepts. Therefore the response to temperature of the allometric intercepts is different from the allometric coefficients. The correlational analysis of both parameters (except at D40/N20°C and at D40/N30°C) found that the intercept was just positively correlated with the coefficient ($r^2=0.44$, $p<0.001$).

Table 2.3. Comparing the effects of temperature on allometric intercepts of asparagus.

Temperature	Brocks	Larac	Tainan No.1	UC157	Mean
D20/N20°C ¹	0.282 ^{ab}	0.360 ^a	0.379 ^a	0.322 ^a	0.336 ^a
D25/N25°C	0.250 ^{ab}	0.279 ^b	0.278 ^{ab}	0.279 ^b	0.217 ^{bc}
D30/N20°C	0.274 ^{ab}	0.300 ^b	0.310 ^{ab}	0.314 ^a	0.299 ^{ab}
D30/N30°C	0.219 ^b	0.239 ^c	0.256 ^b	0.241 ^c	0.239 ^c
D35/N15°C	0.259 ^{ab}	0.276 ^b	0.370 ^a	0.330 ^{ab}	0.309 ^a
D35/N25°C	0.288 ^{ab}	0.296 ^b	0.331 ^{ab}	0.331 ^{ab}	0.311 ^a
D35/N35°C	0.314 ^a	0.268 ^{bc}	0.336 ^{ab}	0.337 ^a	0.314 ^a
D40/N20°C ²	0.101	0.080	0.151	0.213	0.136
D40/N20°C³	0.157	0.166	0.393	0.330	0.284
D40/N30°C ²	0.524	0.584	0.564	0.637	0.577
Mean ⁴	0.269 ^c	0.288 ^{bc}	0.323 ^a	0.308 ^{ab}	

¹ Within a temperature treatment, numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

² The allometric intercepts for D40/N20°C and D40/N30°C are excluded from the analysis of variance.

³ The allometric intercepts (in bold) indicate that fern weight less than 0.018 g was excluded from the allometric analysis.

⁴ The mean of allometric intercepts over all temperature regimes excluding both D40/N20°C and D40/N30°C. Within cultivars (row), numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

2.3.5.3. Analysis of Variances of the Influences of Temperature and Cultivar on Allometric Intercepts

If it is assumed that the allometric coefficient is constant (0.939), then the corrected allometric intercepts were significantly influenced at the 5% level by day temperature, the interaction between day and night temperature; and cultivar (Appendix 8A). Moreover, for each cultivar the day temperature and the interaction between day and night temperature had significant effects on allometric intercepts, while night temperature had no significant effect except on UC157 at 2.3% level (Appendix 8B). Generally it might be concluded that night temperatures had no significant effect on the allometric intercepts.

2.3.5.4. Comparison of the Corrected Allometric Intercepts between Temperatures and Cultivars

The comparison of the corrected allometric intercepts at the various temperature regimes (derived by using an allometric coefficient of 0.939 excluding D40/N20°C and D40/N30°C) (Table 2.5) or the corrected allometric intercepts (derived by using the overall allometric coefficients 0.932) (Table 2.4), showed that at the 10°C and 20°C day/night temperature differential plants produced higher intercepts than at constant temperature when they had the same mean temperatures (Fig. 2.11A). Generally Brocks had lower intercepts compared to other cultivars at all regimes except at D35/N15°C, D35/N35°C and D40/N20°C, while at most temperature regimes Tainan No.1 and UC157 produced high intercepts (Fig. 2.11B-1, 2.11B-2, 2.11B-3). Moreover, at D20/N20°C Larac and Tainan No.1 produced high intercepts (Table 2.4, 2.5). The mean of allometric intercepts over all temperature showed that Tainan No.1 was significantly higher than Brocks.

Table 2.4. Comparing the effects of temperature on predicted allometric intercepts of asparagus, when they are assumed to have same allometric coefficient (0.932).

Temperature	Brocks	Larac	Tainan No.1	UC157	Mean
D20/N20°C ¹	0.287 ^{bcd}	0.345 ^{ab}	0.359 ^a	0.321 ^{bc}	0.328 ^b
D25/N25°C	0.249 ^{de}	0.278 ^c	0.279 ^{cd}	0.278 ^{cde}	0.271 ^{de}
D30/N20°C	0.277 ^{cde}	0.301 ^{bc}	0.312 ^{abcd}	0.317 ^{bcd}	0.302 ^{bc}
D30/N30°C	0.227 ^e	0.255 ^c	0.266 ^d	0.248 ^e	0.249 ^e
D35/N15°C	0.288 ^{bcd}	0.276 ^c	0.347 ^{ab}	0.321 ^{bc}	0.308 ^{bc}
D35/N25°C	0.282 ^{bcd}	0.291 ^{bc}	0.329 ^{abcd}	0.346 ^b	0.312 ^{bc}
D35/N35°C	0.340 ^{ab}	0.299 ^{bc}	0.342 ^{abc}	0.291 ^{cde}	0.318 ^{bc}
D40/N20°C	0.322 ^{abc}	0.283 ^{bc}	0.292 ^{bcd}	0.267 ^{de}	0.291 ^{cd}
D40/N20°C²	0.219	0.179	0.198	0.238	0.208
D40/N30°C	0.371 ^a	0.395 ^a	0.366 ^a	0.402 ^a	0.384 ^a
Mean ³	0.294 ^b	0.303 ^{ab}	0.321 ^a	0.310 ^{ab}	

¹ Within a temperature treatment, numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

² The allometric intercepts (in bold) indicate that fern weight less than 0.018 g was excluded from the allometric analysis.

³ The mean of allometric intercepts over all temperature regimes. Within cultivars (row), numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

Table 2.5. The effects of temperature on predicted allometric intercepts of asparagus, when they are assumed to have same allometric coefficient (0.939).

Temperature	Brocks	Larac	Tainan No.1	UC157	Mean
D20/N20°C ¹	0.290 ^b	0.347 ^a	0.362 ^a	0.323 ^{ab}	0.331 ^a
D25/N25°C	0.250 ^{bc}	0.279 ^{bc}	0.281 ^{bc}	0.279 ^{bc}	0.272 ^c
D30/N20°C	0.277 ^b	0.301 ^{bc}	0.313 ^{abc}	0.317 ^{ab}	0.302 ^b
D30/N30°C	0.228 ^c	0.257 ^c	0.267 ^c	0.249 ^c	0.250 ^c
D35/N15°C	0.292 ^b	0.279 ^{bc}	0.351 ^a	0.324 ^{ab}	0.312 ^{ab}
D35/N25°C	0.283 ^b	0.292 ^{bc}	0.331 ^{ab}	0.347 ^a	0.313 ^{ab}
D35/N35°C	0.348 ^a	0.305 ^b	0.348 ^a	0.297 ^b	0.324 ^{ab}
Mean ²	0.281 ^c	0.294 ^{bc}	0.322 ^a	0.305 ^{ab}	

¹ Within a temperature treatment, numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

² The mean of allometric intercepts over all temperature regimes excluding both D40/N20°C and D40/N30°C. Within cultivars (row), numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

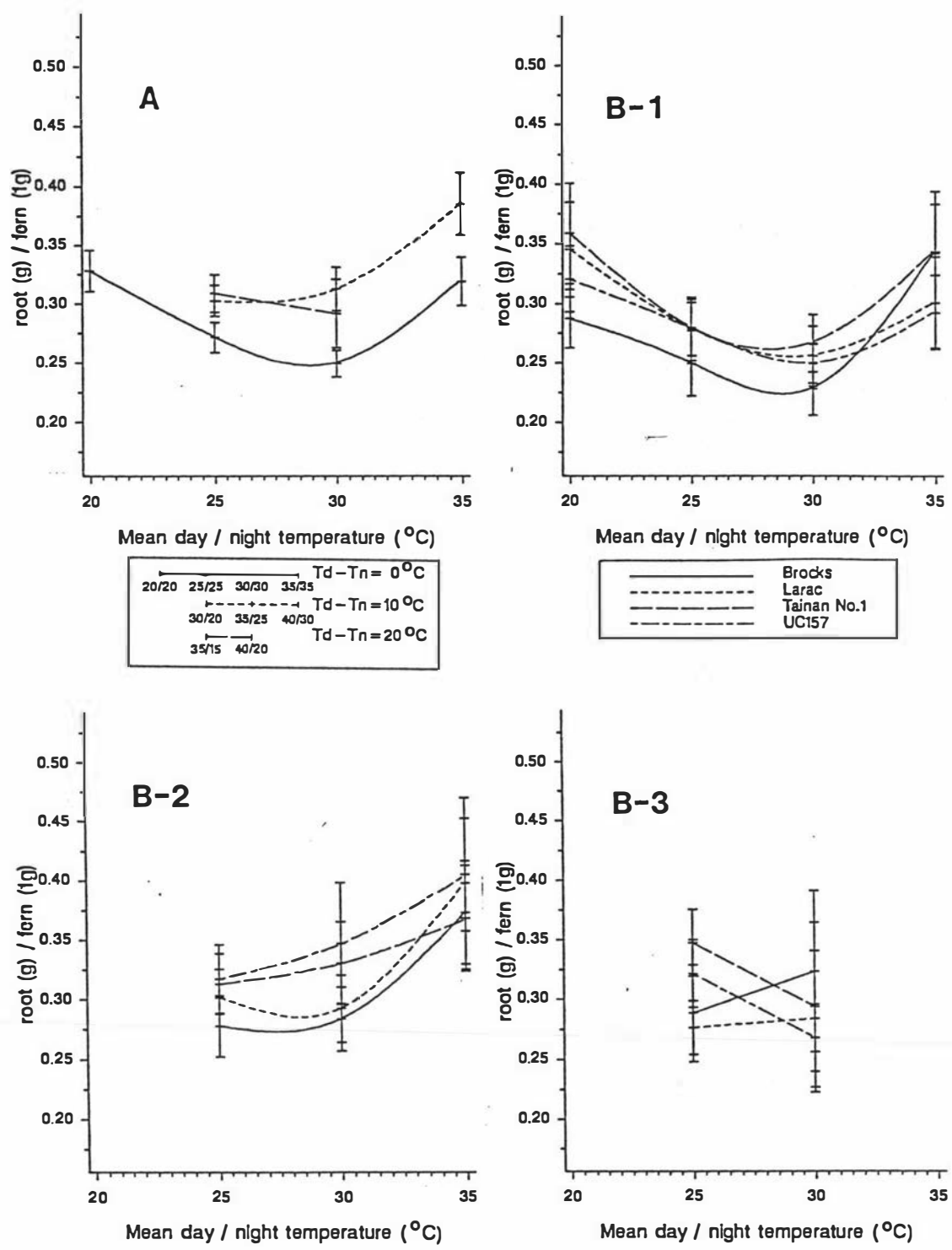


Fig. 2.11. The comparison of the root weights when the fern weight 1 gram between

- A : plants at various temperature regimes (means of four cultivars).
- B-1 : cultivars at the constant day/night temperatures.
- B-2 : cultivars at the 10°C day/night differential.
- B-3 : cultivars at the 20°C day/night differential.

(Vertical lines indicate standard errors of the means)

2.3.6. Analysis of Component Production Rates

2.3.6.1. Interrelationship between Component Production Rates

Relative total plant dry weight growth rate (RPGR) is a sum of the component production rates, so an analysis of component production rates can reveal the extent that individual components contribute to RPGR. In my experiment, the leaf, stem, rhizome and root production rates comprise RPGR. The leaf production rate was the largest contributor throughout the experiment period, root and stem production rates were the second and third largest contributors respectively, and rhizome production rate was the smallest contributor (Fig. 2.12), e.g. the maximal leaf, stem, rhizome and root production rates were 0.12, 0.032, 0.0068, 0.033 per day respectively. In fact, the contributive magnitude of individual component varied with age, with the stem and the rhizome production rates declining with increasing age. Conversely, the leaf production rate increased with increasing age, but the root production rate was maintained nearly constant except at D40/30°C, D30/N30°C and D40/N20°C (Fig. 2.13, 2.14, 2.15, 2.16).

2.3.6.2. Comparison of Component Production Rates between Temperatures

Leaf production rates showed that plants at D30C/N30°C had the highest leaf production rate, followed by D25/N25°C. At D30/N30°C the leaf production rate was similar to D25/N25°C at the early stage, but increased with age (Fig. 2.13). At D20/N20°C, D30/N20°C, D35/N15°C and D35/N25°C the leaf production rates were similar (between 0.081 and 0.089 per day) at the later stage, but had different leaf production rates at the early stage. In general, the contribution of leaf production rates to RPGRs increased with age when the plants were grown at optimum temperature regimes. At D35/N35°C, D40/N20°C and D40/N30°C, however plants had poor leaf growth and low leaf production rates, but the leaf production rates were still the major contributors to RPGR (Fig. 2.12, 2.13).

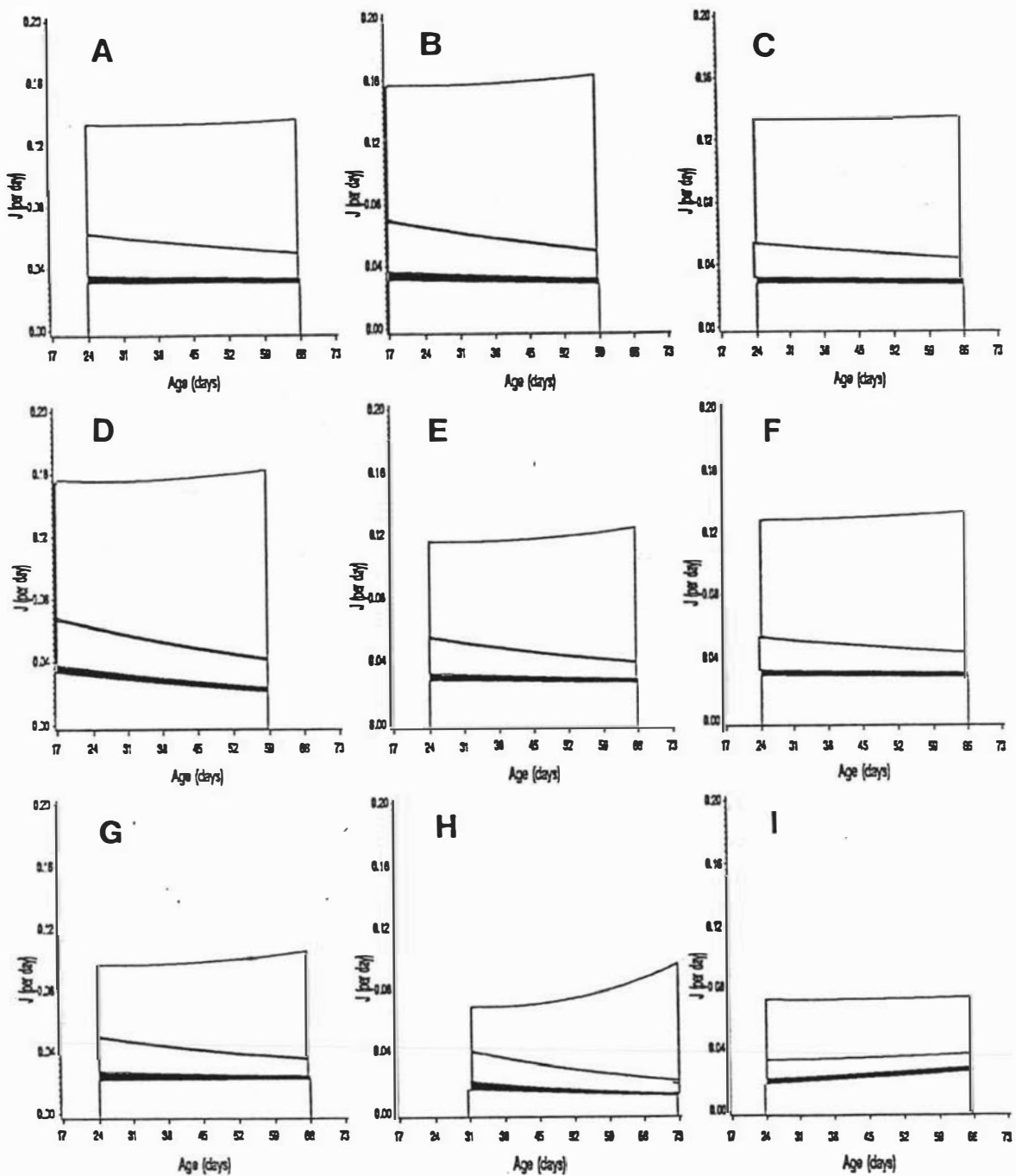


Fig. 2.12. The interrelationship of leaf, stem, rhizome, and root production rates (means of four cultivars)

A : D20/N20°C.	D : D30/N30°C.	G : D35/N35°C.
B : D25/N25°C.	E : D35/N15°C.	H : D40/N20°C.
C : D30/N20°C.	F : D35/N25°C.	I : D40/N30°C.

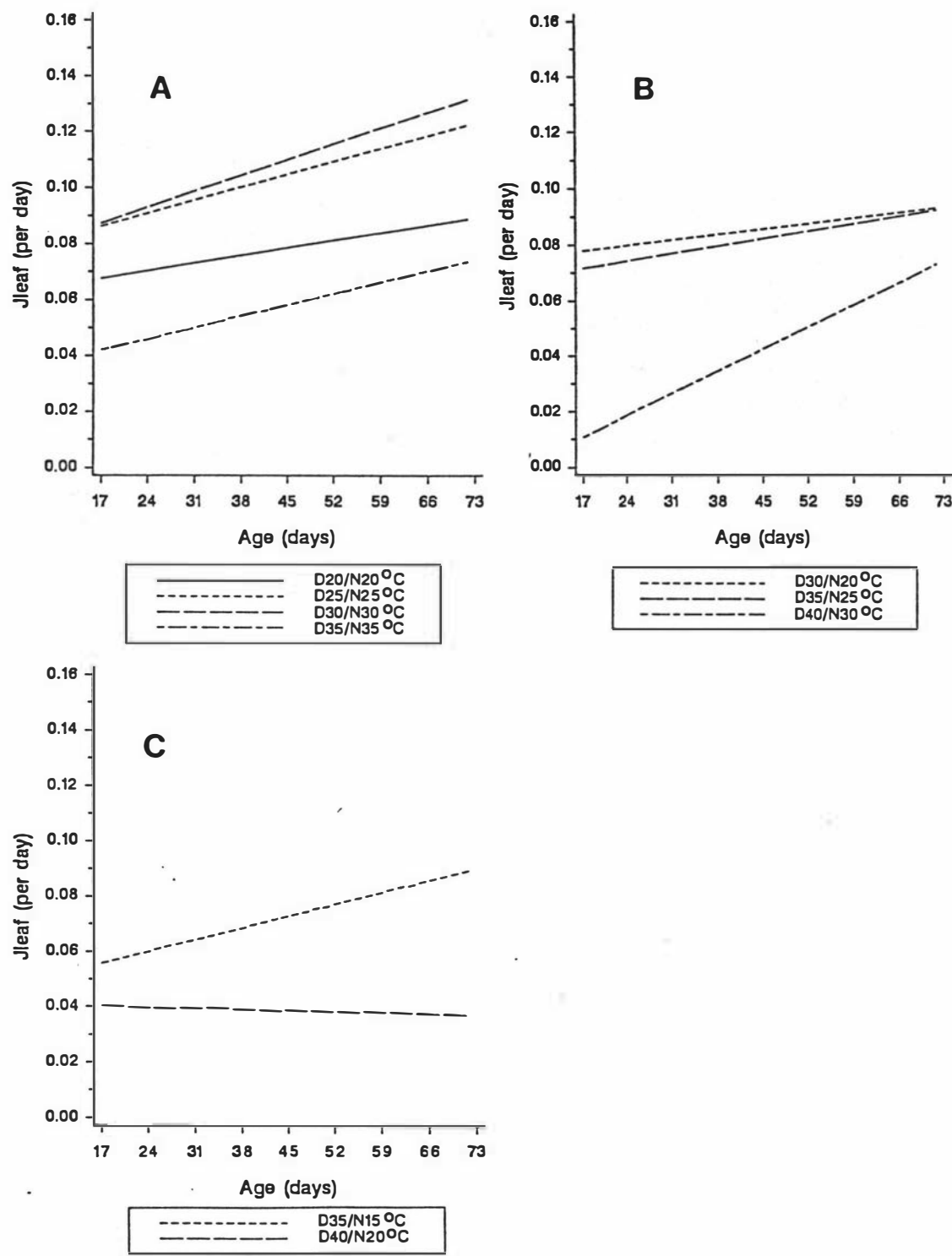


Fig. 2.13. The comparison of the leaf production rates against age (means of four cultivars)

A : at the constant day/night temperatures.

B : at the 10°C day/night differential.

C : at the 20°C day/night differential.

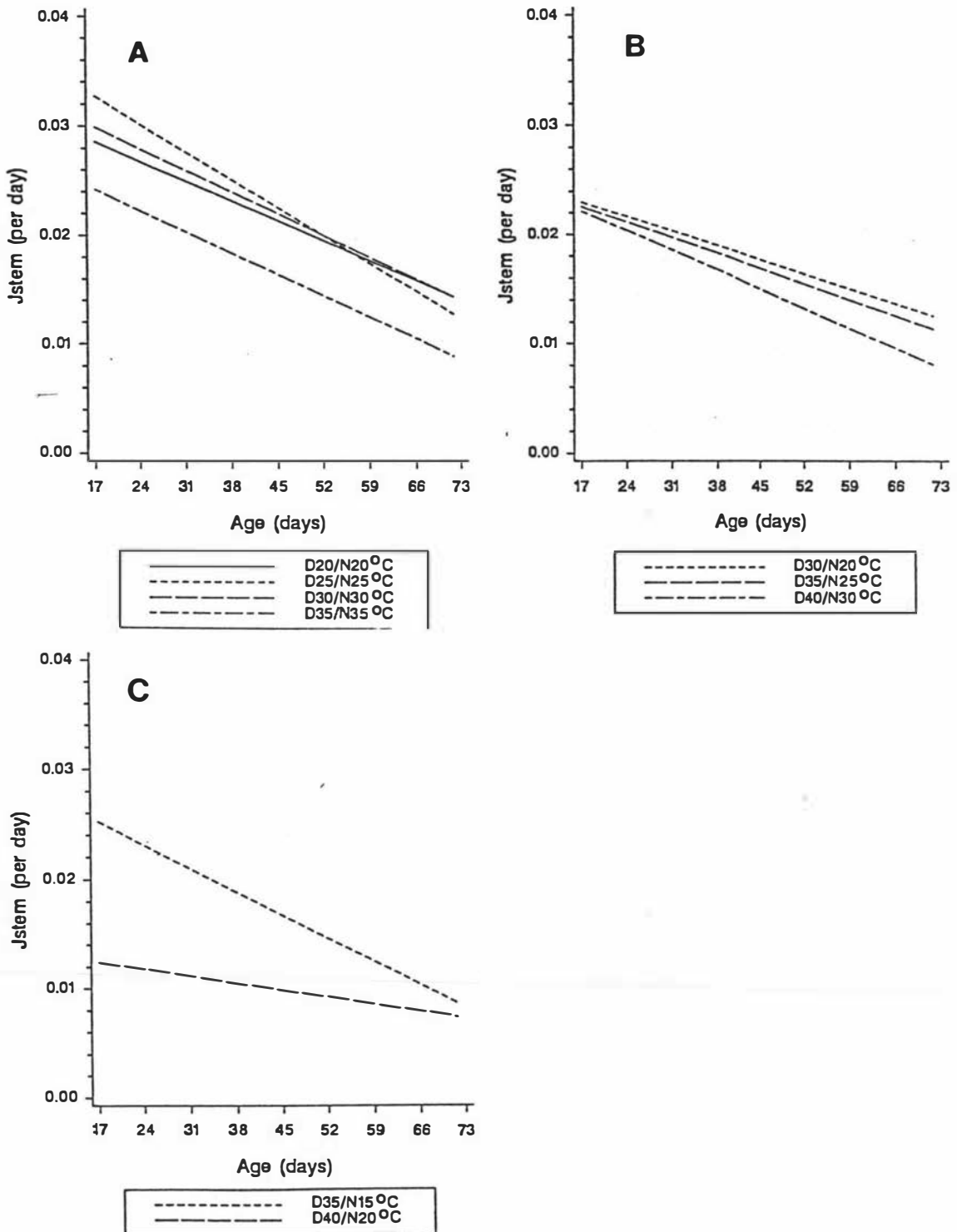


Fig. 2.14. The comparison of the stem production rates against age (means of four cultivars)

A : at the constant day/night temperatures.

B : at the 10°C day/night differential.

C : at the 20°C day/night differential.

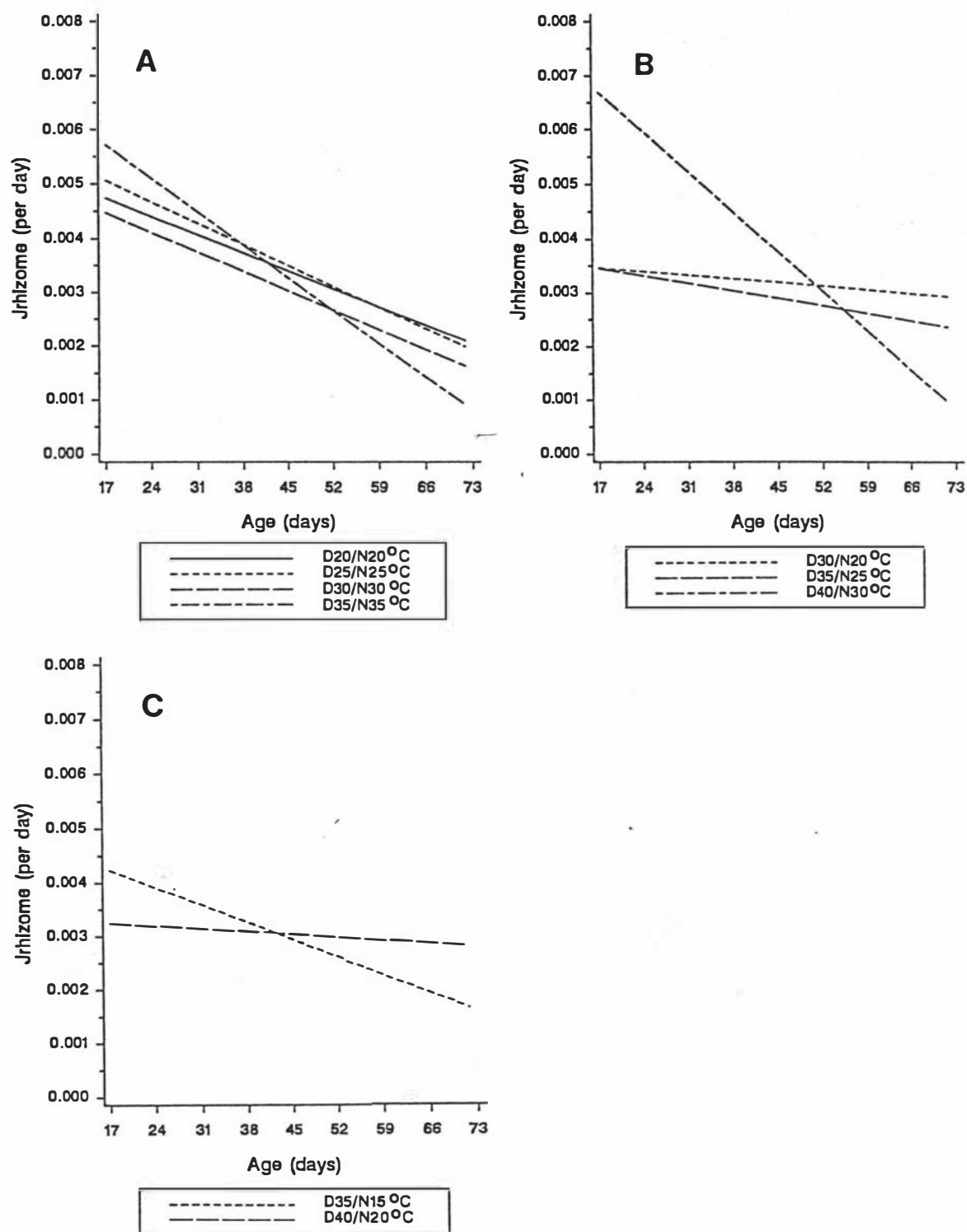


Fig. 2.15. The comparison of the rhizome production rates against age (means of four cultivars)

A : at the constant day/night temperatures.

B : at the 10°C day/night differential.

C : at the 20°C day/night differential.

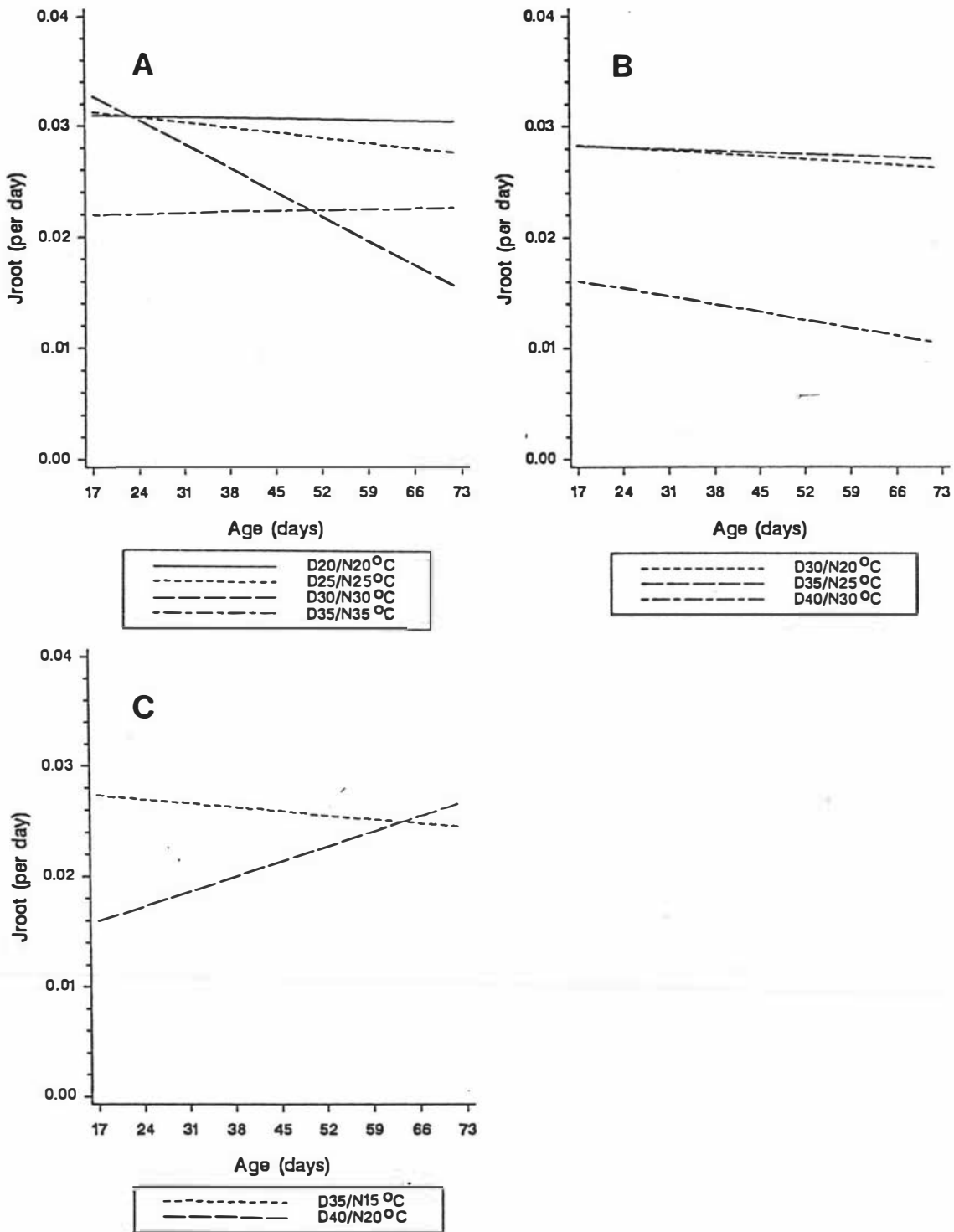


Fig. 2.16. The comparison of the root production rates against age (means of four cultivars)

A : at the constant day/night temperatures.

B : at the 10°C day/night differential.

C : at the 20°C day/night differential.

Generally, the stem production rates declined dramatically with increasing age. They were inversely related to the leaf production rates (Fig. 2.14), except at D40/N20°C which had fairly stable leaf and stem production rates. At D20/N20°C, D25/N25°C and D30/N30°C the plants at the early stage had higher stem production rates compared with rates under other temperature regimes.

The rhizome production rates were similar to the stem production rates, and also declined with increasing age (Fig. 2.15). At D40/N30°C and D35/N35°C rhizome production rates were high at the early stage, but dramatically declined to become the lowest at the later stage. At D20/N20°C, D25/N25°C, D30/N30°C and D35/15°C the rhizome production occurred at an intermediate rate throughout. At D30/N20°C, D35/25°C and D40/N20°C the rhizome production rates were low at the early stage, but declined only slightly with increasing age thus leading to high production rates at the later stages. Generally at high day and night temperature (D35/N35°C or D40/N30°C) the plants had a high rhizome production rate at the early stage, but this declined with increasing age, thus resulting in poor rhizome growth overall.

Plants at most temperature regimes except at D30/N30°C, D40/30°C and D40/N20°C had stable root production rates (Fig. 2.16). At D30/N30°C the plants had the highest root production rate at the early stage which dramatically declined with increasing age. This is associated with the plants being "femy". At D40/N30°C the plants had the lowest root production rate which declined with increasing age, and root growth was poor. On the contrary, at D40/N20°C the plants had low root production rate at the early stage, but this increased with age. At D35/N15°C root production rate decreased with increasing age. At D20/N20°C, D25/N25°C, D30/N20°C and D35/N25°C the root production rates were similar, thus the plants had similar root growth. However, the root production rates were less sensitive to temperature compared to other component production rates, a result consistent with the allometric coefficients which were also insensitive to temperature.

Thus when asparagus is planted at warm climates, root growth maintains more normal growth than the other components.

2.3.7. Prediction of Asparagus Growth

A response surface function was applied to predict the growth indices of RLGR, RFGR, RCGR and RPGR. Although the response to higher night temperatures than day temperatures may be predicted with this function, as my experiment was only carried out with day temperatures higher or equal to night temperatures, the predicted RGRs are limited to a range of day temperatures higher than or equal to the night temperatures.

The contour profiles of RPGR, RLGR and RFGR are similar, but differ considerably for RCGR. The predicted contours of the growth indices vary with temperature (Fig. 2.17), for example, the predicted RLGRs have more contours than other components, which suggests that the RLGR is more temperature sensitive. In contrast, the predicted RCGRs have fewer contours, so the RCGR is less sensitive to temperature compared to other RGRs. Thus the predicted growth response to temperature varies with components. At the temperature regimes of day 35 - 40°C / night 25 - 35°C the predicted RPGR, RLGR and RFGR have great changes of contours. Similarly at day 35 - 40°C / night 15 - 35°C for the predicted RCGR. Therefore all components are particularly sensitive to these regimes.

2.3.7.1. Predicted Growth of Plant Components

The predicted optimal temperatures for RLGR, RFGR, RCGR and RPGR are D26.8/N26.7°C, D26.5/N26.4°C, D26.6/N23.8°C and D26.3/N26.2°C, respectively (Table 2.6). Hence day and night temperature around 26.5°C are optimal for RLGR, RFGR and RPGR, while a lower night temperature of 23.8°C is the optimal temperature for RCGR. This is a reduction of 2.8°C between day and night. The maximum RLGR, RFGR, RCGR and RPGR are

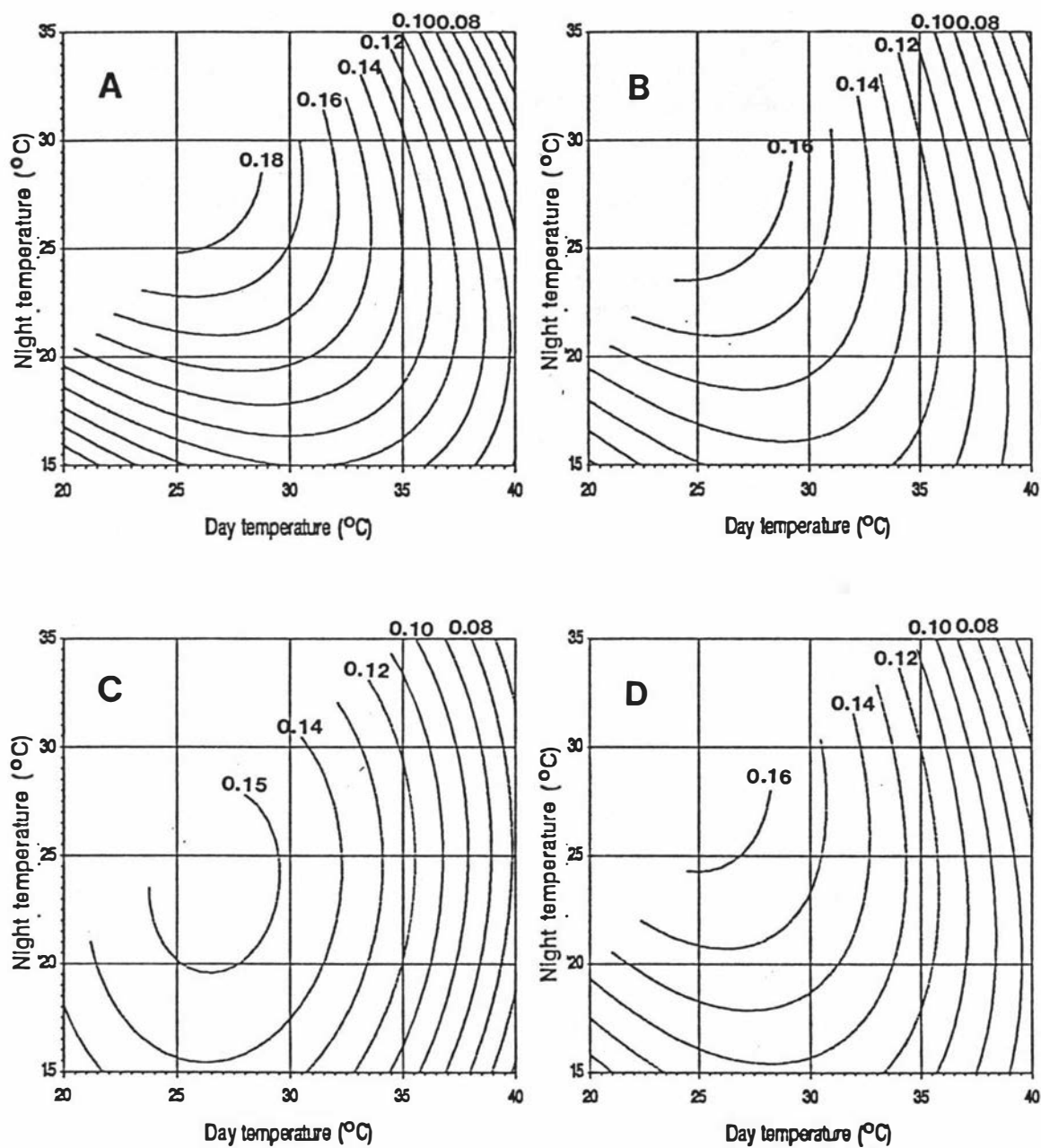


Fig. 2.17. The predicted contours of the relative growth rate (means of four cultivars) of

- A : leaf area (RLGR, per day).
- B : fern dry weight (RFGR, per day).
- C : crown dry weight (RCGR, per day).
- C : total plant dry weight (RPGR, per day).

0.184, 0.166, 0.153 and 0.163 per day respectively. At constant temperature regimes RLGR has the highest and RCGR the lowest growth rates (Fig. 2.18). Although at constant temperature regimes RFGR and RPGR are higher than RCGR, at the day/night temperature differential regimes (5°C, 10°C, 15°C) they have rarely different.

Table 2.6. The predicted optimal temperatures and corresponding RGRs (means of four cultivars).

Plant components	RGR (day ⁻¹)	Day (°C)	Night (°C)	Difference (°C)
Leaf area	0.184	26.8	26.7	0.1
Fern weight	0.166	26.5	26.4	0.1
Crown weight	0.153	26.6	23.8	2.8
Total plant weight	0.163	26.3	26.2	0.1

2.3.7.2. Prediction of Relative Total Plant Growth Rates

Brocks, Larac and UC157 have fewer contours than Tainan No.1 (Fig. 2.19) for RPGR, thus the RPGR of Tainan No.1 is more sensitive to temperature than the others. The maximum RPGR of Brocks, Larac, Tainan No.1 and UC157 are 0.161, 0.166, 0.166 and 0.158 per day at the optimal temperature regimes D26.4/N26.3°C, D26.3/N26.2°C, D26.4/N26.3°C, D26.3/N26.2°C, respectively (Table 2.7). Although the optimal temperatures for all cultivars are very similar, Larac and Tainan No.1 have the highest RPGR's.

The constant and the 5°C day/night differential regimes have higher RPGRs compared to the 10°C and 15°C temperature differential regimes (Fig. 2.20). Generally, at the differential temperature regimes (5°C, 10°C, 15°C) UC157 has low and Brocks has high RPGR's, while both Larac and Tainan No.1 have high

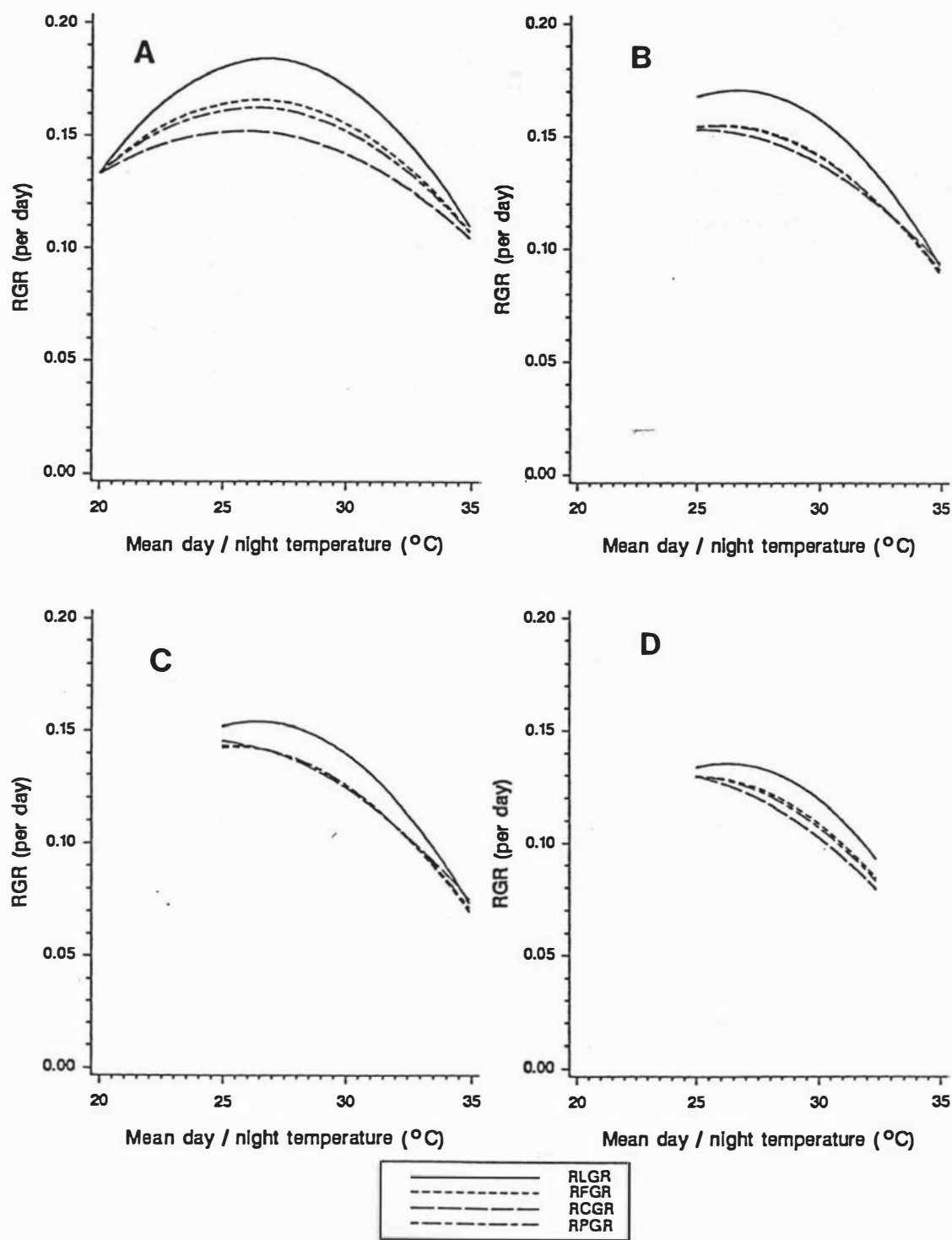


Fig. 2.18. The comparison of the predicted relative growth rates among RLGR, RFGR, RCGR, and RPGR (means of four cultivars) at

- A : the constant day/night temperature.
- B : the 5°C day/night differential.
- C : the 10°C day/night differential.
- D : the 15°C day/night differential.

RPGR's at constant temperature regimes.

2.3.7.3. Prediction of Relative Leaf Area Growth Rates

The predicted contours of RLGR vary with temperature and cultivar, with temperature having a greater influence than cultivar (Fig. 2.21). At high day/high night temperatures or low day/low night temperatures there is a greater change of contours, thus RLGR is very sensitive to supra- or sub-optimal temperature. Tainan No.1 had a greater number of contours than the other cultivars, and Brocks the least, therefore the leaf growth of Brocks is the least sensitive and Tainan No.1 is the most sensitive to temperature shift. The maximum RLGR for Brocks, Larac, Tainan No.1 and UC157 are 0.180, 0.188.

Table 2.7. The predicted optimal temperatures and corresponding RGRs.

Plant components	RGR (day ⁻¹)	Day (°C)	Night (°C)	Difference (°C)
<u>Leaf area</u>				
Brocks	0.180	26.5	26.4	0.1
Larac	0.188	26.9	26.8	0.1
Tainan No.1	0.191	26.9	26.8	0.1
UC157	0.178	26.9	26.8	0.1
<u>Fern weight</u>				
Brocks	0.164	26.5	26.4	0.1
Larac	0.170	26.6	26.5	0.1
Tainan No.1	0.170	26.5	26.4	0.1
UC157	0.161	26.3	26.2	0.1
<u>Crown weight</u>				
Brocks	0.157	28.0	22.1	5.9
Larac	0.161	26.1	20.7	5.4
Tainan No.1	0.157	25.9	25.8	0.1
UC157	0.147	25.9	25.8	0.1
<u>Total plant weight</u>				
Brocks	0.161	26.4	26.3	0.1
Larac	0.166	26.3	26.2	0.1
Tainan No.1	0.166	26.4	26.3	0.1
UC157	0.158	26.3	26.2	0.1

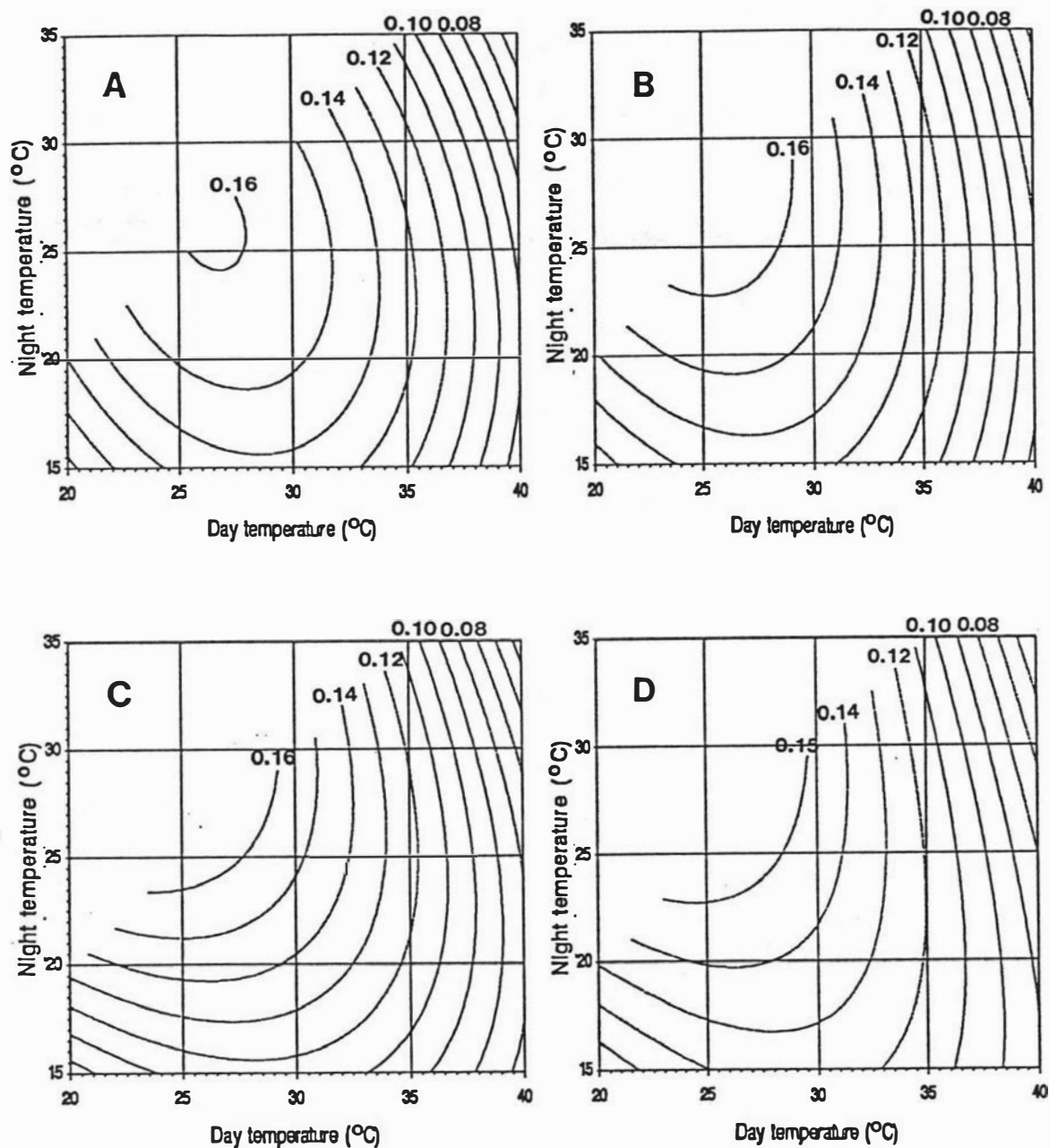


Fig. 2.19. The predicted contours of RPGR (per day)

A : Brocks. B : Larac. C : Tainan No.1. D : UC157.

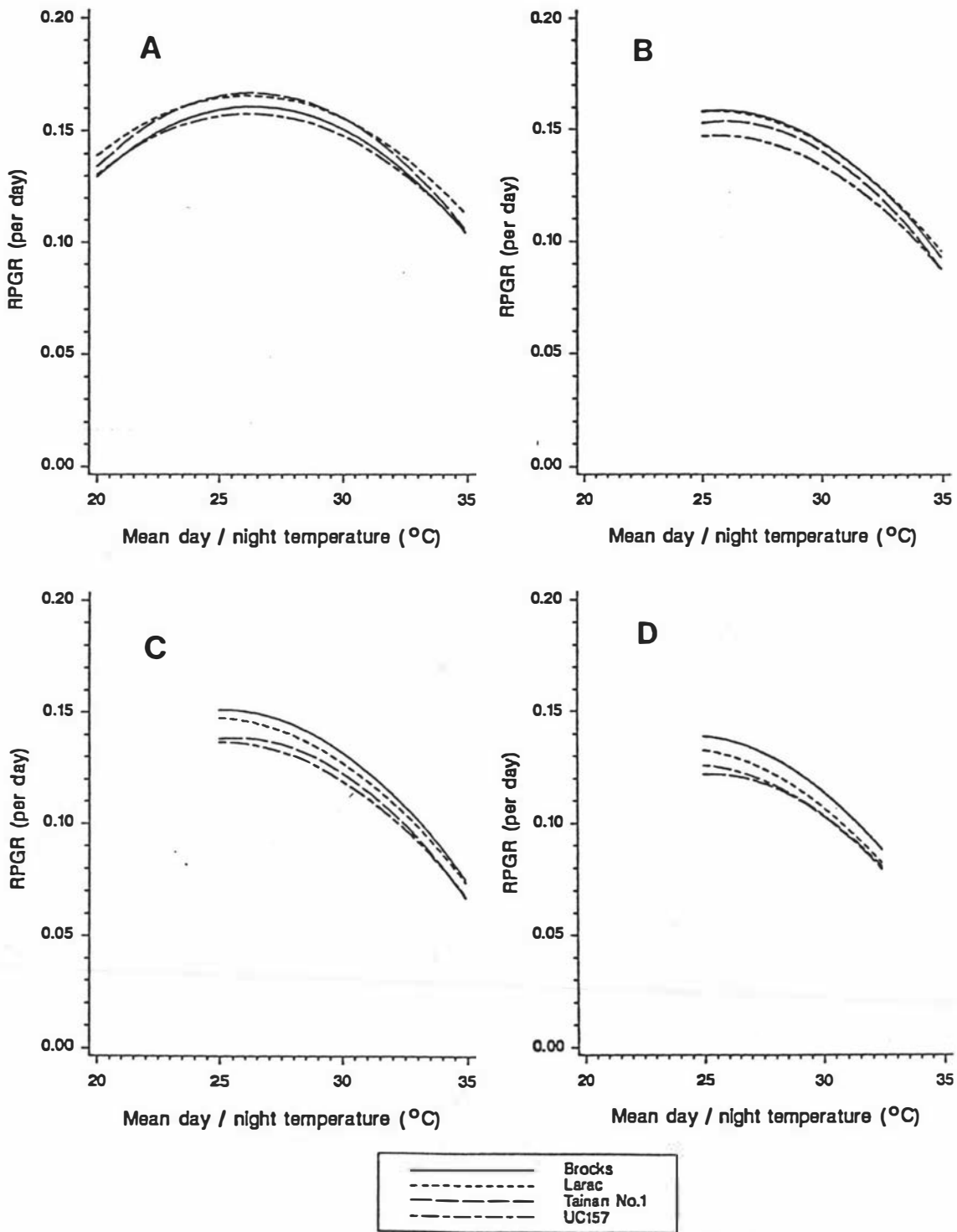


Fig. 2.20. The comparison of the predicted RPGRs

A : constant day/night temperature

B : the 5°C day/night differential.

C : the 10°C day/night differential.

D : the 15°C day/night differential.

0.191 and 0.178 per day at the optimum temperature regimes D26.5/N26.4°C, D26.9/N26.8°C, D26.9/N26.8°C and D26.9/N26.8°C, respectively, thus RLGR's are higher than RPGRs (Table 2.7). Tainan No.1 exhibits the highest RLGR at the optimal temperature. The optimal temperatures for RLGR are slightly higher than for RPGR. Brocks requires a slightly lower day and night temperatures than the other, cultivars all of which require a nearly constant day and night temperature to produce the optimal RLGRs.

- Under both constant and temperature differential regimes of 15°C the RLGRs have the greatest variation among cultivars, while at temperature differential regimes of 5 and 10°C there is less variation (Fig. 2.22). At constant temperature regimes Larac has a higher RLGR compared to Brocks and UC157, but from 25 to 30°C Tainan No.1 was higher than Larac.

2.3.7.4. Prediction of Relative Fern Growth Rates

The profiles of RFGR contours are similar to the RPGR contours. The RFGR of Brocks is less sensitive to temperature while Tainan No.1 is more sensitive (Fig. 2.23). The maximum RFGR of Brocks, Larac, Tainan No.1 and UC157 are 0.164, 0.170, 0.170 and 0.161 per day at the optimal temperatures D26.5/N26.4°C, D26.6/N26.5°C, D26.5/N26.4°C and D26.3/N26.2°C, respectively (Table 2.7). The optimal RFGR are similar to RPGR, and the optimal temperatures for RFGR are near constant, and show little difference among cultivars.

The RFGRs at constant temperature are higher than at the temperature differential regimes, and the greater the differential the lower the RFGR. At the 10 and 15°C temperature differential regime Brocks has the highest RFGR (Fig. 2-24).

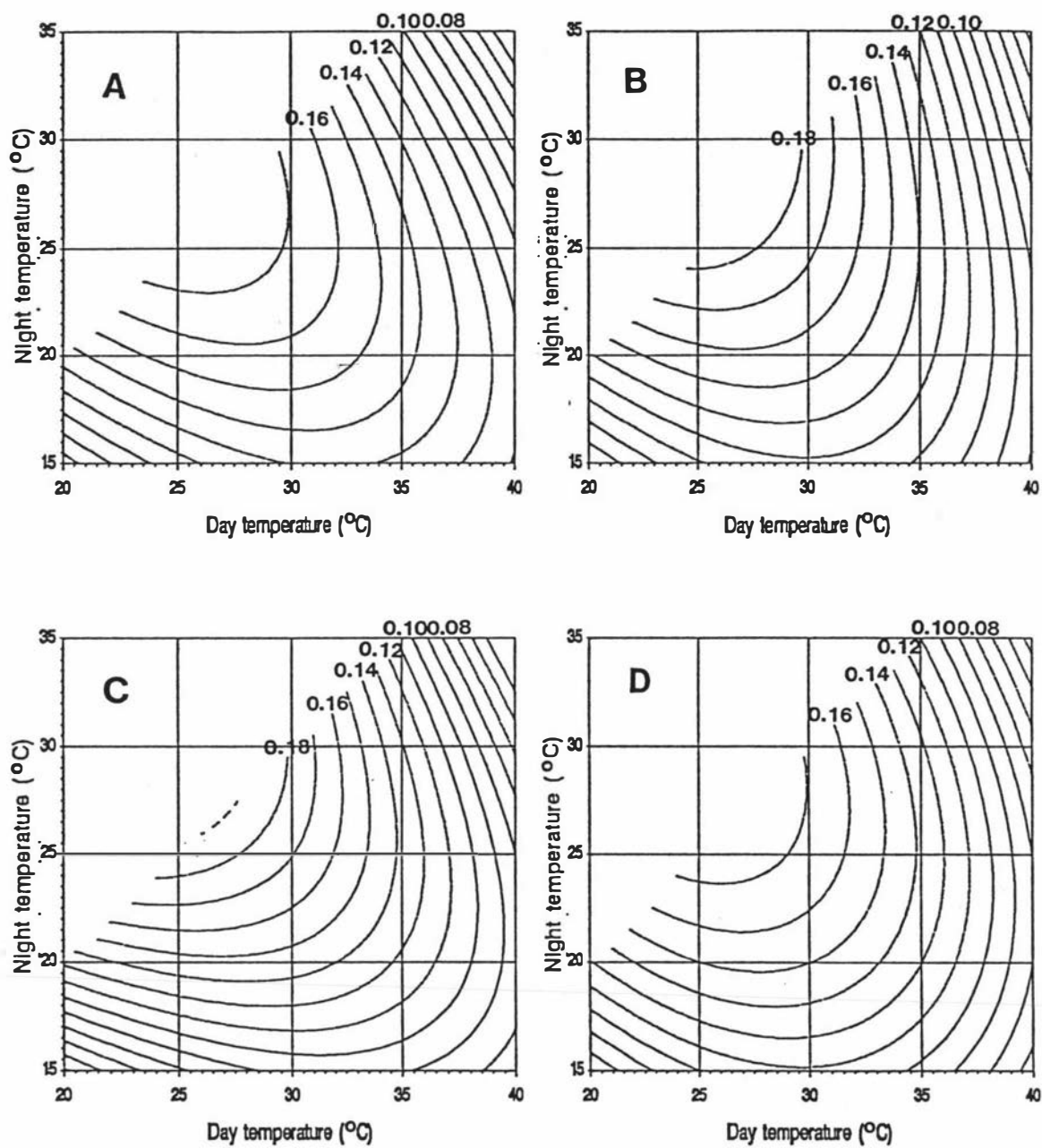


Fig. 2.21. The predicted contours of RLGR (per day)
A : Brocks. B : Larac. C : Tainan No.1. D : UC157.

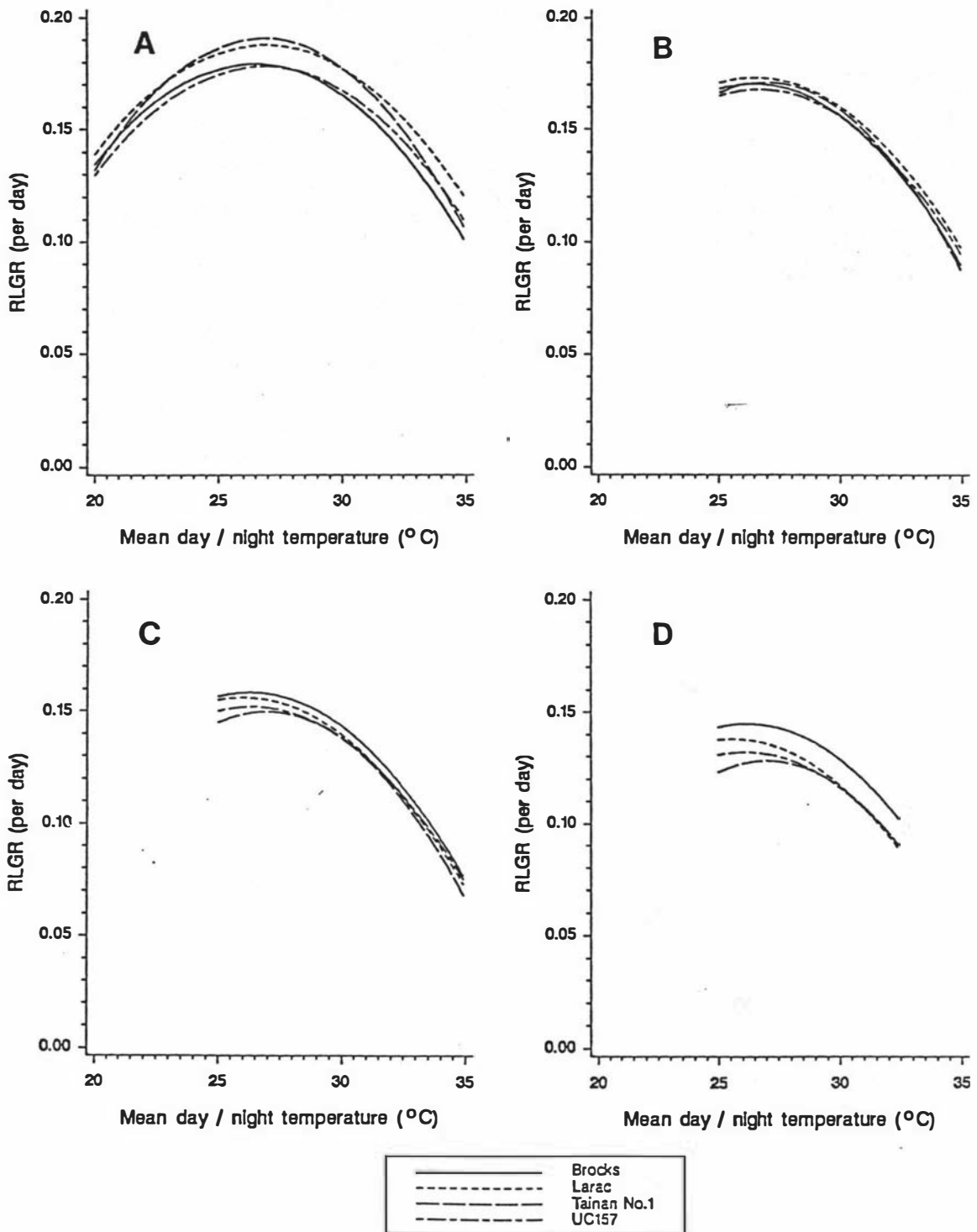


Fig. 2.22. The comparison of the predicted RLGRs

A : the constant day/night temperature.

B : the 5°C day/night differential.

C : the 10°C day/night differential.

D : the 15°C day/night differential.

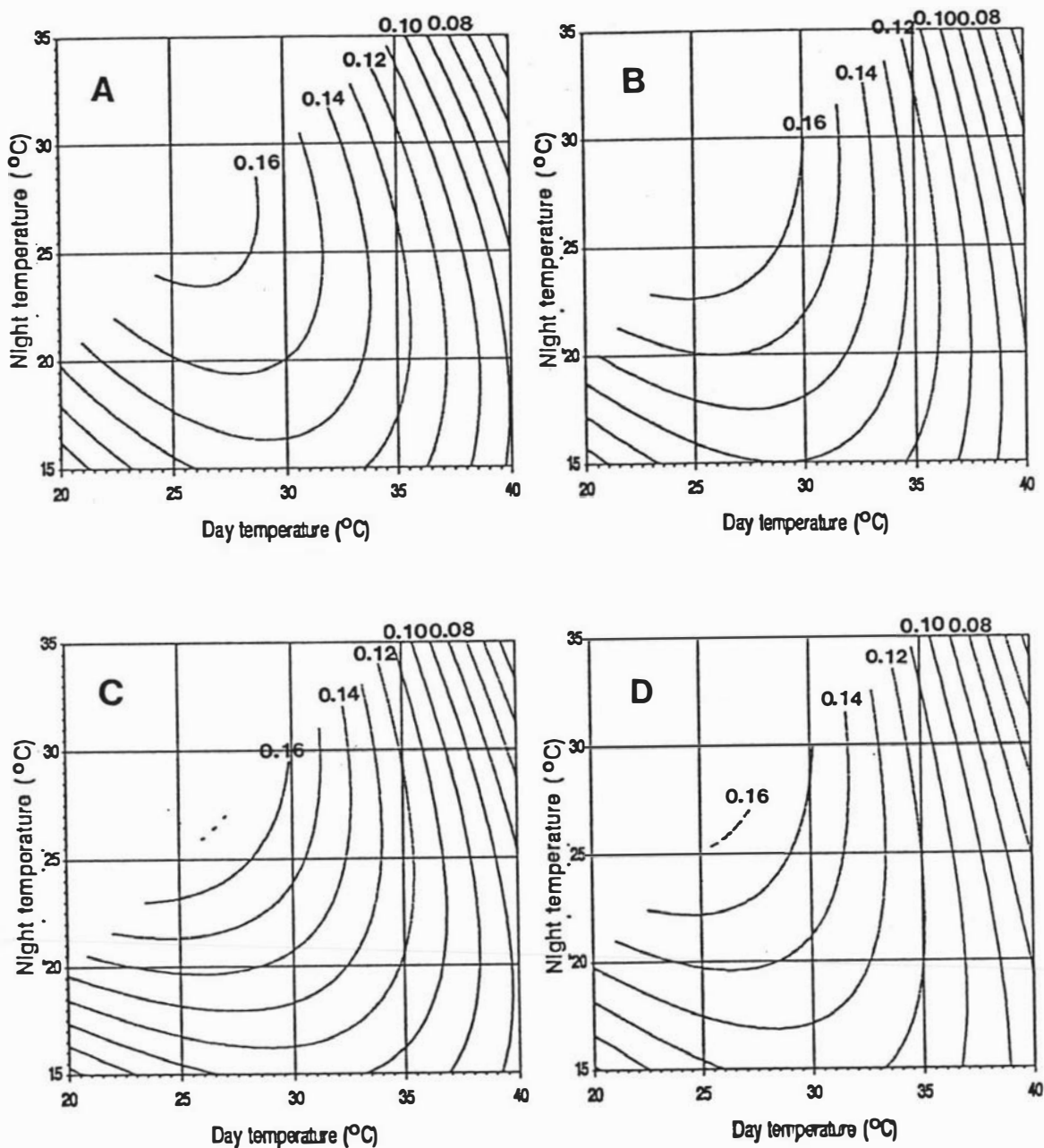


Fig. 2.23. The predicted contours of RFGR (per day)
A : Brocks. B : Larac. C : Tainan No.1. D : UC157.

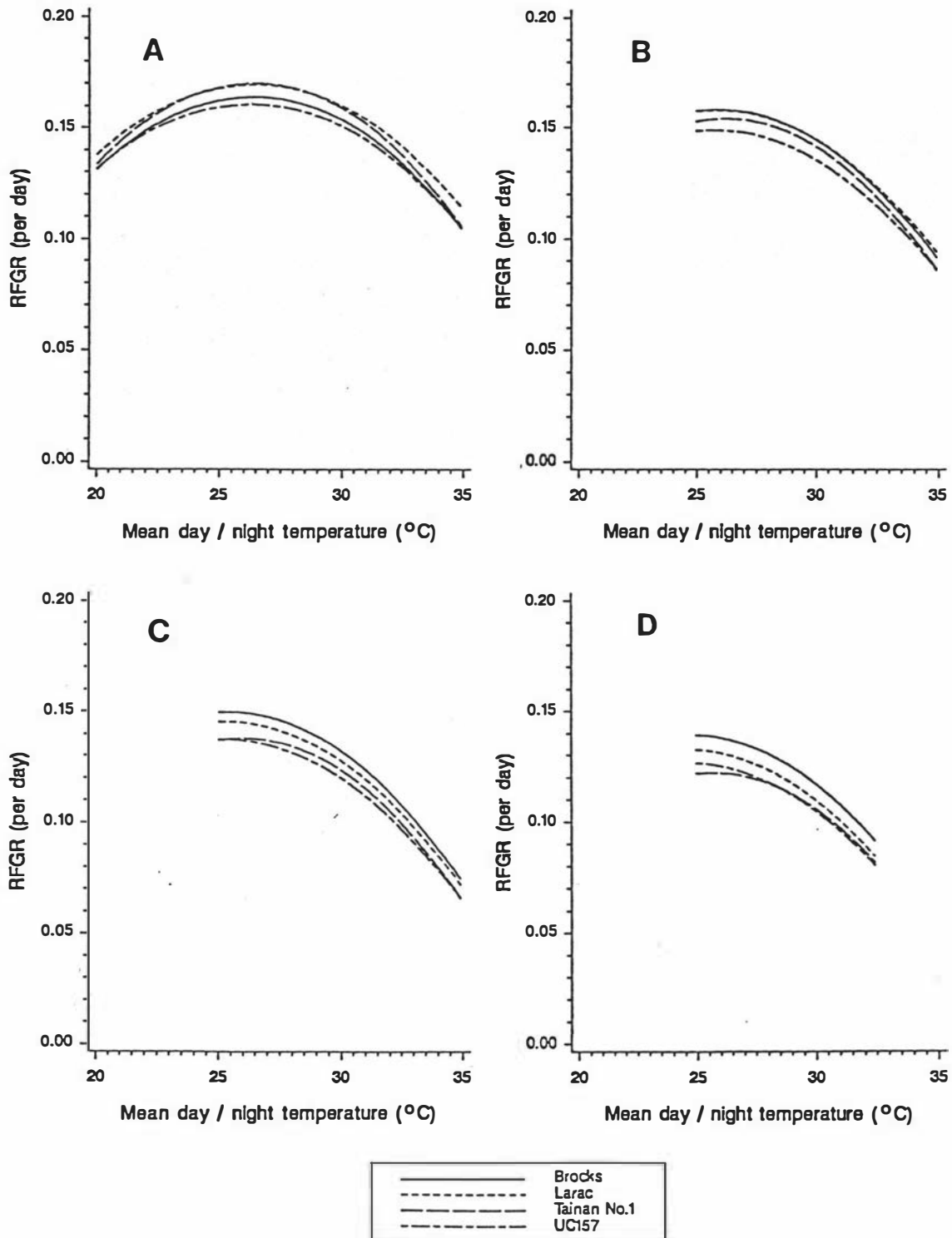


Fig. 2.24. The comparison of the predicted RFGRs

A : the constant day/night temperature.

B : the 5°C day/night differential.

C : the 10°C day/night differential.

D : the 15°C day/night differential.

2.3.7.5. Prediction of Relative Crown Growth Rates

The profiles of RCGR contours are very different to the profiles of RLGR, RFGR, RPGR contours. Thus the response of crown growth to temperature is very different from that of the other components. The RCGR contours rapidly change at high temperature, and rates are poor above day 35°C / night 15-35°C (Fig. 2.25). The maximum RCGRs of Brocks, Larac, Tainan No.1 and UC157 are 0.157, 0.161, 0.157 and 0.147 per day at D28.0/N22.1°C, D26.1/N20.7°C, D25.9/N25.8°C and D25.9/N25.8°C, respectively (Table 2.7). Therefore, the optimal temperatures show great variation among cultivars. This is markedly different from these optimal temperatures of RLGR, RFGR and RPGR. Brocks requires the highest day temperature (28°C), while the other cultivars require lower day temperature (26.1 - 25.8°C). In addition, Brocks and Larac require low night temperatures and Tainan No.1 and UC157 require high night temperatures for maximum RCGR.

At the 5°C temperature differential plant produces a higher RCGR than under the constant temperature regimes, but at the 10°C and 15°C temperature differentials the plants produced lower RCGR's than at the constant regimes (Fig. 2.26). These are different from RLGR, RFGR and RPGR. At constant temperatures Larac and Tainan No.1 have higher RCGR's than Brocks and UC157, but at the 5°C temperature differential Larac and Brocks have the highest RCGR's. Generally, under the 5°C, 10°C, 15°C temperature differential treatments Brocks has the highest RCGR's; and UC157 has the lowest.

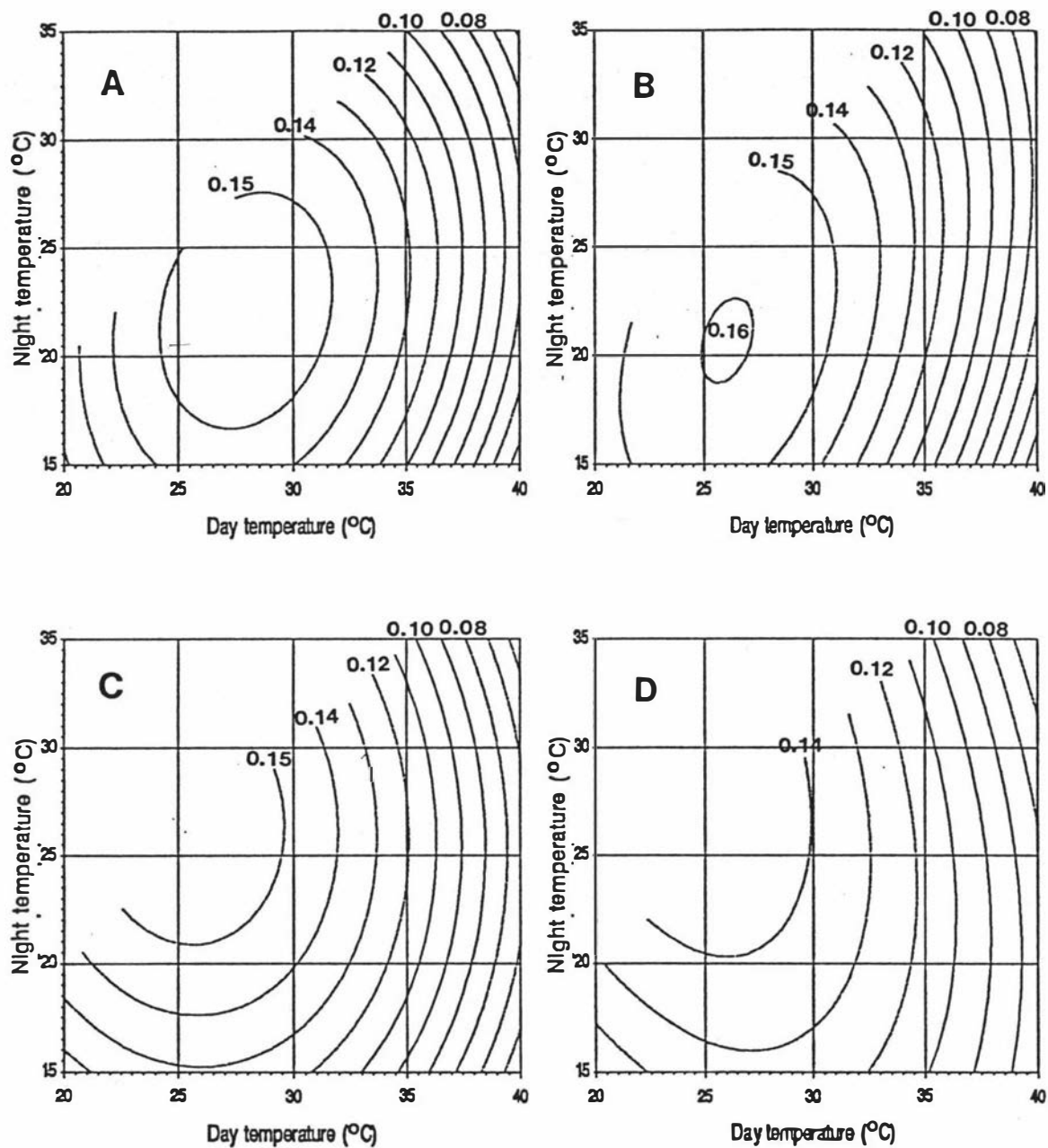


Fig. 2.25. The predicted contours of RCGR (per day)
A : Brocks. B : Larac. C : Tainan No.1. D : UC157.

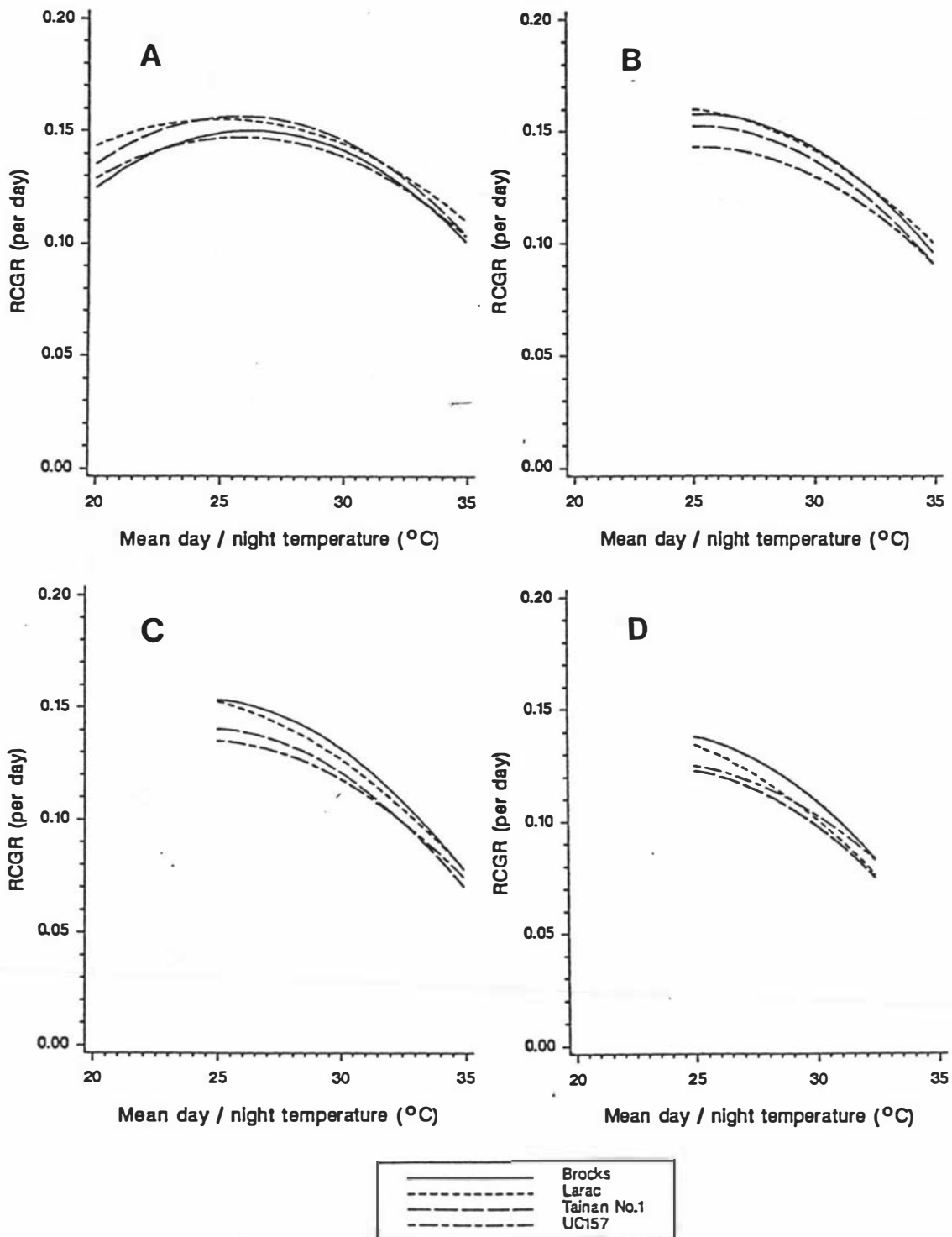


Fig. 2.26. The comparison of the predicted RCGRs

A : the constant day/night temperature.

B : the 5°C day/night differential.

C : the 10°C day/night differential.

D : the 15°C day/night differential.

2.4. Discussion

2.4.1. Influence of Temperature on Asparagus Growth

Plant growth depends on photosynthesis, respiration, partitioning and ontogeny. These physiological activities can vary dramatically with temperature (*Davidson and Milthorpe 1965; Eagles 1967; Wardlaw 1968; Ryle 1970; Hardacre and Turnbull 1986*), hence temperature plays a major role, and interacts with other factors, e.g. light, nutrients, water, genotypes (*Hughes and Evans 1962; Rajan et al. 1971, 1973; Rajan and Blackman 1975*). In my experiments, it was found that temperature strongly influenced the growth of young asparagus plants.

According to the analysis of variance (Appendix 6A, 6B, 6C, 6D), day and night temperatures strongly influenced the RLGR, RFGR, RCGR and RPGR, furthermore the interaction between day and night temperature significantly influenced all the relative growth rates except RCGR. Cultivars and the interaction between cultivars, day temperature and night temperature did not significant influence every RLGR, RFGR, RCGR, RPGR at the 1% level, while the interaction between cultivar and night temperature was significant for RLGR, RFGR, RPGR at the 5% level, it is concluded that RLGR, RFGR, RCGR, and RPGR were strongly influenced by temperature and, to a lesser extent, by cultivar. These results agree with the work of *Hughes et al. (1990)* that temperature has a major influence on the dry matter accumulation in asparagus seedlings. Furthermore day temperature significantly influenced all plant components of each cultivar, but the night temperature and the interaction between day and night temperature did not significantly influence all plant components of each cultivar. So it appears that there are different responses to temperature between asparagus cultivars and plant components.

Most of the experiments were carried out with high day temperatures, the effects of the differences in day and night temperature may be due to the

influence of high day temperature stress on chemical composition being counterbalanced by the sub- or optimum night temperatures. *Baker and Jung* (1968) (citing previous work) showed that leaf growth of orchardgrass does not appear to be influenced by diurnal temperature variations, whereas with Kentucky bluegrass a low night temperature seemed to be able to counterbalance the effect of a high day temperature, and thus night temperature could be important in determining yields.

Duncan and Hesketh (1968) showed that the size differences between maize races were apparent even when the measurements of growth rate were very slight. They suggested that, where due attention was paid to time of germination and to seedling leaf area, a simple observation of the growing plants would probably provide the best criteria of plant adaptation to any particular environment. This is consistent with the results of my asparagus study, where differences in asparagus plant size were noted even though relative growth rates were similar for some temperature regimes or cultivars.

Although there has been a number of studies comparing growth differences between species or genotypes at various temperatures, there are no reported growth differences between cultivars of asparagus due to the influence of temperature. For example, *Scurfield* (1962) showed that different strains of *Phalaris tuberosa* L. possessed varying degrees of adaptability to low and high temperature. The Algerian and Australian strains of *P. tuberosa* appeared to be less sensitive to night temperature and were out yielded by the Israeli strain at a cool regime. In a warm regime (D28/N23°C) however the Algerian and Israeli strains were adversely affected. *Duncan and Hesketh* (1968) also reported major differences in the growth indices of maize races at various temperatures including changes in net assimilation rates, relative leaf growth rates, and leaf numbers. At low temperatures, high altitude races had higher leaf growth rates and higher dry weights at harvest, while at high temperatures, high altitude races had lower net assimilation rates. In another study, it was reported that the rate of growth and development in maize was

strongly influenced by temperatures between 10 and 28°C and the growth responses of strains of maize to temperature depended on the genetic origin (*Stamp et al.* 1983; *Warrington and Kanemasu* 1983a,b,c; *Hardacre and Eagles* 1986; *Hardacre and Turnbull* 1986).

Asparagus is believed to have originated in the eastern Mediterranean region (*Nonnecke and Reinhold* 1989), and thus most cultivars have been developed in temperate zones. Generally, the growth responses of asparagus to temperature is similar to that of other temperate crop in that the influence of cultivar on growth is less than the effect of temperature. The cultivar Tainan No.1 although selected from UC309 for warm Taiwan conditions, still behaves like a classic temperate plant. However, the RPGR, RFGR, RCGR of Tainan No.1 were not influenced significantly by night temperature (Appendix 7A, 7C, 7D) and it appears, therefore that Tainan No.1 might have been selected for minimum response to night temperature.

2.4.2. Growth Analysis of Asparagus at High Temperatures

RGR is the product of NAR and LAR, thus while RGRs are compared in the analysis of growth rate, the parameters of NARs and LARs should be considered at the same time. If the RGRs are constant and NARs or LARs vary with plant ontogeny over the experimental period, then the trends in LAR and NAR may reveal the difference of responses on plant ontogeny. Previous work has shown that NAR is an important parameter in relation to growth and LAR is of less importance (*Watson* 1947a,b; *Hughes and Evans* 1962; *Evans and Hughes* 1962; *Wilson* 1966; *Buttery and Buzzell* 1974). These reports are different from the work of *Potter and Jones* (1977) and *Tollenaar* (1989b), who showed that plant growth was not well correlated with NAR. My studies also agreed with their work that the parameter LAR was found to be reasonably related to the relative growth rate, because high RGRs were related with high LARs. This may be because my experiment was carried out at stress temperatures from 20 - 40°C day and 15 - 35°C night temperatures, whereas

the cited earlier work was carried out at less stressful temperatures.

In my studies most NARs fell, and most LARs increased with the ontogeny of asparagus plants. These results are the opposite of that of *Ivory* and *Whiteman* (1978a,b) who showed that LAR markedly decreased during ontogeny in the controlled environment, and that a range of night temperatures (4 - 20°C) along with a constant 20°C day temperature does not cause any significant difference in LAR. One of the reasons that my experiments had opposite results compared with the work of *Ivory* and *Whiteman* might be because the latter was carried out at low temperature regimes, while my experiments were carried out at high temperature regimes. Furthermore, *Hughes* and *Evans* (1962) reported that LAR increased initially and then fell, reaching a maximum value earliest at the highest light intensity. *Eagles* (1967) also reported that plants of *Dactylis glomerata* grown in a low light intensity glasshouse produced a high LAR, and that the changes in LAR at different temperatures were correlated with changed in leaf morphology and distribution of assimilates within the plant and were positive correlated with RGR at different temperatures.

In my experiment NAR was inversely related to LAR, with most (but not all) the NARs decreasing, and most LARs increasing with plant ontogeny (Fig. 2.6, 2.8). The NAR trend in asparagus is similar to that found with soybean, sugarbeet and mangold, and is the reverse of that found with wheat and barley (*Watson* 1947b; *Buttery* and *Buzzell* 1974; *Seddigh* and *Jolliff* 1984). *Watson* (1947a) reported that NARs of potato decline with time in 1937 and 1938 in the field, but there was a preliminary rise of NAR in 1938. Therefore, not only does the trend in NAR and LAR depend on species, but it also fluctuates with environment and plant ontogeny. It may be concluded that NAR and LAR vary between species, between varieties of the same species, between years, between seasons of the years, and probably plant ontogeny. However in my experiment NARs and LARs were not constant and this agreed with the work of *Evans* and *Hughes* (1962). Furthermore at the late growth stages the

magnitudes of LAR and NAR might represent the actual growth rate, because the seedlings at the early stage still had carbohydrate reserves from the seed, therefore the patterns of LAR and NAR at the early stage were different from at the late stage.

Essentially LAR can be considered to be a measure of the 'balance of payment' between assimilation and respiration, because assimilation occurs in the leaf and respiration throughout the whole plant, thus a higher LAR means a higher ratio of net assimilation (*Hunt 1990*). My studies showed that temperature appeared to have more influence than cultivars on LAR. The LAR varied with temperature regimes and that the more suitable regimes had the higher LAR's (Fig. 2.8). Furthermore, although the profile of LAR of each cultivar was similar, differences existed among cultivars (Fig. 2.9). For example, at the D35/N15°C regime the LARs of Tainan No.1 and UC157 were lower than Brocks and Larac, while at D30/N30°C Brocks and Tainan had lower LARs than other cultivars, and at D25/N25°C Tainan No.1 had the highest LAR. It is concluded that the cultivar having high LARs did not result in it having high RLGR, RFGR, RCGR, or RPGR.

NAR is the unit rate of assimilation, so that total assimilation is the product of NAR and leaf area. Leaf expansion is very slow while plants are at a supra-optimal temperature (i.e. D35/N35°C) and this leads to a small leaf area and poor growth, even though the NAR is high throughout the experimental period. Consequently, it is suggested that RGRs are closely correlated with leaf area partitioning and poorly correlated with NAR. The product of NAR and leaf area partitioning was shown to be equal to RLGR (*Potter and Jones 1977*). *Hanson (1971)* has reported that the principle correlated response to selection of high stalk volume of maize was leaf area, and that variation in net photosynthetic rate did not play a major role in determining productivity within the population. *Duncan and Hesketh (1968)*, however, reported that RLGR and net photosynthesis in maize were closely correlated with temperature except that they were negatively correlated with the altitude of plant source at 36°C.

Hence plant growth rate at supra-optimal temperature may rarely correlate with NAR.

In my experiment the NARs of plants growing at a suitable temperature regime were high during the early stage of growth and tended downward during the late stage. This may be due to the self-shading effect during the later growth stage. Consequently it is postulated that vigorous plant growth can lead to early canopy development and thus lead to the NAR declining early. Thus plant growth over the entire period is considered to depend on : (a) the initial leaf area; (b) NAR; (c) RLGR; (d) relationship of leaf area to leaf weight; (e) the duration of the growing period (*Jackson 1963*).

The longevity of asparagus "leaves" appeared quite long in the field (*Lin and Hung 1978; Lin 1979*). In my studies at the optimal and sub-optimal regimes the "leaves" also showed good vigour at 73 days old. Conversely, the "leaves" at the supra-optimal regimes had deteriorated at this stage, therefore the plant at supra-optimal temperature regimes may not develop a significant canopy due to the rapid deterioration and short longevity of the leaves. This can lead to a decrease in dry matter accumulation (*Fisher and Maurer-O 1976; Badu-Apraku et al. 1983*). The high NAR at some supra-optimal temperatures may thus be explained due to the lack of self shading. Furthermore *Midmore and Prange (1992)* suggested that the greater rate of photosynthesis per unit leaf area for high temperature produced plants was most likely due to biochemical enzymic adaptation of photosynthesis to high temperature and to the greater stomatal density.

As expected, the growth indices showed fluctuations among cultivars, especially for plants at the regimes D35/N15°C, D35/N25°C and D35/N35°C. The causes may be due to a day/night temperature differential of supra-optimal day temperature and sub-optimal or supra-optimal night temperature triggering the characteristics of heat adaptation. The order of magnitudes of RLGR, RFGR, RCGR, RGR, LAR and NAR between the three regimes were

$D35/N25^{\circ}\text{C} > D35/N15^{\circ}\text{C} > D35/N35^{\circ}\text{C}$, this may be explained because the stress day temperature of 35°C may be counterbalanced by a night temperature of 25°C , but counterbalanced less by a low night temperature of 15°C . How the sub-optimum night temperature (15°C) governs growth is not clear (*Badu-Apraku et al.* 1986), but possibly the sub-optimum night temperature depresses the translocation of assimilate. While at high temperature ($D35/N35^{\circ}\text{C}$) the uncoupling phosphorylation from respiration would increase the amount of carbon dioxide produced per unit of dry matter accumulation and lead to poor growth (*Chowdhury and Wardlaw* 1978). High temperatures also depress sink activity and this can lead to a decline in photosynthetic activity (*Dinar and Rudich* 1985a; *Sepúlveda et al.* 1986a).

Stevenson and Goodman (1972) showed that when the upper temperature limit of maize growth (about 36°C) was reached, the maize plants induced tiller production and the apical meristem died. Therefore, the temperature regimes of $D35/N35^{\circ}\text{C}$, $D40/N20^{\circ}\text{C}$, $D40/N30^{\circ}\text{C}$ regimes may be at the upper temperature limit of asparagus growth, because at these regimes the shoot apices died and thus increased new shoot production. Furthermore, in asparagus at these regimes, the responses varied with cultivar, with Larac having more shoots and dead apices. Hence in this aspect heat tolerance among asparagus cultivars appears to be different.

When the growth rate is classified according to the parameters NAR, LAR and RGR, the temperature regimes can be grouped into high : ' $D25/N25^{\circ}\text{C}$ and $D30/N30^{\circ}\text{C}$ ', normal : ' $D20/N20^{\circ}\text{C}$, $D30/N20^{\circ}\text{C}$, $D35/N15^{\circ}\text{C}$, $D35/N25^{\circ}\text{C}$ ', and poor growth rates : ' $D35/N35^{\circ}\text{C}$, $D40/N20^{\circ}\text{C}$, $D40/N30^{\circ}\text{C}$ '. According to this criterion, it is suggested that plant growth is greatly reduced by a relatively small temperature increase above the optimum. This results are consistent with the work of *Duff and Beard* (1974).

High night temperature stress appears more inhibiting than low night temperature stress, for example, in the comparison between the D35/N15°C and the D35/N35°C regimes. Although they have the same day temperature, the regime of D35/N35°C is 8.5°C higher than the estimated optimal night temperature (26.5°C) and the regime of D35/N15°C is 11.5°C lower than this night temperature, but the D35/N35°C plants were much smaller than the D35/N15°C plants. Consequently it is inferred that this is due to the deterioration of growth respiration and physiological process at supra-optimal night temperatures, and that the high maintenance respiration at supra-optimal night temperatures may not completely deplete assimilates produced during the day (*Chowdhury and Wardlaw 1978; Gerik and Eastin 1985; Gary 1989*). Thus the causes of growth differences at supra-optimal temperatures between cultivars may be due to the different adaptability growth of respiration and physiological processes rather than maintenance respiration.

The growth depends on the size of the RGRs, NARs and LARs. At the supra-optimal temperature regimes, D35/N35°C, D40/N20°C, and D40/N30°C, plants having a high NAR and low LAR produce a low RGR and consequently poor growth. Conversely, for plants at D25/N25°C and D30/N30°C, the NARs were dramatically decreased and LARs were upward, but the RGRs were still high and plant size was large. The results showed that the parameters of RGR and LAR are sensitive to temperature but that NAR is less sensitive. This agrees with the work of *Watson (1947a)* who showed that NAR was much less variable between years and between varieties of the same species than LAR. Consequently the differences between years and varieties in yield of dry matter reflected mainly the differences in leaf area, and showed no close relation to the smaller differences in NAR. In addition *Davidson and Milthorpe (1965)* reported that the reduction in RGR of *Dactylis glomerata* with time at 22°C and at 26°C was associated with size of leaf weight which was partly reflected by reductions in LAR. In contrast *Eagles (1967)* reported that the difference in RGR between populations of *Dactylis glomerata* were the result of differences in NAR and not LAR. However, the importance of NAR or LAR to RGR varies

with environment. At moderate to warm conditions NAR varies little with temperature, thus temperature is generally less important than light except in cool climates, and hence in natural climates RGR is more temperature dependent. Therefore, RGR is more closely related to temperature than NAR (*Wilson 1966*). Thus RGR may be a good parameter to measure growth rate, but for further information, NAR and LAR should be analyzed, however plant ontogeny and environments should be considered to avoid misleading conclusions.

2.4.3. Assimilate Partitioning in Relation to Ontogeny

There are two theories to improve yield:

- (1) that any remaining scope for further improvement in carbon allocation must be small, so it would be better now to aim at increasing photosynthetic and growth rate.
- (2) that since partitioning is where flexibility has been in the past, it is better to aim for further increases in harvest index (*Gifford and Evans 1981*).

Asparagus growth may be represented by the component production rate, which also represents the distribution of growth activity of plant components (*Hunt and Bazzaz 1980*). The growth activity of components may be inferred as a unit partitioning magnitude of assimilate to components, and may represent a measurement of sink activity (*Marinus and Bodlaender 1975; Dinar and Rudich 1985a*).

According to the above principle, a well adapted cultivar of asparagus will have a high level of J_{root} , even though RPGR fluctuates with environment, especially temperature. This is because the carbohydrate resource for growing spears come from roots, and therefore the high root / shoot ratio (and the capacity to accumulate more carbohydrate) is desirable. In my experiments, the leaf

production rate contributed about half the rate of relative growth of total plant weight except at supra-optimal temperature regimes. It also increased with ontogeny (Fig. 2.14). This means that half the rate of unit increase of total plant weight is committed to leaves and increasing commitment to leaves with ontogeny, even though the RLGR is constant. This is supported by the increasing LAR with plant ontogeny.

Plants at the temperature regimes of D30/N30°C and D25/N25°C have a high level of J_{leaf} and this increases dramatically with plant ontogeny. Consequently, at both regimes plants have a vigorous leaf growth. Plants at D20/N20°C, D30/N20°C, D35/N15°C, D35/N25°C had different rates of leaf production at the early stage and nearly the same magnitudes at the later stages. Therefore at these regimes, their leaf production rate is similar which means plants have similar leaf growth activity at these regimes. The dry weight increase of leaves is the product of J_{leaf} and instantaneous total plant weight, consequently even though the leaf production rate is the same, due to possible differences in instantaneous total plant weight, there may be different leaf dry matter increments. However the similar growth activity of leaves at both sub- and supra-optimal regimes (i.e. D20/N20°C and D35/N35°C) means the asparagus plant is able to adapt at these regimes and to maintain a similar rate of leaf production at the later stages, even though there are different RGRs at these regimes.

The response of root production rate to temperature was different from that of leaves. In the other words, the influence of temperature on growth activity varied between leaf and root. Temperature is believed to affect root metabolic activity and consequently root sink strength (*Hurewitz and Jane 1983*). At D40/N30°C and D35/N35°C regimes plants had a low root production rate. Conversely plants at D40/N20°C also had a low root production rate but this rate increased with plant ontogeny. The cause may be due to the abnormal root sink strength at these supra-optimal temperatures. *Hurewitz and Janes (1983)* showed that temperatures above 35°C stimulate assimilates to

accumulate in the root, because supra-optimal temperatures induce the inability of retranslocation and thus lead to assimilate deposition in roots. One of the possible causes is that at high temperatures increasing inhibitor content leads to more assimilate moving to the roots. Conversely, the rate of root production at D30/N30°C decreased with increasing ontogeny, because the suitable temperature induces increasing GA content (or a decrease in inhibitor) and thus leads to the decrease in partitioning of the assimilate to the roots, because GA may increase the sink activity of the shoot components (*Atkin et al.* 1973).

There was also a significant difference in basipetal assimilate transport between the heat tolerant and heat sensitive cultivars when observed under heat stress conditions (*Dinar and Rudich* 1985b). Hence the effect of temperature on assimilate distribution is complex and can not be described in uniform terms for all parts of the plant or for all cultivars. *Dinar and Rudich* (1985b) confirmed in their experiment that the response of assimilate partitioning to temperature depends on plant components and temperature regimes, and that there were also cultivar differences.

The rate of root production at D20/N20°C was high throughout, and this agrees with previous work (*Barta* 1978; *Theodorides and Pearson* 1981) which showed that low temperature is a favourable factor for root growth and that high temperature depress root growth, or in other words, low temperature depresses shoot growth. *Atkin et al.* (1973) have explained this by suggesting that the depression of shoot growth by low temperatures was due to an altered production of growth substance in the root system, and that this growth substance is exported to the shoots. Furthermore, *Pardales et al.* (1991) suggested that heat stressed plants have a reduced ability to acquire water and nutrients from the soil, as the root system is small at high temperature. They also cite previous work to show that temperatures of 36 - 38°C sharply reduced the number and length of roots of taro and a temperature of 36°C

inhibited the root elongation of cotton. In my experiment, the supra-optimal temperature regimes, D35/N35°C, D40/N20°C and D40/N30°C significantly decreased RCGR and root production rate of asparagus. The cause is that supra-optimal temperature can lead to root deterioration.

The analysis of component production rate, confirmed that the assimilates had a greater partitioning to the roots at D20/N20°C even though the RGR at that regime is lower than at D25/N25°C and D30/N30°C. It confirmed that at mild high temperature, the assimilates were rapidly used in the expansion of new leaf tissues while at low temperature there was less leaf expansion. In addition, mild high temperature is associated with rapid stem elongation and a fast rate of canopy formation (*Ku and Hunt 1972; Ong 1983a*).

The RCGR and RPGR in D25/N25°C and D30/N30°C were the highest of all the temperature regimes. The root production rate at D30/N30°C was higher than at D25/N25°C during the early stage, the former decreased dramatically and the latter decreased slowly, thus leading to the latter (D25/N25°C) having a higher rate of root production at the later stages. The allometric intercept at D30/N30°C was lower than at D25/N25°C. Previous work suggests that in allometric analysis, the slope is not significantly different between cultivars of asparagus, but the intercept is different (*Nichols and Woolley 1985*). My results supported these findings that the allometric coefficients between cultivars were not significantly different in each regime (except at D40/N20°C), but the allometric intercepts between cultivars were significantly different at most temperature regimes. In the experiment, Tainan No.1 was found to have the highest allometric intercept, and Brocks the lowest. It is concluded that the allometric intercept was very dependent on genotype or environment, and thus may be a useful index for evaluating asparagus genotypes or plant environments.

The work of *Stanhill (1977b)* on the allometric analysis of carrot showed that the effect of different growth temperatures and of added farmyard manure

significantly affected the coefficients, but the results from irrigated and unirrigated carrot crops were too variable for accurate conclusion about the allometric coefficients. *Currah and Barnes (1979b)* cited previous works to conclude that neither light intensity, or water availability could be expected to significantly influence the allometric coefficients, whereas temperature and soil nutrient levels did effect the coefficients. *Currah and Barnes (1979a)* suggested that the allometric intercepts were only slightly affected by plant density but changed significantly with harvest age, whereas density also had a very small effect on the coefficient. The coefficient also changed during the early harvests, but after the first 4 weeks of growth, coefficients showed only slight changes due to plant ontogeny. Plant ontogeny, therefore, influenced the intercept more than the coefficients, and variation of the intercept was more important than that of the coefficients in the analysis of effects of time and plant density on the shoot and root weight of carrot. Hence the above allometric analysis agreed with the results of my experiment which showed that temperature and cultivars had an important influence on the allometric intercept.

In fact, the allometric analysis of one component in relation to another, (for example, the analysis of crown in relation to fern) only shows whether plants grow "rooty" or "ferny", it does not reveal growth activity. In fact due to a strong sink activity of root, the growth of the shoot is often suppressed, therefore, at low temperatures, more assimilates are distributed toward the roots in contrast to the above ground parts.

Conversely, the analysis of component production rates can represent the growth activity and growth trend. It is a sensitive analysis able to reveal growth differences at plant component level between treatments. In my experiment, the analysis revealed differences of component production rates between various temperature regimes or cultivars. For instance, the leaf production rates under each regime of D20/N20°C and D25/N25°C were similar among cultivars, but the root production rate were different. Furthermore, at

D35/N25°C or D35/N35°C regimes, the differences in leaf and root production rates were revealed with the analysis. It is expected that these results can be applied to the field to predict asparagus growth, and to screen genotypes, even though the environment in the field can not be simulated completely by growth room studies.

2.4.4. Growth Prediction

Because the growth experiments can not be carried out at all possible temperature combinations, the data from my experiments were fitted to an equation to predict plant growth at all temperature regimes. Although there are a large number of models which could be used to fit these data, the response surface was used because a lot of previous work has successfully used the model to predict growth and morphologic ontogeny (*Hammer and Langhans 1976; Armitage et al. 1981; Armitage and Carlson 1981; Armitage et al. 1981; Karlsson and Heins 1986; Erwin et al. 1989; Leith et al. 1991; Hopper and Hammer 1991*). When these data were fitted to the response surface model, the correlation coefficient ($0.85 > r^2 > 0.81$) showed it to be a favourable equation to predict RLGR, RFGR, RCGR, RPGR.

From the equation of the response surface, the highest magnitudes of RLGR at the optimal temperature of asparagus is less than in other crops, e.g. corn (*Blacklow 1972; Hunter et al. 1974; Hunter et al. 1977*). Moreover, the optimal temperature is a near constant day and night temperature between 26.2°C to 26.9°C (Table 2.6, 2.7). This is very similar to the work of *Robson (1973)*, who showed that the optimum temperature for leaf growth of tall fescue is about 25°C, with a night temperature equal to or slightly less than that of the day temperature. The optimal temperature of the terms RLGR, RFGR, RPGR showed little difference among cultivars, and the optimum RLGR, RFGR, RCGR, RPGR are also different between cultivars. In fact, asparagus has a high optimal temperature compared to D25/N25°C for tomato, tall fescue, french beans, ryegrass, and 20-21°C for north temperate grasses, such as the

temperate Norwegian race of cocksfoot (*Robson 1973*). Most physiological processes achieves a maximum at 25 - 30°C for temperate crops (*Ong and Monteith 1985*), hence asparagus appears to be a temperate crop as the optimal temperature for growth is at 26.2 - 26.9°C.

In fact, the optimal temperature and relative growth rate can be expected to fluctuate with other environmental factors, e.g. light intensity, nutrition, plant component and plant ontogeny, hence the optimum is not a specific temperature, but a range dependent upon other variables (*Duff and Bread 1974*). However, the optimal temperature for asparagus is lower than for a tropical crop of maize which has a optimal temperature of about 31°C. Thus asparagus has a higher optimal temperature than the common temperate crops, but a lower optimal temperature requirement than the tropical crops, so it is postulated that asparagus may be adapted to warm climates.

The optimal temperature for the RCGR requires a lower night temperature than the other components. This is consistent with the work of *Lahav and Trochoulis (1982)* who in avocado plants showed that warm temperatures were more advantageous in all the growth parameters measured except for root dry matter production. Roots were more active at a lower temperature than the optimal temperature for leaf area, fern weight and total plant weight whereas these regimes reduced root growth. Possibly this is associated with the characteristic of the deep root system of asparagus, since soil temperature decreases with increasing depth. When comparing the night temperature required to produce the optimum RCGR between cultivars Tainan No.1 and UC157 required a constant and high night temperature, while Larac and Brocks required a lower night temperature 5.4 - 5.9°C lower than day temperature. *Lahav and Trochoulis (1982)* showed that the better adaption of the cv. Hass of avocado plant (heat tolerance) was due to a higher optimal temperature for total dry matter production and similarly Tainan No.1 and UC157 appeared to be better adapted to warmer temperatures due to having a higher optimal night temperature compared with the other cultivars. It can be

concluded that Tainan No.1 and UC157 have a broader adaptation to warm temperature.

Plants at day/night temperature differentials are predicted to produce lower RGRs than at constant temperatures (Fig. 2.11, 2.12), thus the phenomenon of thermoperiodicity (*Went* 1944) may not exist for asparagus because RLGR, RFGR and RPGR show no evidence of thermoperiodicity. These results agree with the work of *Robson* (1972, 1973) with tall fescue who showed that the optimum regime for growth was one in which the day and night temperatures were equal (D25/N25°C). Moreover when a mean temperature of 25°C was imposed, the growth at the D25/N25°C regime exceeded that at both the D30/N20°C and D20/N30°C regimes. This may be because the day/night temperature differential leads to plants gaining less assimilate than at constant temperatures (*McCree and Amthor* 1982).

In fact, the effect of thermoperiodicity may depend on plant ontogeny, environmental conditions and species. The responses of plants to a day/night temperature differential may depend on physiological condition, e.g. plant age, plant size (*Went* 1944), hence one attribute is not necessarily similar to other growth attributes (*Bendix and Went* 1956). In my experiments, low temperatures favoured root growth and the day/night temperature differentials produced high allometric intercepts. However, the response of the allometric intercept to day/night temperature differential may not be the effect of thermoperiod, because, at the day/night temperature differential regimes plants had higher allometric intercepts than at the equivalent constant temperatures. Furthermore although the analysis of variance of the effects of temperature on the allometric intercept showed that night temperature had no significantly effect on the allometric intercept, there was a strong interaction between day and night temperature. These results agree with the work of *Robson* (1973), who showed that the leaves of tall fescue were affected more by changes in day than night temperature, and do not agree with the absence of a thermoperiodic response due to a day/night temperature differential.

Conversely, *Tollenaar et al.* (1979) cite work that night temperature exerts a greater influence on rate of leaf development than day temperature. They found that when night temperatures were in the range where the rate of development increases linearly with temperature (i.e. from 12 to 26°C), the rate of development may change much less at maximum day temperatures (i.e. from 26 to 36°C). However, the extent that day and night temperature influences growth depends on stage of plant, organs, species and day/night temperature differential (*Thomas and Raper* 1978).

On the other hand, it is often postulated that the effects of day and night temperature on RLGR, RFGR, RPGR are equal, and the optimal constant temperature is also postulated to lead to high rates of growth due to accelerated rates of development (*Bonaparte* 1975). But in my study day temperature and night temperature is not interchangeable, and they depend on each other, and the relationships between day and night temperature affect each other (*Robson* 1973). Possibly the day/night temperature differential may influence the balance of endogenous hormone and lead to changes in the growth pattern and rate. In work on lilies grown in a number of day/night temperature differentials, the influence of the day and night temperature differential on stem elongation suggested that thermomorphogenesis was not a function of total plant carbohydrate translocation. Instead, The temperature differential appeared to influence the endogenous gibberellin content or the response of plant tissue to gibberellin (*Erwin et al.* 1989). In fact, growth is affected by light intensity, soil nutrient status, soil texture, and plant water potential (*Tollenaar et al.* 1979). Hence, environmental factors other than temperature can also influence growth rate.

From the results of my studies, it is concluded that the relationship of asparagus growth to temperature was curvilinear and that the effects of temperature on growth were stronger than that of cultivar or plant component. There were thermotolerant differences among cultivars and plant components. The optimal temperature for the growth of underground components (roots) is

lower than that for above ground components. Thus in warm climates assimilate partitioned to roots may be less than in cool climates, but at warm temperatures (no heat stress) plants have high RPGR which will result in the RCGR being higher than at cool temperatures due to parallel growth between components, as even at mild high temperature (such as at D35/N25°C) plants can still maintain a high RCGR.

Growth parameters from day/night temperature were fitted to the response surface function to predict growth. Even if field environments can not be completely simulated in climate rooms, this prediction reveals information about asparagus growth at various temperature regimes. These may be applied to screen genotypes or to evaluate plant environments.

CHAPTER THREE

Growth of Asparagus Spears and Fern at High Temperatures

3.1. Introduction

Asparagus is now planted and produces spears in tropical regions, but most cultivars of asparagus were developed and released for temperate areas. Adaptation to high temperature therefore is a most important characteristic when asparagus is planted in tropical climates. Cultivars used in tropical climates are not only evaluated for plant growth, but also for spear yield and spear quality. Vigorous growth only ensures that the roots accumulate plenty of carbohydrates. The later remobilization, spear production and fern development are strongly influenced by temperature. Spear quality is very important for commercial production, because only compact spears of adequate size are accepted by the market.

Temperature can influence bud flush and later fern development, therefore spear numbers, spear size, spear yield, fern development and fern characteristics closely relate with temperature. Yield is the product of spear number and spear size (weight), while the height to the first branch of fern is frequently associated with spear quality (*Ellison* 1986). Therefore these characteristics will be used to evaluate the adaptation of cultivars to tropical climates. In this experiment, established plants of cvs. Brocks, Larac, Tainan No.1 and UC157 were grown in growth cabinets at 28°C, 33°C, and 36°C to produce spears and ferns. Spear number, spear size, fern development, fern weight and first branch height of fern were collected to evaluate the adaptation of these 4 cultivars of asparagus to high temperature.

3.2. Materials and Methods

3.2.1. Plant Materials

Seeds of 4 cultivars (Brocks, Larac, Tainan No.1 and UC157) were sown in trays, and then 4 weeks later transplanted into plastic pots (12.5 cm diameter x 10 cm high) filled with a 50:50 v/v mixture of pumice and peat. The media was mixed with Osmocote 2.25 kg, superphosphate 1.5 kg, lime 1.5 kg, Dolomite 3 kg, and Micromix 0.6 kg per m³ of media. No nutrient solution was used during the first 3 months, then the nutrient solution (appendix 5) was applied to the plants once per week. The plants were then grown for about one and half years in a greenhouse.

3.2.2. Growing Conditions for Evaluating Spear Yield and Quality at High Temperatures

All the fern was cut from the plants prior to their being placed in the growth cabinets. The spears were harvested when above 10 cm height and spear numbers, spear diameter (butt), and spear weight (cut at 10 cm length) were measured every day for 60 days. The plants were watered six times per day via an automatic drip system to prevent water stress, but no nutrients were supplied during the harvest period.

The experiment consisted of 3 temperatures and 3 cultivars (Brocks, Larac, UC157), and each treatment had 12 plants. The measurement of spear production started one week after the plants were placed in the growth cabinets. The 3 growth cabinets were set at 12 hours day / 12 hours night, light intensity 750 $\mu\text{mol s}^{-1}\text{m}^{-2}$, at constant $28\pm 1^\circ\text{C}$, $33\pm 1^\circ\text{C}$, and $36\pm 1^\circ\text{C}$.

3.2.3. Growing Conditions for Evaluating Fern Development and Characteristics at High Temperatures

The potted plants were prepared as in 3.2.1., and all the ferns were cut down

before transferring the plants to the growth cabinets. The top of the crown was exposed, and then covered with pumice to provide accessibility in order to measure spear height from the crown and yet to prevent heat damage to the crowns. The height of each spear was measured twice per day from the start of spear growth to the development of the fern. The height of the ferns were then measured once per day, and then every two days at the later growth stages. These measurement were taken for 2 months. Finally the plants were left to grow for a month, and then fern stem diameter, fern dry weight, fern height, fern stem numbers and height of fern to the first branch were measured.

The experiment consisted of 3 temperatures (28°C, 33°C, 36°C) and 4 cultivars (Brocks, Larac, Tainan No.1, UC157). There were 8 plants per treatment, and the measurement of spear / fern development started 1 week after the plants were placed in the growth cabinets. The growth cabinets were set as in 3.2.2. The potted plant were irrigated six times per day via automatic drip system and watered with nutrient solution (appendix 3) once every week.

3.2.4. Analysis of Spear Growth at High Temperatures

Relative spear growth rate (RSGR) was essentially exponential between 10 and 200 mm, thus when the natural logarithm of spear height is plotted against age (D), a straight line was obtained in which RSGR, (the slope of the line), represents the relative spear growth rate (*Nichols and Woolley, 1985*), thus the growth of spear length from 10 to 200 mm is represented by the equation;

$$H = ae^{RD}$$

$$\log_e H = \log_e a + RD$$

Where,

H : Height of spear.

a : constant of equation.

R : RSGR₁₀₀, relative spear growth rate during spear from 10 to 100 mm.

or RSGR₂₀₀, relative growth rate during spear from 10 to 200 mm.

D : age of spear, in days.

3.2.5. Fern Growth at High Temperatures

The Richard's Growth Equation was used to fit the developing fern (height) (Richard, 1959; Causton *et al.* 1978; France and Thornley, 1984). The equation is;

$$H=f(D)$$

H : fern height.

D : Plant age (days).

$$H = \frac{H_f H_i}{(H_i^N + (H_f^N - H_i^N) e^{-KD})^{\frac{1}{N}}}$$

where,

H_i : first time fern height measured. In this case of above 10 mm and 1 day old.

H_f : final height of fern.

N : the curve type parameter.

K : the rate at which the value of the function of H (fern height) changes.

Differentiation of the equation gives the inflexion point

$$\frac{d^2H}{dD^2} = \frac{K}{NH_i^N} \frac{dH}{dD} (H_i^N - (n-1)H^N)$$

and equating this to zero at time $D=D^i$ gives the height of inflexion point H^i as

$$H_{(D=D^i)}^* = H_i^* \left(\frac{1}{N+1} \right)^{\frac{1}{N}}$$

and the age at the inflexion point D^i as

$$D^* = \frac{1}{K} \log_e \left(\frac{H_i^N - H^N}{N H_i^N} \right)$$

The weighted mean relative growth rate (WMRGR) over the whole period of growth is

$$WMRGR = \frac{K}{N+1}$$

and the corresponding mean absolute growth rate (MAGR) is

$$MAGR = \frac{H_i^* K}{2 * (N+2)}$$

In each case, the weighting is proportional to the absolute growth rate.

3.2.6. Experimental Design and Statistical analysis.

The experiment on the effects of temperature on yield were carried out at 3 temperatures (28°C, 33°C, 36°C) x 3 cultivars (Brocks, Larac, UC157) x 12 replications (plants).

The experiment on the effects of temperature on fern and spear development, fern diameter, fern height, fern dry weight and the height to the first branch height of fern was carried out with 3 temperatures (28°C, 33°C, 36°C) x 4 cultivars (Brocks, Larac, Tainan No.1, UC157) x 8 replications.

The analysis of variance and Duncan's multiple range test was used for the treatment mean comparisons. This statistical analysis used the PROC GLM of SAS. Fern height against age was fitted to the Richard Growth's Equation used the PROC NLIN, and the natural logarithm spear height against age was fitted to a simple linear regression used the PROC REG of SAS software released 6.04.

3.3. Results

3.3.1. Spear Yield and Quality at High Temperatures

Overall my results clearly show that the higher the temperature the lower was the spear yield and size as follows,

- 1). Spear yield decreased with increasing temperature (Fig. 3.1A);
- 2). Spear numbers were reduced at 36°C but not at 33°C compared to 28°C (Fig. 3.1B).
- 3). The higher the temperature the lower the mean spear weight (Fig. 3.1C).
- 4). Spear diameter decreased significantly at 36°C compared to 28°C (Fig. 3.1D).

The sensitivity of spear production to temperature varied with cultivars. All three cultivars had a similar yield at 33°C, but Larac produced the lowest spear yield at 36°C and UC157 the highest yield at 28°C (Fig. 3.2A). Brocks, in particular, had a similar spear yield at all 3 temperatures, and had the highest yield at 36°C (though not significantly different from UC157). Moreover, Larac producing the most spears at 28°C and the least spears at 36°C. Both UC157 and Brocks produced stable spear numbers from 28°C to 36°C, and Brocks, Larac and UC157 produced almost the same number of spears at 33°C (Fig. 3.2B).

There were large differences among cultivars at 28°C and 36°C when comparing spear weights and spear diameters among cultivars. Brocks produced the heaviest spear at 36°C and UC157 the heaviest spear at 28°C; Larac the smallest at both 28°C and 36°C (Fig. 3.2C, D), but all cultivars produced similar sized spear at 33°C.

High temperature had the greatest overall effect on the quality of Larac, which must be regarded as the most sensitive to high temperatures, although all

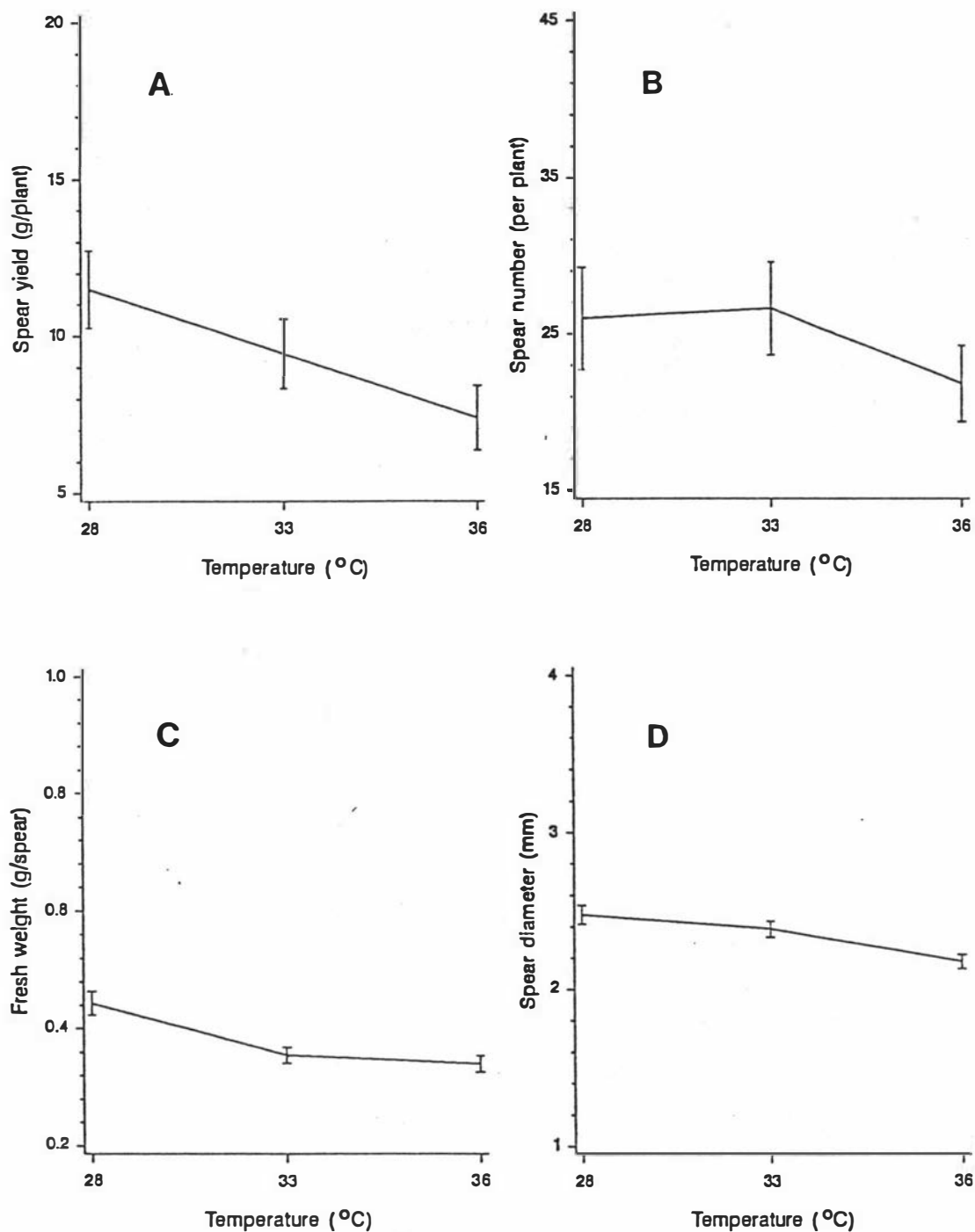


Fig. 3.1. The effects of temperature on (means of three cultivars) (A) spear yield. (B) spear number. (C) spear weight. (D) spear diameter. (Vertical lines indicate standard errors of the means)

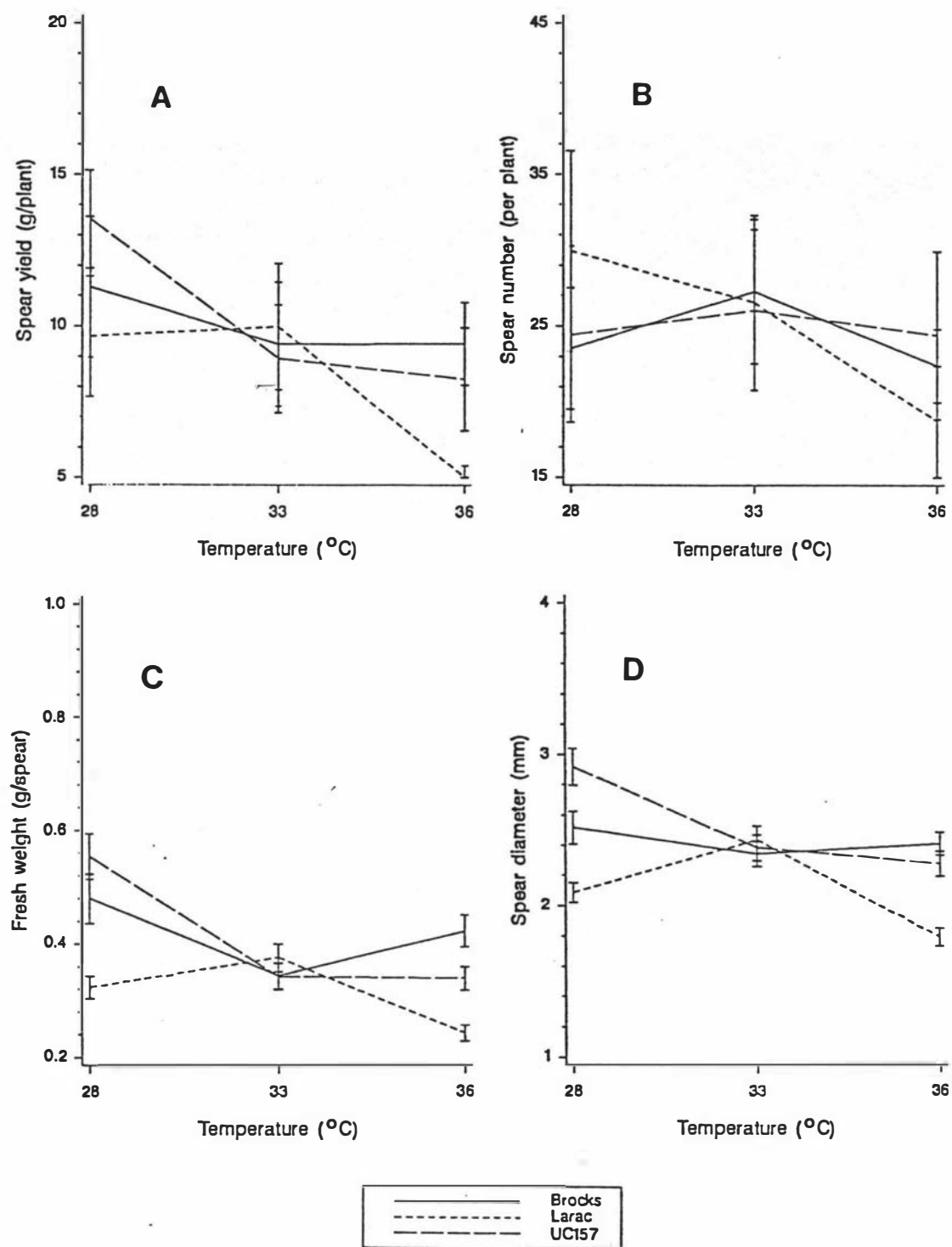


Fig. 3.2. The comparison of cultivars of (A) spear yield. (B) spear number. (C) spear weight. (D) spear diameter.
(Vertical lines indicate the standard errors of the means)

three cultivars produced similar spear quality and yield at 33°C. The spear tips of Larac were not compact at 33°C and 36°C, compared to Brocks and UC157.

In general, plants produced large spears during the early stages and spear size declined with increasing harvest time (Fig. 3.3). This was particularly true for Brocks and UC157 grown at 28°C, where the spears were large during the early stages and small at the later stages, though Larac had a more stable spear size throughout the harvesting period at 28°C. When the plants were grown at 33°C, the spear sizes also declined with increasing harvesting time, but Brocks maintained a stable spear size throughout harvesting periods at 33°C and 36°C. In contrast, at 36°C, the spears of Larac were very small throughout the harvesting period.

3.3.2. Fern Development Characteristics at High Temperatures

3.3.2.1. Fern Characteristics at High Temperatures

When plants were grown at high temperature (36°C), all fern characteristics were very poor (Table 3.1), the first branch height and fern height were low, fern stem diameters were small, fern number was low and the mean individual fern dry weights and total fern dry weights were low. The higher the temperature the greater the depression, but the sensitivity to temperature varied with the parameters. For example fern diameters and total fern dry weights at 33°C were not significantly different from those at 28°C, but were lower at 36°C. The first branch height and individual fern dry weights at 33°C were significantly different from at 28°C, hence the latter (first branch height and fern dry weight) were more sensitive than the former (fern diameter and total fern dry weight) at 33°C. However individual fern weights at 33°C were lighter than at 28°C and heavier than at 36°C, because the plants at 33°C produced more ferns than at 28°C and the total fern weight at 33°C was not different from that at 28°C.

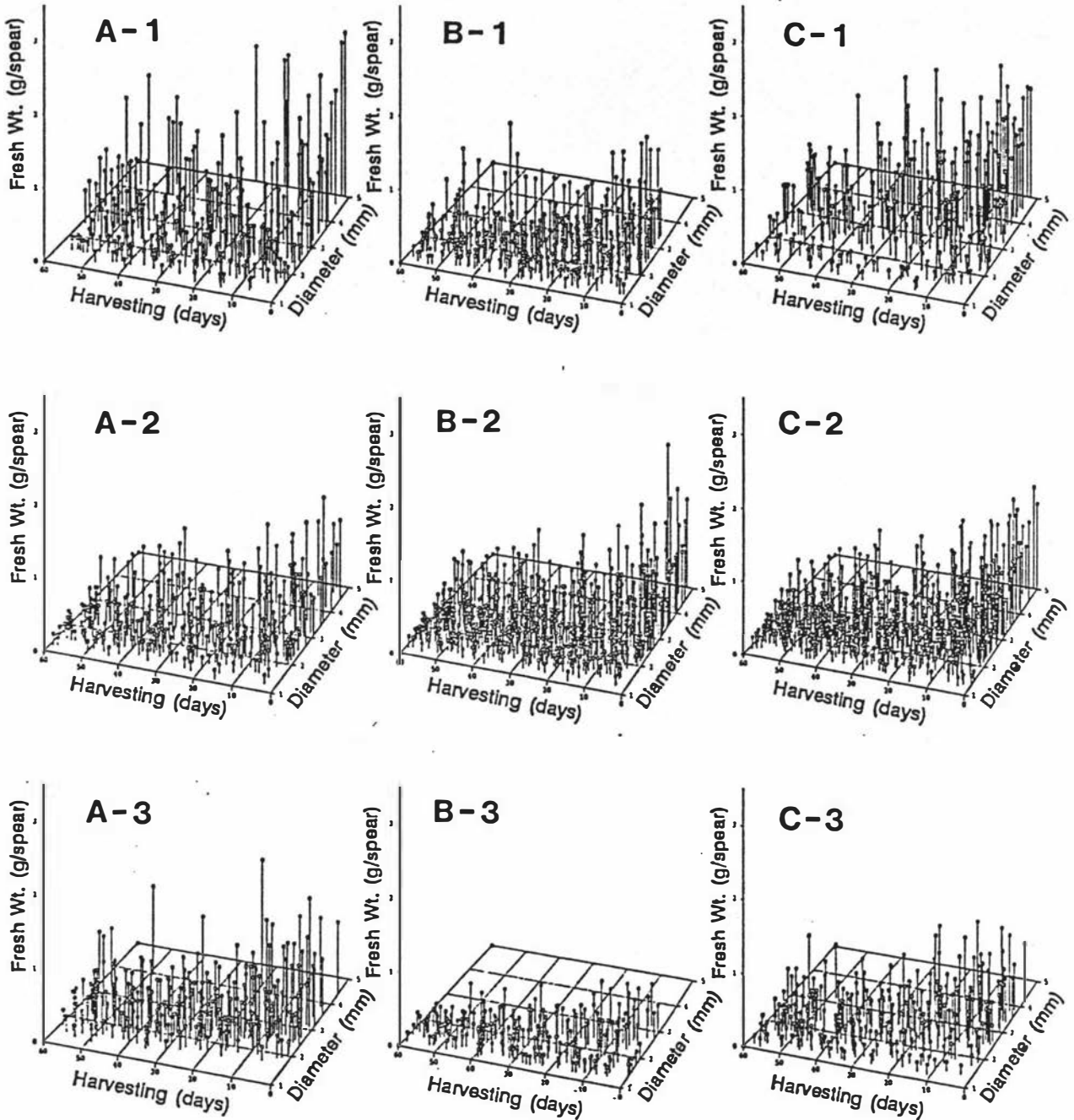


Fig. 3.3. The distribution of spear sizes of harvesting time of

A-1 : Brocks at 28°C. B-1 : Larac at 28°C. C-1 : UC157 at 28°C.

A-2 : Brocks at 33°C. B-2 : Larac at 33°C. C-2 : UC157 at 33°C.

A-3 : Brocks at 36°C. B-3 : Larac at 36°C. C-3 : UC157 at 36°C.

Cultivar comparisons at each temperature showed that the fern characteristics between cultivars at each temperature were also different, except that the individual fern weights were not different among cultivars at either 28°C, 33°C or 36°C. There were also no differences among cultivars for fern diameter, fern height, total fern weight at 28°C, 33°C, 33°C, respectively (Table 3.1). In fact, the fern diameters of all cultivars decreased with increasing temperature except for Brocks which had similar fern diameter from 28°C to 36°C.

Table 3.1. Comparison of the influences of temperature on fern characteristics and weight at 28, 33, 36°C.

Treatment	Diameter of fern (mm)	Height of 1st branch (mm)	Height of fern (mm)	Dry wt. of fern (g/fern)	Total fern dry wt. (g/plant)
Mean of four cultivars					
28°C	2.61 ^a	343 ^a	1048 ^a	1.80 ^a	5.08 ^a
33°C	2.48 ^a	220 ^b	971 ^{ab}	1.26 ^b	4.66 ^a
36°C	2.12 ^b	187 ^b	897 ^b	0.88 ^c	3.37 ^b
28°C					
Brocks	2.70 ^a	342 ^{ab}	975 ^{ab}	1.47 ^a	4.38 ^b
Larac	2.32 ^a	275 ^b	916 ^b	1.48 ^a	5.00 ^{ab}
Tainan No.1	2.68 ^a	320 ^b	1120 ^{ab}	2.20 ^a	4.60 ^{ab}
UC157	2.76 ^a	435 ^a	1180 ^a	2.08 ^a	6.35 ^a
33°C					
Brocks	2.80 ^a	247 ^{ab}	1047 ^a	1.47 ^a	4.12 ^a
Larac	2.30 ^b	169 ^c	942 ^a	1.20 ^a	4.74 ^a
Tainan No.1	2.33 ^b	253 ^a	985 ^a	1.38 ^a	4.60 ^a
UC157	2.48 ^{ab}	212 ^b	911 ^a	1.00 ^a	5.17 ^a
36°C					
Brocks	2.65 ^a	210 ^a	1051 ^a	1.05 ^a	3.01 ^b
Larac	2.05 ^b	147 ^b	858 ^b	0.79 ^a	2.72 ^b
Tainan No.1	1.71 ^b	184 ^a	776 ^b	0.76 ^a	3.10 ^b
UC157	2.08 ^b	209 ^a	902 ^{ab}	0.92 ^a	4.64 ^a

Within an experiment, numbers followed by the same letter are not different at the 5% level using Duncan's Multiple-range test.

In general, the first branch height of fern of all cultivars gradually decreased from 28°C to 36°C but Brocks dramatically decreased at 33°C. At 28°C UC157 had the highest first branch, while Larac had the lowest first branch at all three temperatures. UC157 had the largest decreasing height of first branch from 28°C to 36°C, but as UC157 had a very high first branch at 28°C, it still had a high first branch at 36°C. These results are consistent with Larac producing poor quality spears and UC157 still producing good quality spears at high temperature.

The height and weight of ferns of Tainan No.1 and UC157 were higher than other cultivars at 28°C, but Brocks produced the tallest and heaviest ferns at 33°C and 36°C. Because Brocks produced a smaller number of ferns, the total fern weight of UC157 was heavier than for Brocks. In general, Brocks and UC157 appeared to have fairly stable fern characteristics at supra-optimal temperature (36°C). Although Tainan No.1 produced good quality spears and high first branch of fern, the fern height became very low, and individual fern weight and total fern weight also dramatically decreased at supra-optimal temperatures compared with UC157.

3.3.2.2. Spear Growth at High Temperatures

The $RSGR_{100}$ of spear from 10 to 100 mm and $RSGR_{200}$ of spear from 10 to 200 mm represent spear development over a definitive development phase. It appeared that spears from 10 to 100 mm or from 10 to 200 mm showed simple exponential growth, because the natural logarithm spear height against age was a simple linear regression ($r^2 > 0.92$). The $RSGR$ decreased with increasing height because $RSGR_{200}$ was slightly lower than $RSGR_{100}$ (Table 3.2). The $RSGR_{100}$ at 33°C was significantly higher than at 28°C and at 36°C. The $RSGR_{200}$ at 36°C was significantly lower than at 28°C and 33°C, thus the differences between $RSGR_{100}$ and $RSGR_{200}$ are larger at 33°C and at 36°C than at 28°C. This means that the $RSGR$ from 100 to 200 mm at 33°C and at 36°C had more inhibition compared to that at 28°C. When comparing $RSGR_{100}$

and $RSGR_{200}$ among cultivars at each temperature Larac showed a smaller difference between $RSGR_{100}$ and $RSGR_{200}$ than the other cultivars at both 28°C and at 33°C. However, the difference between $RSGR_{100}$ and $RSGR_{200}$ for Larac at 36°C was similar to the other cultivars (Table 3.2.). The differences between $RSGR_{100}$ and $RSGR_{200}$ were similar for Brocks, Tainan No.1 and UC157 at 28°C and 33°C. At 28°C and 36°C these $RSGR_{100}$ or $RSGR_{200}$ there were no significant differences between cultivars, but at 33°C there were significant differences between cultivars, as Tainan No.1 had the highest $RSGR_{100}$ and $RSGR_{200}$, while Larac and UC157 had the lowest at 33°C.

Table 3.2. Comparison of the influences of temperature on $RSGR$ of spear growth from 10 to 100 mm or 10 to 200 mm at 28, 33, 36°C.

Treatment	$RSGR_{100}$ (per day)	$RSGR_{200}$ (per day)	Difference $RSGR_{100} - RSGR_{200}$
<u>Means of four cultivars</u>			
28°C	0.88 ^b	0.82 ^a	0.06
33°C	0.98 ^a	0.86 ^a	0.12
36°C	0.87 ^b	0.73 ^b	0.14
<u>28°C</u>			
Brocks	0.86 ^a	0.78 ^a	0.08
Larac	0.84 ^a	0.81 ^a	0.03
Tainan No.1	0.97 ^a	0.90 ^a	0.07
UC157	0.86 ^a	0.77 ^a	0.09
<u>33°C</u>			
Brocks	1.05 ^{ab}	0.92 ^{ab}	0.13
Larac	0.85 ^b	0.77 ^{bc}	0.08
Tainan No.1	1.21 ^a	1.06 ^a	0.15
UC157	0.85 ^b	0.72 ^c	0.13
<u>36°C</u>			
Brocks	0.84 ^a	0.71 ^a	0.13
Larac	0.92 ^a	0.77 ^a	0.15
Tainan No.1	0.94 ^a	0.77 ^a	0.17
UC157	0.79 ^a	0.68 ^a	0.11

Within an experiment, numbers followed by the same letter are not significantly different at the 5% level using Duncan's Multiple-range test.

RGR_{100} : RGR of spear growth from 10 to 100 mm.

RGR_{200} : RGR of spear growth from 10 to 200 mm.

3.3.2.3. Fern Growth at High Temperatures

The Richard's Growth Equation provided a good fit of fern height against age. In the analysis of fern growth, these curves reveal the differences between temperatures and cultivars, and the parameters K , N , H_t , H^i , and D^i can be used to interpret fern growth. The relationships (r^2) between parameters of K , N , H_t are for K and N , K and H_t , N and H_t ; 0.930 to 0.909, -0.865 to -0.727, -0.690 to -0.553, respectively, hence K and N are strongly positively correlated, and K and H_t are negative correlated and N and H_t are weakly negatively correlated.

The mean parameter N of fern at 28, 33 and 36°C are 0.214, -0.189 and -0.242, respectively, thus the fern growth is close to the Gompertz model ($N=0$), but the higher the temperature the lower the magnitude of N . Also the magnitude varied with cultivar. The fern at 28°C is predicted to give the highest (H_t) and have the highest inflexion point of fern (H^i) and (D^i) (Table 3.3. Fig. 3.4). The comparison of fern development between cultivars at each temperature shows that Tainan No.1 and UC157 are predicted to produce taller fern than other cultivars at 28°C, while Brocks and Tainan No.1 produce taller ferns than others at 33°C, and Brocks grows the tallest fern at 36°C. As well it was found that the final height of fern is associated with a high inflexion point (H^i), but the time to the inflexion point (D^i) is not closely associated with H_t and H^i .

In fact, the time to the inflexion point (D^i) decreased with increasing temperature for Brocks, hence the fern growth of Brocks reached the inflexion point in 2.3 days at 36°C. Although the D^i for UC157 also decreased with increasing temperature, it was not reduced as much as for Brocks. In fact, the fern growth of all cultivars except cv. Brocks reached the inflexion point at 4.3 to 4.8 days at 36°C.

At 33°C the mean WMRGR of ferns was higher than at 28°C and at 36°C, but the mean MAGR of ferns decreased with increasing temperature. In fact WMRGR and MAGR changed with temperature and genotypes, for example, at 33°C the WMRGR and MAGR of fern of Tainan No.1 were extreme high and of UC157 were low, this result coincided with spear growth. Generally the MAGR of ferns of each cultivars decreased with increasing temperature, but at 33°C all cultivars had a higher WMRGR than at 28°C and 36°C. Furthermore, the MAGRs of Larac and UC157 markedly decreased from 28°C to 33°C, while Brocks and Tainan No.1 markedly decreased from 33°C to 36°C. At 28°C Tainan No.1 and UC157 had high a MAGR. Therefore it

Table 3.3. The parameters of K, N, H_i (mm) and predicted inflexion points of fern developing (H^i , mm), aging (D^i , day), weighted mean relative growth rates (WMRGR per day) and mean absolute growth rates (MAGR mm / day) from Richard growth equation for each temperature.

Cultivars	K	N	H_i	H^i	D^i	WMRGR	MAGR
<u>28°C</u>							
Brocks	0.389	0.281	934	387	7.0	0.304	79.65
Larac	0.423	0.307	878	367	6.5	0.324	80.54
Tainan No.1	0.304	-0.062	1185	422	5.6	0.324	92.89
UC157	0.373	0.330	1130	476	7.6	0.280	90.37
Mean	0.372	0.214	1032	413	6.7	0.308	85.86
<u>33°C</u>							
Brocks	0.291	-0.120	1043	359	5.3	0.330	80.63
Larac	0.249	-0.227	874	281	3.2	0.332	61.24
Tainan No.1	0.295	-0.277	1009	313	3.8	0.407	86.23
UC157	0.253	-0.131	860	295	5.9	0.291	58.12
Mean	0.272	-0.189	947	312	4.6	0.338	71.56
<u>36°C</u>							
Brocks	0.215	-0.242	999	318	2.3	0.284	61.05
Larac	0.191	-0.407	750	208	4.3	0.322	44.89
Tainan No.1	0.298	-0.131	757	259	4.9	0.342	60.27
UC157	0.236	-0.187	833	275	4.8	0.290	54.10
Mean	0.235	-0.242	835	265	4.1	0.310	55.08

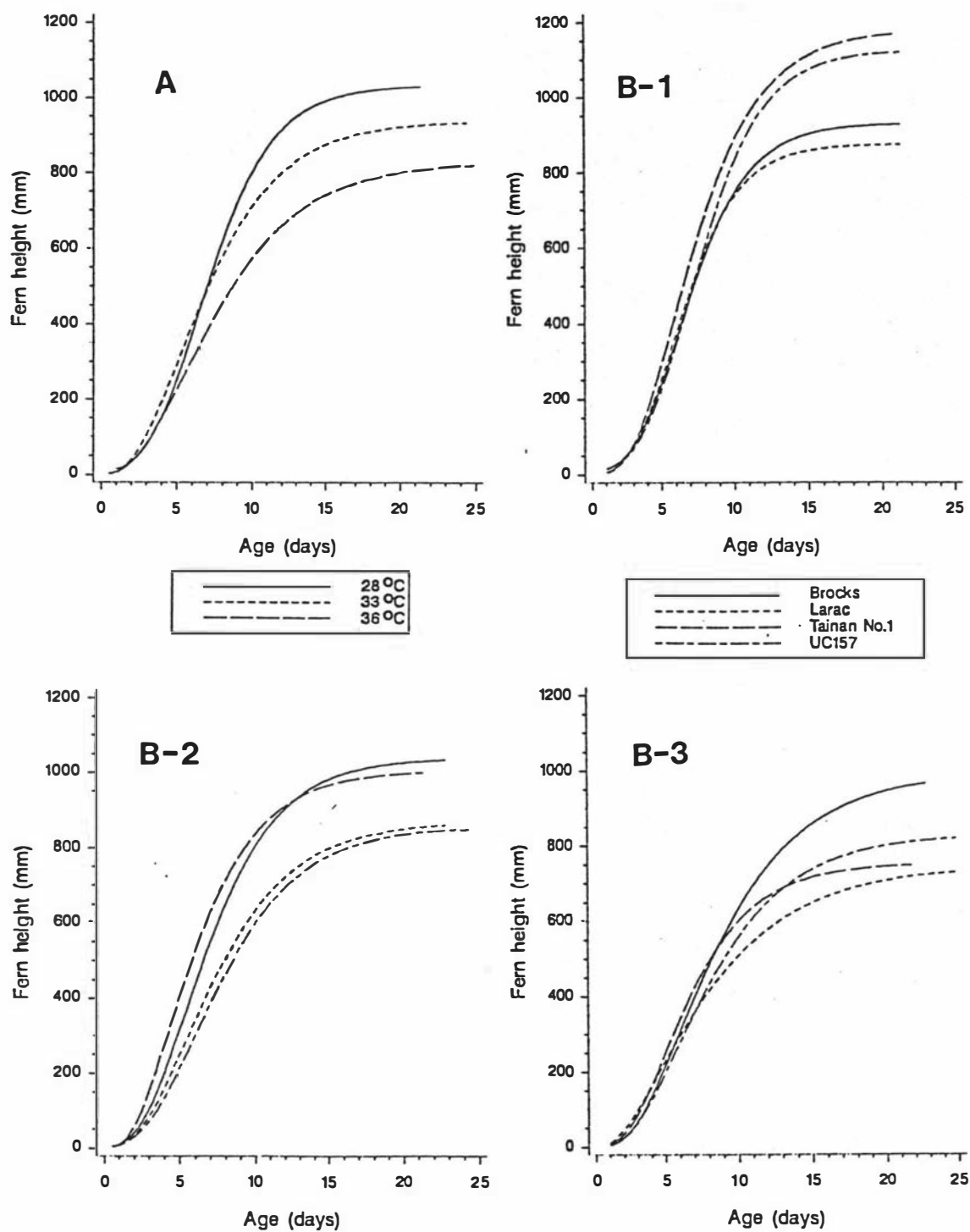


Fig. 3.4. Comparison of the effects of temperatures on fern development (A) at 28, 33, 36°C (means of four cultivars). (B) between cultivars at 28°C. (C) between cultivars at 33°C. (D) between cultivars at 36°C.

appeared that the fern growth of Brocks and Tainan No.1 could tolerate a higher temperature than Larac and UC157. Moreover Tainan No.1 had a higher WMRGR and MAGR than Brocks from 28°C to 36°C although at 36°C both had a similar MAGR, thus, generally, Tainan No.1 had faster growth than Brocks at all temperature regimes.

3.4. Discussion

In the conventional cultivation of asparagus the plant is without fern during the harvesting period, therefore carbohydrates for spear production and for the initial fern growth after harvest come entirely from the storage roots, and the yield of spears is determined mainly by reserve carbohydrate, carbohydrate remobilization, and bud flush. Usually only a small number of the buds will grow into spears and the remaining buds remain 'dormant'. Therefore the number of buds is not usually a limiting factor on yield. In my experiment, the one year old plants had plenty of buds on the crown, so consequently, the bud flush and the following development were the main factors which determined the yield at various temperature regimes. Previous work has shown that temperature influences bud flush and fern development of asparagus (*Culpepper and Moon 1939a,b; Blumenfield et al. 1961; Bouwkamp and McCully 1975; Lampert et al. 1980; Hartmann 1985; Nichols and Woolley 1985*). Even alfalfa plants were significantly depressed in shoot number and shoot length by high temperature (*Pulgar and Laude 1974*). The reason why plants have fewer shoots at high temperature may be due to the older ferns producing hormones, e.g. abscisic acid, to inhibit shoot flush and growth (*Stewart et al. 1980*). In my experiment, the results of bud flush and development were found to be similar to the work of *Culpepper and Moon (1939a,b; Blumenfield et al. (1961); Bouwkamp and McCully (1975) and Hartmann (1985)*, who showed that temperature strongly influenced spear number, yield, average weight and diameter. The responses of these components to temperature also varied with cultivars (Table 3.1).

In fact, the duration of spear development is strongly determined by temperature, hence spear diameter and weight, fern diameter, weight and height are associated strongly with temperature. Spear numbers may be associated with RSGR, because spears having a high RSGR (at 33°C) reached the harvesting height early and were then harvested. This stimulated the next spear flush. This result may be the reason why spear numbers at

33°C did not decrease compared to those at 28°C and at 36°C.

In general the 28°C regime appeared the most favourable temperature for asparagus to grow spears for all cultivars in the experiment, because at that temperature asparagus plants produced the highest yield and size of spears. However the yield and size of spears depends strongly on the cultivar (Fig. 3.1, 3.2), because spear yield is the product of spear number and spear weight. For example, at 28°C UC157 produced the largest spears, and thus the highest yield, even though it had a smaller number of spears than Larac. At 33°C the plants of all cultivars produced nearly the same yield and size of spears, but at 36°C Larac dramatically decreased in both spear yield and size. Hence, Larac represents a poor adaptation to the high temperature of 36°C, while Brocks and UC157 are well adapted to high temperatures of 33°C and 36°C. It is postulated that when plants have adequate reserves of carbohydrates in the crown, the genotype and environment become important, for example Brocks and UC157 maintain nearly the same yield and size of spears at 36°C as at 33°C, but Larac decreased dramatically in both yield and size. This is probably because UC157 and Brocks were developed in warmer California rather than in cooler France (Larac), and thus Brocks and UC157 are better adapted for use in the tropics. However, the reason why Brocks produced the heaviest spear at 36°C is a genetic characteristic, because Brocks produces large spears at all temperatures.

Because fern growth follows spear development, the responses of fern diameter, individual fern dry weight, total fern weight to temperature are similar to the responses of spear yield and size to temperature, i.e. high temperatures depress all the above terms. This is similar to results with the work in alfalfa and tall fescue (*Smith 1969; Pulgar and Laude 1974; Smith 1977*), who found that high temperature stress depressed shoot number and length, leaflets, stem and total herbage.

The responses of spear production and fern growth to temperature may be similar to those of grain growth. Grain size depends on mechanisms of carbohydrate importation and utilization, which are genetically inherited, and on the duration of this carbohydrate flow, which is very dependent on temperature (*Fussell et al.* 1980). Consequently, it is proposed that spear yield and size, fern diameter, height and weight are also very dependent on the genotype and temperature. High temperature stress may shorten the duration of fern growth and thus decrease the weight and height of fern (Table 3.1, 3.3). The results are similar to the work of *Arnold* (1969) on stem growth of corn, who showed that the higher the temperature the greater the depression of elongation of internodes. Though high temperature increased the rate of grain filling, it greatly reduced the duration of the grain filling period, whereas low temperature exerted a reverse response (*Wardlaw* 1970; *Wardlaw et al.* 1980; *Jones et al.* 1981; *Badu-apraku et al.* 1983; *Johnson and Kanemasu* 1983; *Al-Khatib and Paulsen* 1990). In my experiment, spear growth exhibited the highest rate of elongation at 33°C, and at supra-optimal temperature of 36°C the rate of spear elongation was depressed and caused the early cessation of growth.

It has been proposed that high temperature can accelerate the synthesis of starch and proteins, and the rate of cell division (*Egli and Wardlaw* 1980; *Jones et al.* 1981; *Nicolas et al.* 1984; *Carberry and Abrecht* 1990; *Muchow* 1990; *Dombos and Mullen* 1991). Thus at high temperature ferns had a fast rate of elongation, but the duration of the fern elongation period was shorter as shown by the smaller inflexion points of fern development (H^i) and age (D^i), resulting, therefore, in a small fern at maturity. The cessation of fern growth at 36°C may, in part, be related to the problem of unloading sucrose from the root into the fern cells, because the rate of activity of a bud is influenced mainly by the supply of assimilate and its mobilization into new units, which is determined by temperature (*Thiagarajah and Hunt* 1982).

It has been suggested that heat stress inhibits sucrose import from source, and retards the supply of available glucose necessary for processes requiring carbohydrate (*Dinar et al.* 1983; *Dinar and Rudich* 1985b). *Dinar and Rudich* (1985b) showed that heat tolerant cultivars of tomato are able to hydrolyse sucrose more efficiently than heat sensitive cultivars, because its invertase activity was heat stable. Hence it may be postulated that the difference of either spear yield or fern production between temperature regimes or among cultivars is due to a difference in invertase activity. Thus it is suggested that the effect of temperature on fern may be directly on the plant itself, due to temperature affecting the ability of the plant to supply carbohydrates to new fern, because the carbohydrate pool in the root is adequate. On the contrary, the work of *Thomas and Raper* (1977) and *Ong* (1983b) showed that growth reduction at high temperatures was caused by depressing cell numbers, and not to lack of carbohydrates.

The responses of fern characteristics to temperature varied with cultivars. Characteristics such as the first branch height and fern height were very sensitive and depressed at high temperature. Brocks and UC157 maintained good fern characteristics at 36°C, thus both cultivars had a good adaption to high temperature. Conversely Larac was the poorest adapted while Tainan No.1 was also well adapted except for its small fern diameter and short fern. In particular it produced a high first branch and good spear quality.

It is concluded that all cultivars, with the exception of Larac, are adapted to tropical climates and are able to produce marketable spears at high temperature, possibly because Brocks, Tainan No.1 and UC157 were selected in warmer areas than Larac. It is, therefore, postulated that asparagus possesses a capability to adapt to warm climates and the characteristics of spear and fern can also be improved by genetic selection. In fact, it is well known that asparagus can adapt to other stressful environments, e.g. salinity, drought, and possibly high temperature tolerance may also be associated with the ability to tolerate other stresses.

The Richards growth equation is empirical and has a generality that may be an advantage. When the parameter N = negative : near 0 : or positive the function encompasses the monomolecular: Gompertz : logistic equations, respectively (*France and Thornley 1984*). Therefore the growth of fern may be designated to any growth model by the parameter N . In the logistic equation, it is assumed that the quantity of growth machinery is proportional to size, and growth is modified by substrate availability. In the Gompertz equation, it is assumed that the quantity of growth machinery is proportional to size, and the effectiveness of the growth machinery decays with time; this decay may be due to senescence or development. In the monomolecular equation, it is assumed that the quantity of growth machinery is constant and independent of size, and the growth is also modified by substrate availability. In simple exponential growth, it is assumed that the quantity of growth machinery is proportional to size, and the growth works at maximal rate so long as there is substrate available (*France and Thornley 1984*). In my studies the fern growth of asparagus overall follows the Gompertz growth model due to the parameter N being close to 0, whereas spear growth (up to 200 mm) follows simple exponential growth from 28 to 36°C as the logarithm of spear height against age was a simple linear regression. Thus spear growth rate (up to 200 mm) decreased at high temperature due to factors such as hormone levels rather than the availability of carbohydrate, as the roots of asparagus had adequate carbohydrate reserves and spear growth appeared very close to simple exponential growth ($r^2 > 0.92$). In contrast, the decline of fern growth with increased temperature was probably due to hastened development.

Fern growth (from spear to full expansion) generally followed the Gompertz growth model (as N was close to 0). However at the lower temperature fern growth followed the logistic growth model ($N \rightarrow$ positive), while at high temperature the fern growth followed the monomolecular growth model ($N \rightarrow$ negative). Furthermore, the type of fern growth varied with genotype, for example at high temperature (36°C) the fern growth of Larac tended to the

monomolecular growth model ($N = -0.407$) compared to other cultivars (N around -0.2), but at cooler temperature (28°C) the fern growth of Brocks, Larac and UC157 tended to the logistic growth model (N around 0.3), while Tainan No.1 followed the Gompertz growth model ($N = -0.062$). However the growth rate of both was proportional to height.

The inflexion point for both height and time of fern development might reveal the responses of fern development to high temperature, as high temperatures led to a much lower inflexion height and earlier inflexion age, perhaps because fern at high temperature reaches its maximum rate of cell division early, then a slow down in cell division leads to this final low height of fern.

Generally the changes in RSGR from 28 to 36°C were similar to the changes in WMRGR, but not similar to MAGR. Therefore WMRGR might be associated with RSGR. The higher the temperature the lower was the final fern height, the lower was the inflexion point of fern development (height and age). This coincided with the changes of MAGR from 28 to 36°C , therefore MAGR might provide a good description of fern development dynamics.

The patterns of fern development changed with temperature. Generally, the higher the temperature the lower was the H_f , H^i , D^i and MAGR of fern in this experiment. Thus a stable H_f , H^i , D^i , WMRGR and MAGR of fern at high temperature means that the cultivars had good adaptive ability. Overall Tainan No.1 had good parameters derived from the Richards equation, and Brocks also had stable parameters from 28 to 36°C . Thus from the parameters of Richard's Equation and of the RSGRs, the ability of adaptation to high temperature was in order: Tainan No.1 > Brocks > UC157 > Larac.

CHAPTER FOUR

Influences of Exogenous ABA on Asparagus Seedling Growth at High Temperatures

4.1. Introduction

Heat tolerance in plants involves physiological adaptation. ABA may regulate physiological processes and thus adapt plants to heat stress environments. In fact, ABA is considered to be a plant stress hormone, but the effect and mechanism of ABA on heat tolerance is still not clear. The damage from heat stress is different to that from other stresses, e.g. cold stress, or water stress, as heat stress accelerates physiological processes, while cold stress slows them down (*Levitt 1980*). Previous work has shown that exogenous ABA may protect plants against heat stress or enhance a plant's recovery from heat damage (*Bonham-Smith et al. 1988a,b; Itai et al. 1978*). Moreover it was suggested that the elevated ABA content (exogenous application or stress induced) has little effect on growth of an ABA insensitive genotype but enhanced heat tolerance, and decreased the growth of the ABA sensitive plants. Thus a genotype, which is ABA insensitive, may produce a better performance compared to a sensitive genotype under stress environments (or with exogenous ABA), as ABA triggers the mechanism of heat resistance and results in less phenotypic changes in ABA insensitive genotype. Thus genetic differences in ABA insensitivity are apparently associated with stress resistance, and heat tolerance may be increased with insensitivity to ABA (*Lu et al. 1989*). Hence the role of ABA in heat tolerance should be concerned with genetic insensitivity and the level of high endogenous ABA content in plants.

In my experiment, the effect of exogenous ABA on asparagus seedling growth at high temperature was investigated to see whether genetic differences to ABA sensitivity might be associated with stress resistance, and whether low ABA concentrations would improve seedling growth in heat stress environments.

4.2. Materials and Methods

4.2.1. Plant Materials

Four cultivars of asparagus, Brocks, Larac, Tainan No.1 and UC157 were used for the experiment. The seeds of the 4 cultivars were soaked in distilled water for 2 days in an incubator at 24°C, then placed on several layers of tissues (moistened with distilled water), in a box to germinate for 8 - 12 days.

4.2.2. Seedling Growth with ABA at High Temperatures

When the roots of the seedlings had grown 2-3 cm, they were placed on germination paper¹, which had previously been moistened with the desired concentration of ABA solutions. After loading with 10 seedlings, each germination paper was rolled up immediately, enclosed in a plastic bag to ensure high humidity, and then placed in incubators set at 28±1°C, 33±1°C, 34.5±1°C and 36±1°C. 10 days later the seedlings were separated into components of shoot and root. and the length and weight of shoot and root were measured.

ABA² was pre-dissolved in ethanol (95%) and then diluted with distilled water to the desired concentrations of 0 µM, 0.1 µM, 1 µM, 10 µM, 100 µM ABA and then the solution was adjusted to 0.1% ethanol. The germination papers were then moistened with the desired concentrations.

4.2.3. Experimental Design and Statistic Analysis.

The experimental design was 3 temperatures x 5 ABA concentrations x 4

¹ Anchor paper, code 612-298-1311, 480 Broadway, P. O. Box 3648, Saint Paul, N 55165, USA

² purchased from Aldrich, (±)-2-cis, 4-trans-abscisic acid, 98%, synthetic.

replications except that the treatment at 36°C was replicated six times. Each replication had 10 seedlings. 5 seedlings were sampled from each replication to measure weight and length of shoot and root-crown.

The inhibition ratio was based on the absolute measurement of the treatment compared to the control (at 28°C and 0 μ M ABA).

An analysis of variance was used to determine the influences of high temperature and ABA concentration on seedling growth. The PROC GLM of SAS software release 6.04 was used for statistical analysis.

4.3. Results

4.3.1. Analysis of Variance of the Influences of ABA, Temperature and Cultivars on Seedling Growth

Root weight and length, and shoot weight and length were significantly influenced by cultivar, ABA, and temperature as well as by all interactions except that between ABA, temperature and cultivar on shoot weight (Appendix 9A, 9B, 9C, 9D). Specifically, temperature and ABA were very significant.

Furthermore, the effects of temperature, ABA and their interaction on seedling growth for each cultivar were also significant (Appendix 10A, 10B, 10C, 10D) except the interaction between ABA and temperature on the root weight of Brocks and Larac. Therefore it is concluded that the growth of seedlings was strongly influenced by ABA, temperature, cultivar and their interactions.

4.3.2. Comparison of Seedling Growth at High Temperatures and Exogenous ABA

Generally the higher the temperature or the higher the ABA concentration the greater the inhibition of seedling growth. However the extent of inhibition of seedling growth at high temperature varied with the plant component. Overall ABA inhibited seedling growth, although improved seedling growth occurred at some ABA concentrations. The effects of inhibition or improvement varied with temperature, plant component and cultivar. For example, root weight was less inhibited than root length, shoot weight and shoot length which were all strongly inhibited. Root weight was relatively insensitive to ABA treatments (Fig. 4.1). At 36°C and 34.5°C the extent of inhibition showed little difference between root lengths or shoot lengths irrespective of ABA treatments (except that at 34.5°C shoot length was significantly more sensitive than root length at 1.0 μ M ABA). At 28°C and 33°C the inhibition of shoot length was more than for root length irrespective of ABA treatment. In addition, at 34.5°C there was

a great variation of inhibition between components, for example at 34.5°C the inhibition of 0.1 μM ABA on root weight was significantly more than shoot weight, whereas the extent of root weight inhibition was significantly less at 1.0 to 100 μM ABA.

4.3.2.1. Root Growth at High Temperatures and Exogenous ABA

The effect of temperature on root weight was greater than that of ABA or cultivar (Fig. 4.2). The higher the temperature the greater were root weights inhibited, and root weight was insensitive at 0.1 to 10 μM ABA. Root weights were increased by specific ABA concentration from 0.1 μM to 10 μM depending on cultivar and temperature. Thus the responses of root weight to ABA varied with temperature and cultivar.

Comparing the root weight inhibited ratio between cultivars at each temperature showed that UC 157 was the least inhibited by temperature and ABA (Fig. 4.3). At 34.5°C the root weight of Tainan No.1 was greatly inhibited (similar to at 36°C) irrespective of ABA treatments, therefore the root weight of Tainan No.1 was more sensitive than other cultivars. On the contrary, UC157 was less sensitive to 0.1 to 1.0 μM ABA than other cultivars except at 36°C. At 34.5°C UC157 and Brocks root weight was enhanced by 1.0 μM ABA, while Tainan No.1 was severely inhibited and could be not improved by ABA treatment. At 33°C Tainan No.1 and Brocks were improved by 0.1 μM ABA. At 33°C, 34.5°C, 36°C Larac was improved by 1 - 10 μM , 0.1 - 10 μM , 1.0 μM respectively. Generally, the root weight markedly declined with the 100 μM ABA treatment.

The effect of ABA and high temperature on root length was greater than on root weight, because root elongation was strongly inhibited by temperature and ABA, leading to short roots (Fig. 4.4). In general, the seedlings grown at 28°C had the longest roots, and the root length decreased with increasing temperature. Root length also decreased with increasing ABA concentration.

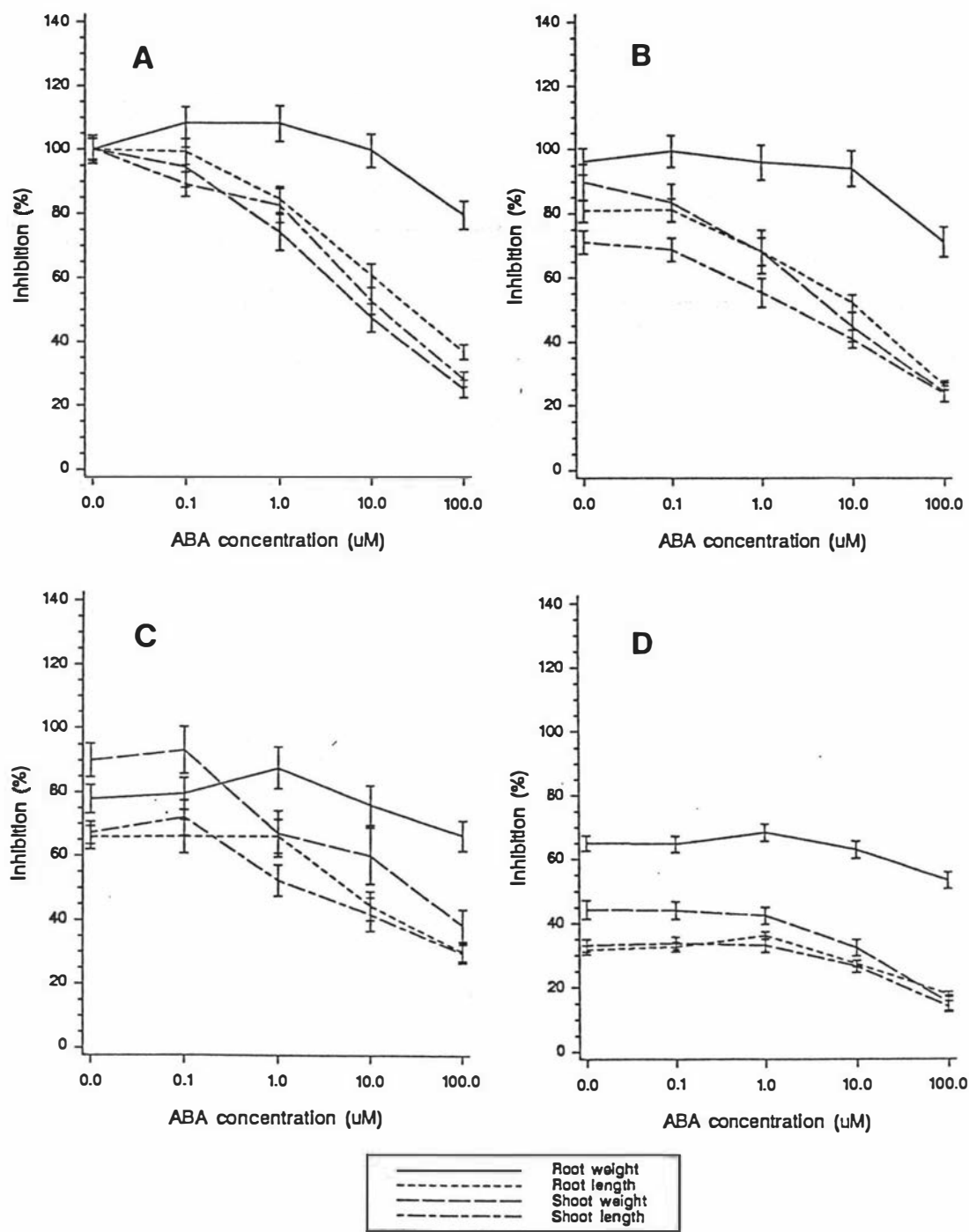


Fig. 4.1. Comparison of relative growth among plant components (means of four cultivars) at high temperatures and exogenous ABA (A) at 28°C. (B) at 33°C. (B) at 34.5°C. (D) at 36°C. (Vertical lines indicate the standard errors of the means; 100 = 0.0 μM ABA at 28°C)

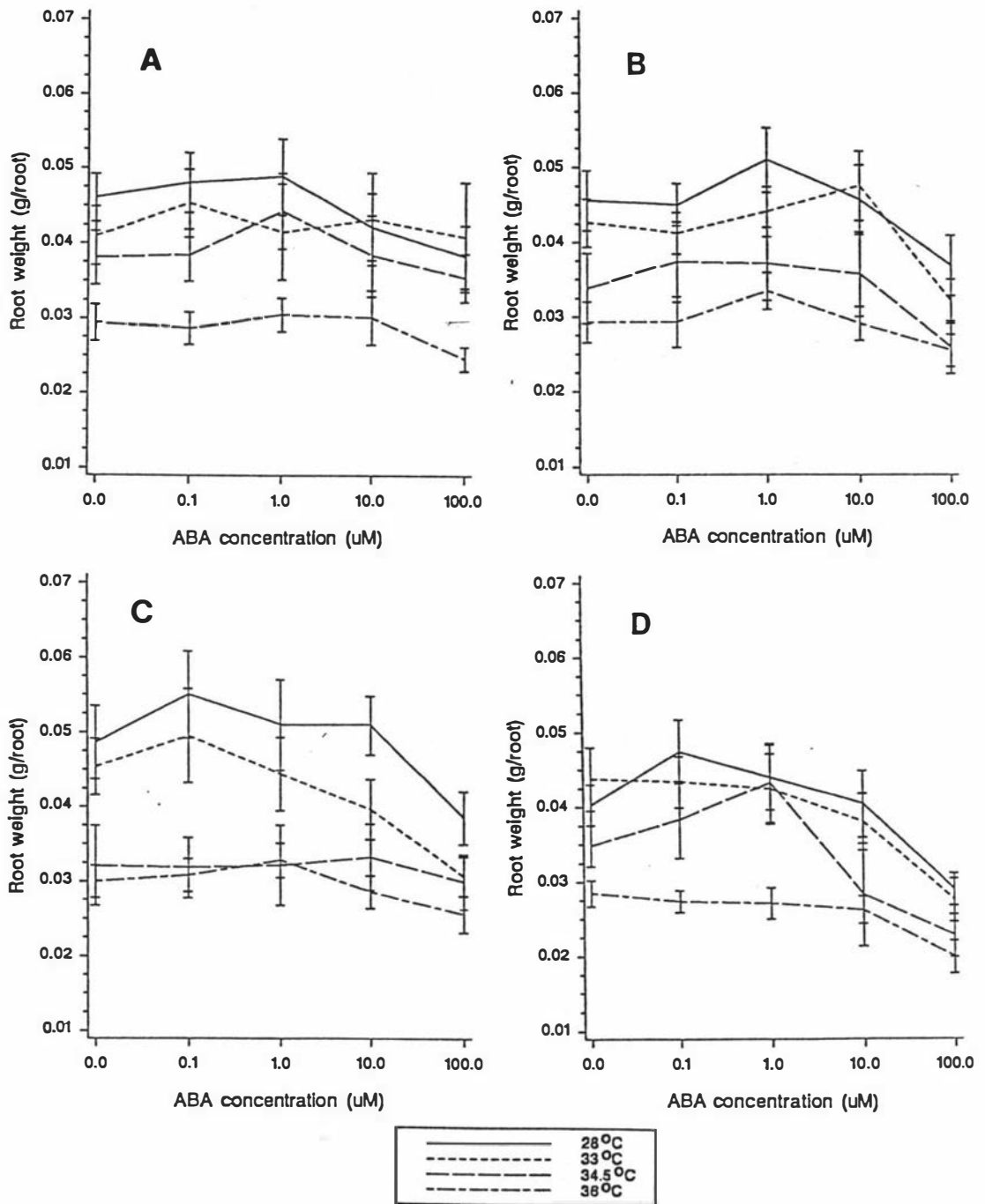


Fig. 4.2. The effects of high temperature and exogenous ABA on root weight of (A) Brocks. (B) Larac. (C) Tainan No.1. (D) UC157. (Vertical lines indicate the standard errors of the means)

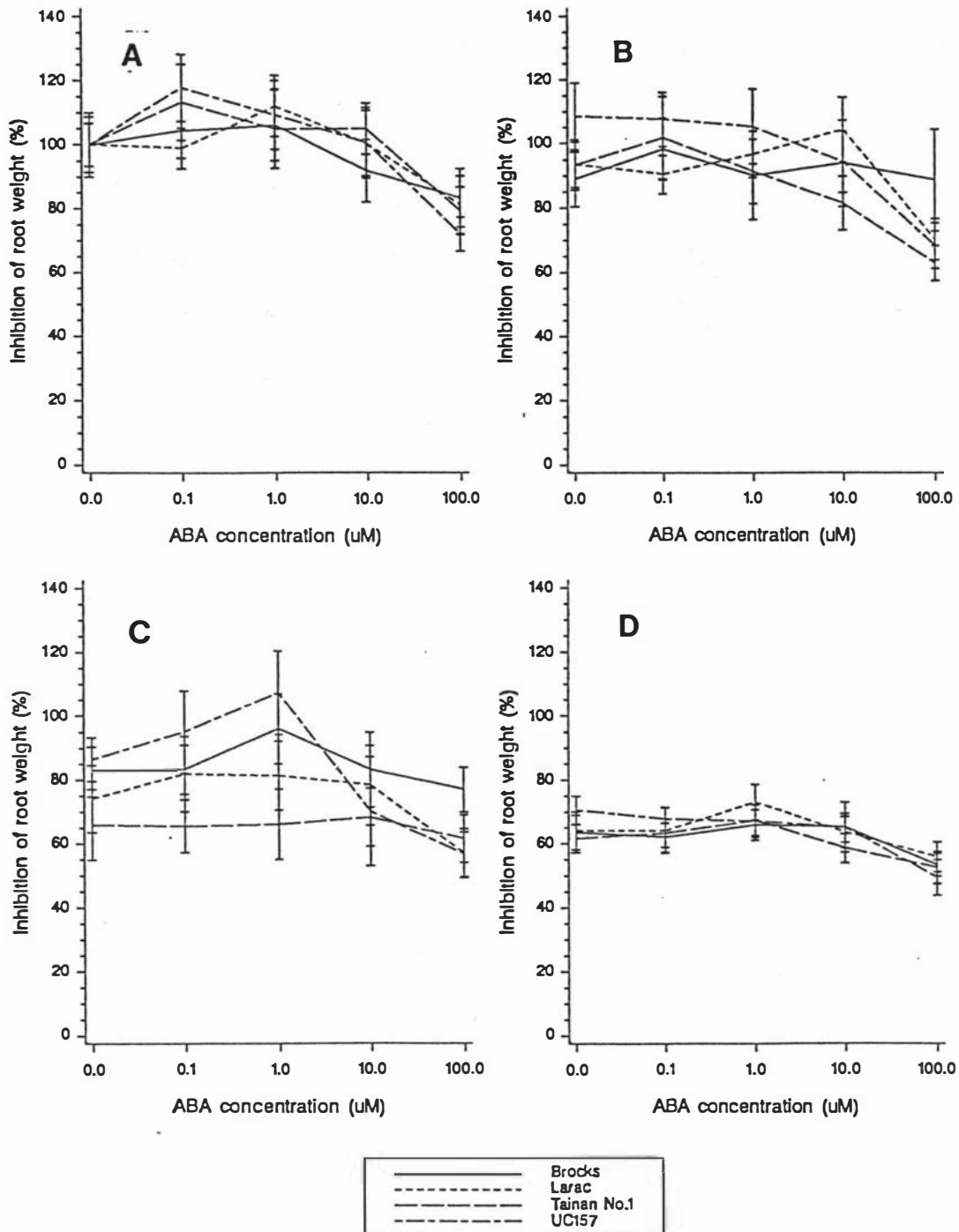


Fig. 4.3. Comparison of root weight among cultivars (A) at 28°C. (B) at 33°C. (C) at 34.5°C. (D) at 36°C. (Vertical lines indicate the standard errors of the means; 100 = 0.0 μM ABA at 28°C)

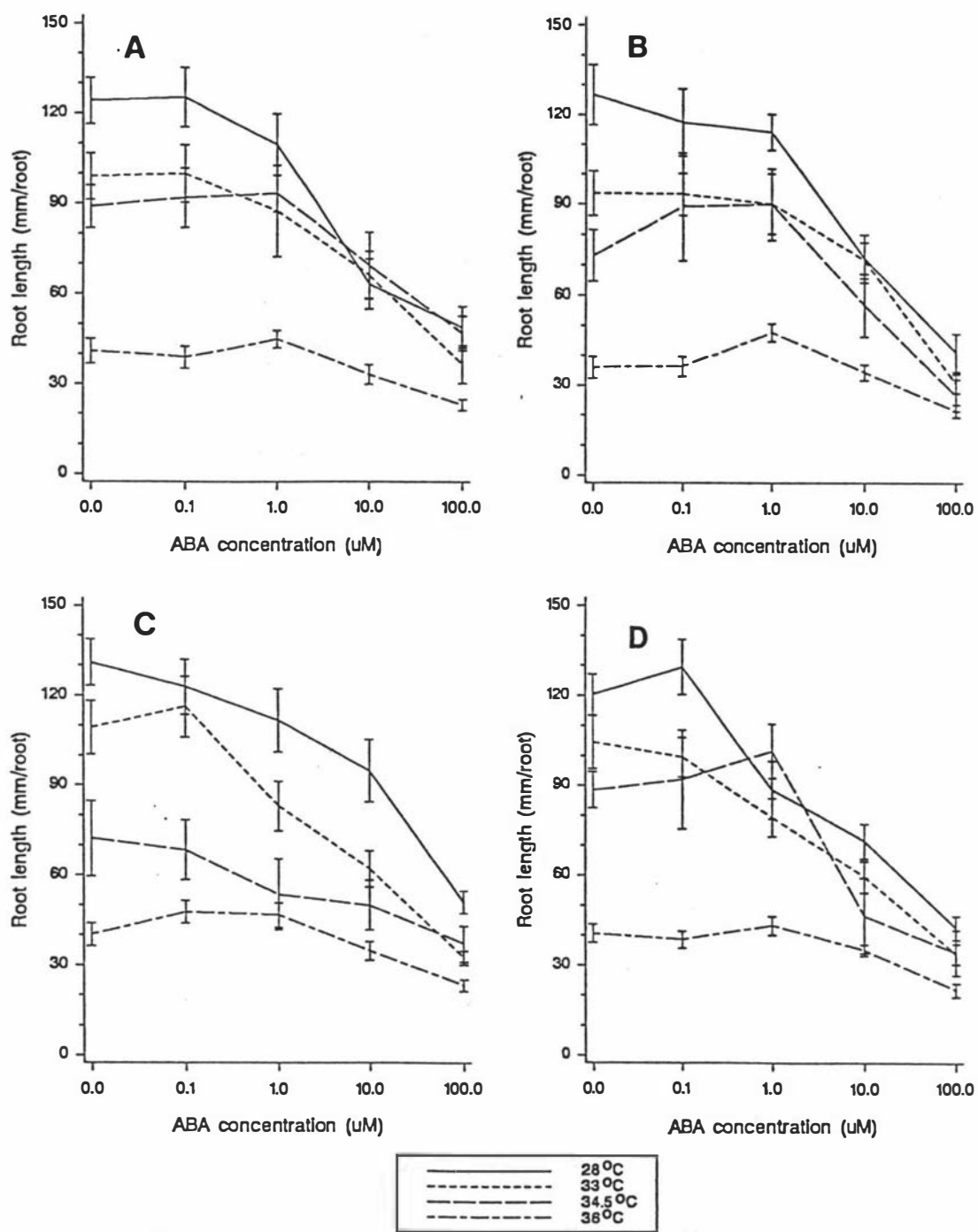


Fig. 4.4. The effects of high temperatures and exogenous ABA on root length of (A) Brocks. (B) Larac. (C) Tainan No.1 (D) UC157. (Vertical lines indicate standard errors of the means)

Root elongation of Tainan No.1 was more sensitive than other cultivars at 34.5°C. In particular Tainan No.1 was significantly more inhibited by 1.0 μM ABA, and UC157 was less sensitive to 1.0 μM ABA (Fig. 4.5c). At 34.5°C and 36°C the root length of Larac was improved by 0.1 - 1.0, 1 μM ABA respectively (Fig. 4.5). At 33°C and 36°C Tainan No.1 were improved by 0.1 μM , 0.1 - 1.0 μM ABA respectively. At 28°C, 34.5°C and 36°C UC157 was improved by 0.1, 1.0 and 1.0 μM ABA respectively. Generally, at 34.5°C root length was improved by 0.1 - 1 μM ABA, at 36°C root length was improved by 1 μM ABA. Thus root elongation was improved by low ABA concentrations and dramatically decreased by higher ABA concentrations.

4.3.2.2. Shoot Growth at High Temperatures and Exogenous ABA

The effect of temperature and ABA on shoot weight was similar to root length. The higher the temperatures or the ABA concentrations the more were shoot weights inhibited (Fig. 4.6).

At 36°C the shoot weights of all cultivars were much reduced compared to 28°C, 33°C and 34.5°C irrespective of ABA treatments (Fig. 4.7). UC157 especially was very sensitive to high ABA concentrations (1.0 - 100 μM) and was not improved by low ABA concentration. The shoot weight inhibition ratios among cultivars without ABA were not significantly different except that at 34.5°C UC157 was smaller than Tainan No.1. In general, the shoot weight of Larac was less sensitive to 1.0 μM and 10 μM . On the contrary, at 28°C and 34.5°C UC157 was significantly more inhibited by 1.0 μM ABA than other cultivars. At 34.5°C and 36°C Larac was improved by 0.1 μM and 1.0 μM ABA respectively, and Tainan No.1 was improved by 0.1 μM ABA. Thus the effect of ABA on shoot weight varied with temperature and cultivar. In general the effect of low ABA concentrations in improving shoot weight was smaller than for root length, and at 34.5°C there was a large variation among cultivars in the sensitivity of shoot weight to ABA.

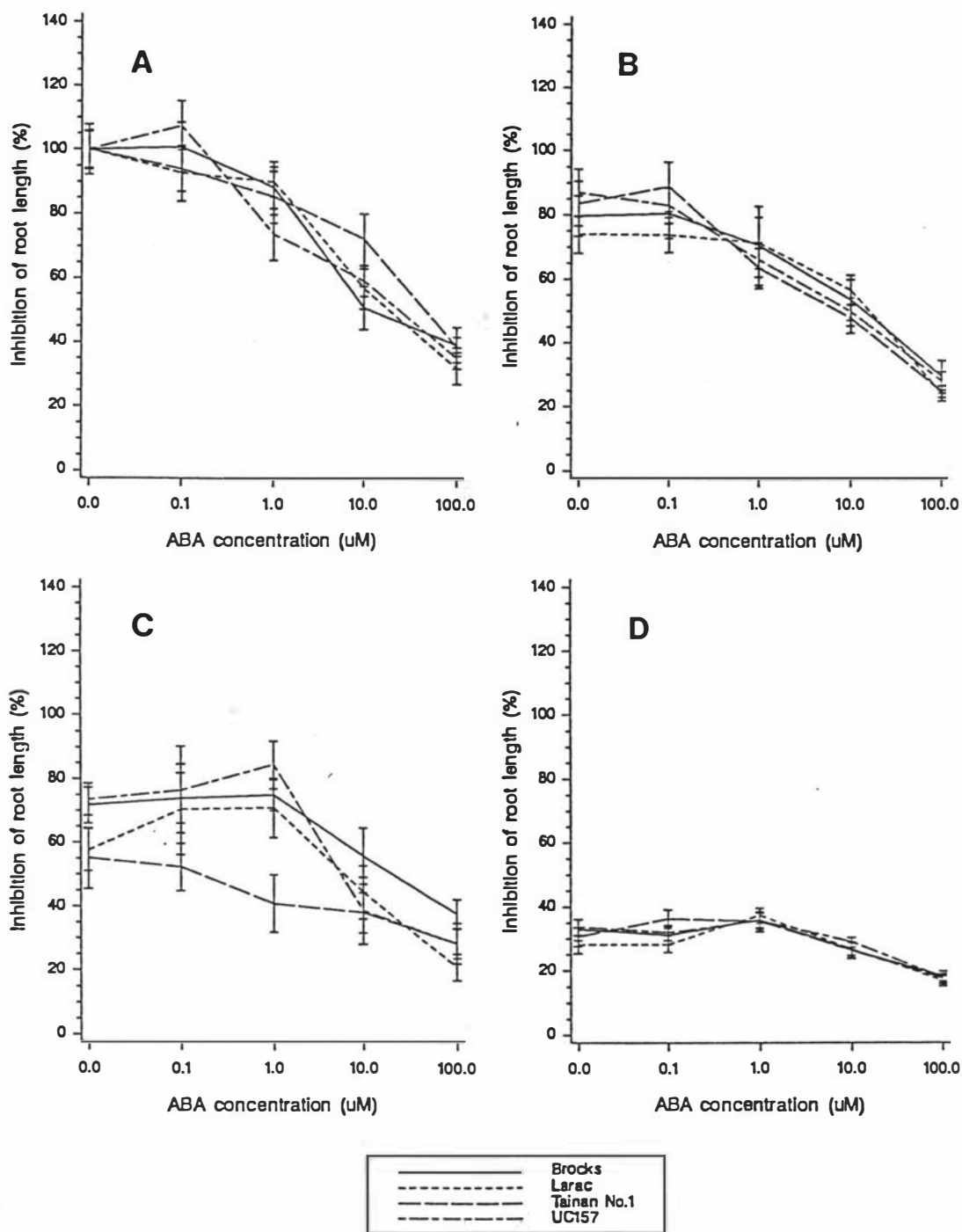


Fig. 4.5. Comparison of root length among cultivars (A) at 28°C. (B) at 33°C. (C) at 34.5°C. (D) at 36°C. (Vertical lines indicate the standard errors of the means; 100 = 0.0 μ M ABA at 28°C)

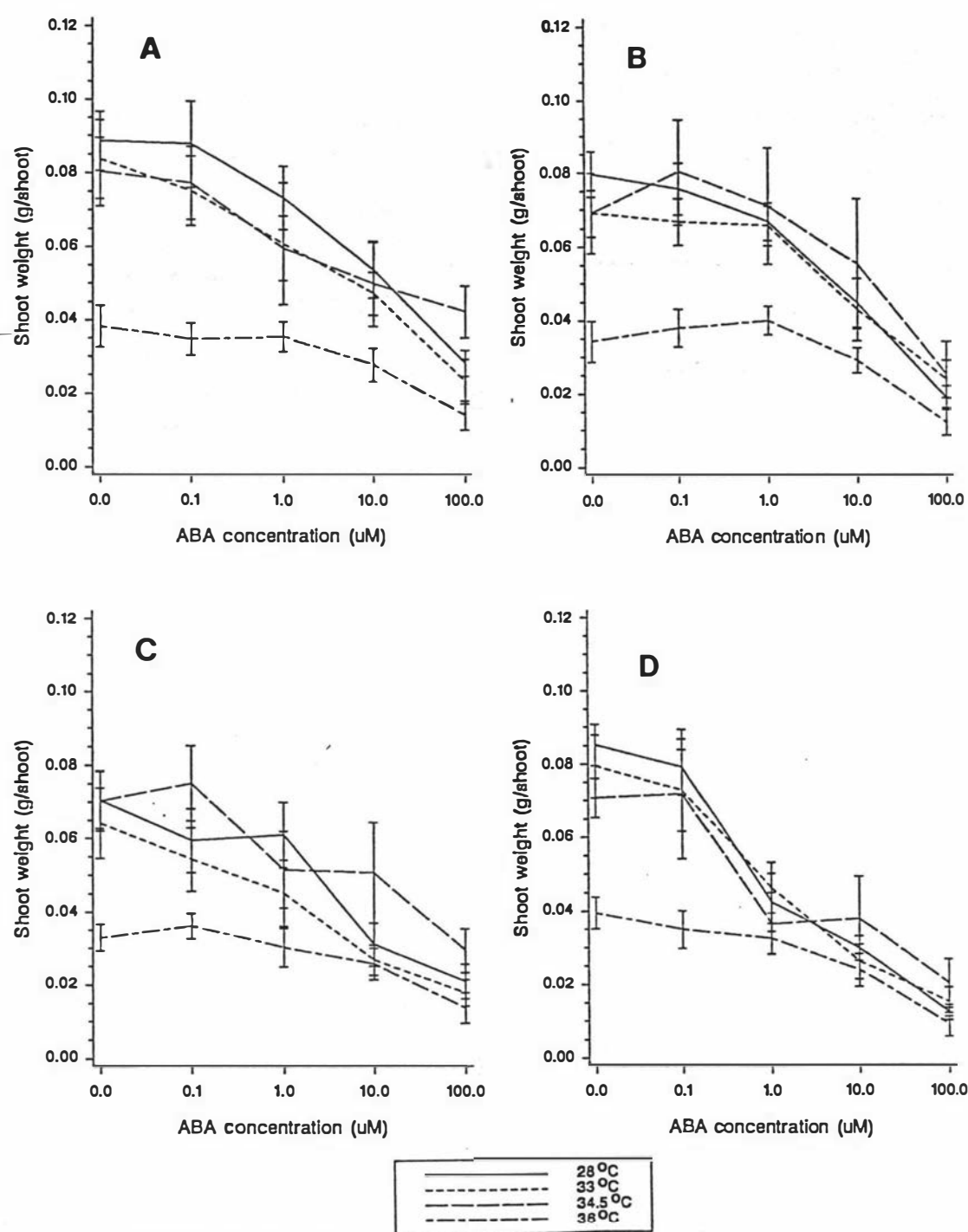


Fig. 4.6. The effects of high temperatures and exogenous ABA on shoot weight of (A) Brocks. (B) Larac. (C) Tainan No.1. (D) UC157. (Vertical lines indicate the standard errors of the means)

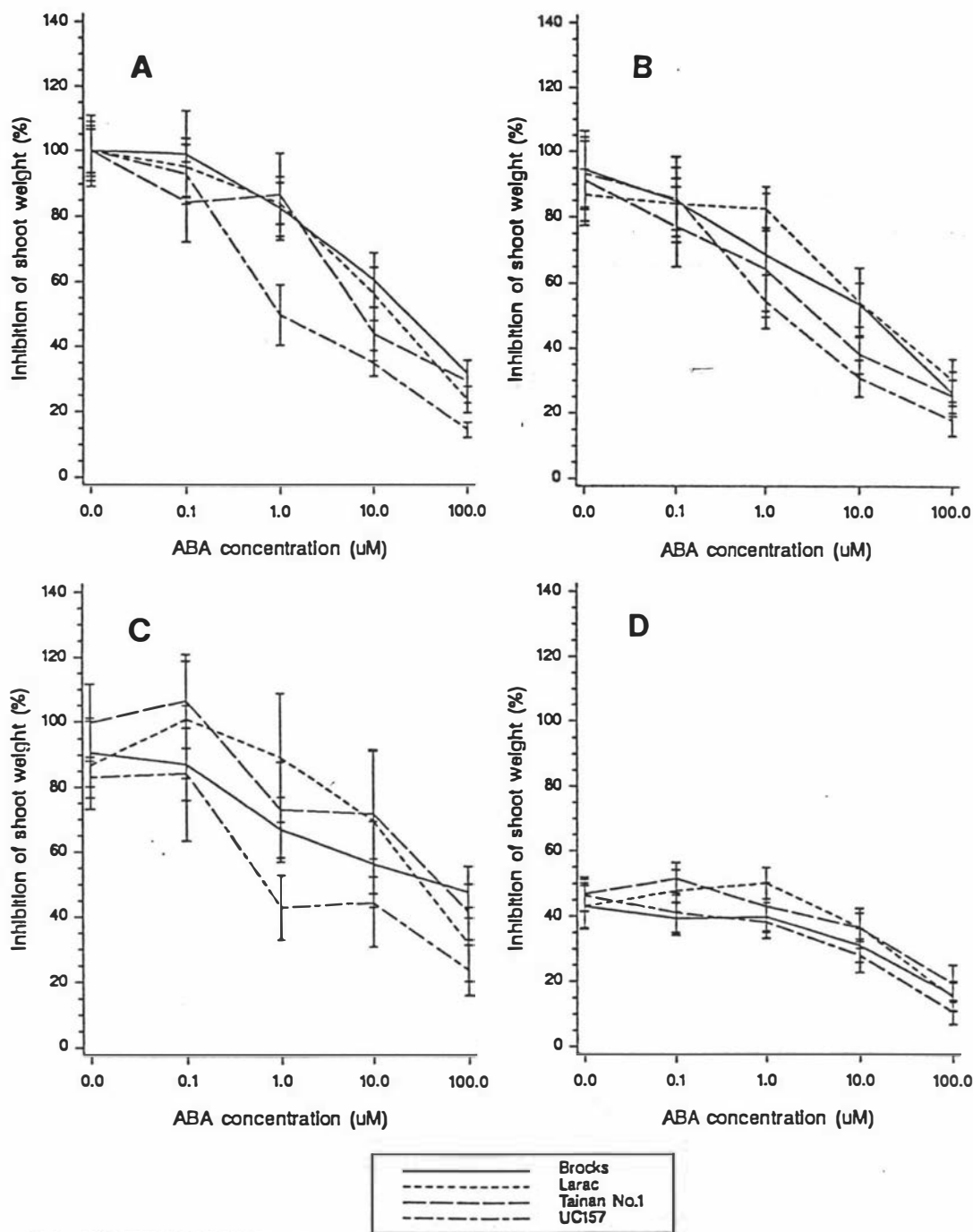


Fig. 4.7. Comparison of the inhibited ratios of shoot weight among cultivars (A) at 28°C. (B) at 33°C. (C) at 34.5°C. (D) at 36°C. (Vertical lines indicate the standard errors of the means; 100 = 0.0 μ M ABA at 28°C)

Higher temperatures or higher ABA concentrations greatly inhibited shoot length (Fig. 4.8). In general, at 33°C the inhibition ratios of shoot length were similar to those at 34.5°C irrespective of ABA concentration or cultivar. In general the shoot length of Larac was less sensitive to 0.1 to 10 µM ABA except at 28°C. On the contrary, UC157 was inhibited more extensively than other cultivars with 1.0 - 100 µM ABA except at 36°C (Fig. 4.9). At 34.5°C Brocks and Larac was improved by 0.1 µM ABA. At 28°C, 33°C, 34.5°C and 36°C Larac were improved by 1.0, 1.0, 0.1 - 1.0 and 1.0 µM ABA respectively. At 33°C and 34.5°C Brocks was improved by 0.1 µM ABA.

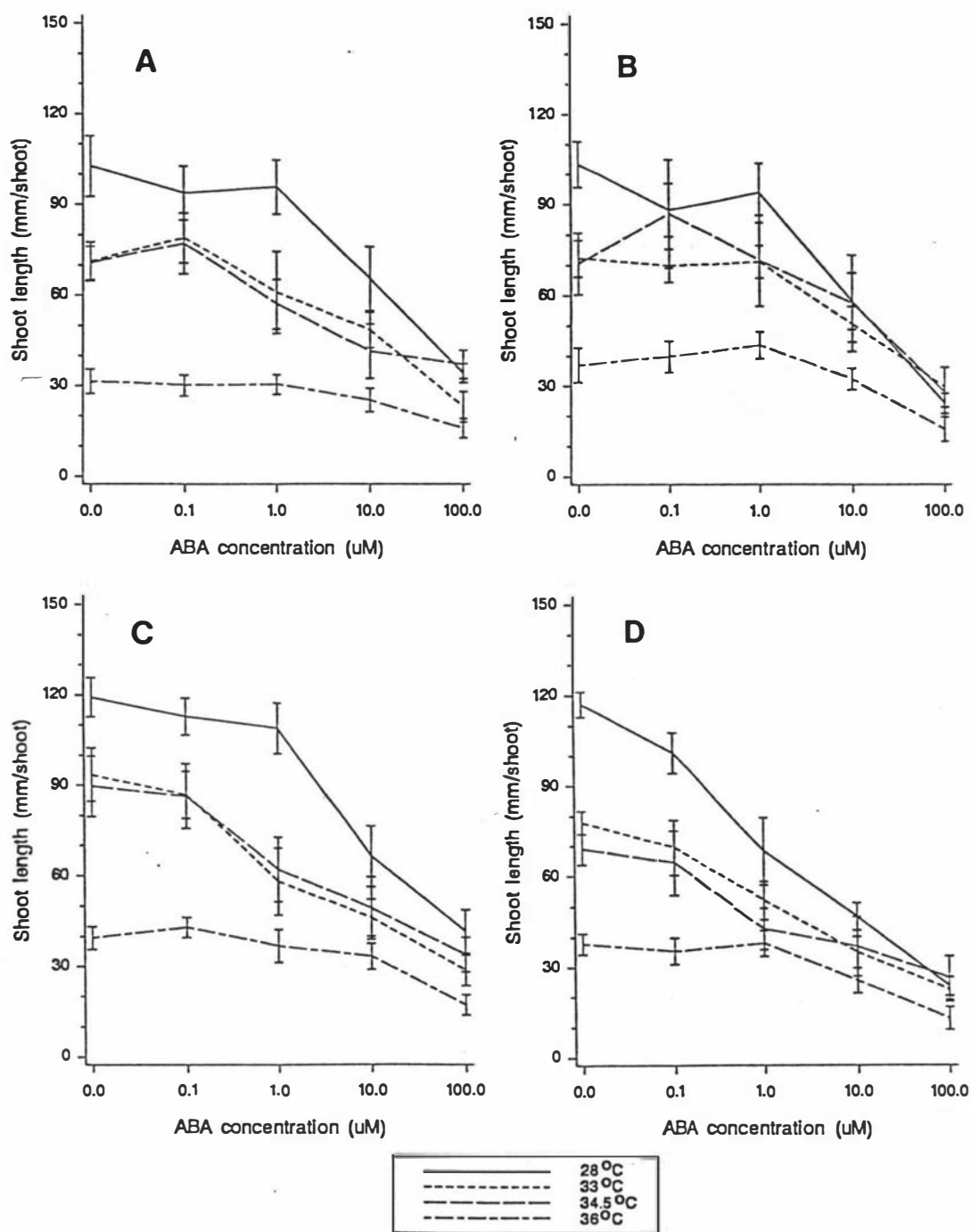


Fig. 4.8. The effects of high temperature and exogenous ABA on shoot length of (A) Brocks. (B) Larac. (C) Tainan No.1 (D) UC157. (Vertical lines indicate the standard errors of the means)

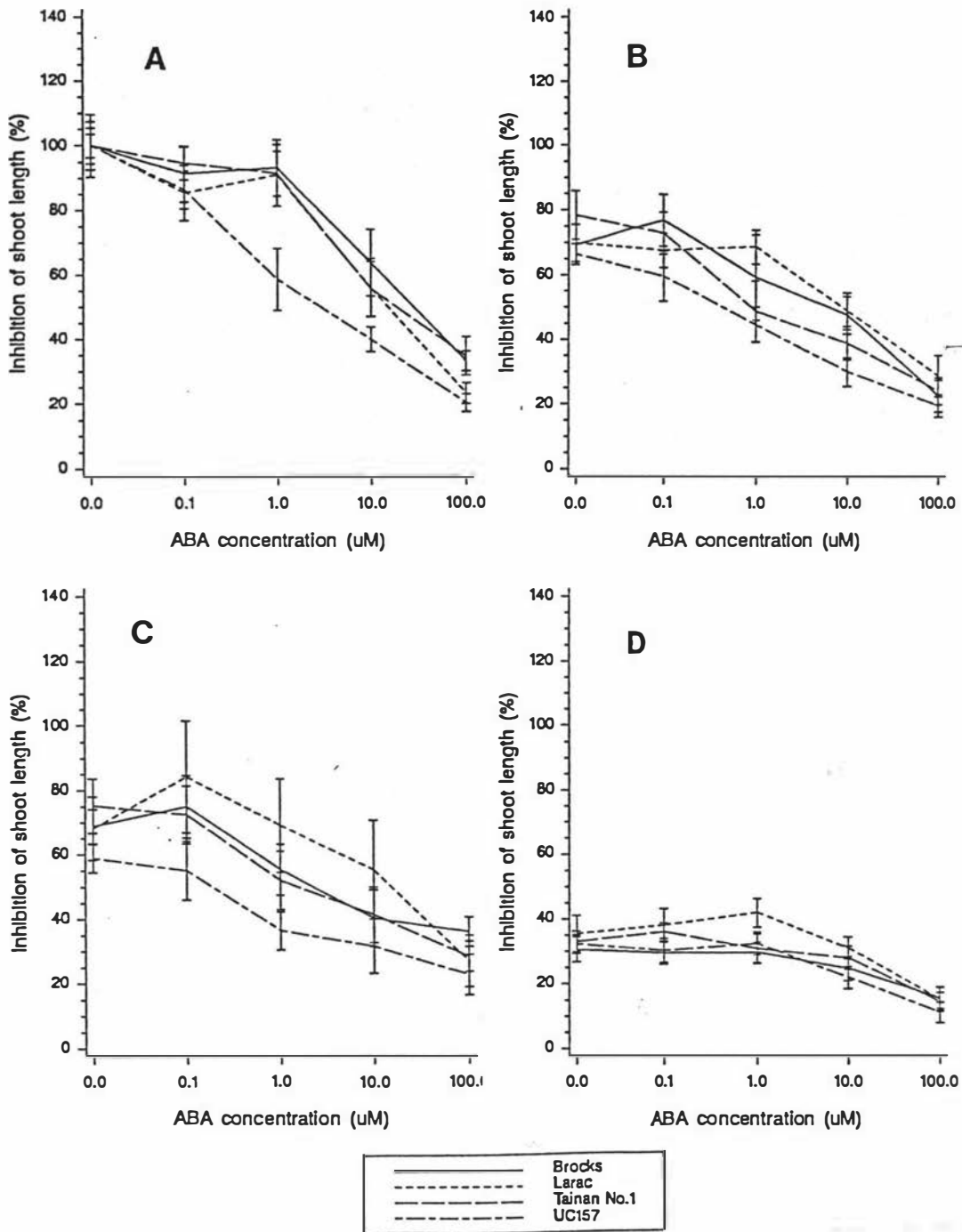


Fig. 4.9. Comparison of shoot length among cultivars (A) at 28°C. (B) at 33°C. (C) at 34.5°C. (D) at 36°C. (Vertical lines indicate the standard errors of the means; 100 = 0.0 μ M ABA at 28°C)

4.4. Discussion

4.4.1. Effect of High Temperatures on Seedling Growth

The results showed that temperature, ABA, cultivar and their interactions significantly influenced shoot length, shoot weight, root length and root weight. These results confirmed previous work (*Gaither et al.* 1975; *Biddington and Dearman* 1982). Thus high temperature inhibited not only young plant growth and spear yield (see chapter 2, 3), but also inhibited seedling growth. The difference in seedling growth among cultivars was significant, but on young plants (Chapter 2) was of little significance. A possible cause may be that the source of carbohydrate for the growth of seedlings comes from storage reserves in the seed, whereas the carbohydrate source for the growth of young plants is from photosynthesis. Consequently it is suggested that differences in available carbohydrate between seedlings and young plants may lead to these different results, because in seedlings, growth is limited by physiological processes at high temperature rather than by the availability of carbohydrate, whereas in young plants, growth may be limited by physiological processes and/or the low availability of carbohydrate due to low rates of assimilation at high temperature.

The results (Fig. 4.1) showed that the higher the temperature the more stunted the growth of seedlings due to heat stress. This agreed with the work of *Onwueme and Laude* (1972); *Hurewitz and Janes* (1983); and *Bugbee and White* (1984). Because a temperature of 34.5°C seriously stunted the growth of asparagus seedlings, temperatures above 34.5°C must be regarded as extreme heat stress temperatures for asparagus. The extent of the inhibition of root weight at high temperature was different from root length, shoot weight and shoot weight, because at high temperature the seedlings had short and swollen primary roots and a smaller number of secondary roots. Thus high temperature severely depressed root length, and had a lesser effect on root weight due to the thicker primary root.

Generally the extents of inhibition of shoot length and shoot weight were similar to that of root length. The extent of inhibition due to heat stress on shoot length was however more than for shoot weight (Fig. 4.7, 4.9), because the shoot became swollen and short at high temperature. Basically, the extent of the heat stress inhibition on growth among cultivars was similar except that 34.5°C strongly depressed root weight and 33°C slightly depressed shoot weight of Tainan No.1. Perhaps Tainan No.1 has a genetic factor which leads to a different depression of growth at high temperature than other cultivars. The work of *Allan et al.* (1962), and *Burleigh et al.* (1964) also showed a progressively shorter coleoptile length in wheat with increasing high temperature. The more heat tolerant the cultivar the lower the depression in the length of the coleoptile.

4.4.2. Influences of ABA on seedling growth at high temperatures

Many plants are able to develop resistance to apparently unrelated stresses and it has been proposed that such a common mechanism of adaptation is regulated by plant hormones, especially by ABA (*Boussiba et al.* 1975; *Chen and Gusta* 1983; *Lachno and Baker* 1986). In my experiments, generally, it appeared that ABA application inhibited growth except at low concentration, and that high concentrations dramatically depressed growth. These results confirmed previous work (*Torre et al.* 1972; *Abou-mandour and Hartung* 1980; *Bonham-Smith et al.* 1988b). The causes are that high ABA concentrations depress cell size, cell number and meristematic cell number leading to a decrease in growth (*Torre et al.* 1972).

Moreover, there is an additive effect of heat stress and high ABA concentration on the growth of seedlings. While there is an ameliorative effect with exogenous application of low ABA concentration on heat stressed plant (*Watts et al.* 1981), because the application of low ABA concentration enhanced growth, high ABA concentration reduced the growth of seedlings at high temperature. Consequently, low ABA concentration may play a role of

protection / or enhancement from heat stress. This confirmed the work of *Bonham-Smith et al.* (1988b), which showed a protective effect of low ABA concentration at mildly high temperature. The effect mainly occurred when the asparagus seedlings were grown at 33°C and 34.5°C when combined with 0.1 - 1 μM ABA application. Conversely, seedlings grown at 28°C rarely showed any significant enhancement of growth with ABA application. The results were similar to the effect of ABA on the growth of water stressed plants, in that ABA has little effect on well watered plants, but had an ameliorative effect on water stressed plants (*Watts et al.* 1981). However the ameliorative effect of ABA on heat damage appeared to be not as great as water stress.

The effect of the application of low ABA concentration to enhance growth may be different from the protection against water stress, chilling stress, etc. *Itai et al.* (1978) showed that ABA pretreatment did not reduce heat damage to tomato leaves, and concluded that ABA affected no protection from heat stress, but the effect of enhancing plant recovery from heat damage might provide protection to other stresses. It is proposed that ABA may trigger the genetic system to express stress resistance (*Chen and Gusta* 1983; *Klee and Estelle* 1991). Thus at appropriate temperature regimes exogenous ABA would enhance growth. Moreover, it is postulated that seedlings at extremely high temperature (e.g. 36°C) have deteriorating physiological processes which lead to the loss of the ability to trigger the mechanism of heat resistance with exogenous ABA. For example at 36°C ABA provided less protection against heat stress. Also perhaps the extremely high temperature results in a reduced availability of a receptor or a reduced binding capacity of receptor to ABA (*Koomneef et al.* 1984). Moreover, heat stress may change the ultrastructure of the cell (*Mansfield et al.* 1988), and thus loses the acquisitive ability on heat tolerance by elevated levels of endogenous ABA.

The response of asparagus root elongation to ABA was similar to the growth of pea root tips enhanced by low ABA concentration (1 μM) (*Gaither et al.* 1975), in that low ABA concentration promoted root elongation and root weight

of asparagus. Conversely, high ABA concentrations ($>10\ \mu\text{M}$) caused a significant reduction in root length and shoot dry weight of asparagus which was similar to the work in cauliflower (*Biddington and Dearman, 1982*), and of both lentil and corn (*Gaither et al. 1975*). In fact, it has been reported that very low ABA concentrations (about $0.01\ \mu\text{M}$ ABA) could enhance the elongation of corn segments, soybean root, runner bean root and shoot and pollen tube, but above this concentration there was inhibition (*Rehm and Cline 1973; Abou-mandour and Hartung 1980; Pilet and Chanson 1981; Mulkey et al. 1983; Pilet and Rebeaud 1983; Pilet and Saugy, 1987; Gude et al. 1988*). Moreover, *Evans and Mulkey (1982)* showed that when $1\ \text{mM}$ ABA was applied on corn roots the growth was initially enhanced then inhibited. A low level of ABA being absorbed initially led to root growth promotion, but then, as ABA accumulated growth inhibition occurred. Overall my results and previous work clearly showed that low ABA concentration has growth promotion, but high ABA inhibition.

The root weight of UC157 was less inhibited by high temperature, indicating that UC157 was less sensitive to high temperature and maintained more normal growth at high temperature. Moreover the root weight of UC157 was also insensitive to ABA application, which may partially explain why it is more heat tolerant than other cultivars (see chapter 6). *Koomneef et al. (1984)* and *Lu et al. (1989)* have suggested that the more the insensitivity to ABA the more the heat tolerance. Root weight of Brocks was also markedly insensitive to ABA at $34.5\ ^\circ\text{C}$ and was improved by $1.0\ \mu\text{M}$ ABA, therefore the root weight of Brocks represented an extensive tolerance at that temperature regime. Conversely, the root weight and root length of Tainan No.1 were sensitive to high temperature. Furthermore, the shoot weight of UC157 was very sensitive to ABA (Fig. 4.7), and therefore ABA did not improve shoot weight. Conversely the shoot weight of Larac was more insensitive to high temperature than other cultivars, and improved by $0.1 - 1.0\ \mu\text{M}$ ABA at high temperature. The endogenous concentration of ABA inhibits between $0.1\ \mu\text{M}$ and $1\ \mu\text{M}$ for corn roots, and - promotes at $0.01\ \mu\text{M}$ (*Pilet and Saugy, 1987*). This is similar to the

exogenous levels of applied ABA used to enhance heat tolerance / growth for asparagus seedlings.

Only a few examples of ABA improving growth were significant in my experiment, e.g. 1.0 μM ABA on root length at 36.5°C for all cultivars, 1.0 μM ABA on root weight at 34.5°C for UC157 and 1.0 μM ABA on root length at 36°C for Larac. Therefore examples of ABA improving growth in my experiment were not as clear as the work of *Gaither et al.* 1975; *Yamaguchi and Street* 1977; and *Pilet and Rebeaud* 1983, who showed that low ABA concentration clearly stimulated growth. Perhaps the causes were that their work was carried out with excised tissues. The effect of exogenous ABA on intact roots and on root segments might be different from that on excised segments which usually release plant hormones from tissue (*Pilet and Saugy* 1987), and the source of the plant hormones might be blocked, or the later synthesis inhibited (*Lacho and Baker* 1986). Thus excised tissues changes the content and balance of plant hormones. However *Abou-Mandour and Hartung* (1980) showed that the effects of ABA on the growth of intact plants or excised segments of runner bean were similar.

The interpretation of the effect of ABA enhancing growth at heat stress is complicated by various unknown factors arising as a result of the exogenous ABA; for example ABA may involve an interaction with other endogenous growth regulators (*Sidhu et al.* 1986). But the work of *Bonham-Smith et al.* (1988b) suggested that elevated ABA content and increased heat tolerance were not associated with GA concentration or cytokinins (*Biddington and Dearman*, 1982; *Asare-Boamah and Fletcher.* 1986). *Onwueme and Laude* (1972) showed that heat stressed plants had decreased cell expansion leading to growth retardation, but neither IAA or GA application relieved the stunting effect of heat stress, consequently, the stunting effect due to heat effect was probably not caused directly by the inactivity of endogenous IAA or GA. *Weidner and Ziemens* (1975) proposed that a low ABA concentration may increase enzyme content (or activity) to increase growth. While *Torre et al.*

(1972) stated that the effect of ABA may be on the metabolism of nucleic acids.

Lu et al. (1989) assumed that genetic differences to ABA insensitivity are apparently associated with stress resistance. In my studies the inhibition of ABA on root growth or shoot growth among cultivars was different, especially at 34.5°C. This means that at high temperature the sensitivity of asparagus seedlings to ABA had considerable variation, thus the sensitivity differences between genotypes were well expressed and became clear. Therefore at high temperatures the difference in heat tolerance of asparagus may be determined by ABA insensitivity, as the root growth of UC157 was the most ABA insensitive. This agrees with the membrane thermostability results in which both the roots of UC157 and the shoots of Larac showed ABA insensitivity, and yet the membranes were fairly thermostable (Chapter 6). The explanation is that exogenous ABA induced plant responses similar to those of abiotic stress, thus genetic difference in ABA insensitivity are apparently associated with the difference of stress resistance (*Lu et al.* 1989). The work of *Quarrie* (1978) (cited by *Lu et al.* 1989) showed that drought stress resistance could be improved by reducing sensitivity to ABA, because applying ABA had little effect on yield of an ABA insensitive wheat line, but decreased the yield of a sensitive line.

Although little is known about the mechanism of ABA insensitivity, *Lu et al.* (1989) have stated that the insensitivity of plants to ABA is more beneficial for drought or osmotic stress resistance than for heat stress resistance. This is because drought stress induces higher ABA levels than heat stress. Furthermore ABA is unlikely to act alone on stress resistance and the effect of ABA on growth promotion for the heat stressed plant may be quite complex. Especially when it was found that overall the 0.1 - 1.0 μM ABA application could be used to examine the difference in ABA insensitivity between cultivars, as the growth of ABA insensitive cultivars could be improved while ABA sensitive cultivars did not. Therefore it is suggested that in ABA insensitive

genotypes ABA triggers the mechanism of heat tolerance, while in the ABA insensitive line there is less growth depression in elevated ABA due to heat stress or applying ABA, because the phenotypic changes of the ABA in the insensitive line to stress responses are similar to the responses of the ABA deficient line (*Koornneef et al.* 1984). Hence the determination of ABA insensitivity may be an effective approach to screen lines for heat resistance.

CHAPTER FIVE

Endogenous ABA In the Response of Asparagus Plant to High Temperatures

5.1. Introduction

Absciscic acid is thought to play an important role in increasing resistance to drought and cold stress and for acclimation (Zeevaart and Creelman 1988; Klee and Estelle 1991). Plants exposed to stress react by producing ABA, which may regulate physiological processes to increase stress tolerance. Endogenous ABA content is usually elevated at high temperature, although the elevation by heat stress is lower compared with that of other stresses, e.g. cold, drought (Daie and Campbell 1981; Eze *et al.* 1983; Cammue *et al.* 1989). Itai *et al.* (1978) assumed that ABA increases resistance to other environmental stresses, but not to heat stress. Heat shock may elevate the endogenous ABA level and therefore affect the recovery from heat damage. However Bray (1991) showed that elevated levels of endogenous ABA are not involved in the tomato heat shock response. Several investigators believe that ABA can increase a plant's tolerance to any sub-optimal conditions, therefore ABA may be a common mediator for all plant stresses (Boussiba *et al.* 1975; Daie and Campbell 1981; Daie *et al.* 1981; O'Connor *et al.* 1991). It has been proposed that ABA induced heat tolerance is due to ABA inducing some HSPs (Sachs and Ho 1986, see chapter 7).

Physiological stresses at high temperature are different from those of other stressful environments, because the former accelerates processes and the latter slows them down, thus the metabolism of ABA at high temperature may be different. For example, turnover, synthesis and interconversion between free and conjugated ABA may lead to the endogenous ABA levels between genotypes at high temperature being different. Furthermore genetic diversity may also include variations in ABA insensitivity (see Chapter 4). These conditions may be associated with the different levels of heat tolerance. In fact,

the heat tolerance of asparagus seedlings and young plants showed variation with genotypes (see chapter 2 and 3) and some ABA concentrations enhanced seedling growth at high temperature (see chapter 4). Therefore endogenous ABA contents of the seedlings and spears at high temperature may be associated with thermostability.

5.2. Materials and Methods

5.2.1. Seedlings

5.2.1.1. Plant Material

The 4 cultivars were the same as used earlier, namely Brocks, Larac, Tainan No.1 and UC157. Seeds of the 4 cultivars were soaked in distilled water for 2 days in an incubator at 24°C, and then laid on several layers of moistened tissues (with distilled water) in a box to germinate for about 8 - 12 days.

5.2.1.2. Growing and Conditioning

The seedlings were grown until the roots were about 2-3 cm, and then placed on germination paper¹, which had been previously moistened with distilled water. Each germination paper was rolled up immediately after it was loaded with 10 seedlings, enclosed in a plastic bag to ensure high humidity, and stood in an incubator at 28°C for 7 days. The bags were transferred for 24 hours to a series of incubators which were set up at 28±1°C, 33±1°C, 36±1°C, 38±1°C and 40±1°C. After the heat shock, the seedlings were separated into shoot and root. The top, 3 cm length of shoots, and the whole roots were immersed in liquid nitrogen. These samples were either analyzed immediately or kept at -20°C and analyzed within 3 days.

5.2.2. Spears

5.2.2.1. Plant Material

The potted plants of the 4 cultivars of asparagus (Brocks, Larac, Tainan No.1,

¹ Anchor paper, code 612-298-1311, 480 Broadway, P.O. Box 3648, Saint Paul, MN 55165, USA

UC157) were grown as in chapter 3. The ferns were cut off before these plants were placed in the growth cabinets, and prior to sampling the spears, the plants were acclimated at the desired temperature for 4 weeks.

5.2.2.2. Growing and conditioning

There were 12 plants of each cultivars at each temperature. These were grown in growth cabinets which were set at $28\pm1.5^{\circ}\text{C}$, $33\pm1.5^{\circ}\text{C}$ and $36\pm1.5^{\circ}\text{C}$ constant with a 12 hours photoperiod at $750\ \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Plants were watered once every 4 hours to prevent water deficit. All spears were sampled 3 hours after lights on, at which time the top 5 cm of the spears was removed and immersed in liquid nitrogen as soon as possible. These samples were kept at -20°C and were analyzed within 10 days.

5.2.3. Determination of ABA by an Indirect Enzyme Linked Immunoassay (ELISA)

5.2.3.1. ABA Extraction

Frozen samples (spears 0.5 g, shoot 0.2 g or root 0.2 g) were homogenized in 5 ml of methanol (methanol containing 100 mg/L butylate hydroxytoluene and 0.5 g/L citric acid monohydrate) at 3500 rpm for 3 minutes in an ice bath (KINEMATICA GmbH, LITTAU-LUZERN, Switzerland). Extracts were kept and shaken overnight, then spun at 3000 g for 5 minutes. 0.2 ml of the supernatant was taken from the spear sample and 0.5 ml supernatant was taken from the shoot and root sample. The supernatants were then dried in a vacuum oven. The dried samples were dissolved with 200 μl 10% methanol and diluted with TBS buffer to a suitable concentration for ELISA assay. There were at least 4 replications for each treatment.

5.2.3.2. ELISA Assay Materials

The procedure follows the immunoassay method of *Walker-Simmons* (1987).

TBS Buffer:

Per litre buffer contains TBS 6.05 g Tris, 0.20 mg MgCl_2 , 8.80 g NaCl (pH 7.8).

Washing buffer:

TBS containing 0.05% (v/v) Tween 20, and 0.1% (w/v) BSA (ELISA grade, Sigma).

ABA-4'-BSA conjugate:

The conjugate was prepared according to *Weiler* (1979) and lyophilized. Conjugate was suspended in 0.05 M NaHCO_3 (pH 9.6), at a concentration of 7 mg/ml and stock aliquots of 60 μl were frozen at -20°C . Before ELISA assay a 60 μl aliquot was thawed and diluted with 0.05 M NaHCO_3 (pH 9.6), to final volume of 16 ml, which was sufficient to coat the assay wells of a microtitration plate.

MAb (Monoclonal antibody):

MAb to free cis, trans(+)ABA was purchased from Idetech, Inc., 1057 Sneath Lane, San Bruno, CA 94066. Two mg MAb was mixed into 135 ml TBS, containing 0.2% (w/v) BSA. Aliquots of 2.25 ml of the MAb solution were stored at -20°C . Before ELISA assay a 2.25 ml aliquot was thawed and diluted in TBS buffer to a final volume of 11.25 ml, which is sufficient for the assay wells of a microtitration plate.

Second antibody:

Rabbit antimouse alkaline phosphatase conjugate (Sigma Chemical Co.) was diluted 1:1000 in TBS.

Alkaline phosphatase substrate:

The substrate, *p*-nitropheny phosphate was prepared at a concentration of 1 mg/ml in 0.05 M NaHCO_3 (pH 9.6).

ABA standard:

($\pm$)cis-trans ABA purchased from Sigma Chemical Co. was dissolved in

TBS in concentrations ranging from 25 to 400 pg/100 μ l.

Microtitration plates:

Immulon 2 flat bottom, 96-well microtitration plates, Dynatech Laboratories, Inc., Alexandria, VA were utilized. The upper and lower rows of wells of the plate were not used.

5.2.3.3. ELISA Assay Procedures

All samples and solutions were kept in the dark during incubations.

1. Coating of well with ABA-4'-BSA conjugate:

A 200 μ l aliquot of the conjugate was added to each well of the microtitration plate, excluding the upper and lower rows. Plates were incubated at 4°C overnight.

2. Incubation of ABA samples with MAb:

A 120 μ l aliquot of ABA sample was pipetted into a test tube, then 120 μ l of MAb solution was added and mixed. The solutions were incubated overnight at 4°C.

3. Addition of ABA samples incubated with MAb:

Plate wells coated with conjugate were washed three times with washing buffer. For the final step only, the final washing solution was left in the plate for 10 minutes and then discarded. A 200 μ l aliquot of the samples incubated with MAb was added to each well. Plates were incubated for 2.5 hours. This incubation and all the following steps were performed at 25°C.

4. Addition of the second antibody:

Wells were washed three times with washing buffer. Rabbit antimouse alkaline phosphatase conjugate (200 μ l) was added to each well. Plates were incubated for 2 hours.

5. Measurement of alkaline phosphatase:

Wells were washed three times with washing buffer. *p*-Nitrophenyl phosphate substrate (200 μ l) was added to each well. Plates were

incubated for 2 hours. The sample absorbance measured at 405 nm with Dynatech, Minireader. The absorbance of the sample is inversely proportional to the amount of ABA in the original sample incubated with MAb.

5.2.3.4. Estimating ABA Concentrations in Plant Samples

A standard curve is illustrated in Fig. 5.1. The plot of *logit* (B/B_0) is linear in respect to $\ln [12.5 - 200 \text{ pg/well } (\pm \text{ABA})]$, then the amount of ABA in the plant extract samples calculated based on the linear regression of the ABA standard for each plate.

$$\text{logit} \frac{B}{B_0} = \ln \frac{\frac{B}{B_0}}{1 - \frac{B}{B_0}}$$

B_0 is the $A_{O.D.}$ in the absence of any ABA

B is the $A_{O.D.}$ in the presence of ABA standard or samples

5.3. Results

5.3.1. Immunoassay for Asparagus Tissue ABA

This quantitative ABA immunoassay is an indirect ELISA. The assay was found to be linear from $\ln [12.5 \text{ to } 200 \text{ pg/well } (\pm)\text{ABA}]$ (Fig. 5.1). A major advantage of this indirect ELISA assay is that only a small amount (0.3 $\mu\text{g/assay well}$) of a commercially available MAb to (+)ABA is required. Additionally, the sensitivity of the assay is as low as 12.5 pg thus enabling the measurement of ABA on very small samples. Also the simple extraction method (with MeOH) may avoid loss and ensure a good recovery of ABA.

5.3.2. Influences of High Temperatures on Endogenous ABA of Seedlings

The level of endogenous ABA was determined in the shoots and roots of Brocks, Larac, Tainan No.1 and UC157 incubated at 28°C, 33°C, 36°C, 38°C and 40°C for 24 hours. In general, the profiles of endogenous ABA contents against temperature were similar for shoots and roots (Fig. 5.2A, 5.2B). Endogenous ABA decreased with temperature and then increased to a peak around 38°C for Larac and Tainan No.1, but peaked at around 36°C or lower for Brocks and UC157 for both roots and shoots.

A comparison of ABA contents between cultivars for each temperature showed that when seedlings were grown at 28°C for 24 hours, Tainan No.1 had the highest ABA content in both roots and shoots, Larac the second highest, and Brocks and UC157 lowest. Generally, the seedlings of Brocks and UC157 had high ABA content at 33°C and at 36°C, especially, Brocks which had a very high ABA content at 36°C. The roots of Brocks had higher ABA content than shoots at 33°C and roots of UC157 had higher ABA content than shoot at 33°C and at 36°C. Furthermore, the ABA contents of shoots and roots of seedlings of Brocks and UC157 dramatically decreased at 38°C and 40°C. Even though the ABA content on shoots of Brocks decreased at 38°C and 40°C ABA was

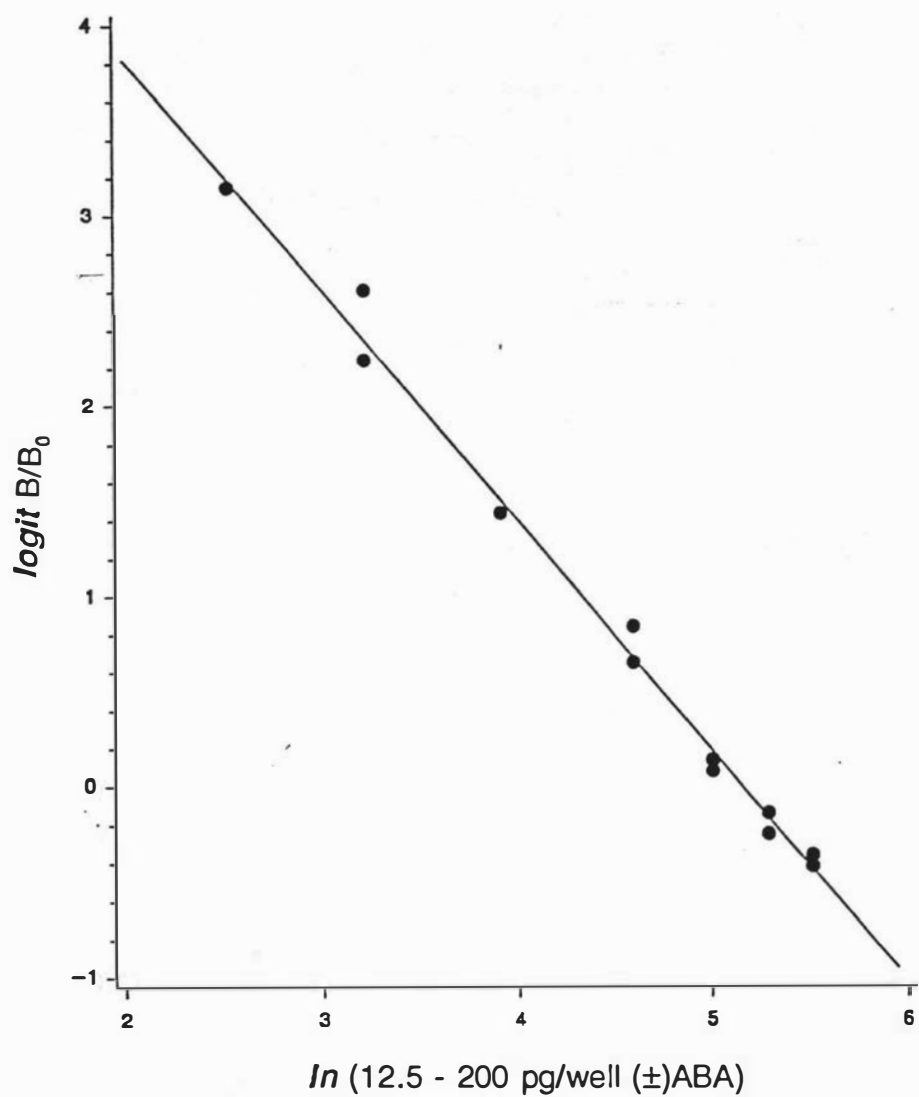


Fig. 5.1. The standard curve of $\text{logit } (B/B_0)$ against $\ln (12.5 - 200 \text{ pg/well } (\pm)\text{ABA})$ fitted to a linear regression.

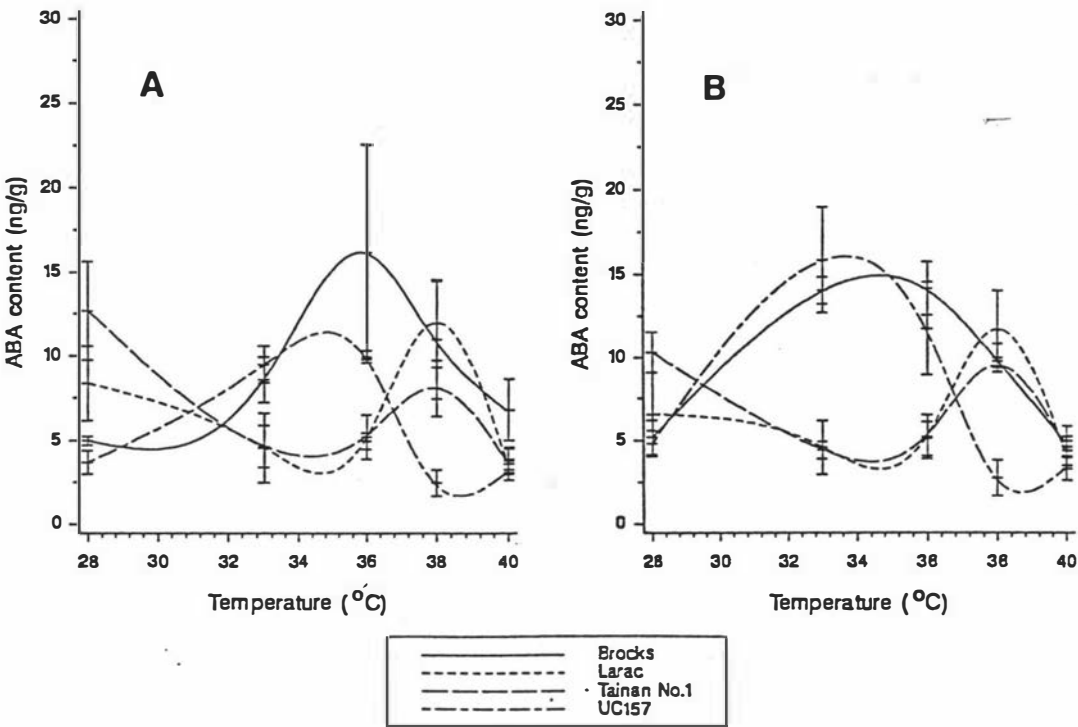


Fig. 5.2. The effect of high temperatures on the endogenous ABA levels of (A) seedling shoots. (B) seedling roots.

still high compared to the other cultivars. The shoots and roots of Larac had the highest ABA content at 38°C due to the ABA content peak occurring at that temperature. In contrast, although Tainan No.1 also had a peak of ABA content at 38°C, it was lower than Larac. In general, the roots and shoots of seedlings for all cultivars had quite low ABA contents at 40°C except for the shoot of Brocks.

The most important generalization is that Larac and Tainan No.1 had high levels at 28°C which decreased with temperature until about 36°C, while Brocks and UC157 had low levels at 28°C which increased markedly between 33°C and 36°C.

5.3.3. Endogenous ABA In the response of spears to high temperatures

Endogenous ABA was obtained from the upper spear (5 cm length) which was dissected from 10 cm high spears of Brocks, Larac, Tainan No.1 and UC157, produced from crowns grown in growth cabinets at 28°C, 33°C and 36°C and acclimated for at least 1 month before the spears were sampled. The results suggest that the endogenous ABA contents of spears, for all cultivars, were much higher than for seedlings (Fig. 5.3). Moreover, the spears of Tainan No.1 had a extremely high ABA content at 28°C and 33°C and fell to similar levels to the other cultivars at 36°C. The patterns in ABA content against temperature for Tainan No.1 and Larac were similar, in that ABA content increased at 33°C and went down at 36°C, while Brocks and UC157 had nearly equal ABA contents at 28°C and 33°C, their ABA contents increased at 36°C. Brocks had the highest ABA content among all cultivars at 36°C. The results showed that endogenous ABA content varied in response to high temperature for spears and seedlings. This might be due to different heating duration, plant components or environment (lighting), or due to differences in ABA metabolism between plant components. Water deficit was not a factor due to the plants in pots being watered once per 4 hours and the seedlings enclosed in plastic bag to maintain high humidity.

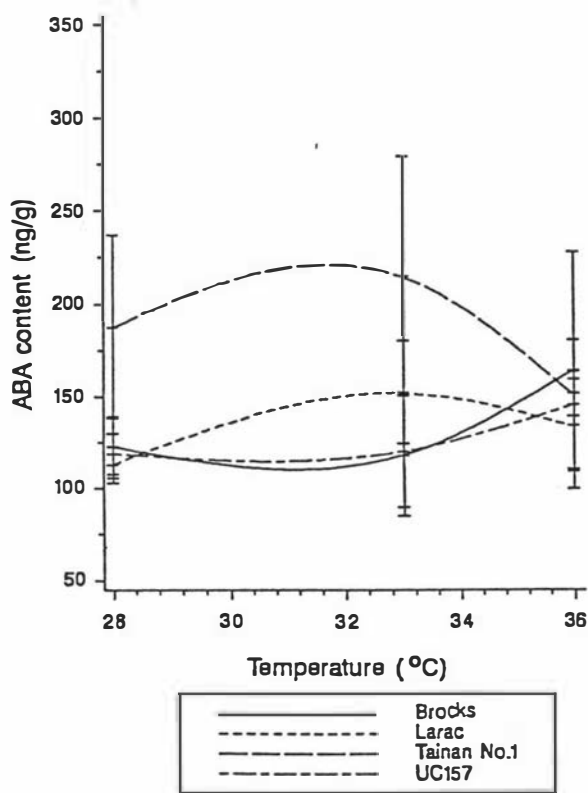


Fig. 5.3. The effect of high temperatures on the endogenous ABA levels of pears.

5.4. Discussion

The endogenous ABA levels (2 - 17 ng/g) of asparagus seedlings were much lower compared to spears (100 - 220 ng/g). The ABA levels in spears in my experiment are similar to those found by *Matsubara* (1980), who showed that the ABA levels in spears was 70 - 210 ng/g. However the ABA levels of asparagus tissue is low compared with the 200 - 1800 ng/g fresh weight found in well watered *Xanthium strumarium* or the 4000 ng/g fresh weight in water stressed *Xanthium strumarium* (*Zeevaart* 1980). Generally the level of endogenous ABA was elevated at higher temperatures and this result coincides with the work of *Itai et al.* (1973); *Daie and Campbell* (1981) and *Eze et al.* (1983). It is proposed that not only does ABA protect plants during period of severe stress, but it also appears to enable the plant to withstand smaller stresses for long periods by adaptation (*Hiron and Wright* 1973), because ABA can act on many genes (such as *rab*) (*Finkelstein et al.* 1985; *Skriver and Mundy* 1990).

The endogenous ABA content of the shoot was similar to that of the roots except for the roots of Brocks and UC157 at 33°C (Fig 5.2A, 5.2B). These results confirm the work of *Itai et al.* (1973), who showed that heat treatment reduced cytokinin levels and increased ABA levels of shoots and roots, and also reduced root and shoot growth. They suggested that heat treatment changes the root hormone levels, and that these hormones are translocated in the xylem exudate to the shoot, and thus affect shoot growth. The magnitudes of the elevated ABA content depends on the stress temperature and cultivars. The maximum increase observed in my experiment was about 3-times the level in seedlings at 28°C. These results coincide with the work of *Daie and Campbell* (1981). Consequently it is concluded that the genetic diversity of asparagus might lead to the differences of ABA contents induced by stress temperatures, which varies with genotype and plant component (Fig. 5.2A, 5.2B, 5.3). Such an adaptation, brought about by an increased ABA level, is believed to occur in tomato, dwarf bean and wheat plants when they

are flooded (*Hiron and Wright 1973*).

The response of plants to ABA may be different among cultivars / tissues. Thus the ability of a plant's adaptation to stress can not be assessed simply will reference to ABA levels, but the sensitivity of plants to ABA should be taken into account to assess the adaptation ability, because large difference between cultivars were noted in sensitivity to ABA (*Perry and Hellmers 1973; Walker-Simmons 1987, 1988*). In addition, *Lu et al.* (1989) showed that the ABA contents in wheat plants increased during heat stress, and was associated with stomatal closure, low photosynthetic rate and accelerating senescence. An ABA insensitive line is able to reduce the effects of elevated ABA level at high temperature, thus it is concluded that the ABA insensitive lines are heat tolerant. In the present results the growth of the heat tolerant cultivar UC157 was insensitive to ABA, but ABA appeared to give some protection / enhancement against high temperature (see 4.3.2.1). This protection / enhancement effect of ABA was possibly through effects on heat shock proteins or ABA induced proteins (Chapter 7).

In fact, the causes of differences of endogenous ABA levels may involve ABA turnover and *de novo* synthesis, or interconversion between free and conjugated ABA. Thus the interpretation of the endogenous ABA levels in the tissues of asparagus should be concerned with all the above factors. Seedlings at the extreme temperature of 40°C had a low endogenous ABA level, similar to the levels at 28°C, thus confirming the report of *Itai et al.* (1973). Perhaps the cause is low ABA synthesis due to the effect of deleterious physiological processes at extreme temperature, (*Eze et al. 1983; Radin and Hendrix 1986*) or due to the rapid metabolism of ABA leading to a low ABA level (*Daie et al. 1981*). At mildly high temperatures (about 33°C to 36°C depending on cultivars / tissues) stresses may increase the rate of turnover of ABA leading to a much lower endogenous ABA level compared to that at 28°C (*Radin and Hendrix 1986*). Furthermore, plants at 35°C are known to synthesise heat shock proteins, and the metabolism of ABA may be related to the appearance of

these proteins. The function of these proteins is still not known, but may involve the conversion from ABA to phaseic acid (*Ho and Uknes 1982; Uknes and Ho 1984*). The roots and shoots of both Tainan No.1 and Larac at 38°C, and UC157 and Brocks at 33°C / 36°C had high endogenous ABA levels. These results showed that the endogenous ABA content of asparagus was elevated at these high temperature regimes, and that the inducing temperature regime depended on cultivar.

However the magnitude of the elevated ABA levels induced by high temperature stress is much lower compared with that induced by other stresses, e.g. drought stress (*Eze et al. 1983; Gamble and Mullet 1986; Guerrero and Mullet 1986*); low temperature (*Daie et al. 1981; Daie and Campbell 1981; Taylor et al. 1990*); mineral deprivation and salinity (*Boussiba et al. 1975*). In fact, the increase in ABA level at low temperature is not as high as the increase caused by drought stress, but the former elevated ABA level is still higher than the increase caused by high temperature stress (*Daie and Campbell 1981; Daie et al. 1981*). Perhaps the rapid accumulation of ABA during high temperature stress is due to moisture stress rather than heat stress, because a function of high temperature is moisture stress. Thus elevated ABA level may not result only from high temperature stress (*Eze et al. 1983*). In my experiment the seedlings were enclosed in moist germination paper inside a plastic bag, and it is unlikely that a water deficit occurred in these conditions. Consequently the elevated ABA levels in asparagus seedlings subjected to high temperature, was most likely due to heat stress.

Previous work proposed that the function of ABA in protecting plants from heating stress may differ from drought stress or low temperature stress, because the function of ABA may only induce adaptation and not maintain the growth of plants at sub-optimal temperatures (*Taylor et al. 1990*). Furthermore, the function of ABA does not play a role in increasing the resistance of plants to heat stress, but enhances recovery after heat damage (*Itai et al. 1978*). In addition, *Lafuente et al. (1991)* showed that the ABA level is not associated

with membrane leakage. However, although the function of ABA and heat stress is still not clear, it may be concluded that elevated ABA level implicates these responses including heat stress and several other stresses (*Hiron and Wright 1973; Daie et al. 1979; Abou-mandour and Hartung 1980; Daie and Campbell 1981; Daie et al. 1981; Eamus and Wilson 1983; Hwang and VanToai 1991*). There is a qualitative resemblance between the effect of ABA and water stress on wheat development and morphology (*Quarrie and Jones 1977*), thus ABA application may induce adaptation to water stress without a water stress condition. Furthermore, heat tolerance may be induced by heat acclimation without elevating endogenous ABA level. It has been proposed that heat tolerance is associated with heat shock proteins rather than endogenous ABA level (*Bray 1991*). Hence ABA may provide growth enhancement rather than protection at high temperature. There is clearly a need to distinguish the physiological processes between enhancement and protection at high temperature.

The endogenous ABA concentrations in spears were much higher compared with seedlings. The probable explanations are that the endogenous ABA level of spears increases with age (*Matsubara 1980*), and because the spears are exposed to light, they have a high chlorophyll content which may lead to increased ABA synthesis. Thus the ABA levels in leaf and stem increase with increasing plant height (*Chen and Chen 1988*). Also ABA levels increase in leaves of plant subjected in heat stress (*Hocking et al. 1972 and Hoad 1973* cited by *Watts et al. 1981*). In my experiment the heat stress treatment of spears and seedlings were different in that the seedling treatment was a 24 hours heat shock while the spears were subjected to over 4 weeks of heat stress. Thus the responses of endogenous ABA levels in both seedlings and spears to heat stress might be different. High temperature initially decreased ABA levels and then increased then, with the final ABA level increasing 3 to 10 fold, depending on the species/variety, when plants were subjected to stress temperature for long period (*Daie and Campbell 1981*). This coincides with the results of my experiment where spears flushing from crowns subjected

to stress temperatures over a 4 week period, the ABA levels in spears were much higher compared with seedlings subjected to stress temperature for only 24 hours.

Because the synthesise of both ABA and chlorophyll share the same pathway in carotenoid biosynthesis (*Moore and Smith 1974; Gamble and Hullet 1986*), it is often proposed that ABA is synthesized in chloroplast leading to green tissue having a high ABA content (*Radley 1976*). However *Aghofack-Nguemezi et al. (1991)* showed that environmental conditions influence carotenoid and ABA levels independently, even if they share parts of the same biosynthetic pathway. Other workers also reported that other parts of the plant, not only chloroplasts, also synthesise ABA (*Moore and Smith 1984; Quarrie and Lister 1984*). In my experiment the seedlings enveloped by germination paper in a dark incubator, still synthesised ABA. This result confirms the conclusion that ABA is synthesised in all parts of the plant (*Creelman and Mullet 1991*). *Makus and Guinn (1992)* also showed that the ABA and IAA levels in green spears were much higher than in white spears. However because green spears had a much higher ABA level than seedlings, it appears that the green tissue are a major contributor of ABA synthesis.

Spear development is rapid even at high ABA levels (Fig. 5.3). This result coincides with the work of *Matsubara (1980)*, who showed that the apices of asparagus spears grew very rapidly, but contained high concentrations of ABA and GA-like substances. *Makus and Guinn (1992)* reported that green spears had higher ABA and IAA levels than white spears, and had a higher growth rate than white spears. In addition, *Chen and Chen (1988)* showed that alfalfa contained high ABA levels and yet still maintained rapid growth. Therefore growth is likely to be regulated by the balance between promotive hormones (such as GA) and inhibitive hormones (such as ABA) /or insensitivity to ABA (*Radley 1976; Daie and Campbell 1981; Krauss and Marschner 1982*). The growth of spears was most rapid at 33°C (Table 4.3). This may be explained in that the mildly high temperature may increase GA content and decrease

auxin content, and accelerate plant development (*Radley 1976; Hadid et al. 1986*). Furthermore there may be a differences in the binding capacity of the receptor site for the hormone or in the reaction of the ABA receptor complex to heat stress. Changes of sensitivity to GA may also contribute to the observed ABA response differences (*Walker-Simmons 1988*), therefore it is possible that plants can contain high ABA levels and still maintain rapid growth.

Heat tolerance involves a variety of physiological processes rather than simply a maintenance of growth rate. For example a high auxin level may prevent the abscission of newly set tomato fruit at high temperature, because heat susceptible lines at high temperature stress have decreased auxin levels (*Kuo and Tsai 1984*). If the criterion of heat tolerance of asparagus is spear flush and fern development, then the four asparagus cultivars studied have no tolerance above 38°C. In fact, the preliminary experiment also showed that seedlings could not survive above 38°C and died at 40°C. These results correspond with the low ABA levels of seedlings at these regimes, because the elevated ABA level may play a key role in resisting high temperatures (*Hellali and Hester 1979*), hence it is proposed that when heat stress is high enough to depress ABA level, this then leads to a cessation of growth and death of the asparagus plant.

CHAPTER SIX

Membrane Thermostability and Heat Tolerance of Asparagus

6.1. Introduction

High temperature depresses plant growth and development in asparagus (see chapters 2, 3, 4, 5). Growth depression is generally thought to be the result of change in enzymatic activation energy, protein denaturation, inactivation of photosystem II, cessation of cytoplasmic streaming and respiration, inhibition of translocation and leakage of cell electrolytes. Many methods of measuring these changes have been developed to assay heat injury, and in particular, the measuring of electrolyte leakage of membranes is an inexpensive, sensitive and reliable assay, applicable to many plants and tissues (*Lester 1985*). Because heat injury increases the permeability of cell membranes, electrolyte leakage from injured cells can be correlated with membrane thermostability which corresponds with heat tolerance (*Paull 1981; King and Ludford 1983; Saadalla et al. 1990a,b*).

Thermotolerance is not only related to plant species, genotype and plant tissue, but is also related to the pre-conditioning temperature condition; i.e. plant response to heat tolerance also depends on acclimation to high temperature (*Saadalla et al. 1990a; Vierling 1991*). *Chen et al. (1982)* suggested that when breeding for high yield under high temperature conditions, it is preferred to select genotypes with high heat adaptability. The heat adaptability between genotypes cannot be distinguished unless plants are acclimated at a reasonably high temperature. Therefore in my experiment the thermostabilities of spears with different maturities and from different acclimation treatments were measured to distinguish differences in heat tolerance among cultivars.

A mathematical model has been developed (*Ahrens and Ingram 1988*) that characterizes the response to high temperature as sigmoidal. The inflection point in the response curve is the critical high temperature (T_m) for a given

exposure duration. In my studies *Ingram's* equation (*Ingram* 1985) was used to predict T_m as the critical high temperature of membrane thermostability in asparagus.

6.2. Materials and Methods

6.2.1. Plant Materials

One year old potted plants of four asparagus cultivars, namely Brocks, Larac, Tainan No.1 and UC157, were prepared as previously described (Chapter 3). All ferns were cut from these plants before they were placed in growth cabinets or greenhouse. The plants were pre-acclimated for the first 2 weeks in growth cabinets, then all spears were cut. After acclimation the middle size of spears (about 25 cm height) were sampled to estimate membrane thermostability.

Plants were placed in growth cabinets at (A) day $25 \pm 1.5^{\circ}\text{C}$ / night $17 \pm 1.5^{\circ}\text{C}$, (B) day $35 \pm 1.5^{\circ}\text{C}$ / night $25 \pm 1.5^{\circ}\text{C}$. A 12 hours photoperiod at PFD of $750 \mu\text{mol s}^{-1}\text{m}^{-2}$ was synchronous with day/night temperature treatment, or (C) were grown in a greenhouse (Plant Growth Unit, Massey University) in September 1990. All spears from the growth cabinets were sampled 4 hours after the start of the light period or at 10 am in the greenhouse grown plants.

6.2.2. Heat Injury Treatment

Three cm sections from the butt (older), middle or upper (younger) parts of the spears were used to measure the effect of spear maturity on membrane thermostability. For comparison of thermostabilities among cultivars only the upper portion of the spears was used. Each section was dissected into 8 segments of about 2.5 mm thickness, then the 8 segments from the same section were used in a series of heat treatments at 25°C , 35°C , 40°C , 45°C , 50°C , 55°C , 60°C and 65°C . There were 10 replications (sections) for the series of heat treatments to minimize genetic and maturation variation.

These dissected segments were washed in deionized water 4 times for 20, 30, and 60 minutes to remove exogenous electrolytes, then, after the water was

decanted off, each segment was placed into an individual plastic centrifuge tube. Each tube contained 2 drops of deionized water to maintain humidity during heat injury treatments, and was sealed with a plastic stopper.

Spear segments exposed to heat injury were placed in thermostatically controlled water baths for 30 minutes. The water baths were pre-set at $25 \pm 0.5^{\circ}\text{C}$, $35 \pm 0.5^{\circ}\text{C}$, $40 \pm 0.5^{\circ}\text{C}$, $45 \pm 0.5^{\circ}\text{C}$, $50 \pm 0.5^{\circ}\text{C}$, $55 \pm 0.5^{\circ}\text{C}$, $60 \pm 0.5^{\circ}\text{C}$, and $65 \pm 0.5^{\circ}\text{C}$. After the heat treatment 8 ml water was added to tube, which was then incubated at 15°C for 1 hour. Then samples were shaken gently (to prevent damage tissue) and the electrical conductivity (R_1) measured immediately with a conductivity meter (Lriac, Model: CM 100/E, New Zealand).

After these measurements were taken the tubes were immediately frozen with liquid air for 10 minutes to completely kill the cell membranes. The tubes were then stood in a water bath at 25°C for 1 hour and the electrical conductivity (R_2) again measured. Electrolyte leakage (El) of each segment was expressed as the ratio of incubation solution conductivities R_1/R_2 .

6.2.3. Analysis of Membrane Thermostability with Mathematic model

The electrolyte leakage values, El , were fitted to sigmoidal response curves (*Ingram* 1985). The *Ingram's* equation is described as following:

$$El=Z+\frac{X-Z}{1+e^{-K(T-Tm)}}$$

where

- Z the baseline level of electrolyte leakage.
- X the maximum proportion of electrolyte leakage.
- Tm the temperature corresponding to the midpoint. i.e. inflection point of the response curve.
- K a function of the slope at the inflection point.
- T heat injury temperature.

A least squares approach of this non-linear regression, using PROC NLIN (SAS Institute Inc.), was employed to determine the best fit for each treatment and derived these parameters (T_m , Z , X , K) of *Ingram's* equation.

6.3. Results

6.3.1. Effects of Spear Maturity and Duration of heat Injury on thermostability

The results (Table 6.1) showed that the midpoint temperatures (T_m) varied with the maturity of spears and duration of heat injury. The upper spear always had a low T_m , while the spear butt had a high T_m . In addition, heating for 60 minutes generally led to lower T_m s than heating for 20 or 30 minutes. The order of T_m was butt > middle > upper, and this pattern did not change with heating time. The spears heated for 20 minutes were shorter spears than those heated for 30 or 60 minutes, thus the former had higher Z than the latter. By contrast, both X and K values were rarely related to spear maturity. The correlation between coefficients (T_m , K, X, Z) ranged between -0.5 to 0.5 indicating weak interrelationships.

6.3.2. Comparing Membrane Thermostabilities between Cultivars and Growing Temperatures

When spears were sampled from plants grown at D35°C/N25°C and D25°C/17°C in growth cabinets, UC157 had the highest T_m , whereas when spears were sampled from the plastic greenhouse, UC157 had the lowest T_m (Table 6.2). Generally the T_m increased with increasing temperature at which the spear was grown, i.e. for UC157 the increasing magnitude of T_m from 46.03°C at D25°C/N17°C to 46.95°C in the greenhouse was less than for Brocks, Larac and Tainan No.1. from about 44.74°C to 47.75°C. Furthermore at D35°C/N25°C the T_m of 50.40°C for UC157 was higher than the others which were about 49.0°C. Generally the heat tolerance of Brocks, Larac and Tainan No.1 increased with increasing growth temperature, but not as much as UC157.

Table 6.1. The effect of heating time on the Ingram's equation parameters of different sections.

Section	Tm (°C)	K	Z	X
<u>Heating 20 minutes</u>				
Butt	50.58 ± 0.56	0.302 ± 0.047	0.250 ± 0.022	0.911 ± 0.024
Middle	49.58 ± 0.51	0.370 ± 0.063	0.326 ± 0.019	0.891 ± 0.018
Upper	46.68 ± 0.61	0.357 ± 0.067	0.531 ± 0.016	0.891 ± 0.012
<u>Heating 30 minutes</u>				
Butt	50.87 ± 0.29	0.423 ± 0.048	0.164 ± 0.014	0.914 ± 0.016
Middle	49.26 ± 0.29	0.321 ± 0.026	0.174 ± 0.024	0.910 ± 0.011
Upper	47.41 ± 0.71	0.295 ± 0.053	0.270 ± 0.030	0.922 ± 0.024
<u>Heating 60 minutes</u>				
Butt	49.39 ± 0.57	0.335 ± 0.057	0.173 ± 0.027	0.917 ± 0.026
Middle	47.33 ± 0.78	0.270 ± 0.050	0.196 ± 0.035	0.930 ± 0.030
Upper	44.49 ± 0.54	0.289 ± 0.069	0.265 ± 0.024	0.928 ± 0.017

Table 6.2. Comparison of the Ingram's equational parameters between cultivars at various growth temperatures.

Section	Tm (°C)	K	Z	X
<u>Plastic house</u>				
Brocks	47.52 ± 0.86	0.372 ± 0.101	0.582 ± 0.018	0.942 ± 0.018
Larac	47.58 ± 0.99	0.435 ± 0.148	0.577 ± 0.022	0.942 ± 0.023
Tainan	47.75 ± 0.88	0.465 ± 0.145	0.582 ± 0.020	0.945 ± 0.020
UC157	46.95 ± 0.99	0.357 ± 0.109	0.600 ± 0.020	0.942 ± 0.020
<u>Day 35°C / night 25°C</u>				
Brocks	48.71 ± 1.46	0.315 ± 0.129	0.478 ± 0.033	0.913 ± 0.038
Larac	49.04 ± 1.03	0.370 ± 0.128	0.447 ± 0.027	0.910 ± 0.031
Tainan	49.00 ± 1.15	0.321 ± 0.108	0.486 ± 0.026	0.915 ± 0.031
UC157	50.40 ± 1.09	0.317 ± 0.100	0.437 ± 0.027	0.933 ± 0.037
<u>Day 25°C / night 17°C</u>				
Brocks	44.46 ± 0.74	0.515 ± 0.211	0.454 ± 0.029	0.921 ± 0.022
Larac	44.74 ± 1.20	0.361 ± 0.142	0.472 ± 0.036	0.913 ± 0.028
Tainan	44.55 ± 1.04	0.428 ± 0.184	0.430 ± 0.038	0.919 ± 0.030
UC157	46.03 ± 0.74	0.373 ± 0.091	0.472 ± 0.021	0.919 ± 0.019

6.4. Discussion

Many techniques are available to assay plant heat tolerance, but the assay of membrane thermostability is the preferred technique due to its reliability and time saving features (*Onwueme 1979; Martineau et al. 1979; Ingram and Buchanan 1984; Chen et al. 1982; Bouslama and Schapaugh 1984; Marsh et al. 1985; Saadalla et al. 1990a,b; Shanaha et al. 1990*). The results of my experiments suggest that membrane thermostability is an appropriate assay to measure heat tolerance of asparagus. The differences of heat tolerance between various portions of spear (maturity), spears grown at various temperatures and cultivars is revealed by the coefficient T_m (Table 6.1, 6.2). These results showed that T_m varied with genotype, age, environment and their interaction as also reported in spring and winter wheat, soybean and *Cucumis melo* (*Martineau et al. 1979; Bouslama and Schapaugh 1984; Lester 1985; Shanaha et al. 1990; Saadalla et al. 1990a,b*). It is therefore concluded that membrane thermostability is a reliable test for heat tolerance, and that it has application to asparagus as it has for other crops.

In my studies younger (upper) spear sections were more sensitive to high temperature than older (butt) sections (Table 6.1). Similarly, *Chen et al. (1982)* showed that heat tolerance was positively related with acclimating temperature and heat adaptability was negatively related with age. The upper portion of each spear should therefore be selected as the most useful indicator of heat tolerance.

Heat acclimation may increase the membrane thermostability of young tissues perhaps due to the increasing membrane developmental rate rather than simply increasing maturity. The work of *Lester (1985)* showed that the young leaves of *Cucumis melo* may alter membrane thermostability within 24 hours heat acclimation, but the older leaves had a lesser ability to acquire heat tolerance. Acclimation to high temperature may involve a change in development and composition of cell membranes. It has been shown that

plants grown at high temperature have increased saturated fatty acids in membranes, and that increased lipid saturation is correlated with the greater thermostability (*Pearcy* 1978). Moreover, *Lin et al.* (1985) proposed that the induced heat shock protein (HSP) 15 kD (kilo-Dalton) may play a role in preventing leakage of electrolyte. Although ABA appeared responsible for part of the HSPs induction (*Heikkila et al.* 1984; *Radin and Hendrix* 1986; *Sachs and Ho* 1986), the work of *Lafuente et al.* (1991) showed that there was not a direct relationship between ABA and the electrolyte leakage of membrane.

The profile of electrolyte leakage with increasing temperature in asparagus spears showed a sigmoidal shape that was consistent with the work of *Ingram and Buchanan* (1984) and *Inaba and Grandall* (1988;). The mathematic model developed by *Ingram* (1985) provided a better approach to derived T_m than the method of direct interpolation used by *Martinean et al.* (1979), *Chen et al.* (1982), *Ingram and Buchanan* (1984) and *Lester* (1985), because *Ingram's* model can estimate T_m more accurately than the interpolation method (*Ahrens and Ingram* 1988). The reason is that the spears are very sensitive at the range around T_m , thus a dramatic change in magnitude of electrolyte leakage occurs over a narrow temperature range, therefore the temperature causing 50% relative injury can not be accurately estimated by the interpolation method, unless the electrolyte leakage is measured at very small intervals of temperature. Thus the data fitted to a sigmoid-shaped model is the preferred method to predict T_m .

Nonlinear regression analysis, when the parameters of Z and X represent the lowest and the highest extent of electrolyte leakage respectively, and K represents the slope at T_m , indicated that these coefficients (K , Z , X) were not strongly correlated with heat tolerance. Only T_m was directly associated with thermostability and was a meaningful index, as T_m was only weakly correlated with the other 3 coefficients. Furthermore, *Ingram* (1986) and *Inaba and Crandall* (1988) showed that T_m is negatively correlated with the natural logarithmic of exposure time, and the T_m may be predicted from the linear

relationship of T_m and exposure time. My study showed that the T_m was a useful index of thermotolerance, thus supporting the work by *Chen et al.* (1982); *Ingram* (1985) and *Inaba and Crandall* (1988).

The work of *Chen et al.* (1982) showed that heat tolerance dramatically increased when it reached a certain growth temperature. Thus difference in heat tolerance between genotypes becomes clear. For example, when bean, potato, soybean and tomato were grown at 35 to 37°C there were dramatic increases in heat tolerance, but above the temperature the effect of acclimation declined. Therefore it is proposed that when the environmental temperature reaches a certain level, over a narrow range, an acclimation mechanism is activated which increases heat tolerance. When the extent of heat tolerance between species or cultivars is to be compared, this should only be when the plants are grown in acclimating conditions (*Chen et al.* 1982).

Using T_m as a criterion of heat stress tolerance showed that asparagus can acquire heat tolerance when growth temperature increased from D25/N17°C < greenhouse < D35/N25°C (the temperature regime of greenhouse was between D25/N17°C and D35/N25°C). UC157 had a similar heat tolerance response to the other cultivars when plants were grown at D25/N17°C or in the greenhouse, but at high heat stress condition, e.g. D35/N25°C, UC157 was superior to the other cultivars. Therefore UC157 may be expected to be best adapted to a tropical climate, because UC157 had the highest T_m . In addition, Tainan No.1, Larac and Brocks also have increased heat tolerance when grown at high temperature.

CHAPTER SEVEN

Heat Shock Proteins, ABA and Heat Tolerance in Asparagus

7.1. Introduction

Environmental conditions, such as temperature, light and water, may regulate gene expression in plants, with a the modification of gene expression leading to adaptation to stressful environments. Thus plants may develop a tolerance to normally lethal temperatures, if they are first subjected to certain treatments at a high (but non-lethal) temperature. It appears that the production of heat shock proteins (HSPs) is an essential component of thermotolerance development. Usually HSP synthesis begins when temperature exceeds 32 - 33°C, and increases with increasing temperature (*Lindquist and Crag 1988; Vierling 1991*). *Kimpel and Key (1985a)* divided these HSPs into two groups: the high-molecular-weight (HMW) and the low-molecular-weight (LMW) HSPs.

Although it is not yet possible to define precisely how HSPs contribute to a plant's ability to survive high temperature, the importance of the accumulation of HSPs for protection from thermal killing has been demonstrated. For example, *Oughum and Stoddart (1986)*, *Fender and O'Connell (1989)* and *Krishnan et al. (1989)* identified several unique HSPs as only occurring in thermotolerant lines. Thus genetic differences in the high-temperature susceptibility of crop plants may be correlated with variation in the temporal development of the capacity to synthesis HSPs and acquire thermotolerance (*BrodI 1989*). In addition, *Hwang and Zimmerman (1988)* showed that the HSPs between cell lines showed not only qualitative, but also quantitative differences . Thus this work was an attempt to link the genetic diversity of asparagus to thermotolerance and HSP synthesis.

ABA is thought to play an important role in a plant's response to a number of stress environments, and may be a mediator in adaptation (*Zeevaart and Creelman 1988; Klee and Estelle 1991*). Thus an elevated endogenous ABA

level under stress conditions or exogenous application of ABA may cause enhanced tolerance to stress environments. Moreover *Radin* and *Hendrix* (1986) also showed that the metabolites of abscisic acid may relate to the appearance of HSPs. At high temperature ABA concentrations are low compared to those at other stress conditions, such as water stress and cold stress and *Bray* (1991) showed that the elevated endogenous ABA levels did not involve a heat shock protein response in tomato. However, several inducers which induced all or part HSPs other than those induced by high temperature have been found, such as in response to water stress, metal, ABA, etc. (*Heikkila et al.* 1984; *Sachs and Ho* 1986; *Burke and Orzech* 1988). Thus whether ABA is involved in heat tolerance is not clear. In my studies, the ABA induced proteins are compared to heat induced proteins to examine any correlation between ABA and heat tolerance.

7.2. Materials and Methods

7.2.1. Heat Shock and ABA Induced Proteins

Two cultivars of asparagus, Larac and UC157, were used to measure heat shock induced proteins. Seeds of the 2 cultivars were germinated for 15 - 20 days (about 3 - 5 cm long shoot) at 24°C, then placed on filter papers moistened with distilled water and incubated at 28°C for 2 days. Subsequently half the seedlings were transferred to fresh filter paper (previously moistened with distilled water), and the remainder were transferred to fresh filter papers previously moistened with 1 μ M ABA¹. Both lots of seedlings were then incubated at 28°C for a further 24 hours, and then a 0.5 cm shoot segment from the tip was removed from each seedling for protein analysis.

7.2.2. *In Vivo* Protein Labelling

Proteins were labelled with L-S³⁵-methionine (>1000 Ci/mmol, Amersham), as described by *Krishnan et al.* (1989). The samples, with or without 1 μ M ABA, in 20 mM Tris-HCl buffer (pH 7.5) were placed in a water bath for 2 or 6 hours at 28°C, 34°C or 37°C.

7.2.3. Protein Extraction and Electrophoresis

7.2.3.1. Protein Extraction and One-dimension Gel Electrophoresis

The proteins were extracted as described by *Damerval et al.* (1986). The pellet was solubilized in 80 μ l SDS sample buffer solution (Appendix 15) plus 0.8 μ l bromophenol blue tracking dye (Appendix 16) and placed in boiling water for 5 minutes followed by centrifugation at 10000 g for 15 min. 30 μ l supernatant was then taken for one-dimensional SDS-PAGE.

¹ purchased from Aldrich, ($\pm$)-2-cis,4-trans-abscisic acid, 98%.

One-dimensional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) was performed as described by *Laemmli* (1970) with the following modifications. The gel was 10 - 20% (w/v) linear polyacrylamide gradient gel 11 cm long and 0.75 mm thick (Appendix 11). Electrophoresis was carried out at a constant current 25 mA for 1100 volt-hours (V-H).

7.2.3.2. Protein Extraction and Two-Dimension Gel Electrophoresis

The pellet was solubilized in 50 μ l UKS solution (Appendix 13), centrifuged at 10000 g for 15 minutes, and then 20 μ l of supernatant taken for two-dimensional IEF/SDS-PAGE (isoelectric focus/sodium dodecyl sulfate polyacrylamide gel electrophoresis).

Two-dimensional IEF/SDS-PAGE was performed as described by *O'Farrell* (1975). The IEF gels were 13.5 cm long (Appendix 12) and after running for 8000 V-H (400 volts X 16 hours + 800 volts X 2 hours) were equilibrated in the equilibration solution for 15 minutes (Appendix 14). The second electrophoresis was performed as for one-dimensional gel electrophoresis except that the gel length was 13.5 cm and run at 1200 V-H (Appendix 11).

7.2.3.3. Fluorographic procedures

Fluorography was carried out as described by *Skinner* and *Griswold* (1983). The slab gel was soaked in acetic acid for 5 min, then soaked in 20% (w/v) PPO (2,5-diphenyloxazole) for 90 min, and finally soaked in distilled water for 30 min. Sequently the gel was laid on thick filter paper and covered with a cellophane membrane and dried with a Slab Dryer (model 443, Bio-Rad, Richmond, CA) under high vacuum at 60°C for 5 hours. It was cooled before releasing the vacuum.

The dried gels were exposed to pre-flashed Kodar X-Omat AR5 film at -70°C in Cassette (Cronex, intensifying screens, lightning plus, DuPont).

7.3. Results

Asparagus shoots exhibited changes in the pattern of protein synthesis when heat shocked at 34°C and 37°C for 2 and 6 hours. HSPs were observed in asparagus shoots after one-dimensional SDS-PAGE and two-dimensional IEF/SDS-PAGE analysis. Basically, the protein patterns between 2 hours and 6 hours were similar, but the protein patterns between plus and minus ABA were different.

7.3.1. Heat Shock Induced Pattern of Protein Synthesis

Proteins of 70 and 48 kD (kilo-Dalton) bands were induced and a 45 kD band disappeared in UC157 at 34°C for 2 hours compared to 28°C for 2 hours, but 2 protein bands of 90, 17 kD were induced and a band of 13.5 kD disappeared at 34°C for 6 hours compared to 28°C for 6 hours in UC157. The patterns of UC157 at 37°C and 34°C were similar. five protein bands of 97, 90, 70, 48 and 36 kD were induced and a 45 kD band disappeared for Larac at 34°C for 6 hours compared to 28°C (Plate 7.1; Table 7.1). The patterns of Larac between 37°C and 34°C were similar except that bands 97 and 90 kD disappeared at 37°C. A 97 kD protein was induced at 34°C in Larac, but not in UC157.

When comparing the protein patterns following heat shock and normal temperatures, it was found that heat shock not only induced HSPs, but also changed the amount of normal proteins. Comparing HSPs between cultivars it was found that most LMW HSPs were similar, but a few were cultivar specific, such as the 5 LMW HSPs (numbers 13, 23, 31, 32, 34) only observed in UC157 and the 5 LMW HSPs (numbers 17, 18, 19, 20, 24) in Larac. four HMW HSPs (No. 3, 5, 6, 8) were found in UC157 while these HSPs were absent in Larac (Plate 7.2, 7.3; Table 7.2). Heat shock induced more HSPs in UC157 (21 HSPs) than in Larac (17 HSPs).

7.3.2. ABA Induced Patterns of Protein Synthesis

Asparagus shoots also exhibited changes in the patterns of protein synthesis when subjected to 28, 34, 37°C for 2 and 6 hours in presence of ABA (Plate 7.4). The protein pattern of UC157 at 34°C plus ABA for 6 hours and of Larac at 37°C plus ABA for 2 and 6 hours showed a great number of new bands. The ABA induced protein bands between 2 and 6 hours at 28°C or at 37°C were similar. The shoots of UC157 subjected to 34°C plus ABA for 2 hours lacked 4 bands (70, 19, 17, 16.5 kD) compared to 28°C plus ABA for 2 hours. Larac also lacked 2 bands (70 and 17 kD) and produced 2 bands (80 and 48 kD) (Table 7.3). UC157 at 34°C plus ABA for 6 hours induced 4 bands (80, 36,

Table 7.1. Comparison of the heat shock induced protein patterns using one-dimensional SDS-PAGE.

M.W. kD	Larac						UC157					
	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C
97	-	-	+	+	-	-	-	-	-	-	-	-
90	-	-	+	+	-	-	-	-	?	+	+	+
80	-	-	-	-	-	-	?	-	-	-	-	-
70	-	-	+	+	+	+	-	+	+	+	+	+
48	-	-	+	+	+	+	-	+	+	+	+	+
45	+	+	-	-	-	-	+	-	-	-	-	-
42	?	?	+	+	+	+	?	+	-	-	-	-
38	?	+	+	-	-	-	?	?	-	-	+	-
36	-	-	+	+	+	+	-	+	-	-	-	+
20	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
17	?	?	+	+	+	+	?	-	+	+	+	+
16.5	?	?	+	+	+	+	?	+	+	+	+	+
15	?	?	+	+	+	+	+	+	+	+	+	+
13.5	+	+	+	+	+	+	?	+	-	-	+	+

+ presence
- absence
? not clear

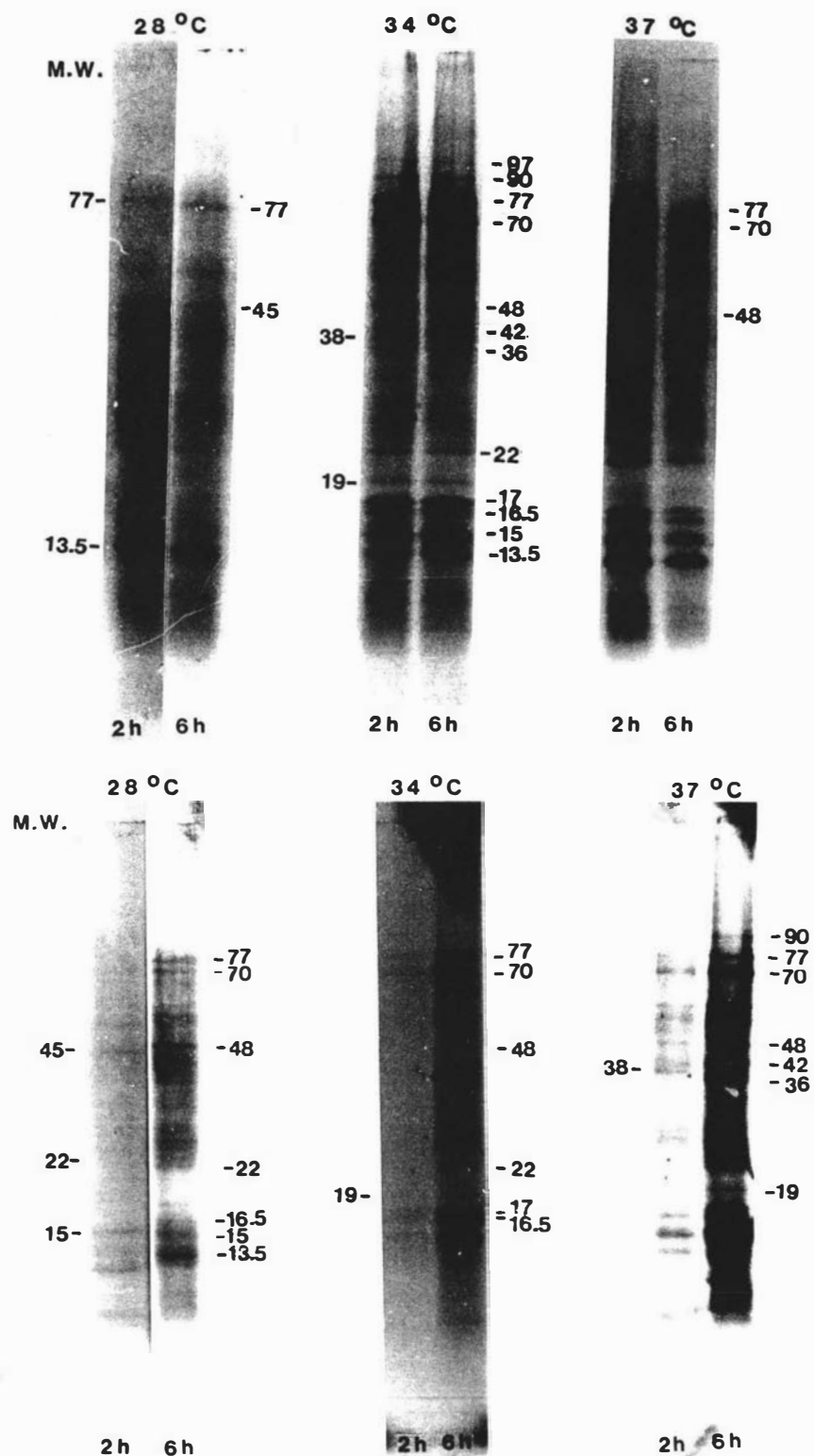


Plate 7.1. Comparison of protein patterns at 28°C, 34°C and 37°C for 2h (hours) or 6 h of Larac (Top) and UC157 (Bottom) using one-dimensional SDS-PAGE

Table 7.2. Comparison of heat shock plus / minus ABA induced protein patterns using two-dimensional IEF/SDS-PAGE.

HSP No.	Larac				UC157			
	28°C	28°C+ABA	37°C	37°C+ABA	28°C	28°C+ABA	37°C	37°C+ABA
1	+	+	-	-	+	+	-	+
2	-	+	-	-	-	-	-	+
3	-	-	-	+	-	-	+	+
4	-	-	-	-	-	-	-	+
5	-	+	-	-	-	+	+	+
6	+	+	-	-	-	+	+	-
7	-	-	-	-	-	+	-	+
8	-	+	-	-	-	+	+	-
9	-	+	-	-	-	+	-	+
10	-	+	+	-	-	+	+	+
11	-	-	+	+	-	-	+	+
12	-	+	+	+	-	-	+	+
13	-	-	-	-	-	-	+	+
14	-	-	+	+	-	-	+	+
15	-	-	+	+	-	-	+	+
16	-	-	+	+	-	-	+	+
17	-	-	+	+	-	-	-	-
18	-	-	+	-	-	-	-	-
19	-	-	+	+	-	-	-	-
20	-	-	+	+	-	-	-	-
21	-	-	-	+	-	-	-	-
22	-	-	+	+	-	-	+	+
23	-	-	-	-	-	-	+	+
24	-	-	+	+	-	-	-	-
25	-	-	+	+	-	-	+	+
26	-	-	-	-	-	-	-	+
27	-	-	+	+	-	-	+	+
28	-	-	+	-	-	-	+	+
29	-	-	+	-	-	-	+	-
30	-	+	+	+	-	-	+	+
31	-	+	-	-	-	-	+	-
32	-	-	-	-	-	-	+	-
33	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	+	-
35	-	-	-	+	-	-	-	-
36	-	+	-	-	-	-	-	-
37	-	+	-	-	-	-	-	-
38	-	+	-	-	-	-	-	-

+ presence - absence

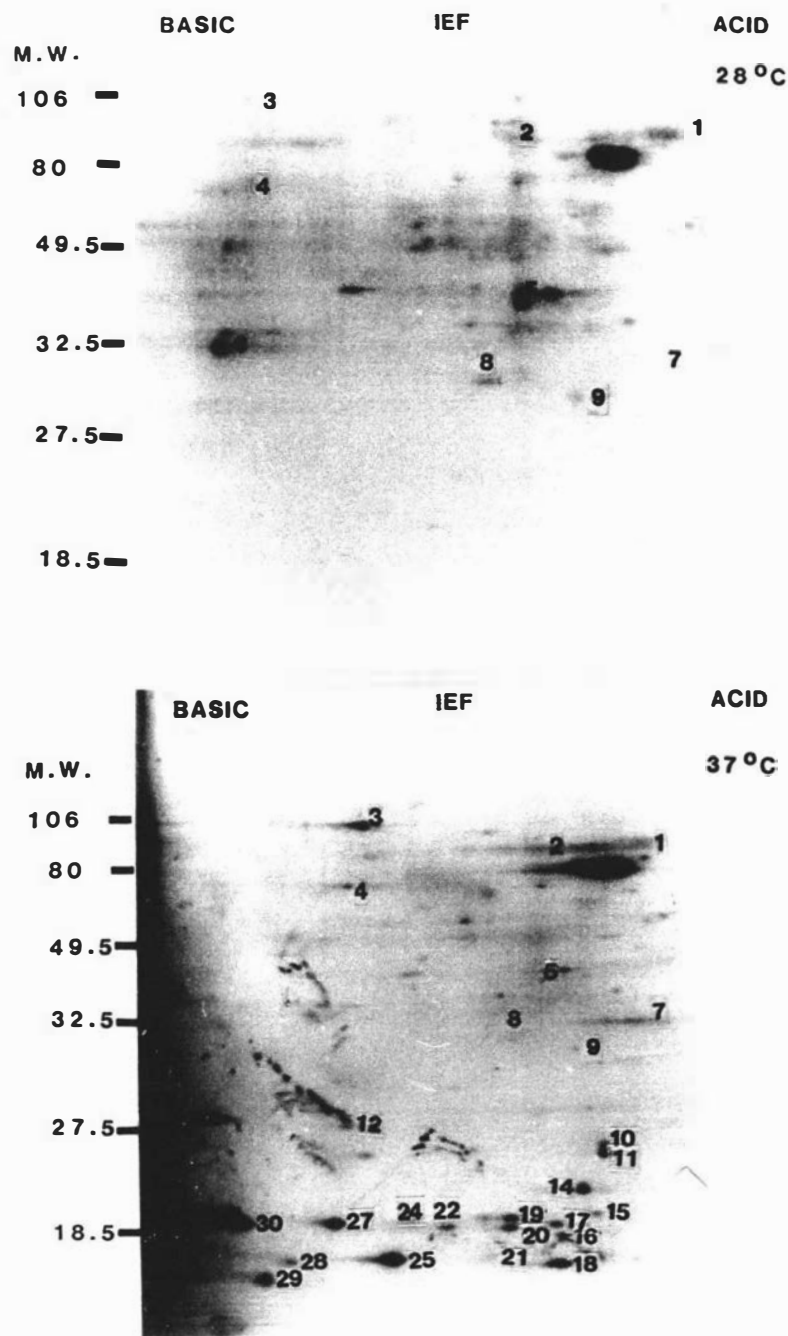


Plate 7.2. Heat shock induced protein patterns of Larac using two-dimensional IEF/SDS-PAGE. (Top : 28°C; Bottom : 37°C)

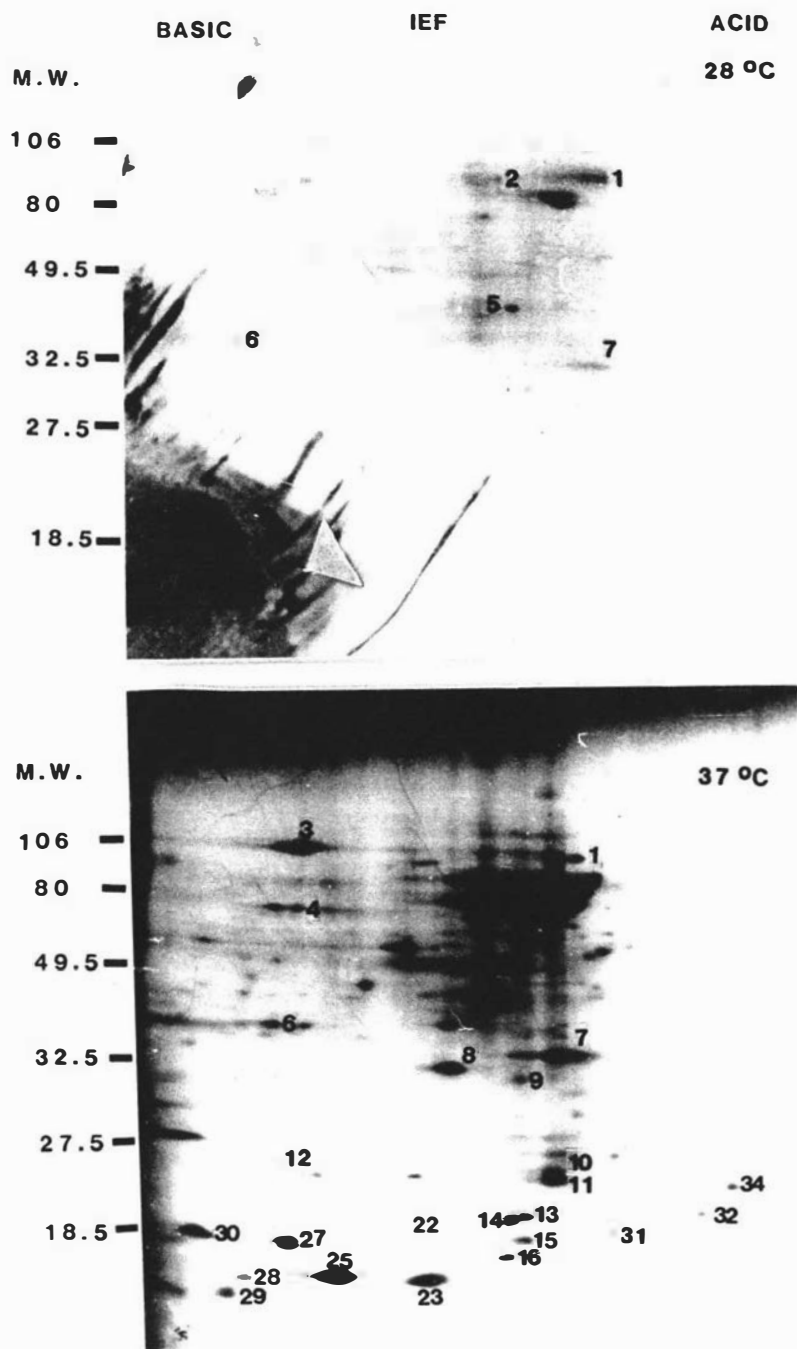


Plate 7.3. Heat shock induced protein patterns of UC157 using two-dimensional IEF/SDS-PAGE. (Top : 28°C; Bottom : 37°C)

20, 18 kD) and 4 bands disappeared (70, 19, 17, 15 kD) compared to at 28°C plus ABA for 6 hours, and Larac at 34°C plus ABA for 6 hours were 3 induced bands (80, 36, 18 kD) and 2 bands disappearing (70 and 17 kD) compared to 28°C plus ABA for 6 hours. Overall the bands induced by ABA at 34°C for 2 hours were less than for 6 hours.

There were many induced proteins at 37°C plus ABA which covered from HMW to LMW (Plate 7.5, 7.6; Table 7.2). The ABA induced proteins varied with cultivars, only a few were present in both cultivars (i.e. No.5, 6, 8, 9, 10 at 28°C and No.3 at 37°C).

Table 7.3. Comparison of the heat shock plus ABA induced protein patterns using one-dimensional SDS-PAGE.

M.W. kD	Larac						UC157					
	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C
97	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	+	-	-	-	-	-	-	-
80	-	-	+	+	-	-	-	-	-	+	-	-
70	+	+	-	-	+	+	+	+	-	-	+	+
48	-	+	+	+	+	+	+	+	?	+	?	?
45	+	-	+	-	-	-	+	-	+	-	-	-
42	-	-	-	-	-	-	-	+	-	+	-	-
38	-	-	-	-	-	-	-	-	-	-	-	-
36	-	-	-	+	-	-	-	-	-	+	-	?
20	-	-	-	-	-	-	-	-	-	+	-	-
19	-	-	-	-	+	?	+	+	-	-	-	-
18	-	-	-	+	-	-	-	-	-	+	-	-
17	+	+	-	-	+	+	+	+	-	-	+	+
16.5	+	+	+	+	+	+	+	+	-	+	+	+
15	+	+	?	+	+	+	+	+	+	-	+	+
13.5	+	+	+	+	+	+	+	+	+	+	-	-

+ presence
- absence
? not clear

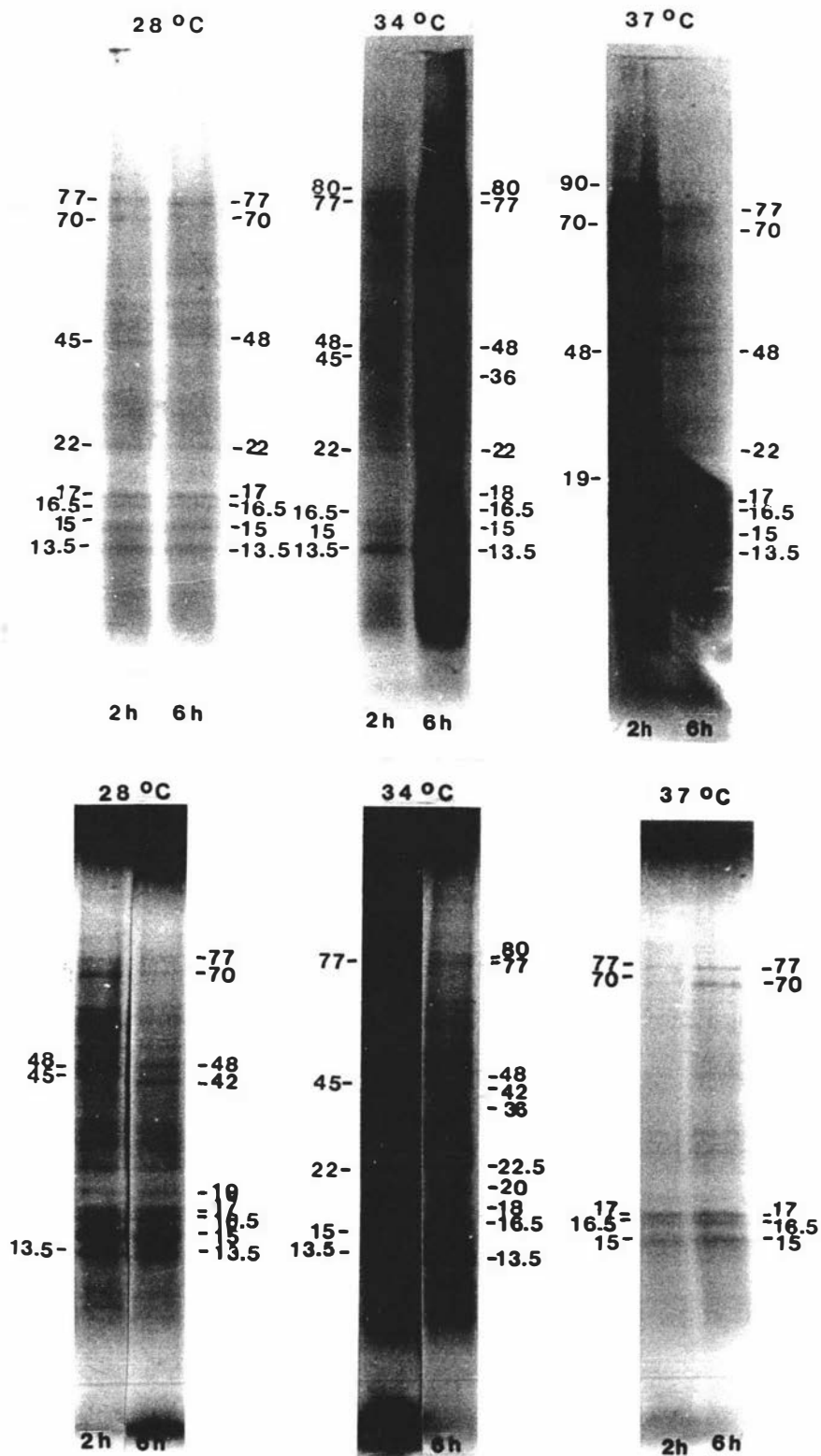


Plate 7.4. Comparison of protein patterns at 28°C, 34°C and 37°C plus ABA for 2h (hours) or 6h of Larac (Top) and UC157 (Bottom) using one-dimensional SDS-PAGE.

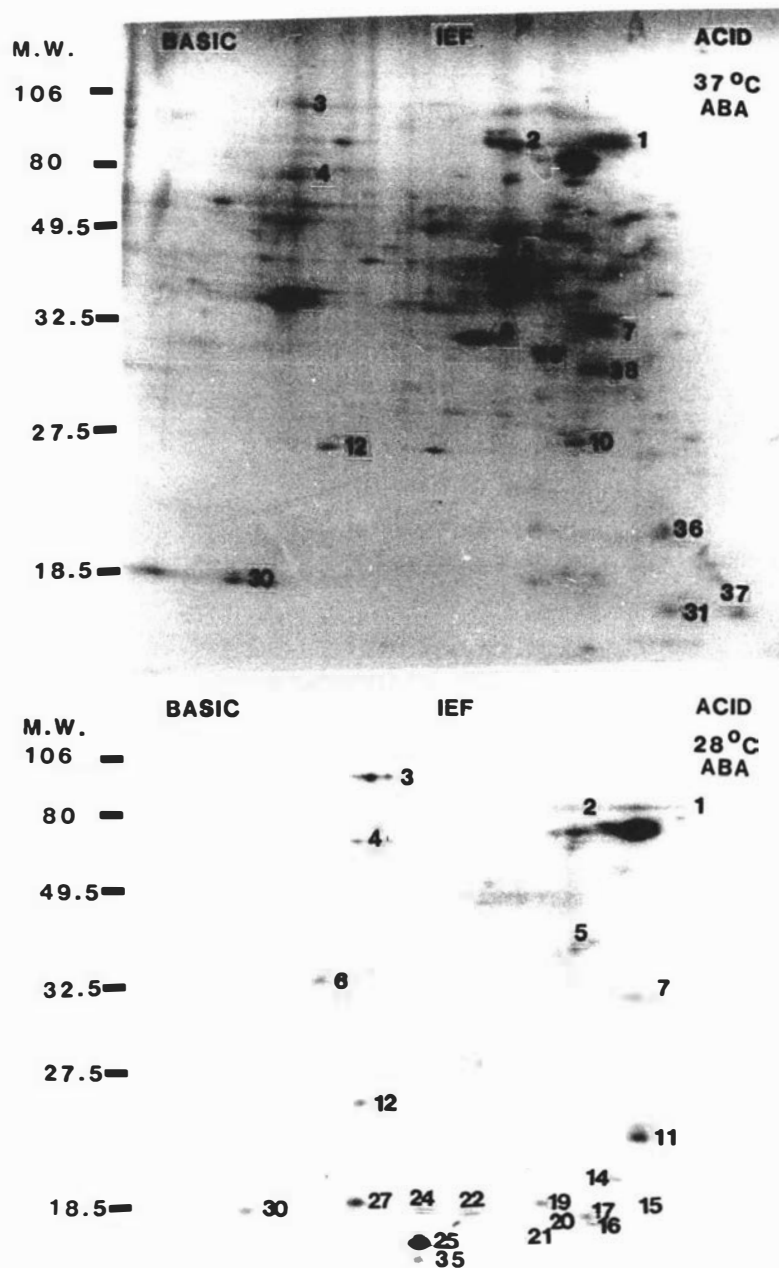


Plate 7.5. Heat shock plus ABA induced protein patterns of Larac using two-dimensional IEF/SDS-PAGE. (Top : 28°C; Bottom : 37°C)

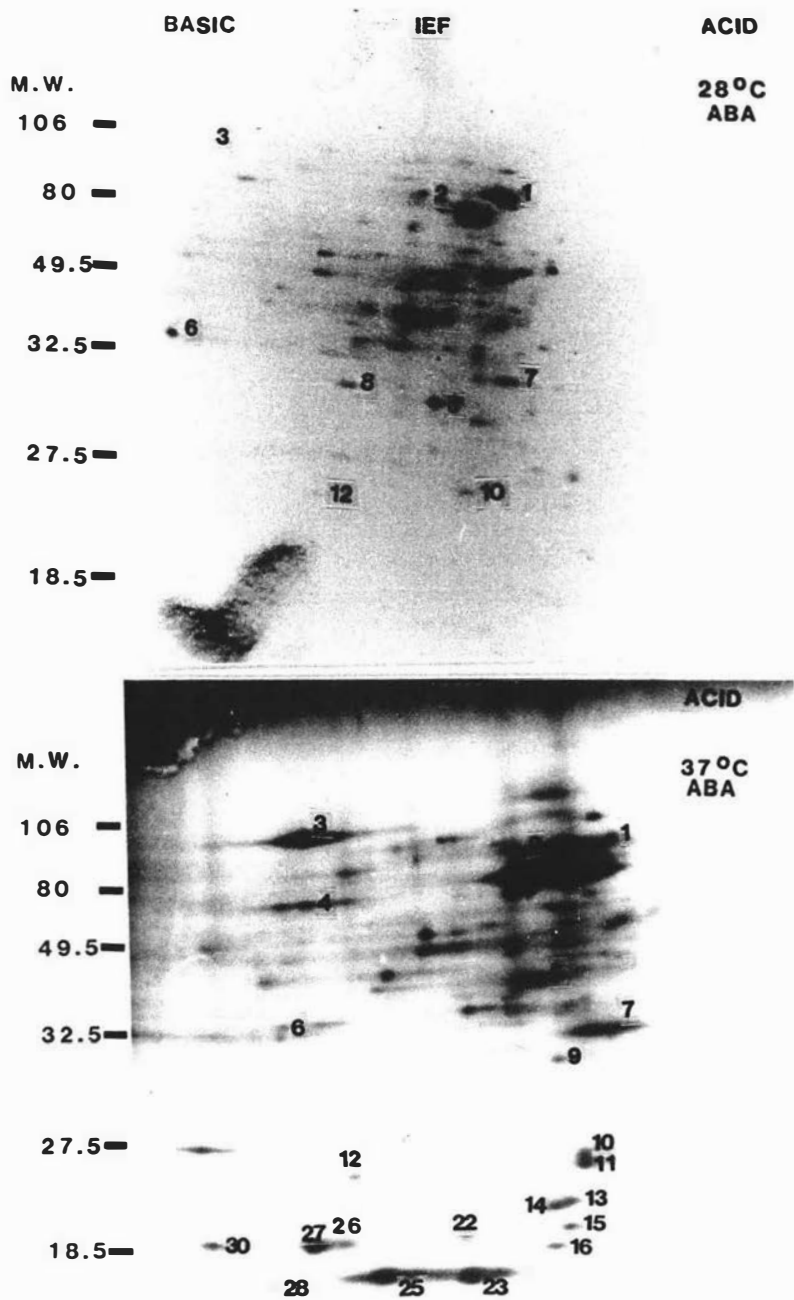


Plate 7.6. Heat shock plus ABA induced protein patterns of UC157 using two-dimensional IEF/SDS-PAGE. (Top : 28°C; Bottom. : 37°C)

7.3.3. Comparing Protein Synthesis Induced by Heat Shock and ABA

The protein pattern at 28°C plus ABA for 2 hours was compared to 28°C minus ABA for 2 hours. The former had 3 induced bands (70, 48, 19 kD) and 1 band disappeared (36 kD for 6 hours ABA) for UC157, and 2 induced bands (70 kD, 48 kD for 6 hours only) and 2 bands disappeared (45 and 38 kD for 6 hours only) for Larac. The patterns of protein synthesis at 28°C minus ABA for 2 hours were different from the patterns of protein synthesis at 34°C and at 37°C plus ABA for 2 or 6 hours.

The induced proteins at 28°C plus ABA were very different from those at 28°C minus ABA and most proteins induced by ABA at 28 °C were different from those HSPs induced at 37°C in two-dimension SDS-PAGE. For example at 37°C and 28°C UC157 had one LMW (No.26) and 5 HMW (No.1, 2, 4, 7,9) proteins induced plus ABA compared to 1 LMW (No.10) and 5 HMW (No.5, 6, 7, 8, 9) minus ABA, while Larac had 2 LMW (No.21, 35) and 1 HMW (No.3) induced plus ABA compared to 6 LMW (No.10, 12, 30, 31, 36, 37) and 5 HMW (No.2, 5, 8, 9, 38) minus ABA.

The protein patterns at 37°C plus ABA were very different from at 28°C, but were similar to the patterns at 37°C minus ABA. There were fewer HSPs at 37°C plus ABA than at 37°C minus ABA, as 6 HSPs (No.6, 8, 29, 31, 32, 34) in UC157, and 4 HSPs (No. 10, 18, 28, 29) in Larac were inhibited by ABA application. On the contrary, ABA application induced 6 proteins (No. 1, 2, 4, 7, 9, 26) in UC157 and 3 proteins (No. 3, 21, 35) in Larac.

7.4. Discussion

7.4.1. Heat Shock Induced Pattern of Protein Synthesis

The normal protein patterns of asparagus found here were similar to those found by *Bracale et al.* (1991). However a variety of new and distinct proteins were synthesized in response to elevated temperature, thus confirming the work of *Key et al.* (1981) and *Lafuente et al.* (1991). These studies also confirm the work of *Hwang* and *Zimmerman* (1988) and *Ristic et al.* (1991), who found both qualitative differences (cultivar specific proteins) and quantitative differences. Thus specific HSPs may be linked to a specific genetic heat tolerance. Generally the patterns of heat induced protein synthesis between cultivars were similar, but a few HSPs were cultivar specific.

The major HSPs were LMW and only a few were HMW. However differences in HSPs between asparagus cultivars occurred in both, while *Bewley et al.* (1983) and *Krishnan et al.* (1989) found changes mainly in LMW HSPs. Similar LMW HSPs tended to increase or decrease together, suggesting that they were encoded by linked genes, as was also found by *Hwang* and *Zimmerman* (1988) using carrot.

The pattern of proteins formed after 2 hours heat shock was slightly different from exposure to heat shock for 6 hours. Thus the patterns of protein synthesis depended not only temperature, but also time, as found also by *Bonham-Smith et al.* (1987). The explanation appears to be that one set of HSPs is maintained at an elevated level after development of high temperature tolerance is completed, and another set of HSPs only occurs during the acclimation process (*Baszczynski et al.* 1983; *Kee* and *Nobel* 1986). Thus it is suggested that heat tolerance of asparagus is triggered by acclimation, and that a suitable acclimation temperature and time may induce a great number of HSPs. *Farkhadi* and *Aliiev* (1990) also showed that heat shock at 37°C may

trigger more extensive heat tolerance than at 34°C.

Two dimensional IEF/PAGE/SDS-PAGE offered improved protein resolution. Thus a greater number of HSPs are likely to be found than with one-dimensional SDS-PAGE. Analysis of two-dimensional IEF/SDS-PAGE of asparagus after heat shock not only revealed a great number of new proteins, but also showed alteration of normal protein synthesis, thus confirming the work of *Key et al.* (1981); *Meyer and Chartier* (1983); *Burke and Orzech* (1988); *Clarke and Critchley* (1990). UC157 has better membrane stability at high temperature than Larac (Chapter 6), and yet the number of LMW HSPs found were similar although 5 out of 17 were different. These results do not support the suggestion of *Kee and Nobel* (1986) and *Krishnan et al.* (1989) that heat stable lines produced a greater number of HSPs.

LMW HSPs below about 18 kD were abundant in asparagus. This agrees with the work of *Mansfield and Key* (1987) and *Hwang and Zimmerman* (1989), that HSPs of 15 - 18 kD are unique to higher plants (*Sachs and Ho* 1986), as many LMW HSPs smaller than 14 kD were found. *Bracale et al.* (1991) also found that there were many small molecular weight proteins in asparagus.

7.4.2. ABA Induced Patterns of Protein Synthesis

Heat shock and other environmental stresses can alter gene expression, and ABA has been identified as an endogenous signal that is involved in the regulation of several environmentally induced genes (*Bonham-Smith et al.* 1987; *Bray* 1988, 1991; *Cohen and Bray* 1990). Whether ABA is involved in heat tolerance is of interest, because several workers (and my experiment) have shown that the application of low ABA concentrations may enhance plant growth or/and heat tolerance. The effect of stress has been shown to concomitantly elevate ABA levels (*Bensen et al.* 1988; *Bray* 1988; *Zeevaart and Creelman* 1988; *Plant et al.* 1991) and this is also confirmed with my results (Chapter 5), in which high temperature elevated the endogenous ABA

levels in asparagus. ABA also induced a pattern of changes in protein synthesis. Thus the effect of ABA induced / inhibited protein synthesis may be linked to enhanced growth at high temperature due to elevated endogenous ABA levels. In fact, ABA induces proteins which involve many physiological processes, such as the metabolic conversion of ABA to phaseic acid, adaptation to salt, and the inhibition of germination in immature embryos (Uknes and Ho 1984; Lin and Ho 1986).

Ried and Walker-Simmons (1990) citing previous works stated that the ABA responsive proteins possess unique biochemical properties which may protect cells during periods of limited growth or environmental stress. Therefore, even though the functions of ABA induced proteins in asparagus are unknown, it is postulated that these proteins are also heat stable and may be stress-related. Moreover in my studies it was found that some ABA induced proteins were similar to HSPs, and ABA inhibited a few HSPs, as some induced proteins at 28°C plus ABA disappeared at 37°C plus / minus ABA. However most HSPs still appeared at 37°C plus ABA. This result nearly agrees with the work of Bonham-Smith *et al.* (1987), who showed that heat shock overrode wound, water stressed or ABA induced protein synthesis, so that only HSPs were synthesized. Thus it is concluded that heat stress has a more extensive modification of protein synthesis than ABA application.

At high temperature the enhancement of seedling growth by applied low ABA concentration was found (see chapter 4). Thus one may speculate that although some HSP synthesis was depressed by ABA, most HSPs were still induced by high temperature with ABA application. Moreover, some specific proteins only appeared at 37°C plus ABA, such as 5 HMW proteins (No. 1, 2, 4, 7, 9) and 1 LMW protein (No. 26) in UC157, and 1 HMW protein (No.3) and 2 LMW Proteins (No. 21, 35). Thus ABA may turn on those genes responsive to ABA, and some of those genes may be responsive to diverse stresses, (such as HSP70 gene, Heikkilä *et al.* 1984), thus resulting in the enhancement of seedling growth.

Unfortunately the functions of HSPs or of ABA induced proteins is almost unknown, as only a few have been identified although some had already have their genes encoded and sequenced (*Lindquist and Craig 1988; Conner et al. 1990; Dietrich et al. 1991*). However it has been proposed that the function of HSPs may be to afford protection against potential lethal temperatures (*Kee and Nobel 1986*). The HSP genes may also include some multiple stress genes activated by other than a heat shock (*Bonham-Smith et al. 1987*). For example ABA may turn on some HSP genes when transferred from normal to high temperature regimes, thus enhancing thermotolerance. The diversity of HSPs may allow classical genetic analysis of allelic variation and gene action (*Vierling and Nguyen 1990*) and the genetic diversity associated with HSPs opens new areas for investigating heat tolerance.

CHAPTER EIGHT

Summary

A series of experiments were carried out to examine the physiological responses of asparagus to high temperature. These included analysis and modelling of growth, and the measurement of heat tolerance of asparagus at high temperatures. Experiments were carried out in climate rooms to measure the influences of high temperatures on young plant growth, and one year old crowns were grown in growth cabinets to measure the influence of high temperature on spear yield and fern development.

ABA is considered as a stress hormone, hence a part of my studies was to gain an understanding of the effects of ABA on heat tolerance and growth of asparagus plants at high temperatures. These studies included the effects of exogenous ABA on seedling growth at high temperature, the effect of high temperature on endogenous ABA levels in seedlings and spears, and the changed pattern in ABA-induced protein synthesis. Determinants of heat tolerance capacity included measurements of membrane thermostability and the changed pattern of heat-induced protein synthesis. The membrane thermostability of asparagus spears was measured to determine differences of heat tolerance between cultivars, while HSPs were analyzed to examine potential heat tolerance between cultivars. Both the patterns of ABA and heat shock induced protein synthesis were compared to reveal the possibility of ABA involvement in heat tolerance. These studies on physiological responses to high temperature are expected to be of value to facilitate asparagus production in tropical climates.

In the first experiment seeds of four asparagus cultivars were sown in climate rooms and were grown for 2 - 3 months for the analysis of plant growth at high temperatures. These growth data were collected weekly and then were fitted against plant age with a semi-log regression, from which were determined parameters such as RGRs, NARs, LARs, allometric coefficients and allometric

intercepts. These results showed that growth of asparagus during the young stage was exponential and thus the parameters RLGR, RFGR, RCGR, RPGR were constant for any one cultivar or temperature regime. The growth rates were classified according to the parameters NAR, LAR and RGR, into high : 'D25/N25°C and D30/N30°C', normal : 'D20/N20°C, D30/N20°C, D35/N15°C, and D35/N25°C', and poor growth rates : 'D35/N35°C, D40/N20°C, and D40/N30°C'.

The growth analysis parameters showed some differences among cultivars, especially for plants at the regimes D35/N15°C, D35/N25°C and D35/N35°C. The causes may be due to day/night temperature differential of supra-optimal day temperature and sub-optimal or supra-optimal night temperature triggering the characteristics of heat adaptation.

The NARs varied with temperature, plant age and cultivar. The effect of temperature on NAR was greater than the effect of cultivar. Generally, NARs decreased markedly with increasing age except that at D20/N20°C, D35/N35°C, D40/N20°C and D40/N30°C the NARs maintained little change throughout the experimental period. At D30/N30°C and at D25/N25°C NAR was highest at the 1st harvest and then declined with increasing age to become lowest at the last harvest.

The LARs also varied with temperature, cultivar and age. The effect of temperature was again greater than for cultivar. Generally, LARs could be classed into 4 groups. Plants in the first group D30/N30°C and D25/N25°C had the highest LAR and increasing LARs, the best growth; the second group D30/N20°C and D35/N25°C had the second highest LAR and increasing LARs, good growth; the third group D20/N20°C and D35/N15°C had nearly stable LAR's ($50 - 70 \text{ cm}^2\text{g}^{-1}$), normal growth; the last group D35/N35°C, D40/N20°C and D40/N30°C had low LAR, very poor growth, except that at D40/N20°C the LAR increased with age from a low initial figure. My studies found that the parameter LAR was reasonably related to the relative growth rate, because

high RGRs were related with high LARs, but comparing LARs between cultivars showed that cultivars having high LARs did not necessarily have high RGRs.

The leaf, stem, rhizome and root production rates comprise the RPGR. The leaf production rate was the largest contributor throughout the experiment period, root and stem production rates were the second and third largest contributors respectively, and rhizome production rate was the smallest contributor. In fact, the contributive magnitude of the individual components varied with age, e.g. the stem and the rhizome production rates declined with increasing age, while leaf production rate increased with increasing age, and the root production rate was maintained nearly constant except at D40/30°C and at D30/N30°C.

The predicted optimal temperatures for RLGR, RFGR, RCGR and RPGR are D26.8/N26.7°C, D26.5/N26.4°C, D26.6/N23.8°C and D26.3/N26.2°C, respectively. Hence a day and night temperature around 26.5°C is the optimal temperature for RLGR, RFGR and RPGR, while a night temperature of 23.8°C is the optimal temperature for RCGR.

Over a wide range of temperatures the plants produce allometric coefficients between 0.9 to 1.0, thus asparagus can be expected to be planted at tropical climates and to still have good balanced growth between root and fern. However the allometric intercepts at various temperatures were significantly different, with in general the lower the temperature, the greater the intercept (higher root : shoot ratio). The allometric intercepts between cultivars were also significantly different, Tainan No.1 having the largest and Brocks the lowest allometric intercepts. It is concluded that the allometric intercept is very dependent on genotype and environment, and thus may be valuable index for evaluating asparagus genotypes or plant environments. Furthermore the comparison of the allometric intercepts between constant temperatures and at 10°C day/night differential temperatures showed that at the same mean

day/night temperature regimes the intercepts of the day/night differential were higher than at the constant temperature.

One-year old crowns of four cultivars were grown in growth cabinets to examine spear yield and fern development at high temperatures (28°C, 33°C and 36°C). In one study, spears were harvested for 2 months and measurement of spear weight, spear yield, spear diameter and spear number were taken. In another study the spears were not harvested, but were left to develop into fern, and the height during development from spear to fern was measured. These results showed that not only did high temperature depress spear yield, but also depressed the total fern weight of the whole plant and individual fern height. This may be explained by high temperature elevating growth inhibitors or lowering growth promoters to decrease spear flush, fern height and spear number. The responses of fern characteristics to temperature varied with cultivars. Characteristics such as the first branch height and fern height were very sensitive and depressed at high temperature. Brocks and UC157 maintained good fern characteristics at 36°C, thus both cultivars represent a good adaption to high temperature, conversely Larac was the poorest adapted while Tainan No.1 was also well adapted except for its small diameter and dwarf fern. In particular it produced a high first branch and good spear quality.

It is concluded that all cultivars, excluding Larac, may adapt to tropical climates and produce marketable spears. When fern height was fitted to the Richard's Growth Equation the Gompertz function was preferred to the monomolecular or the logistic function. The higher the temperature the faster the growth, but fern height did the reverse. The spear growth (up to 20 cm high) was exponential, but spear growth appeared faster at 33°C than at 28°C and 36°C.

The patterns of the developing fern height changed with temperature. Generally, the higher the temperature the lower H_f , H^i , D^i and MAGRs of fern appeared in the experiment. Tainan No.1 had good parameters derived from

the Richards equation, and Brocks also had stable parameters from 28°C to 36°C. The criterion, in general, of fern or spear heat adaption with the parameters of Richard's Equation and RSGRs was in order of Tainan No.1 > Brocks > UC157 > Larac.

In order to understand the effects of ABA and heat tolerance, an experiment was carried out using asparagus seedlings with a 2 - 3 cm long roots. These were enveloped with germination paper previously wetted with ABA solution from 0.1 to 100 μ M, enclosed in a plastic bag and stood in incubators (28°C, 33°C, 34.5°C and 36°C) for 10 days. The results showed that temperature, ABA, cultivar and their interactions significantly influenced shoot length, shoot weight, root length and root weight. The higher the temperature the more stunted the growth of seedlings due to heat stress. Because a temperature of 34.5°C seriously stunted the growth of asparagus seedlings, temperatures above 34.5°C must be regarded as extreme heat stress temperatures on asparagus.

Generally ABA application inhibited growth except at low concentrations, and at high concentrations dramatically depressed growth. While there is an ameliorative effect (enhanced growth) with exogenous application of low ABA concentration on heat stressed plants, high ABA concentration reduced the growth of seedlings at high temperature. Consequently, low ABA concentration may play a role of protection / or enhancement from heat stress, This also occurred when the asparagus seedlings were grown at 33°C and 34.5°C when combined with 0.1 - 1 μ M ABA application. Conversely, seedlings grown at 28°C rarely showed any significant enhancement of growth with ABA application. There is an additive effect of heat stress and high ABA concentration on the growth of seedlings.

In my studies the inhibition of ABA on root growth or shoot growth among cultivars was different, especially at 34.5°C, thus at high temperature the sensitivity differences between genotypes can be clearly demonstrated. The

difference in heat tolerance of asparagus may be determined by ABA insensitivity, as the root growth of UC157 was the most ABA insensitive. This agrees with the membrane thermostability results in which both the roots of UC157 and the shoot of Larac showed ABA insensitivity, and yet the membranes were fairly thermostable.

As low ABA concentrations may enhance / protect seedling growth at high temperatures, it is assumed that heat acclimation is mediated by elevated endogenous ABA levels. An experiment was carried out with 15 - 19 day old seedlings heat shocked for 24 hours from 28°C to 40°C. Endogenous ABA levels from the shoots and roots of seedlings were measured with the ELISA method, and the results showed that endogenous ABA decreased with temperature and then increased to a peak around 38°C for Larac and Tainan No.1, but peaked at around 36°C or lower for Brocks and UC157 for both roots and shoots. The genetic diversity of asparagus seedlings might lead to the differences in ABA contents induced by heat stress. However the magnitude of the elevated ABA levels induced by high temperature stress is very low, as the ABA contents at extreme temperature were low, thus it is proposed that when heat stress is high enough to depress ABA level, this then leads to a cessation of growth and death of the asparagus plant. The endogenous ABA content in spears were much higher compared with seedlings, but spear development is rapid even at high ABA levels. Therefore the growth of spears is likely to be regulated by the balance between promotive hormones (such as GA) and inhibitive hormones (such as ABA) /or insensitivity to ABA.

Heat tolerance is a major characteristic of asparagus cultivar recommendation in tropical climates. In my studies membrane thermostability of spears were measure to determine heat tolerance potential. The spears were produced in a greenhouse and in growth cabinets set at D25/N17°C and D35/N25°C and sampled to measure membrane thermostability by using the spear segments heated from 35°C to 60°C for 20, 30, and 60 minutes. The electrolyte leakage from the cells was used as an index of membrane damage. These data

(electrolyte leakage against heating temperature) were fitted to the *Ingram's* equation to determine the temperature inflection point (T_m). The results showed that the more immature the spears the more sensitive they were to heat damage. Spears heated for 30 minutes induced leakage of electrolytes which could be used to differentiate heat tolerance between cultivars. The results of my experiments suggested that membrane thermostability is an appropriate assay to measure heat tolerance of asparagus. The differences in heat tolerance between various parts of the spear (maturity), spears grown at various temperatures and cultivars could be revealed by the coefficient T_m . These results showed that T_m varied with genotype, age, environment and their interaction. and that heat acclimation may increase the membrane thermostability of young tissues. Using T_m as a criterion of heat stress tolerance showed that asparagus can acquire heat tolerance when growth temperature increased from D25/N17°C < greenhouse < D35/N25°C. UC157 had a similar heat tolerant response to the other cultivars when plants were grown at D25/N17°C or in the greenhouse, but at a high heat stress condition, e.g. D35/N25°C, UC157 was superior to the other cultivars. Therefore UC157 may be expected to be best adapted to a tropical climate, because UC157 had the highest T_m . In addition, Tainan No.1, Larac and Brocks also have increased heat tolerance when grown at high temperature.

The expression of heat tolerance may be mediated by the synthesis of heat shock proteins, thus HSPs are assumed to be associated with heat tolerance. In addition, ABA was found to be a mediator of stress tolerance, but the association of ABA with heat tolerance is not clear. Seedlings about 20 days old were treated with 1 μ M ABA or without ABA for 24 hours, then 0.5 cm shoot segments were pre-incubated for 1 hour and then labelled with S^{35} -methionine at 28°C, 34°C and 37°C for 1 hour or 5 hours. The proteins were then extracted and separated with one-dimension SDS-PAGE and two-dimension IEF/SDS-PAGE.

My studies found both qualitative (cultivar specific proteins) and quantitative differences. Generally the patterns of heat induced protein synthesis between cultivars were similar, but a few HSPs were cultivar specific. The major HSPs were LMW and only a few were HMW. However differences in HSPs between asparagus cultivars occurred in both. The pattern of proteins formed after 2 hours heat shock was slightly different from heat shock for 6 hours. Thus the patterns of protein synthesis depended not only temperature, but also time. Two dimensional IEF/PAGE/SDS-PAGE offered improved protein resolution over one-dimensional SDS-PAGE. Analysis of two-dimensional IEF/SDS-PAGE of asparagus after heat shock not only revealed a great number of new proteins, but also showed alterations in normal protein synthesis. It should be noted however that UC157 has better membrane stability at high temperature than Larac, and yet the majority of LMW HSPs found were similar, although 5 out of 17 were different.

Some ABA-induced proteins were similar to HSPs, although ABA depressed a few HSPs and a number of ABA-induced proteins were depressed by high temperature. However most HSPs were induced at high temperature even with ABA application, thus heat shock overrides ABA-induced protein synthesis. Thus it is concluded that heat stress has a more significant modification of protein synthesis than ABA application.

HSP genes may also include some multiple stress genes activated by other than a heat shock (*Bonham-Smith et al.* 1987). For example ABA may turn on some HSP genes when transferred from normal to high temperature regimes, thus enhancing thermotolerance. The diversity of HSPs may allow classical genetic analysis of allelic variation and gene action (*Vierling and Nguyen* 1990) and the genetic diversity associated with HSPs opens new areas for investigating heat tolerance.

CHAPTER NINE

Conclusions, General Discussions, and Recommendations

9.1. Conclusions and General discussions

Plant growth and development is the result of the interplay between the genetically governed potential of the plant and the plant environment in which it grows. This may be symbolized by the equation:

$$P=f(G,E)$$

where

P phenotype (what actually develops)

G genotype (the genetic potential)

E environment (i.e. temperature, radiation, soil, ... etc.)

Unfavourable environmental conditions for crops minimize the genetic potential (*Lewis and Christiansen, 1981*). In the field the crops can not avoid these unfavourable environments, thus the more suitable the environment the less the reduction in genetic potential. The effect of environmental stress on plants and the adaptation of the plant's genetic potential to the environment are of major interest to plant breeders and physiologists.

Plants are seldom grown for the total biomass but rather for specific plant parts such as grains, fruits, tubers, or fibre. In asparagus it is the spears. The carbohydrate source for spear growth comes from roots and only the marketable spears can be sold. Therefore the equation above should be modified to:

$$M=Q*Y*C*P=f(G,E)$$

where

Q Ratio of marketable spear yield to total spear yield

Y Total spear yield which is a function of the size of the

carbohydrate pool.

C The size of carbohydrate pool is a function of the size of the total asparagus plant.

M Marketable yield.

The parameters of **Q**, **Y**, **C** may be improved by modifying the environment to suit the genotype or by modifying genotype to suit the environment.

The parameters of **Q** and **Y** are primarily associated with the carbohydrate pool (Chapter 3), because the carbohydrate source for spear growth in temperate climates comes from the roots and is the product of last season growth. Therefore asparagus production is different from cereal crops, or fruit trees, whose carbohydrate source comes from the current year's assimilate. Thus the evaluation of asparagus genotypes to different environments or the effect of environment on asparagus production must include considerations of both the carbohydrate pool and of spear quality.

My studies showed that temperature strongly influenced the asparagus plants RGRs and growth trends (allometry, component growth rate, etc), which are major determinants in the size of the carbohydrate pool, which the following season relates to spear growth and marketable spear yield. It is possible that adaptability to high temperature can be shown by such physiological indices of heat tolerance, as membrane thermostability, HSP synthesis, endogenous ABA levels, and sensitivity to exogenous ABA, as these all varied with temperature and cultivar. Overall the greater the temperature stress the poorer was the plant growth, but the response to high temperature stress between different physiological functions was not consistent.

It is concluded that high temperature has a stronger influence on growth than genetic adaptability. Moreover, my studies also revealed that the adaptability to high temperature varied with the individual component or individual physiological process. For example, the variation in the growth between crown,

leaf, and root due to high temperatures. The sensitivity of root to exogenous ABA was also different from that of the shoot. However, any one of these measured terms are not directly related to spear yield, but only represents a specificity to heat tolerance.

The following are the conclusions raised from my studies:

1. The Effect of Suitable Night Temperatures in Tropical Climates

In general the higher the heat stress the more is growth depressed, but this may be modified by the day/night temperature differential which may ensure that plants grown at high day temperatures grow better than at the equivalent constant temperature, due to the counterbalanced effects of suitable night temperatures. In natural environments days are usually hot and nights cool, thus asparagus may be grown in tropical climates, but the longer the exposure to high temperature the more the growth depression.

2. Critical levels of Relative Growth Rates

The effects of high temperature on growth may range from tolerance to growth depression, to plant death (*Levitt* 1980). Linking observations and growth analysis parameters showed that when RLGR, RFGR and RPGR were above about 0.125 per day (and RCGR above about 0.12 per day) growth was "normal", but below 0.11 per day it was very weak. Therefore these levels may be a measure of the limit of adaptability of a genotype to temperature.

3. Growth Response to Temperature by Fitting Growth Parameters to the Response Surface

Although growth analysis can be used to evaluate the heat tolerance adaptability of asparagus, it is clearly impossible to carry out experiments covering all possible temperature regimes. The technique of fitting growth

parameters to a response surface may provide a means to predict growth at any temperature or to represent the response to a defined day/night temperature range. In my studies the growth contours markedly decreased above a specific high temperature (about 33°C constant). The more heat tolerant the lines (or component) the higher is the specific temperature. In addition, a genotype having a lesser number of contours is preferred, as it is more stable over a wider temperature regime. This could be taken into account when screening genotypes for heat tolerance.

4. High Temperatures Involving the Carbohydrate Pool

The work of *Fisher and Maurer-O* (1976) and *Badu-Apraku et al.* (1983) showed that the grains of wheat and maize may cease growth early resulting in small grains due to the effect of high temperatures even though assimilation is still active. On the contrary, large grains result from a long filling period. Hence the size of the carbohydrate pool of asparagus may be greatly modified by high temperatures, as high temperature may depress the activity of roots. This may be a possible cause of the carbohydrate pool at supra-optimal temperatures (i.e. D35/N25°C) being smaller than at sub-optimal temperatures (i.e. D20/N20°C).

5. Carbohydrate Pool at Sub-optimal Night Temperature

The analysis of component growth rate (a measurement of component growth activity) is another measurement associated with carbohydrate accumulation. The regimes of D20/N20°C, D25/N25°C, D30/N20°C and D35/N25°C represented high and stable root growth activity, and showed that at a night temperature of 25°C or below, that growth was "rooty" even at high day temperatures. Therefore in tropical climates asparagus can maintain fairly normal root growth provided that night temperatures are below 25°C.

The optimal day temperatures for RCGR among cultivars are similar, but the optimal night temperatures for Tainan No.1 and UC157 are higher than for Brocks and Larac, thus at warm night temperatures the genetic potential of the carbohydrate pool of the former may be less depressed than the latter as the former are more adaptable to warm night temperatures.

6. Parallel Growth between Components

Although at a sub-optimal temperature plant growth is "rooty", due to the effect of parallel growth between the plant's components the relative growth rate of the crown at optimal temperature is still higher than at a sub-optimal temperature, while at high temperatures root growth is poor because total plant growth is also poor. Thus at high temperatures improving crown growth by improving total plant growth is likely to be more effective than improving crown growth itself.

7. Spear Yield Associated with factors other than Carbohydrate Pool

If the carbohydrate pool is adequate then spears can be produced at temperatures of up to 36°C, but spear quality is low, as the higher the temperature the less compact and the more fibrous the spear. Thus at high temperatures an improvement in spear quality may be a very important method of increasing marketable spear yield.

8. Heat Acclimation and Heat Tolerance

The studies to evaluate heat tolerance between cultivars by measuring membrane thermostability showed that differences between cultivars became clear only when the plants were grown at high temperature. Perhaps the genetic potential for heat tolerance is stimulated by heat acclimation, thus it is proposed that heat tolerance should only be measured after heat acclimation. For example the yield of a heat tolerant line becomes clearly different from that

of a heat sensitive line only at the high temperature regimes (*Chen et al.* 1982; *Saadalla et al.* 1990a,b; *Shanahan et al.* 1990). In tropical areas the potential of heat tolerant lines may be enhanced as in the field the elevated temperatures may result in heat acclimation, in which case a heat tolerant line has better growth than the heat sensitive line.

9. Possible Role of ABA at High Temperature

The role of ABA at high temperature is still obscure because my studies found that low ABA concentration enhanced seedling growth. On the contrary, ABA induced proteins were rarely related to HSPs. Moreover, *Lu et al.* (1989) reported that the ABA insensitive lines appeared to be heat tolerant. *Bray* (1991) suggested that the effect of ABA in improving growth is mediated by other pathways rather than by thermostability. Thus it is postulated that ABA may enable plants to recover from heat damage. As ABA is regarded as a growth inhibitor, the growth of ABA insensitive lines may be due to less inhibition by ABA, but ABA can still have the function of enabling insensitive plants to recover from heat damage. My studies also found that at high temperatures the root weight of UC157 seedlings was least inhibited by ABA, and enhanced by low concentration of exogenous ABA, while Tainan No.1 was severely inhibited and could not be improved by ABA application. On the contrary the shoot weight of UC157 seedlings was very sensitive to ABA and was not improved by ABA application.

10. Linking Heat Shock Proteins to Spear Yield and Quality

The heat tolerance ability does not appear to be associated with the number of HSPs, on the contrary, it may be linked to some specific proteins, because different HSPs may have their different functions in heat tolerance. Moreover, all HSPs are not synthesized and distributed at the membrane, thus the number of HSPs does not completely relate to membrane thermostability. As the HSPs were extracted from the shoots of seedlings, and the membrane

thermostability was measured with young spears, the physiological status between the seedling shoot and the young spear were different. Therefore the heat tolerance measured by membrane thermostability of spear may not completely coincide with the HSPs from the shoots of seedlings, as the heat shock response is tissue specific (*Cooper et al.* 1984).

It is concluded that the appearance of heat tolerance in plants is the integration of many physiological processes, not only membrane thermostability, plant growth, or spear yield, but the whole physiological adaptations to high temperature. The analysis of HSPs is an assay of heat tolerance at the molecular level. Linking HSPs to physiological adaptation, will lead to an understanding of the mechanism of heat tolerance, and eventually the opportunity to manipulate the heat tolerance genes.

9.2. Recommendations

My experiments raise a number of suggestions for future research:

1. Although it can be concluded from my studies that asparagus has the physiological capability to be produced commercially in the tropics, there are thermotolerant differences between genotypes, and heat stresses are different between local environments. These studies (e.g. growth analysis, membrane thermostability, heat shock proteins) may be used to evaluate either thermotolerant genotypes or local environments. However, the agricultural ecology in tropical areas is very different from temperate areas, therefore the physiological adaptability of the plant to high temperatures may not be the sole consideration on whether to plant asparagus in tropical climates. Resistance to diseases, drought, and flooding must also be taken into account.
2. In a breeding program for developing heat tolerance, integrating all heat tolerant characteristics into one plant is desirable, but this may not be

very practical. Therefore only those characteristics directly related to marketable spear yield and carbohydrate pool should initially be transferred into one plant. The measurement of carbohydrate pool and marketable spear yield may be the most direct and simple ways to evaluate either asparagus lines or plant environments. However membrane thermostability, and HSP synthesis are also good potential indices of heat tolerance, which may be used to screen heat tolerant lines from mass populations in the laboratory.

3. The functions of most HSPs are still not clear, therefore, in future, the investigation of specific HSPs linked to the carbohydrate pool or spear yield is of potential interest, because these HSPs may be correlated with spear yield at high temperature. Moreover the genes coding HSPs may be manipulated in a breeding program to enhance heat tolerance.
4. Generally, temperature regimes from slightly low (15°C) to mild high (30°C) are recommended for asparagus production, but longer observations on root growth may be necessary, because the size of the carbohydrate pool is a function of temperature and genetic potential. Because asparagus is a perennial crop, the growth of a mature plant may be different from that of the young plants which were used in my studies. Therefore it may be necessary to analyze the growth of mature plants, and then to compare these results with those for young plants.
5. In temperate climates the carbohydrate in the roots is depleted at the end of the harvest, and then is refilled during the following growth season, while new roots grow from the rhizomes and a part of the old roots die. The dynamic growth of roots is different from fern growth which die in the winter season and produces new ferns from the crown in the next growth season. Therefore the allometry of root in relation to fern in the mature plant may be very different from that of the young plant. The allometric coefficients were constant at the various temperature regimes

and were not significantly different among cultivars, but the allometric intercepts were different. These findings need to be confirmed with mature plants.

6. In tropical climates the asparagus plant does not become dormant and spear production is year around. Thus it is necessarily to understand, whether the carbohydrate source for spear growth still comes from the roots or whether it is derived directly from the current assimilates.
7. When growers decide to plant asparagus in a tropical climate, they must be concerned with selecting heat tolerant cultivars, as the temperature in the field and the interplay between these, will determine the size of the carbohydrate pool. As spear yield and quality are also strongly influenced by temperature, this can be overcome by selecting genotypes having a high spear yield and quality, or the spears should be harvested before they become fibrous (at a shorter length) or the harvesting season adjusted out of the extreme hot season. Clearly there is a need to further examine the effect of temperature on spear quality.
8. High endogenous ABA levels in spears were found in my studies and in previous work (*Matsubara* 1980; *Makus* and *Guinn* 1992). The growth rate of spears was much higher than the normal plant growth rate. Moreover Tainan No.1 had the highest spear relative growth rate and the highest endogenous ABA in the spears. Therefore, although the role of ABA is usually regarded as a growth inhibitor, this does not coincide with my results in which plant with high ABA levels also had high growth rates. Hence the sensitivity of tissue to ABA or other growth promoters may involve spear growth and should be further investigated.

Appendices

Appendix 1A. The number of seeds sown, seedlings after thinning, and harvested plants (D25/N25°C, D30/N30°C, D35/N35°C¹).

No. of Harvest	seeds	<u>No. of seedlings after thinning</u>			Harvested plants
		17days ²	24days	31days	
1st	10	5	-	-	5
2nd	7	4	3	-	3
3rd	7	4	3	-	3
4th	4	2	1	-	1
5th	4	2	1	-	1
6th	4	2	1	-	1
7th	4	2	1	-	1

¹ the seedlings were too weak to be thinned.

² The age of seedlings is the number of days after sowing.

Appendix 1B. The number of seeds sown, seedlings after thinning, and harvested plants (D30/N20°C, D35/N25°C, D40/N30°C¹, D20/N20°C, D35/N15°C, D40/N20°C¹).

No. of Harvest	seeds	<u>No. of seedlings after thinning</u>			Harvested plants
		17days ²	24days	31days	
1st	12	-	5	-	5
2nd	9	-	5	3	3
3rd	9	-	5	3	3
4th	5	-	3	1	1
5th	5	-	3	1	1
6th	5	-	3	1	1
7th	5	-	3	1	1

¹ the seedlings were too weak to be thinned.

² The age of seedlings is the number of days after sowing.

Appendix 2

The Climate Room

The climate room measured 2.75 x 2.75 meters, with an effective growing area of 2 x 2 meters. Conditioned air from ducting along the top of each side wall was passed over the plant trolleys and was recycled via a false floor to the machinery chamber at the rear of each room. The artificial light was supplied from each source in the light rig located in the loft region above each room. Radiation from the rig was passed through a temperature controlled 2.5 cm waterscreen heat-barrier supported on a sheet of plate glass. Mirroring on the walls of each room gave a more even spread of light over the plant growing area and decreased the light gradient from the light loft.

Appendix 3A. The environmental conditions of climate rooms (D25/N25°C, D30/N30°C, D35/N35°C).

Treatment		D25N25°C		D30N30°C		D35N35°C	
		Day	Night	Day	Night	Day	Night
Temperature	± 0.5°C	25	25	30	30	35	35
Humidity	R.H ± 5%	81	81	86	86	89	89
	VPD mb	6	6	6	6	6	6
Day length	hours	12	0	12	0	12	0
PPFD ¹	pre-experiment	714		706		708	
	post-experiment	669		661		612	
	mean	692		684		660	
PAR ²	pre-experiment	151		150		150	
	post-experiment	140		136		127	
	mean	146		143		139	
CO ₂	range ³ ppm	335-405		320-340		330- 420	

The experiments were run from 27/11/1989 to 26/01/1990.

The room conditions were as programmed, within the set limits, for the duration of the experiment.

The lighting system used consisted of 4x1000W Sylvania "metal-arc"high pressure discharge lamps, together with 4x1000W Philips tungen iodide lamps.

The day/night, night/day change overs took 120 minutes the light period started and finished halfway through the change overs.

¹ The photosynthetic photon flux density (PPFD), $\mu\text{molm}^{-2}\text{s}^{-1}$, was measured at standard trolley height using a Li-Cor 185 meter with an LI-190S quantum sensor.

² The photosynthetically active radiation (PAR), Wm^{-2} , in the 400-700 nm wavelength, was measured similarly using an LI-190SE flat response sensor.

³ The carbon dioxide levels were monitored.

Appendix 3B. The environmental conditions of climate rooms (D30/N20°C, D35/N25°C, D40/N30°C).

Treatment		D30N20°C		D35N25°C		D40N30°C	
		Day	Night	Day	Night	Day	Night
Temperature	± 0.5°C	30	20	35	25	40	30
Humidity	R.H ± 5%	76	57	82	68	86	76
	VPD mb	10	10	10	10	10	10
Day length	hours	12	0	12	0	12	0
PPFD ¹	pre-experiment	732		720		724	
	post-experiment	714		732		703	
	mean	723		726		714	
PAR ²	pre-experiment	149		150		148	
	post-experiment	148		149		143	
	mean	149		150		146	
CO ₂	range ³ ppm	330-410		335-395		330-410	

The experiments were run from 19/03/1990 to 25/05/1990.

The room conditions were as programmed, within the set limits, for the duration of the experiment.

The lighting system used consisted of 4x1000W Sylvania "metal-arc" high pressure discharge lamps, together with 4x1000W Philips tungsten iodide lamps.

The day/night, night/day change overs took 120 minutes the light period started and finished halfway through the change overs.

¹ The photosynthetic photon flux density (PPFD), $\mu\text{mol m}^{-2}\text{s}^{-1}$, was measured at standard trolley height using a Li-Cor 185 meter with an LI-190S quantum sensor.

² The photosynthetically active radiation (PAR), W m^{-2} , in the 400-700 nm wavelength, was measured similarly using an LI-190SE flat response sensor.

³ The carbon dioxide levels were monitored.

Appendix 3C. The environmental conditions of climate rooms (D35/N15°C, D40/N20°C, D20/N20°C).

Treatment		D35N15°C		D40N20°C		D20N20°C	
		Day	Night	Day	Night	Day	Night
Temperature	± 0.5°C	35	15	40	20	20	20
Humidity	R.H ± 5%	82	64	86	74	74	74
	VPD mb	10	6	10	6	6	6
Day length	hours	12	0	12	0	12	0
PPFD ¹	pre-experiment	720		732		713	
	post-experiment	710		736		702	
	mean	715		734		702	
PAR ²	pre-experiment	150		149		151	
	post-experiment	147		152		144	
	mean	149		151		148	
CO ₂	range ³ ppm	375-500		370-455		360-445	

The experiments were run from 18/06/1990 to 05/10/1990.

The room conditions were as programmed, within the set limits, for the duration of the experiment.

The lighting system used consisted of 4x1000W Sylvania "metal-arc"high pressure discharge lamps, together with 4x1000W Philips tungen iodide lamps.

The day/night, night/day change overs took 120 minutes the light period started and finished halfway through the change overs.

¹ The photosynthetic photon flux density (PPFD), $\mu\text{molm}^{-2}\text{s}^{-1}$, was measured at standard trolley height using a Li-Cor 185 meter with an LI-190S quantum sensor.

² The photosynthetically active radiation (PAR), Wm^{-2} , in the 400-700 nm wavelength, was measured similarly using an LI- 190SE flat response sensor.

³ The carbon dioxide levels were monitored.

Appendix 4. The formula : a modified half-strength Hoagland's a nutrient solution.

Chemical	g/litre	ppm in final solution
<u>Stock solution A</u>		
Calcium nitrate		Ca : 100.20
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	295.9	N : 70.03
Sequestrene 330		Fe : 2.08
10% DTPA	10.4	Na : 1.02
<u>Stock solution B</u>		
Potassium phosphate		K : 19.55
KH_2PO_4	34.02	P : 15.49
Potassium nitrate		K : 97.75
KNO_3	126.39	N : 35.02
Magnesium sulphate		Mg : 24.32
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	123.24	S : 32.06
Boric acid	0.715	B : 0.250
H_3BO_3		
Manganese chloride		Mn : 0.251
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.4525	Cl : 0.324
Zinc sulphate		Zn : 0.025
ZnSO_4	0.055	S : 0.012
Cooper sulphate		Cu : 0.010
CuSO_4	0.020	S : 0.005
Sodium molybdate		Na : 0.003
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.0067	Mo : 0.005
Potassium chloride		K : 1.652
KCl	1.575	Cl : 1.498

pH of final solution = 6.5 - 7.5.

Appendix 5. The rates of half-strength Hoagland's a solution were applied to pots via an automatic microtube system.

Growth room	Rate
D20/N20°C	3 x 1.5 min/pot/day.
D25/N25°C	3 x 1.5 min/pot/day.
D30/N20°C	3 x 1.5 min/pot/day.
D30/N30°C	3 x 1.5 min/pot/day for the first 6 weeks, followed by an increase to four applications till the end of the experiment.
D35/N15°C	3 x 1.5 min/pot/day for the first four weeks with an additional application pot ⁻¹ day ⁻¹ till the end of the experiment.
D35/N25°C	3 x 1.5 min/pot/day.
D35/N35°C	3 x 1.5 min/pot/day for the first four weeks with an additional application of nutrient after that also a 1 x 1.5 min application of water pot ⁻¹ day ⁻¹ till the end of the experiment.
D40/N20°C	3 x 1.5 min/pot/day for the first four weeks with an addition application pot ⁻¹ day ⁻¹ till the end of the experiment.
D40/N30°C	3 x 1.5 min/pot/day for the first six weeks with an additional application pot ⁻¹ day ⁻¹ till the end of the experiment.

Appendix 6A. The analysis of variance of the influences of day temperature, night temperature, cultivar and interaction on RPGR of asparagus plants.

Source	df	F value	P > F
Cultivar (C)	3	2.61	0.055
Day temperature (D)	3	275.71	0.001
Night temperature (N)	3	23.95	0.001
C * D	9	0.71	0.700
C * N	9	2.13	0.033
D * N	1	31.26	0.001
C * D * N	3	0.99	0.398

Appendix 6B. The analysis of variance of the influences of day temperature, night temperature, cultivar and interaction on RLGR of asparagus plants.

Source	df	F value	P > F
Cultivar (C)	3	2.22	0.090
Day temperature (D)	3	232.52	0.001
Night temperature (N)	3	27.90	0.001
C * D	9	0.99	0.456
C * N	9	3.97	0.001
D * N	1	75.44	0.001
C * D * N	3	1.02	0.387

Appendix 6C. The analysis of variance of the influences of day temperature, night temperature, cultivar and interaction on RFGR of asparagus plants.

Source	df	F value	P > F
Cultivar (C)	3	2.99	0.034
Day temperature (D)	3	244.52	0.001
Night temperature (N)	3	17.74	0.001
C * D	9	0.61	0.788
C * N	9	2.29	0.021
D * N	1	59.13	0.001
C * D * N	3	0.59	0.622

Appendix 6D. The analysis of variance of the influences of day temperature, night temperature, cultivar and interaction on RCGR of asparagus plants.

Source	df	F value	P > F
Cultivar (C)	3	1.32	0.273
Day temperature (D)	3	244.70	0.001
Night temperature (N)	3	32.01	0.001
C * D	9	1.77	0.083
C * N	9	1.39	0.201
D * N	1	0.90	0.344
C * D * N	3	2.54	0.060

Appendix 7A. The analysis of variance of the influences of day temperature, night temperature and interaction on RPGR of asparagus plants.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Day temperature (D)	3	67.46	0.001
Night temperature (N)	3	13.54	0.001
D * N	1	2.57	0.120
<u>cv. Larac</u>			
Day temperature (D)	3	201.34	0.001
Night temperature (N)	3	13.60	0.001
D * N	1	15.43	0.001
<u>cv. Tainan</u>			
Day temperature (D)	3	47.98	0.001
Night temperature (N)	3	2.36	0.094
D * N	1	8.29	0.008
<u>cv. UC157</u>			
Day temperature (D)	3	56.90	0.001
Night temperature (N)	3	8.05	0.001
D * N	1	11.82	0.002

Appendix 7B. The analysis of variance of the influences of day temperature, night temperature and interaction on RLGR of asparagus plants.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Day temperature (D)	3	45.91	0.001
Night temperature (N)	3	12.53	0.001
D * N	1	13.49	0.001
<u>cv. Larac</u>			
Day temperature (D)	3	177.53	0.001
Night temperature (N)	3	13.08	0.001
D * N	1	56.57	0.001
<u>cv. Tainan</u>			
Day temperature (D)	3	39.32	0.001
Night temperature (N)	3	5.94	0.003
D * N	1	19.49	0.001
<u>cv. UC157</u>			
Day temperature (D)	3	58.05	0.001
Night temperature (N)	3	12.72	0.001
D * N	1	11.72	0.002

Appendix 7C. The analysis of variance of the influences of day temperature, night temperature and interaction on RFGR of asparagus plants.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Day temperature (D)	3	54.42	0.001
Night temperature (N)	3	10.46	0.001
D * N	1	7.77	0.010
<u>cv. Larac</u>			
Day temperature (D)	3	146.74	0.001
Night temperature (N)	3	6.47	0.002
D * N	1	33.65	0.001
<u>cv. Tainan</u>			
Day temperature (D)	3	46.18	0.001
Night temperature (N)	3	2.26	0.104
D * N	1	14.50	0.001
<u>cv. UC157</u>			
Day temperature (D)	3	53.57	0.001
Night temperature (N)	3	7.31	0.001
D * N	1	15.38	0.002

Appendix 7D. The analysis of variance of the influences of day temperature, night temperature and interaction on RCGR of asparagus plants.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Day temperature (D)	3	76.78	0.001
Night temperature (N)	3	18.43	0.001
D * N	1	3.76	0.063
<u>cv. Larac</u>			
Day temperature (D)	3	287.90	0.001
Night temperature (N)	3	37.21	0.001
D * N	1	14.27	0.001
<u>cv. Tainan</u>			
Day temperature (D)	3	36.15	0.001
Night temperature (N)	3	2.58	0.074
D * N	1	0.12	0.735
<u>cv. UC157</u>			
Day temperature (D)	3	42.57	0.001
Night temperature (N)	3	7.12	0.001
D * N	1	1.61	0.215

Appendix 8A. The analysis of variance of the influences of day temperature, night temperature, cultivar and interaction on allometric intercept of asparagus plants.

Source	df	F value	P > F
Cultivar (C)	3	3.32	0.022
Day temperature (D)	3	19.58	0.001
Night temperature (N)	3	1.62	0.183
C * D	9	1.36	0.200
C * N	9	1.41	0.180
D * N	1	58.05	0.001
C * D * N	3	1.48	0.218

Appendix 8B. The analysis of variance of the influences of day temperature, night temperature and interaction on allometric intercept of asparagus plants.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Day temperature (D)	3	8.88	0.001
Night temperature (N)	3	1.90	0.130
D * N	1	6.72	0.010
<u>cv. Larac</u>			
Day temperature (D)	3	4.69	0.003
Night temperature (N)	3	1.12	0.340
D * N	1	15.48	0.001
<u>cv. Tainan</u>			
Day temperature (D)	3	3.95	0.009
Night temperature (N)	3	0.27	0.844
D * N	1	8.63	0.004
<u>cv. UC157</u>			
Day temperature (D)	3	6.88	0.001
Night temperature (N)	3	3.23	0.023
D * N	1	38.53	0.001

Appendix 9.A Analysis of variance of the influences of cultivar, temperature and ABA on root weight of asparagus seedlings.

Source	df	F value	P > F
Cultivar (C)	3	20.45	0.0001
Temperature (T)	3	328.96	0.0001
ABA	4	69.34	0.0001
C * T	9	5.44	0.0001
C * ABA	12	3.88	0.0001
T * ABA	12	3.28	0.0001
C * T * ABA	36	1.69	0.0067

Appendix 9B. Analysis of variance of the influences of cultivar, temperature and ABA on root length of asparagus seedlings.

Source	df	F value	P > F
Cultivar (C)	3	28.47	0.0001
Temperature (T)	3	1143.98	0.0001
ABA	4	601.98	0.0001
C * T	9	12.96	0.0001
C * ABA	12	4.19	0.0001
T * ABA	12	52.11	0.0001
C * T * ABA	36	3.80	0.0001

Appendix 9C. Analysis of variance of the influences of cultivar, temperature and ABA on shoot weight of asparagus seedlings.

Source	df	F value	P > F
Cultivar (C)	3	25.63	0.0001
Temperature (T)	3	279.50	0.0001
ABA	4	385.21	0.0001
C * T	9	4.98	0.0001
C * ABA	12	4.36	0.0001
T * ABA	12	18.86	0.0001
C * T * ABA	36	1.27	0.1322

Appendix 9D. Analysis of variance of the influences of cultivar, temperature and ABA on shoot length of asparagus seedlings.

Source	df	F value	P > F
Cultivar (C)	3	46.52	0.0001
Temperature (T)	3	701.45	0.0001
ABA	4	510.17	0.0001
C * T	9	6.67	0.0001
C * ABA	12	4.50	0.0001
T * ABA	12	40.26	0.0001
C * T * ABA	36	2.16	0.0001

Appendix 10A. Analysis of variance of the influences of temperature and ABA on root weight of asparagus seedlings.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Temperature (T)	3	68.83	0.0001
ABA	4	5.93	0.0001
T * ABA	12	1.18	0.2939
<u>cv. Larac</u>			
Temperature (T)	3	84.67	0.0001
ABA	4	21.45	0.0001
T * ABA	12	1.56	0.1021
<u>cv. Tainan</u>			
Temperature (T)	3	105.30	0.0001
ABA	4	14.92	0.0001
T * ABA	12	2.77	0.0012
<u>cv. UC157</u>			
Temperature (T)	3	94.36	0.0001
ABA	4	42.85	0.0001
T * ABA	12	3.07	0.0004

Appendix 10B. Analysis of variance of the influences of temperature and ABA on root length of asparagus seedlings.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Temperature (T)	3	253.17	0.0001
ABA	4	120.79	0.0001
T * ABA	1	11.12	0.0001
<u>cv. Larac</u>			
Temperature (T)	3	292.96	0.0001
ABA	4	170.88	0.0001
T * ABA	12	15.51	0.0001
<u>cv. Tainan</u>			
Temperature (T)	3	307.39	0.0001
ABA	4	127.72	0.0001
T * ABA	12	14.84	0.0001
<u>cv. UC157</u>			
Temperature (T)	3	381.98	0.0001
ABA	48	214.07	0.0001
T * ABA	12	25.72	0.0001

Appendix 10C. Analysis of variance of the influences of temperature and ABA on shoot weight of asparagus seedlings.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Temperature (T)	3	92.94	0.0001
ABA	4	73.58	0.0001
T * ABA	12	4.08	0.0001
<u>cv. Larac</u>			
Temperature (T)	3	83.26	0.0001
ABA	4	117.48	0.0001
T * ABA	12	4.62	0.0001
<u>cv. Tainan</u>			
Temperature (T)	3	58.83	0.0001
ABA	4	74.40	0.0001
T * ABA	12	4.85	0.0011
<u>cv. UC157</u>			
Temperature (T)	3	65.78	0.0001
ABA	4	186.02	0.0001
T * ABA	12	11.79	0.0001

Appendix 10D. Analysis of variance of the influences of temperature and ABA on shoot length of asparagus seedlings.

Source	df	F value	P > F
<u>cv. Brocks</u>			
Temperature (T)	3	199.22	0.0001
ABA	4	93.48	0.0001
T * ABA	12	9.38	0.0001
<u>cv. Larac</u>			
Temperature (T)	3	123.21	0.0001
ABA	4	119.78	0.0001
T * ABA	12	8.26	0.0001
<u>cv. Tainan</u>			
Temperature (T)	3	204.35	0.0001
ABA	4	140.91	0.0001
T * ABA	12	12.07	0.0001
<u>cv. UC157</u>			
Temperature (T)	3	207.28	0.0001
ABA	4	213.03	0.0001
T * ABA	12	21.99	0.0001

Appendix 11. The formulae of 1-Dimension polyacrylamide electrophoresis gel.

Compositions (cc) gel	Running gel				stacking
	<u>11 cm slat</u>		<u>14 cm slat</u>		
Acrylamide concentration	10%	20%	10%	20%	4.5%
Lower Tris buffer ¹	5.0	5.0	6.0	6.0	-
Upper Tris buffer ²	-	-	-	-	2.5
Acrylamide (running gel) ³	6.7	13.0	8.0	16.0	-
Acrylamide (stacking gel) ⁴	-	-	-	-	1.5
Distilled water	8.3	-	10.0	-	6.0
Ammonium persulfate (μl) ⁵	100	100	120	120	100
TEMED ⁶ (μl)	10	10	12	12	10
Total (approx) (cc)	20	20	24	24	10

¹ Tris-HCl 1.5 M, SDS 0.4%
² Tris-HC 0.5 M, SDS 0.4%
³ Acrylamide 30%, Methylene-bis-acrylamide 0.5%
⁴ Acrylamide 30%, Methylene-bis-acrylamide 1.6%
⁵ Ammonium persulfate 10% fresh
⁶ N,N,N¹,N¹-Tetramethylethylenediamine

Appendix 12. Isoelectric focus gel solution

acrylamide	3.78% (w/v)
N,N',-methylenebis-acrylamide	0.22% (w/v)
Urea	9.2 M
Triton X-100	3% (v/v)
Carrier ampholytes	4% (v/v)
Bio-Lyte pH 5-7 (Bio-Rad)	3.2% (v/v)
Bio-Lyte pH 1-10 (Bio-Rad)	0.4%(v/v)
Pharmalyte pH 1-10 (Phmarcia)	0.4% (v/v)

Appendix 13. UKS solution

Urea	9.5 M
SDS (Sodium dodecyl sulfate)	1.25%
K ₂ CO ₃	5 mM
Dithiothretiol	0.5% (w/v)
Carrier ampholytes	4% (v/v) :
Bio-Lyte pH 5-7 (Bio-Rad)	3.6% (v/v)
Bio-Lyte pH 1-10 (Bio-Rad)	0.2%(v/v)
Pharmalyte pH 1-10 (Phmarcia)	0.2% (v/v)
Triton X-100	6%(v/v)

Appendix 14. Equilibration solution

SDS	2.3% (w/v)
Glycerol	10.0% (v/v)
2-Mercaptoethanol	0.1% (v/v)
Bromophenol blue	0.05% (w/v)
Tris-HCl 62.5 mM	pH 6.8

Appendix 15. SDS sample buffer.

2-mercaptoethanol	10% (v/v)
SDS	6% (w/v)
Upper Tris buffer	25% (v/v)

Appendix 16. Bromophenol blue tracking dye solution.

Bromophenol	0.05% (v/v)
Glycerol	40.00% (v/v)

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