ENVIRONMENTAL REGULATION OF PROCESSES CONTROLLING YIELD

IN PEANUT (*Arachis hypogaea* L.)
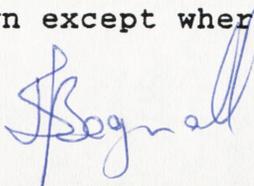
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STATEMENT

The work presented in this thesis
is my own except where stated

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Table 3.6 and the discussion of it in Chapter 3 will appear in Bell, M.J., Bagnall, D.J. and Harch, G. 1991. Effects of photoperiod on reproductive development of peanut (*Arachis hypogaea* L.) in subtropical Australia. II Temperature interactions. Aust. J. Agric. Res.

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Abstract

The environmental factors photoperiod, irradiance,
humidity and temperature all influence growth and
development of peanut (*Arachis hypogaea* L.) although the
relative importance of each to yield change through the
life of the crop. Daylength does usually not affect time
to first flower appearance, although short days enhanced
subsequent flower and fruit production and the species
can be described as a quantitative short day plant.
Twelve hour short days doubled flower and peg numbers
compared with 16 hour long days, while pod numbers
increased 3 to 12 fold with SD for a range of peanut
cultivars. Photoperiodically induced changes in flower
and fruit numbers were independent of plant dry weight
although for individual plants, flower and fruit numbers
were always correlated highly with plant dry weight. The
photoperiodic sensitivity of peanut fruit formation
disappeared at low growth temperatures (24/19°C), and
this interaction between temperature and photoperiod
might well over-ride predicted low yields due to long
days in the sub-tropical growing season. Differences in
flowering pattern between cultivars bred in the sub-
tropics and tropical varieties may also contribute to
yield differences due to latitude. Varieties that
partition virtually all assimilate to fruit during pod-
filling in the sub-tropics have a more synchronous
pattern of flowering than "primitive" varieties and these

differences in flowering pattern may explain differences in adaptability to equatorial environments.

Irradiances above 500 µmol $m^{-2}s^{-1}$ did not decrease the time to first flower appearance although subsequent flower numbers were highly correlated with photon flux density (PFD). The flower number versus irradiance response was approaching saturation at a PFD of 1000 µmol $m^{-2}s^{-1}$. Growth was similarly highly correlated with PFD and flower numbers appeared to be dependent on assimilate availability.

Low atmospheric VPD (~1.0 kPa) at the time of canopy closure reduced flower and subsequent fruit number in the cultivar Early Bunch, and this was related to reduced transpiration at a high relative humidity.

Temperature was found to be of overriding importance to peanut growth and development during the first 60 to 70d of growth. Both leaf and flower production were temperature dependent with optimum temperatures of 30°C or higher. Below this optimum the linear thermal time model adequately described first flower appearance, although subsequent flower production was affected by photoperiod and humidity as well as by growth. Growth and unadapted CO_2 assimilation rate (A) had markedly different responses to temperature although in the first 2d of cooling to 19°C, A decreased by 50-70%. This decline in A was not due to either stomatal factors or respiration, but was shown to be associated with carbohydrate accumulation. Clear evidence was obtained of

sink control of A by independently manipulating the temperature of different leaves on the plant. Cooling (to 19°C) most of the plant (the sink) led to a 70% decline in A of the remaining leaves at 30°C after 3d, whereas the converse treatments (30°C sink, 19° source) led to only a small change (17%). Biochemical regulation of A after cooling involved the photosynthetic inhibitor fructose 2,6-bisphosphate and inactivation of ribulose bisphosphate carboxylase. Leaves of all varieties accumulated starch at 20°C, although a cool-tolerant cultivar showed no increase in soluble sugars during cooling. The cool-sensitive cultivars more than doubled their leaf soluble sugar concentrations in the 4d of cooling, and this probably reflects differences in sucrose phosphate synthase activation/inactivation.

Overall, environmental factors regulated yield either via growth (temperature and photon flux density), or via flower numbers and subsequent partitioning (photoperiod and high humidity).

Abbreviations:

A	Rate of net CO_2 assimilation.
ANOVA	Analysis of variance.
A_o	Assimilation rate which would occur if resistance to CO_2 diffusion was zero.
c_i	Substomatal CO_2 concentration.
cv	Cultivar.
d	Day.
df	Degrees of freedom.
DN	Day neutral.
DNP	Day neutral plant.
DW	Oven dried (24h at 80^oC) plant dry weight.
EC	Enzyme Commission.
EU	Enzyme units.
Expt	Experiment number.
f	Days from seedling emergence to the appearance of the coloured corolla of the first flower.
F2,6BP	Fructose 2,6-bisphosphate.
g	Stomatal conductance to diffusion of water vapour.
h	Hour.
IBPGR	International Board for Plant Genetic Resources
ICRISAT	International Crops Research Institute for the Semi Arid Tropics.
L	Long.
LD	Long day.
LDP	Long day plant.
LSD	Least significant difference.
n	Number of replicates in a sample.
NAR	Net assimilation rate.

NHI	Nitrogen harvest index.
ns	Not significantly different.
OD	Optical density.
PCR	Photosynthetic Carbon Reduction (cycle)
p	Probability of an event occurring.
PAR	Photosynthetically active radiation.
pers comm	Personal communication.
PFD	Photon flux density.
r	Correlation coefficient.
Rubisco	Ribulose-1, 5-bisphosphate carboxylase/ oxygenase (EC 4.1.1.39).
RuBP	Ribulose bisphosphate.
RGR	Relative growth rate.
RWC	Relative water content.
S	Short.
SD	Short day.
SDP	Short day plant.
SE	Standard error.
SLW	Specific leaf weight.
SPS	Sucrose-phosphate synthase (EC 2.4.1.14).
TAC	Technical Advisory Committee of the Food and Agriculture Organization Of the United Nations.
T_b	Base temperature.
T_{opt}	Optimum temperature.
θ_f	Thermal time required for flowering.
VPD	Vapour pressure deficit.
v/v	Volume/volume.
Y	Rate to first flower appearance.
Y_{opt}	Rate to first flower appearance at the optimum temperature.

Chapter 1

Introduction

Arachis hypogaea (L.), peanut or groundnut, is a
tropical legume that is grown principally for its edible
oil and protein rich seed. In developing countries it is
frequently grown as a mixed crop (with maize, rice,
pigeon peas or tree crops) while in developed countries,
it is a row crop sometimes grown under irrigation
(Bunting *et al.*, 1985). It is the second most important
source of vegetable oil in the world (after soybean) and
a major source of vegetable protein (Evans, 1975; Bunting
et al., 1985). Peanuts are cultivated between $40^{\circ}N$ and
$40^{\circ}S$ of the equator although yields vary enormously from
3t ha^{-1} in the U.S.A., to 1.5 t ha^{-1} in Australia and 0.8
t ha^{-1} in the developing world (Bunting *et al.*, 1985).
These wide variations in yield partly represent different
inputs (pest and disease control, fertilizer, water,
mechanization) but also in part are due to differing

responses to the environment. Germplasm also differs
greatly, but there are large genotype by environment
interactions. Peanut germplasm introduced from Bolivia
(16-17°S) was the highest yielding in the world (9.6 t
ha^{-1}, under high input conditions) in Zimbabwe (16-18°S),
while North American varieties at the same location
performed poorly (Hildebrand, 1975; Hildebrand and
Smartt, 1980). These Bolivian/African varieties,
including Mani Pintar, Makulu Red and Egret were
conversely, poorly yielding in the U.S.A., relative to
North American varieties. Hildebrand and Smartt suggest
that these differences were because the Bolivian
varieties responded to cooler temperatures or bigger
diurnal temperature ranges better than North American
varieties. An alternative explanation is that there are
differences in photoperiodic response among varieties,
despite peanut generally being described as a Day Neutral
Plant (DNP e.g. Fortanier, 1957). These high yields in
Zimbabwe (9.6 t ha^{-1}) were repeated for locally adapted
Virginia varieties and environmental factors such as
light intensity, vapour pressure deficit and length of
the growing season also contributed to record yields,
although the relative importance of each of these factors
remains hard to assess (Hildebrand, 1980).

Different local environments also have markedly
different effects on the yield of peanut varieties
notwithstading apparently optimal and similar conditions.
In Florida, Duncan et al. (1978) reported yields of five

Virginia-type peanut cultivars that varied from 2.5 to 5.4 t ha^{-1} whereas in Virginia and North Carolina, the yield of 20 similar cultivars were more stable (3.6 to 4.6 t ha^{-1}: Mozingo *et al.*, 1987). Until there is a better understanding of how factors such as photoperiod, temperature, irradiance and humidity affect growth, flowering and partitioning it is very difficult to explain these results or predict which varieties are best suited for particular regions.

Many aspects of the physiological response of peanut to the wide range of environments in which it can be grown are poorly understood. In a recent review of environmental regulation of flowering in peanut, Summerfield and Roberts (1985) commented

> "it is clear that a program of carefully designed factorial experiments will be required in order to resolve more clearly which environmental factors are most important and when during crop life they exert their principal effects on development".

Crop yield is the production of economically desirable plant parts and in this study the meaning of yield is restricted to seed although peanut haulms (vegetative residues) have been used as animal feed and the shells as fuel, compost and hardboard (Bunting *et al.*, 1985). Farm yields are reported as unshelled weights of which 70% is seed. Yield is usually expressed per unit land area although in multiple cropping and with the

increasing pressure on agricultural land it is frequently
defined on a per unit area per unit time basis (Evans,
1975). The yield of legumes has only increased at about
half or less than half the rate of major cereals (Evans,
1980; Specht and Williams, 1984; Mozingo *et al.*, 1987)
and a variety of reasons have been suggested to explain
this poor relative improvement although Evans (1980)
argues that

> "a better understanding of the physiology of yield
> in legume crops is needed before we can assert that
> their yield potential is less than that of cereals"

What is known of the limits to yield in peanuts?

The smaller yield improvement in all legumes
relative to cereals (Evans, 1980; Specht and Williams,
1984) is frequently thought to be due to their
requirement for nitrogen during pod filling (Lawn, 1989).
According to Pate and Minchin (1980) approximately 37% of
final seed nitrogen has been remobilized from vegetative
parts in peanut compared with 43% in chickpea (*Cicer
aritinum* L.), 50% in broad bean (*Vicia faba* L.), 54% in
soybean (*Glycine max* (L.) Merrill) and 58% in cowpea
(*Vigna unguiculata* (L.) Walp.) Peanut does not exhibit
monocarpic senescence, as do some other legumes e.g.
soybean and cowpea, and it is frequently not responsive
to applied nitrogen (Cox *et al.*, 1982). Nevertheless,
some authors suggest yield may be limited by nitrogen
remobilization late in pod filling (Duncan *et al.*, 1978;

Williams, 1979). By contrast, duration of pod filling at lower temperature (Dreyer *et al.*, 1981), leaf area duration (Waggoner and Berger, 1987) and percentage dry weight partitioning into fruit (Duncan *et al.*, 1978) have all been found to be strongly correlated with yield in peanut and these correlations are similar to those observed in wheat (*Triticum aestivum* L.; Evans *et al.*, 1975). The difference between cereal and legume yield potentials is largely due to greater responsiveness to applied nitrogen by the recently developed cereal varieties (Evans, 1980).

According to the model of Duncan *et al.* (1978), the increase in yield potential in peanut varieties over the last 40 years has been principally due to changes in partitioning of assimilate with the high yielding variety Early Bunch allocating 98% of photosynthate into pods during the pod filling period, while lower yielding, less synchronous varieties partitioned proportionately less into fruit growth. Although the underlying assumptions behind this model may be overly simple (i.e. no redistribution of assimilate to fruit from other plant parts and assimilation rate not responsive to changing sink demand), it provides a useful comparison among varieties at a particular location. A shortcoming of the predictive value of the model is that the highest yielding varieties in the world (Hildebrand and Smartt, 1980) have intermediate partitioning co-efficients, and that partitioning co-efficients change at different

locations (McCloud *et al.*, 1980; Bell pers. comm.). The limited number of cultivars (five) in the study of Duncan *et al.* and the choice of those cultivars probably had a significant effect on the subsequent findings.

Similar analyses to that of Duncan *et al.* (1978) were completed in North Carolina and Virginia by Mozingo *et al.* (1987) and Coffelt *et al.* (1989) for plantings of a larger range of cultivars (20 and 14 respectively). The increases in yield reported over the forty years from 1945 to 1985 are of the order of 13 to 18.5 per cent rather than the doubling suggested by Duncan *et al.* (1978). The highest yielding cultivars had generally increased total flower production compared to other cultivars, whereas reproductive efficiency (the conversion of flowers to pegs and pods) "has not played a significant role in yield increases of most cultivars" according to Coffelt *et al.* (1989). Cultivars with larger flower numbers have the advantage of more potential fruiting nodes and this has been identified as an important contributor to yield across a range of grain legumes (Summerfield and Lawn, 1987).

Harvest index and yield of peanut

The suggestion by Donald (1962) that it is possible to increase yield by identifying characters that have an "increased capacity to exploit the positive components of the environment" led to his defining an ideotype as a plant that will out-yield currently available cultivars

through incorporation of ideal physiological characteristics (Donald, 1968). One of the important characteristics included in Donald's concept of an ideotype was an increase in the harvest index which he defined as the proportion of the total plant top dry weight that is seed (Donald, 1962). Increases in wheat yield have been due, principally, to changes in harvest index (Donald and Hamblin, 1976) and several researchers have advocated breeding for increased harvest index of grain legumes (e.g. Lawn, 1989). The problems with identifying an ideotype for peanut can be illustrated by the data of Mozingo *et al.* (1987) and Coffelt *et al.* (1989) where there was no correlation between harvest index and yield (r=0.00) in 17 Virginia cultivars released between 1944 and 1981. The lack of correlation between harvest index and yield in peanut would appear to refute the suggestion by McWilliam and Dillon (1987) that increases in peanut yield over the last 40 years were due to "no increase in biological yield but an increase from 23 to 51% in harvest index". Donald (1962), and Johnson and Major (1979) clearly showed that comparisons of harvest index should not be made across dissimilar maturity types, yet Duncan *et al.* (1978), and subsequently McWilliam and Dillon (1987), compared the late maturing, runner cultivar Dixie Runner with the early maturing, erect cultivar Early Bunch. Coffelt *et al.* (1989) reported marked differences in harvest index depending on maturity type, and whether cultivars were

erect or spreading. Early maturing, erect cultivars had
high harvest indices in the range 50 to 56%, compared
with late maturing, spreading types (42 to 46%). The
small number of cultivars and the relatively small
changes in harvest index within a maturity group limit
the generalizations that can be made about the
contribution of harvest index to peanut yield increase
from these studies.

Harvest index in legumes can be constrained by the
requirement for nitrogen in their protein-rich seed and
Lawn (1989) suggested that the nitrogen harvest index
(proportion of total plant nitrogen recovered in seed)
provided a better measure of potential for yield
improvement in legumes. Lawn observed that legume species
with less seed nitrogen had a greater capacity for
improvement in harvest index, whereas for a species like
soybean with a high nitrogen harvest index "a further
advance in harvest index will be exceedingly difficult".
Pate and Minchin (1980) compared nitrogen harvest index
(NHI) of peanuts with soybean, faba bean, chickpea and
cowpea. Peanut had a higher NHI (0.80) than all of the
other legumes, although this was probably due to leaf
spot (*Cercosporidium personatum* Berk and Curt.) attacking
the crop and reducing the leaf tissue (original data of
Bunting and Anderson, 1960). Until more reliable figures
become available, it is difficult to assess whether yield
improvement in peanut can be achieved with breeding for
increased NHI.

This study contributes to the understanding of peanut yield by examining how environment affects plant processes controlling yield. In Chapters 2 and 3, the environmental regulation of flowering and early fruit formation is examined. Temperature exerts a major control of peanut growth up until fruit formation and in Chapters 4 and 5, regulation of assimilation rate by temperature and its biochemical regulation by feedback inhibition are described. High humidity has occasionally been observed to limit peanut flowering under monsoonal conditions (Smith, 1954; Dart *et al.*, 1983) and in Chapter 6, this limitation to yield is examined.

The relationship between photosynthesis and yield in peanut

There has been a continuing debate as to the relative importance of breeding for higher photosynthetic rate to improve yield in peanuts and other crop plants (see Gifford and Evans, 1981 and Zelitch, 1982, for contrasting views). Comparisons of assimilation rates in modern lines of wheat and their progenitors (Evans and Dunstone, 1970; Khan and Tsunoda, 1970) established that changes in photosynthetic rate were not responsible for the increased yield in modern cultivars. Similar results have been reported for a range of maize varieties (*Zea mays* L.; Duncan and Hesketh, 1968) and for cowpeas (Lush and Rawson, 1979), rice (*Oryza sativa* L.; Cook and Evans, 1983) and cotton (*Gossypium hirsutum* L.; El-Sharkawy *et*

al., 1965). Zelitch (1982) claims that peanut, on the other hand, does show a positive relationship between maximum assimilation rate and yield on the basis of research by Bhagsari and Brown (1976). However, this claim overstates the case somewhat. Bhagsari and Brown do not present yield data and there is a large overlap in the range of photosynthetic rates between cultivated and wild plants. Rao and Rama Das (1981) also suggested that selection for higher net assimilation rate (A) should also lead to higher peanut yield. They found that crop growth rate in the first 35d was strongly correlated with assimilation rate for 6 peanut varieties. However, peanut takes 90-160 d to mature (Gibbons, 1980) and the connection between the first 35 d of growth and final yield was not established by Rao and Rama Das. In reviewing this subject, further doubts about the merit of selection for higher assimilation rate are raised by Ketring *et al.* (1982) who observed that the ranking of photosynthetic rates among peanut lines was not consistent between studies and it appeared likely that varieties were responding differently to growth conditions.

Part of the variation in peanut assimilation rates among varieties may have been due to changes in rate with leaf age. Pallas and Samish (1974) found that, for "unknown reasons", peanut plants in growth cabinets under constant conditions underwent a reduction in assimilation rate after four weeks. This reduction was greatest in the

afternoon and probably involved changes in sink activity feeding back on assimilation rate. Thus, one reason why maximum assimilation rate may be a poor index of final yield is that demand for photosynthate changes during the growing season. The nature and possible mechanism of feedback inhibition are examined in Chapters 4 and 5 of this thesis.

The lack of increase in crop yields with changes in maximum assimilation rate has been explained by de Wit *et al.* (1979) and one of those reasons is that the presence of physiological sinks may modify photosynthetic rates. They also observe that only part of the plant surface is operating at light saturation, so that the benefit of higher A is only a fraction of the potential increase. Increases in maximum assimilation rates (per unit leaf area) may also be associated with reduced leaf size (Evans and Dunstone, 1970) and consequent reduced growth. Canopy photosynthesis, when it is integrated over the growing season, is a better predictor of final yield than maximum leaf photosynthesis. An understanding of the processes limiting yield in peanut must therefore examine not only how photosynthetic rate is modified by the environment, but also how plant factors, like source-sink interactions affect growth. The response of photosynthetic rates to changing sink activity (due to temperature changes) will be examined in Chapter 4.

Temperature and its effect on growth and yield in peanut

The reported optimal temperatures for both growth and yield of peanut range from 23°C to 32°C for growth and from 20°C to 30°C for yield (Bolhuis and de Groot, 1959; Carlson et al., 1975; Williams et al., 1975). Most studies have found that the optimum temperature for growth decreases with plant age. Apart from observing that this phenomenon also occurs in other species (Wood, 1968) no explanation of this change has been proposed. This change in the optimum growth temperature for peanut occurs at about the time when assimilation rate "dropped considerably" (Cox, 1979; Pallas and Samish, 1974) and the changes in both growth and assimilation rate might be explained by changing demand for assimilate. Although the experiments in this study do not look specifically at the changing optimum temperatures for growth, knowledge about temperature mediated sink regulation of photosynthesis (Chapters 4 and 5) may provide an explanation of this phenomenon.

Peanut Botanical types.

The classification of peanuts into Botanical types has been based principally on branching and floral patterns. Krapovickas (1973) divided A. hypogaea L. into two subspecies with the following discriminating characteristics;

Subspecies hypogaea Krap. et Rig.: no floral axis on main
                                    stem, alternating pairs of vegetative

and floral axes on laterals.

var. *hypogaea*: less hairy, branches
short (Virginia type).
var. *hirsuta*: more hairy, branches
long (Runner type)

Subspecies *fastigiata* Waldron: floral axes on main stem,
continuous runs of floral axes
on laterals.

var. *fastigiata*: little branched
(Valencia type).
var. *vulgaris*: more branched (Spanish
type).

Current commercial cultivars have mainly been
derived from intersubspecific hybridizations and the
various Botanical type cultivars (Virginia, Valencia and
Spanish) include phenotypes with intermediate
characteristics from their parental lines. In 1976 more
than 70% of the cultivars in the U.S.A. were products of
hybridization between *A. hypogaea hypogaea* and *A.
hypogaea fastigiata* (Hammons, 1976). One of the products
of those crossings was the early maturing, erect Virginia
bunch type cultivars, like Early Bunch, which were less
indeterminate than traditional long-season Virginia
runner types (Duncan *et al.*, 1978). Hammons (1976)
observed that "in crosses between genotypes from
different subspecies, many qualitative characteristics in
this amphidiploid crop are complexly inherited, with
interacting systems of duplicate and complimentary
genes". The difficulties associated with classification
of these hybrids led the International Board for Plant

Genetic Resources to devise a classification scheme based
on a series of plant descriptors to help characterize
peanut genotypes (IBPGR, 1981). These descriptors
included growth habit (procumbent, decumbent or erect),
branching pattern (alternate, sequential or irregular),
plant, pod and leaflet dimensions, seed color and shape,
and plant hairyness. This classification acknowledges
that the subspecies of *A. hypogaea* L. are interfertile
and that characteristics like seed size and growth habit
are not unique to particular botanical types. The
cultivars used in this thesis are listed under their
Botanical type and growth habit classifications in
Appendix 1, along with their country of origin. In this
thesis comparisons are sometimes made between the
environmental responses of cultivars from different
Botanical types and sometimes between cultivars from the
same type, that have different degrees of Lawn's
"determinateness" (Lawn, 1989). The comparisons between
responses of cultivars from different Botanical types
that have different flowering/branching patterns will not
necessarily be reflected in different physiological
responses to the environment.

Rhizobial versus nitrate fed peanut plants.

In this thesis all plants were grown using standard
Canberra Phytotron nutrient solution except for some
preliminary comparative studies. Appendix 2 sets out the
mineral composition of the nutrient solution. The

concentration of nitrate in the solution (15mM) was found to inhibit rhizobial activity of healthy established nodules (Appendix 3) and completely prevented nodule formation in seedlings.

The decision to apply mineral nitrogen to these experimental plants was based partly on the experience derived from these preliminary experiments and partly because of the need to separate the influence of the environmental factors on the plant from the environmental responses of the rhizobia. Clearly there is a need to examine how factors like temperature and plant carbohydrate status affect rhizobial activity and consequently the plant/rhizobial symbiosis, but these topics are outside the range of this research.

A review of the literature on the effects of providing peanut plants with mineral or rhizobial nitrogen suggests that this choice is unimportant for flowering, peg and pod formation because vegetative growth is altered while flowering and pod formation are not. Several researchers have found that there were decreases in harvest index with increases in nitrogen applied to peanut (Ball *et al.*, 1983; Selamat and Gardiner, 1985; Nambiar *et al.*, 1986), although in none of these studies was pod yield reduced by mineral nitrogen nutrition. The change in harvest index involved an increase in vegetative growth. In some cultivars however, e.g. Robut 33-1, harvest index actually increased with nitrogen application up to 150 kg ha^{-1}

(Nambiar *et al.*, 1986). It is therefore most likely that reproductive processes like flower, peg and pod formation are unaffected by supplying mineral or rhizobial nitrogen, except indirectly as a function of growth. Although remobilization of nitrogen contributes significantly to seed yield at plant maturity (Pate and Minchin, 1980), the experiments in this thesis were conducted on plants that had either not started seed formation (e.g. Chapters 2, 4 and 5) or where seed weight was less than 10 per cent of the vegetative plant weight (Chapters 3 and 6). At this early stage of seed development the data of Bunting and Anderson (1960) suggest that remobilization of nitrogen was unlikely to have been significant.

A preliminary experiment (described in Appendix 4) established whether growing the plants on microbial or mineral nitrogen affected the time-to-first flower appearance.

The decision to grow the plants on mineral nitrogen also has implications for the research on peanut temperature sensitivity in Chapters 4 and 5, because

"the symbiotic system is generally more temperature sensitive (at both extremes) than growth of plants on combined nitrogen"

according to Sprent (1979).
This sensitivity is due to both reduced nodule fomation and efficiency (Sprent, 1979). Delayed commencement of nitrogen fixation at sub-optimal temperatures can lead to

apparent nutrient deficiency symptoms (Gibson, 1980) that would have increased the complexity of any interpretation of results. A deliberate reductionist approach was adopted and the possibility that the rhizobial response to temperature might interact with the plant response was eliminated. An additional benefit of this approach was that plant cultivar responses were due exclusively to plant factors and not to the variable efficiency with which a particular rhizobial strain was able to fix nitrogen when used to inoculate different groundnut genotypes (Wynne *et al.*, 1980).

The chapters in this thesis have been organized to initially resolve how particular phenological stages are affected by environmental factors (Chapters 2 and 3). Then, the most important environmental variable in the vegetative phase (temperature) which regulates growth and photosynthesis is examined (Chapters 4 and 5). Subsequently, the role of high humidity in affecting seed yield is looked at (Chapter 6). Throughout this study it is implicit that the supply of water is adequate and not limiting growth (see Appendix 2 for a description of the plant irrigation in these experiments).

Chapter 2

Environmental control of flowering in peanut.

Introduction:

For the tropical legume *Arachis hypogaea* (L.),
peanut or groundnut, the role of environmental factors in
the control of its flowering is poorly understood. Some
studies have found that daylength affects time to first
flower, although either short days (SDs) or long days
(LDs) may hasten first flower appearance (Tétényi, 1957;
Wynne and Emery, 1974; Sengupta *et al.*, 1977). Other
authors suggest it is a day neutral plant (DNP)
(Fortanier, 1957; Bunting and Elston, 1980; Summerfield
and Wien, 1980; Leong and Ong, 1983). These apparent
inconsistencies may represent differences in response
between varieties. Thus, Wynne *et al.* (1973) showed that
whilst daylength had no effect on time to first flower
for a Valencia and a Spanish variety, a Virginia variety
was significantly slower to first flower in long days,

but only at one of their three temperature regimes. Summerfield and Roberts (1985), when reviewing the environmental regulation of flowering in peanut, concluded that the results reported in the literature were conflicting and that it was impossible to define the daylength responses of peanut because of the interaction of photoperiod, temperature, radiation and humidity in many experiments.

In this study the separate and interacting influences of daylength, temperature and light intensity are shown to affect time to first flower in a number of peanut varieties of the Virginia, Valencia and Spanish Botanical types.

Materials and Methods:

Researchers examining the photoperiodic responses of a range of plant species or varieties follow an established *pro forma* with regard to the degree of environmental control of experimental space in the Canberra phytotron. Initially, photoperiodic experiments are conducted in glasshouse cabinets, which provide accurate photoperiods and good temperature control (within ±0.2°C in these experiments). These cabinets are naturally-lit and are therefore dependent on seasonally varying irradiance. The relatively large number of these cabinets in the phytotron and consequently, the relatively large amount of space available permits several varieties to be screened in a range of

photoperiods at any one time. Experiments that require irradiance to be controlled are conducted in artificially-lit cabinets. The differences in photoperiodic responses between seasons (Table 2.2 versus Table 2.7) provided evidence that the seasonal variation in irradiance was responsible for the inconsistent time-to-first flower in peanut varieties, and the normal progression from glasshouse cabinets to artificially-lit cabinets was followed.

The range of photoperiods that was initially selected to screen time-to-first flower (Table 2.2) was representative of the variation in photoperiod over planting times in Kingaroy, Australia, as the field data of Bell and Shorter (pers. comm.) suggested substantial daylength effects in a range of peanut varieties. When no response to photoperiod was observed in time-to-first flower (Table 2.2), the experiment was repeated with different temperature treatments (Table 2.7, Winter data), and subsequently with a greater range of photoperiods (Table 2.7, Spring data).

The choice of varieties for the experiments in this and the next chapter was based on the reputedly photoperiodically sensitive varieties reported by Witzenberger *et al.* (1985) and Bell and Shorter (pers. comm. and Bell *et al.*, 1990a and b). In the experiments where space and replication requirements reduced the number of varieties that could be included, representative lines of the Spanish and Virginia

Botanical types were selected: either Chico, TMV2 or
White Spanish (Spanish types) and Early Bunch or Robut
33-1 (Virginia types). These were cultivars of
contrasting, reputed daylength sensitivity, that were
relatively fast maturing. They were either erect or bunch
types, which took up less space than runner types and
could therefore meet replication requirements within the
constraints of cabinet dimensions.

Germination and Growth Conditions:

All experiments used a standard germination
technique in which the seed (provided by M. Bell,
Queensland Department of Primary Industries, Kingaroy,
Aust.) were soaked in $30^{\circ}C$ water for 2 hours, then
transferred to 15x25x30 cm seed trays of medium grade
vermiculite (source: Neuchatel, West Melbourne, Aust.)
and watered 3 times per day with tap water in a $30/25^{\circ}C$
glasshouse of the Canberra phytotron (Morse and Evans,
1962). After 5 days the seedlings were selected for
uniformity and grown in 1 litre red pots (125 mm
diameter) of 1:1 mixture of vermiculite:perlite (perlite
source: Australian Perlite Ltd Banksmeadow, Aust.).
Plants were watered three times a day, once with a
modified Hoagland's number two nutrient solution (See
Appendix 2) and twice with tap water. The cotyledons
emerged on the fifth or sixth day after soaking and the
time from this date to the date of the first flower

(appearance of the coloured standard) was taken as the time to first flower (f). For any treatment the time to first flower was the mean value of the first fifty per cent of plants to flower. The rate of progress toward first flower appearance (Y) was defined by Roberts and Summerfield (1987) as 100/f.

Experimental:

(a) The effect of photoperiod on the time to first flower and flower number.

After germination and selection for uniformity, seedlings of the varieties White Spanish and Chico (Spanish types) and Robut 33-1 and Shulamit (Virginia types) were transferred to phytotron glasshouse C-units (Morse and Evans, 1962) running at 30/25°C day/night temperature and photoperiods of 10, 11, 11.5, 12, 12.5 and 14h. Each photoperiodic treatment involved supplementing 10 hours natural light with low intensity (60 µmol m^{-2} s^{-1}) incandescent lamps where required split equally before and after the natural light period (0700-1700 h Eastern Australian Standard Time). During the period of this experiment average daily energy received at canopy level was 12.3 MJ m^{-2}. Duplication of photoperiod treatments involved 10 plants split into replicated photoperiod cabinets to give five plants per variety per cabinet. There were no significant differences between the plants in the replicated photoperiods and so all plants were bulked for the

analysis. A similar experiment was conducted with the variety Early Bunch in which flower numbers were counted daily during the 15 d after first flowering in photoperiods of 10, 12, 14 and 16 h. Each of the 4 photoperiod treatments involved 14 plants split between replicated cabinets (7 plants per cabinet). All regression lines and polynomials in this thesis were fitted using rawsoft computer software (H. Rawson, CSIRO Division of Plant Industry), or Genstat V, a general statistical program (Lawes Agricultural Trust, Rothamsted).

(b) The interaction between photoperiod and temperature in regulating the time to first flower.

After germination and selection for uniformity, seedlings of the Spanish varieties Red Spanish, Chico, TMV2 and White Spanish, Virginia varieties Q18164 (PI270806), VB223, Robut 33-1 and Early Bunch; and Valencia types (*A. hypogaea* subspecies *fastigiata* var. *fastigiata*): Q18657 (Nc Acc 17133), Q18636 (PI393531) and Q18660 (Nc Acc 17132) were subjected to photoperiods of 11, 11.5, 12.25, 13 and 14h under two temperature regimes, 30/25°C (a mean temperature of 26.7°C) and 24/19°C (a mean temperature of 20.7°C). Average daily irradiance at canopy level during this experiment was 13.7 MJ m^{-2}. All photoperiodic treatments were duplicated and each treatment involved 8 plants (4 plants per variety per cabinet). A similar range of varieties were

subjected to two photoperiods (12 and 14h) and three temperature regimes (21/16, 27/22 and 33/28°C in Winter (7.0 MJ m^{-2}d^{-1}), and to two temperatures (30/25 and 33/28°C) and two photoperiods (8 and 14h) in Spring (12.6 MJ m^{-2}d^{-1}). The fresh air exchange rate of 0.1 m^{3}s^{-1} in the glasshouse cabinets maintained CO_2 concentration within 30 μl l^{-1} of ambient (350 μl l^{-1}) for experiments reported in this thesis.

(c) The effect of temperature on the time to first flower

After standard germination and selection for uniformity, plants of the Spanish varieties White Spanish and TMV2, and Virginia varieties Robut 33-1 and Early Bunch were transferred to phytotron glasshouse C units running at 23/18°C, 25/20°C, 27/22°C, 29/24°C, 31/26°C and 33/28°C. The 5°C differential between day and night temperatures corresponded to that in the glasshouses i.e. 8 hour/16 hour, day/night cycle, to maximize temperature control in the cabinets. The "day" thermoperiod was from 0800-1600 h (Eastern Australian Standard Time). Cabinet temperatures were controlled to within ±0.2°C throughout the experiment. All cabinets were set to a 12 hour natural photoperiod (0600-1800 h). During this experiment, the average daily radiation at canopy level was 13.8 MJ m^{-2}. Each temperature treatment was replicated in either two or three cabinets and each treatment involved 18 plants.

(d) The interaction between photon flux density and photoperiod in regulating the time to first flower.

After standard germination and selection for uniformity, peanut plants of the varieties Chico, TMV2 and White Spanish (Spanish types) and Early Bunch, Robut 33-1 and Q18164 (Virginia types) were transferred to artificially lit LB cabinets (Morse and Evans, 1962) running at a constant $30^{o}C$. Illumination was provided by six or eight Wotan Powerstar HQI-T 400W/DH metalarc lamps (Siemens, Munich, FRG) supplemented by two 500 W quartz iodide lamps. Appendix 5 provides additional information about the spectral qualities of the artificial lighting. Photon flux density (PFD) was varied by adjusting the distance between the light source and the plants. In some treatments, the 12 h day was extended by 2 hours low intensity incandescent lamps. All plants (8 per treatment) were included in the calculation of time to first flower. In the initial experiment PFDs were 150 $\mu mol\ m^{-2}\ s^{-1}$ PAR for both 12 and 14h, 500 $\mu mol\ m^{-2}\ s^{-1}$ (12h), 600 $\mu mol\ m^{-2}\ s^{-1}$ (14h), 700 $\mu mol\ m^{-2}\ s^{-1}$ (12h) and 800 $\mu mol\ m^{-2}\ s^{-1}$ (14h). In the second experiment the irradiances were 370 $\mu mol\ m^{-2}\ s^{-1}$ (12 and 14h) and 500 $\mu mol\ m^{-2}\ s^{-1}$ (12 and 14h). The treatment common to both experiments (500 $\mu mol\ m^{-2}\ s^{-1}$ for 12h) confirmed that cabinet conditions had not changed between experimental runs. All cabinets were run at relative humidities in the range 70-80%. The maximum deviation in carbon dioxide

concentration from ambient (350 µl l^{-1}) in artificially

lit cabinets was 30 µl l^{-1}, with an air exchange rate of

0.03 m^{3}s^{-1} (I.A. Dawson, pers. comm.).

Results:

(a) The effect of photoperiod on the time to first flower

and flower number.

At a temperature of 30/25°C there was no effect of

photoperiods between 10 and 14h on the time to first

flower. In Table 2.1, four cultivars were compared in

Table 2.1: Effects of photoperiod on time from emergence to first
flower appearance for four peanut varieties grown at 30/25°C. (Expt
7.2.86). Experiment numbers correspond to germination dates in this
and the next chapter.

	Days from emergence to first flower						
Cultivar	Photoperiod (h)						Mean±SE
	10	11	11.5	12	12.5	14	for all
Spanish types:							
Chico	18.3	18.5	18.2	18.3	18.0	18.7	18.3±0.1
White Spanish	20.2	20.9	20.2	19.8	19.8	20.2	20.2±0.3
Virginia types:							
Shulamit	24.8	25.0	24.5	26.9	25.3	25.4	25.3±0.3
Robut 33-1	24.2	24.8	24.1	25.6	24.9	25.6	24.8±0.3

this experiment, although later experiments reported in this chapter reproduced these results for 12 cvs (see Section b of this chapter). In a further experiment there were no differences in time to first flower for Early Bunch (28.5±1.5 d) over a greater range of photoperiods, however, in the subsequent 14d, plants in a 10h photoperiod had approximately double the flower number of those in a 16h photoperiod (Figure 2.1). Fifteen days after flowering the plants were harvested and there was no significant difference in plant dry weight between treatments. It was not possible to define a critical daylength for flower number from these data. A subsequent experiment with Early Bunch and Robut 33-1 at 2 photoperiods (12 and 16 h) showed similar promotion of flower production by SD (see Chapter 3). In none of the photoperiod experiments in this or the next chapter were any changes in plant morphology apparent.
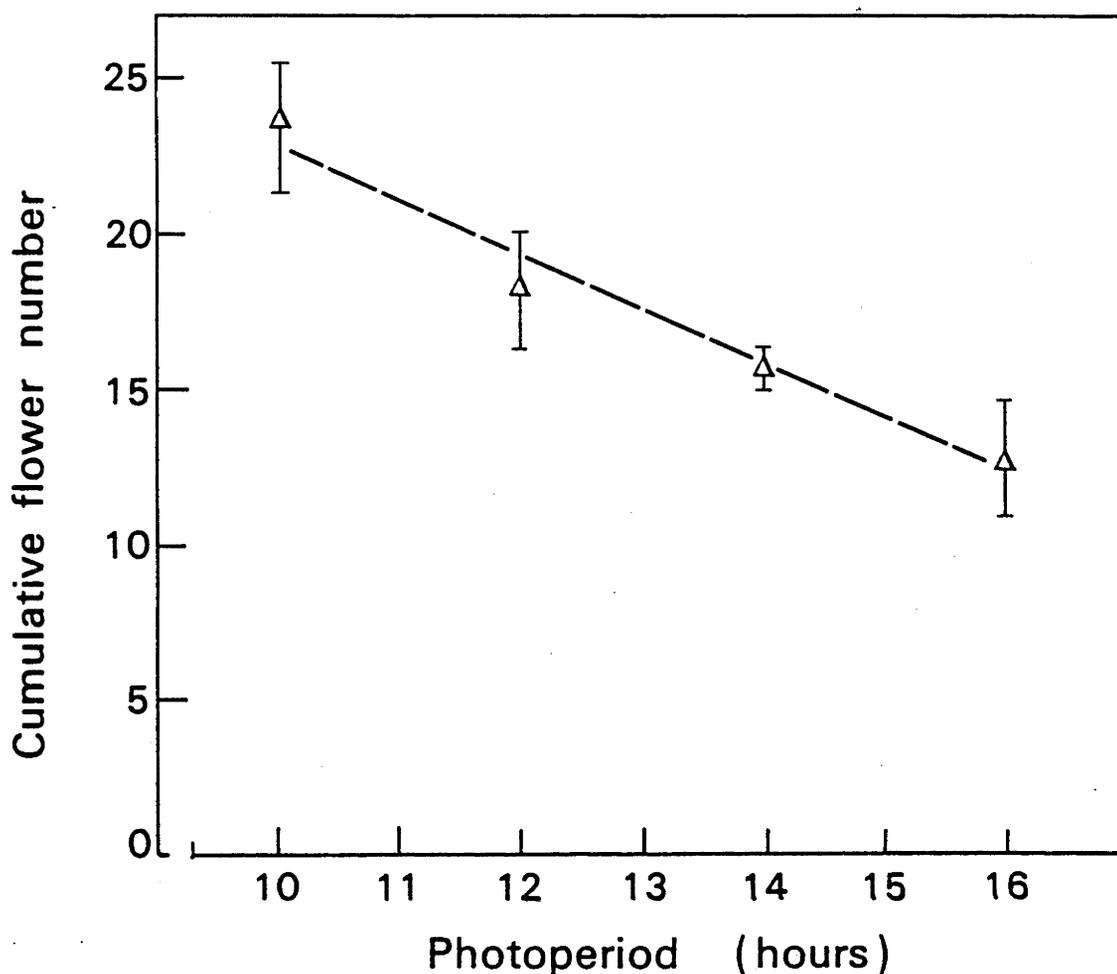
Figure 2.1: Cumulative flower numbers in the first 14 d of flowering at 30/25°C in the variety Early Bunch exposed to four photoperiods. Standard error bars are equal to 2x SE. Expt 30.9.88.

(b) The interaction between photoperiod and temperature in regulating the time to first flower.

Temperature had a major influence on time to first flower for all varieties tested, but there was no evidence of either a photoperiod effect or any interaction between temperature and photoperiod on the time to flower (Table 2.2) in this experiment. The lack of any photoperiodic response simplified modelling the

Table 2.2: Time to first flower at two temperature
regimes and five photoperiods for twelve cultivars of
peanut. Expt 25.10.85.

Temperature ($^{\circ}$C)	Photoperiod (h)			
11	11.5	12.25	13	14

Spanish Types:

Red
Spanish

	11	11.5	12.25	13	14
24/19	43.5	45.0	43.7	40.0	37.3
30/25	24.3	24.0	22.5	21.8	23.7

Chico

24/19	32.8	33.8	34.3	33.5	34.3
30/25	18.7	18.3	18.5	18.2	18.5

TMV2

24/19	42.6	42.0	42.7	45.4	39.0
30/25	20.5	24.0	22.8	22.3	22.0

White
Spanish

24/19	38.6	40.1	39.6	40.1	41.0
30/25	21.7	20.8	21.3	20.9	21.5

Valencia Types:

Q18660

24/19	37.6	39.8	35.7	38.5	38.6
30/25	22.3	22.4	21.2	21.0	21.9

Q18657

24/19	35.7	38.5	35.0	40.7	37.4
30/25	22.8	22.5	22.0	20.9	22.0

Q18636

24/19	38.0	42.5	39.5	41.3	34.0
30/25	23.0	22.8	21.8	21.2	21.3

Virginia Types:

Robut 33-1

24/19	39.3	37.8	36.6	40.2	39.0
30/25	24.7	24.0	24.7	24.5	26.0

Q18164

24/19	46.0	46.8	45.8	42.0	45.7
30/25	28.7	23.8	24.0	27.0	25.3

Early Bunch

24/19	40.0	46.8	44.0	42.0	43.8
30/25	24.7	24.0	24.7	24.5	26.0

VB223

24/19	48.4	48.2	48.0	47.6	50.7
30/25	28.3	29.0	30.4	30.7	30.5

NC17209

24/19	50.1	51.5	51.5	48.1	49.3
30/25	32.3	31.9	27.0	31.1	27.5

flowering response, which conventionally involves fitting
a linear regression to the inverse of time to first
flower against temperature.

The rates of leaf appearance and of progress toward
first flower appearance (Y=100/f) shows a linear
relationship to temperature over a range of 15°C for
several cultivars including Chico and Robut 33-1 (see
Figure 4.1, Angus *et al.*, 1981 and Leong and Ong, 1983).
On this basis a linear regression of the form Y=a+bT
where a and b are constants, was fitted to flowering rate
data for twelve peanut varieties (three or more varieties
per Botanical type). The heat sum or thermal-time model
can be used to distinguish differing temperature effects
on rates of development and establishes an approximate
base temperature (T_b): a value at which rate of progress
towards flowering is zero. The data in Table 2.3, show
that the Virginia varieties had lower rates and generally
lower base temperatures than the Spanish varieties, and
the Valencias intermediate base temperatures, although
these generalisations are based on a relatively small
number of varieties tested in each Botanical type. The
effect of temperature on development can be assessed by
the thermal time required for flowering (θ_f) which is the
amount of "day-degrees" above the base temperature after
which flowering will occur. The three Botanical types
progressed to flowering with differing sensitivities to
temperature. On average Spanish varieties took 280 "day-
degrees" to flower, while Valencia varieties took 310

"day-degrees" and Virginia varieties 391 "day-degrees".

The estimated base temperatures for flowering were

Table 2.3: Time (in days) from seedling emergence to first flower (f) at two temperatures (T in $^{\circ}$C). At each temperature there were 5 photoperiods ranging from 11 to 14 hours. There were no significant differences between photoperiodic treatments at either temperature and the data have been combined to show the mean temperature response ± s.e. The regression equations relate the rate of first flower appearance (Y) to mean temperature. The estimated base temperature (T_b) is calculated as -a/b, while θ_f equals 100/b. Expt 25.10.85.

Variety	Time to first flower at 24/19°C	Time to first flower at 30/25°C	Regression equation	T_b ($^{\circ}$C)	θ_f ($^{\circ}$Cd)
Spanish Type					
Red Spanish	41.9±1.4	23.3±0.5	Y=0.32(T)-4.18	13.2	312
Chico	33.8±0.6	18.4±0.2	Y=0.41(T)-5.56	13.5	244
TMV2	42.3±1.0	22.3±0.6	Y=0.35(T)-4.96	14.0	286
White Spanish	39.3±0.4	21.2±0.2	Y=0.36(T)-5.03	13.8	278
Valencia Type					
Q18660	38.0±0.7	21.8±0.3	Y=0.33(T)-4.14	12.7	303
Q18657	37.5±1.0	22.0±0.3	Y=0.31(T)-3.75	12.1	323
Q18636	39.1±1.5	22.0±0.4	Y=0.33(T)-4.22	12.8	303
Virginia Type					
Robut 33-1	38.5±0.6	24.8±0.3	Y=0.24(T)-2.28	9.6	417
Q18164	44.7±1.2	25.8±0.4	Y=0.27(T)-3.45	12.5	370
Early Bunch	42.1±0.8	24.8±0.4	Y=0.28(T)-3.36	12.1	357
VB223	48.6±0.5	29.8±0.5	Y=0.22(T)-2.43	11.2	454
NC17209	50.1±0.7	30.0±1.1	Y=0.28(T)-3.36	11.7	357

approximately 13.6°C for Spanish, 11.4°C for Virginia,

and 12.5°C for Valencia types, with extremes of 9.6°C and

14.0°C for cvs Robut 33-1 (from Israel) and TMV2 (from

India), respectively.

(c) The effect of temperature on time to first flower.

Despite the application above of a linear thermal-

time model, the plot of time to first flower appearance

versus temperature must diverge from a straight line at

the optimum temperature. Figure 2.2 shows the inverse of
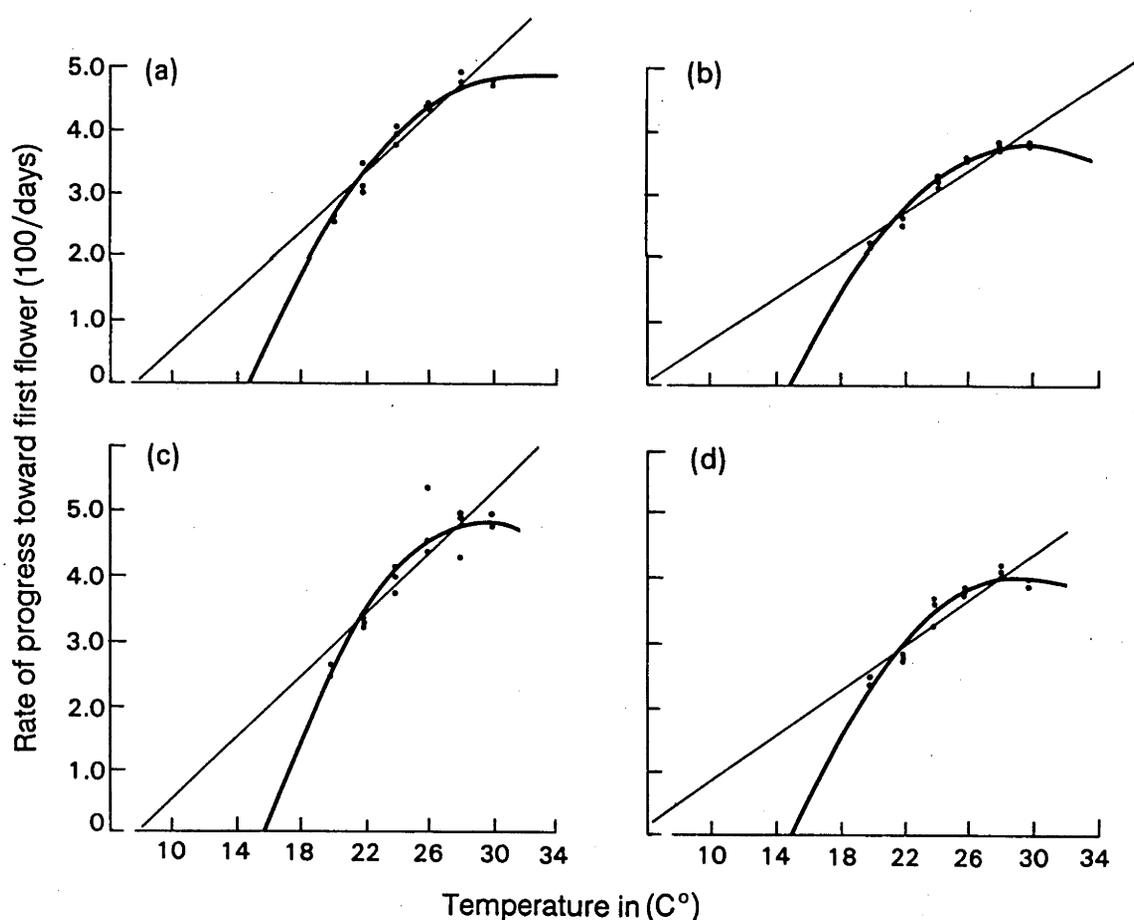
Figure 2.2: The rate of progress to first flower
appearance for the peanut varieties a) TMV2, b) Early
Bunch, c) White Spanish d) Robut 33-1, as a function of
average temperature. Expt 19.11.87.

time to first flower as a function of temperature for four varieties. The standard statistical method (Snedecor and Cochran, 1967) for assessing which of a quadratic or a linear equation provides a better fit of a set of data is to carry out an analysis of variance and compare the sums of squares for both models (Tables 2.4, 2.5 and Appendix 6). If the reduction of the sum of squares, tested against the mean square remaining after curvilinear regression proves to be significant, the hypothesis of linear regression is abandoned. For all four sets of data (Table 2.4, Appendix 6) there was significant curvilinearity. As well as the standard statistical analysis, a non-parametric test of "curvedness" of the data was applied (Wolfe and Bagnall, 1979). This test, which compares the distribution of residuals around the straight line and the curve, showed that the quadratic curve gave a better fit to the data in each case. For all four cultivars' data it was possible to reject the straight line fit (p>0.98).

Table 2.4: Analysis of variance for polynomial curve fitting of rate of first flower appearance as a function of temperature for the peanut variety Early Bunch. Similar analyses of the data from Figure 2.2 of the cultivars TMV2, White Spanish and Robut 33-1 are presented in Appendix 6 and are summarized in Table 2.5. The rate to first flower Y is defined by Y=100/d, where d is the number of days from emergence to first flower. The quadratic and straight line fits for this variety are illustrated in Figure 2.2B. Expt 19.11.87.

REGRESSION ANALYSIS FOR EARLY BUNCH

Model: Y= -1.004+0.1717(T) which accounts for 88.8% of variance

	d.f.	Sum of Squares	Mean Squares
Regression	1	4.4314	4.4314
Residual	13	0.5133	0.0395
Total	14	4.9447	0.3532

Model: $Y=-11.3+1.02(T)-0.01718(T)^2$ which accounts for 97.6% of variation

	d.f.	Sum of Squares	Mean Squares
Regression	2	4.8418	2.4209
Residual	12	0.1029	0.0086
Total	14	4.9447	0.3532
Reduction (due to Quadratic term)	-1	-0.4104	0.4104

$$\text{Significance of fitting quadratic} = \frac{\text{Total change in SS}}{\text{Residual SS}}$$

= 47.86 on 1,12 d.f.
which is a significantly better fit (p<0.001).

The quadratic equation in Table 2.4 can be rearranged (see Appendix 7) to a form that includes physiological parameters

$$Y= Y_{opt}[1-\{(T_{opt}-T)/(T_{opt}-T_b)\}^2]$$

where Y_{opt}, T_{opt} and T_b are the rate to first flower at the optimum temperature, the optimum temperature and the base temperature respectively. The quadratic equations

for all four varieties are presented in this form in

Table 2.5. The quadratic equations extrapolate to T_b

values that are 1 to 5°C higher than those predicted by

the linear model (Table 2.3). T_b and T_{opt} values are

similar for both Botanical types (Spanish and Virginia)

in this experiment although the restricted number of

varieties (two per Botanical type) limits the

generalizations that can be made.

Table 2.5: Comparison of goodness-of-fit for polynomials and
straight lines in each of four peanut varieties grown in glasshouse
cabinets set at mean temperatures from 19.7°C to 29.7°C. Derivation
of data for Early Bunch presented in full in Table 2.4, other
cultivars in Appendix 6. Quadratic equations are presented as
$$Y= Y_{opt}[1-\{(T_{opt}-T)/(T_{opt}-T_b)\}^2]$$

	Percentage variance accounted for by linear equation	Percentage variance accounted for by quadratic equation	Significance of quadratic improvement	T_b	Quadratic Equation
TMV2	91.2	96.4	p<0.001	14.8	$Y=4.64[1-\{(30.9-T)/16.1\}^2]$
White Spanish	79.3	97.6	p<0.01	15.7	$Y=5.43[1-\{(29.3-T)/13.5\}^2]$
Early Bunch	88.8	97.6	p<0.001	14.7	$Y=3.84[1-\{(29.7-T)/15\}^2]$
Robut 33-1	82.0	91.2	p<0.01	14.8	$Y=4.03[1-\{(29.1-T)/14.3\}^2]$

(d) The interaction between PFD, temperature and photoperiod in regulating time to first flower.

Figure 2.3 shows that PFD affects rate to first flower, with saturation at about 500 µmol m^{-2} s^{-1}. Below this irradiance flowering is considerably slowed. After first flower appearance the individual plants were harvested and oven dried. At PFDs of 500 µmol m^{-2} s^{-1} and higher, each variety flowered at a particular dry weight, whereas at the lower PFD plant dry weights were much reduced at the time of flowering (Table 2.6).
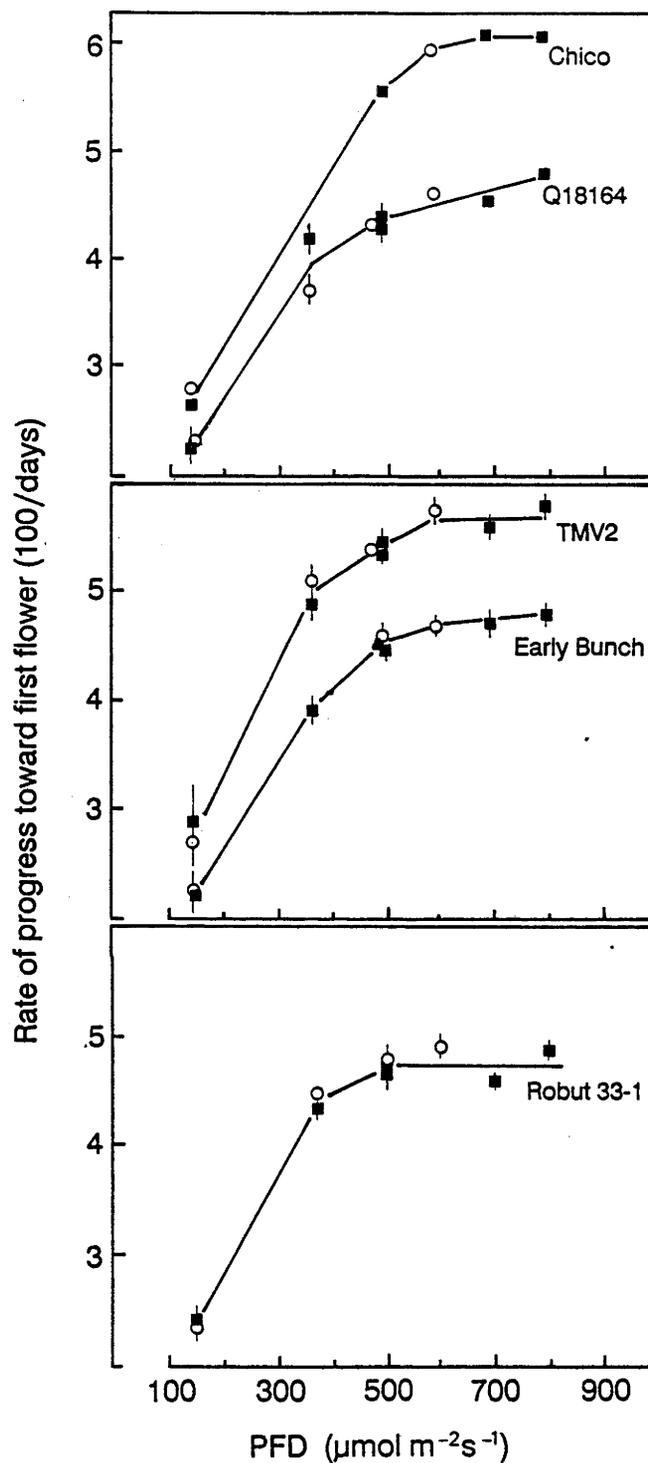
Figure 2.3: Rate of progress to first flower appearance for five peanut varieties at a range of PFDs. Those treatments represented by (■) were short day photoperiods, while those by (O) were long days. Error bars equal 2 x S.E. Where S.E. bars not shown, they are smaller than the symbol. The variety Chico was not grown at 370 μmol m^{-2} s^{-1}, while the 2 treatments at this PFD for the variety Q18164 were significantly different (p<0.05). Expts 18.9.87 and 31.10.87.

Table 2.6: Mean oven dry weights (g) of peanut plant leaves and stems on the first day of flower appearance after being grown under differing irradiances for 12 or 14h photoperiods. These are identical plants to those in Figure 2.3. Significant differences (p<0.05) indicated by * for treatments that are adjacent in the table. Expts 18.9.87 and 31.10.87.

	Photon flux density (μmol m^{-2} s^{-1})						
	150		370		500		600, 700& 800
Chico	0.95±0.12		*		1.54±0.15	ns	1.75±0.06
Q18164	3.02±0.26	*	4.62±0.21	*	5.22±0.27	ns	5.42±0.21
TMV2	1.80±0.11	*	2.04±0.17	*	2.42±0.16	*	2.74±0.10
Early Bunch	2.77±0.31	*	3.59±0.23	*	3.98±0.21	ns	4.14±0.21
Robut 33-1	1.98±0.19	*	3.08±0.09	*	3.21±0.15	ns	3.36±0.22
White Spanish			1.97±0.13	*	2.58±0.26		

Under low PFD there is evidence of a PFD/photoperiod interaction. Under short days at 370 μmol m^{-2} s^{-1}, the variety Q18164 flowered significantly faster than under LD (p=0.05) although there was no evidence of a short day photoperiodic response at other PFDs (Figure 2.3). In confirmation of this observation, times to first flower in a glasshouse in low winter irradiance (Table 2.7) were much greater than under spring and summer irradiances (Table 2.2). Most of the varieties examined (eleven of the twelve) showed a SD photoperiodic response; they flowered faster under SD at the higher temperatures,

Table 2.7: Time (in days) from seedling emergence to first flower for a range of peanut varieties, grown under low (winter) and intermediate (spring) irradiances in naturally lit glasshouses. The mean daily radiation at canopy height was 7.0 MJ m^{-2} d^{-1} (winter: Expt 18.6.87) and 12.6 MJ m^{-2}d^{-1} (spring: Expt 28.8.87). Significant differences (*) between photoperiodic treatments shown at p<0.05.

Winter data; SD are 12 hours and LD are 14 hours.

	33/28°C		27/22°C		21/16°C	
	SD	LD	SD	LD	SD	LD
Spanish Types:						
Red Spanish	30.0 *	55.5	31.5	31.0	71.3	72.0
Chico	20.2 *	22.5	29.0	28.5	55.5	57.8
TMV2	22.0	24.0	30.2	29.5	64.2	65.0
White Spanish	23.2 *	29.0	31.0	29.8	64.5	64.7
Valencia Types:						
Q18660	22.2 *	26.7	26.2	28.0	61.7	60.0
Q18657	23.5 *	25.7	29.8	32.5	62.7	64.3
Q18636	22.2 *	25.2	29.5	29.3	62.2	62.8
Virginia Types:						
Robut 33-1	38.5 *	65.2	38.2	38.2	71.0	71.0
Q18164	27.8 *	78.0	34.5 *	39.7	72.7	71.2
VB187	26.7 *	43.0	35.8	34.0	77.0	77.8
Early Bunch	33.0 *	49.0	34.0 *	40.0	70.0	70.5
NC17209	27.7	28.2	31.8 *	38.0	71.3	72.0

Spring data; SD are 8 hours, LD are 14 hours.

	33/28°C		30/25°C	
	SD	LD	SD	LD
Chico	18.0	17.7	19.3	19.7
White Spanish	21.0	21.3	21.5	21.7
Early Bunch	23.7 *	27.5	25.5	27.0

33/28 or 27/22°C. At low temperature time to first flower was similar under both SD and LD in all varieties. In the Spring experiment under intermediate light, Early Bunch showed a photoperiodic response at 33/28°C, but not at 30/25°C. The delay in first flower appearance in those plants in the replicated LD cabinets compared with the respective SD treated plants in Table 2.7 was not due to differences in temperature, PFD or humidity. The calibrated thermister records of the respective cabinets gave no indication that there was any difference in temperature between the DL treatments at 33/28°C and leaf production rates were similar for the plants of each variety from dissimilar DL treatments. Although PFD and humidity can not be controlled and are not monitored continuously in individual glasshouse cabinets in the Canberra phytotron, measurements made during the course of the experiments did not reveal significant differences in either environmental variable between contrasting DL treatment cabinets. The cabinets that were programmed for contrasting photoperiods were located close to each other in the same glasshouse, so that it was unlikely that humidity or PFD varied greatly between them.

Discussion:

Response to photoperiod:

In peanut time-to-first-flower is generally not responsive to daylength, (Tables 2.1 and 2.2) although subsequent flower numbers are doubled by short days (Figures 2.1 and 3.1). However, in winter, under low PFD at high temperature, peanut does express a SD response for time-to-first-flower (Table 2.7, Figure 2.3). Later in its life cycle peanut appears to be even more sensitive to daylength and, again, it exhibits SDP characteristics. Short days after first flower formation increase flowers, pegs and/or pods compared with LD treatments (Emery et al., 1981). Thus, classical definitions of daylength sensitivity relying on time to first flower appearance (Vince-Prue, 1975) have limited applicability to peanut. With respect to onset of flowering, peanut is strictly a SDP because under some environmental conditions, SD hasten flowering. This definition is also consistent with later stages of the life cycle where peanut exhibits a cumulative or increasing requirement for SD. However, in most practical situations, onset of flowering is unaffected by daylength (Summerfield and Wien, 1980).

Response to temperature:

By contrast with the slight sensitivity to photoperiod, temperature plays a major role in determining the time to first flower in peanut (Tables 2.2 and 2.3). Below 30°C this temperature dependence is close to linearity for both the rates of flower and leaf appearance for the Virginia variety Robut 33-1 (Leong and Ong, 1983). A similar relationship has been observed for leaf production in the Spanish variety Chico (Figure 4.1). Such linearity has allowed Bell *et al.* (1990a) to fit a heat-sum model to flowering data for peanuts grown under fluctuating field temperatures, and they suggested that Spanish cultivars had a slightly higher base temperature (T_b values in the range 13.31 to 14.10°C) than Virginia or Valencia cultivars (10.27 to 12.85°C) and that Spanish cultivars required significantly fewer "day-degrees" (263 for three cultivars) to reach flowering than their Virginia or Valencia counterparts (345 to 476 "degree-days"). The range of θ_f values in this study are Spanish: 244-312, Valencias: 303-323 and Virginias: 357-455 and these are consistent with those reported by Bell *et al* (1990a). The calculated base temperatures imply that Virginia varieties are generally able to develop at lower temperatures than Spanish varieties, although the assumption of linearity and the considerable extrapolation required in both this study and that of Bell *et al.* (1990a) must qualify this finding. The linear model generated T_b values for Spanish

varieties of 13.6°C, for Valencias it was 12.5°C and for the Virginias 11.4°C, whereas quadratic equations predicted T_b values in the range 14.7 to 15.8°C. Some studies have found no evidence for differences between survival of Spanish and Virginia botanical types at low temperatures (Angus *et al.*, 1981; J.H. Williams, pers. comm.) and the T_b values predicted by the quadratic equations appear to support this observation.

At near-optimal temperatures it is not always appropriate to assume linearity of response. At average temperatures near to 30°C, a single straight line does not provide as good a fit as a quadratic curve (Tables 2.4 and 2.5, Appendix 6). These data are therefore outside the linear rate/temperature relationship necessary for accurate use of the linear model. Roberts and Summerfield (1987) overcame this problem by fitting a second line through the supra-optimal data. The difficulty with fitting two straight lines to these data is that curves might be closer approximations than are straight lines (Wolfe and Bagnall, 1979). Broad optima are not easily described by two intersecting straight lines and fitting those lines can involve complex statistical analyses (Chappell, 1989) if the change point is not fitted "by eye". The shortcomings of fitting straight lines after locating the change point "by eye" are: the procedure is not objective and repeatable in that it does not take into account that the change point is estimated from the data and that the procedure

eliminates the mathematically desirable attribute of continuity at the change point (Chappell, 1989). There is no physiological reason why developmental processes should be linear (Roberts and Summerfield, 1987) and there is also a strong likelihood that curves are a more accurate representation of biological processes (Bagnall and Wolfe, 1978). In summary, the choice for those modelling peanut phenology lies between the simplicity of a straight-line fit and the increased accuracy of curves.

The related question of whether it is statistically valid to extrapolate to obtain T_b (i.e. whether there is a linear relationship between rate of development and temperature) has been critically examined by a number of researchers (e.g. Wang, 1960; Lombard and Richardson, 1979). Angus *et al.* (1981) found that for many species including peanut, rate of development is highly correlated with low sub-optimal temperatures in the early stages of growth and that it is valid to assume that a linear relationship exists. Angus *et al.* argue that that the extrapolated T_b has "considerable statistical significance" and that the resulting models can be valid for simulating crop production in diverse environments. The continued use of models involving extrapolated T_b estimates for a range of crops, including peanut (Angus *et al.*, 1981; Bell *et al.*, 1990a), appears to justify this approach.

Response to irradiance:

Time to first flower in peanut can be considerably delayed at low irradiance (Figure 2.3) although PFD would usually not be limiting in subtropical and tropical locations. Given such a requirement for photosynthetically active radiation it is difficult to interpret some of the earlier studies of photoperiodic responses. Some of these studies reported differences in photoperiodic control of time to first flower but the authors changed photosynthetic as well as photoperiodic irradiance (e.g. Sengupta *et al.*, 1977) while other studies appear to have been carried out under low irradiance conditions (e.g. Tétényi, 1957).

When there is an interaction between photoperiod and PFD under very low photon fluxes (7.0 MJ m^{-2} d^{-1}) peanut behaves as a SDP (Table 2.7) with regard to first flower appearance. In all of the experiments, conducted with a range of varieties from all 3 Botanical types, peanut behaved consistently as insensitive or as a SDP and never as a LDP. It would be unusual for a crop of tropical origin to respond as a LDP (Roberts and Summerfield, 1987). However, higher PFDs lead to faster flowering (Figure 2.3) as occurs in natural long days and these differences might explain other aspects of the contradictory responses to daylength reported in the literature. Similarly, field studies involving different sowing dates may confuse differing photosynthetic inputs with photoperiodic differences and thus some varieties

may show apparent but not true LD flowering responses (Bell *et al.*, 1990b).

Considering all of the environmental effects on time to flowering of peanut, under PFDs normally ocurring in subtropical and tropical regions (above 500 μmol m^{-2} s^{-1}) both photoperiod and PFD are non-limiting. Temperature, then, is the dominant influence on time-to-first-flower for peanut in the field. Over a range of temperatures commonly experienced by peanut, days-to-first-flower was approximately halved between 20 and 27°C (Table 2.2). This marked temperature dependence can provide the basis for a linear thermal time model (Bell *et al.*, 1990a). However, in some environmental conditions, the usefulness of this model is limited. As an example, in the monsoon tropics a mean temperature of 30°C is common during early growth (Bell, 1986) and the shortcomings of the linear thermal time model are very apparent at this temperature (Figure 2.2).

Chapter 3

Environmental control of fruit development in peanut.

Introduction:

Although *Arachis hypogaea* (L). has been classed as a day-neutral plant (DNP) with respect to time to first flower (Bunting and Elston, 1980; Leong and Ong, 1983), in Chapter 2 it has been shown to be weakly sensitive to daylength and can be described as a quantitative short day plant (SDP). Other workers using controlled environments showed that fruit development is also affected by photoperiod. Short day (SD) treatments increased peg and fruit numbers across a range of *Arachis* species and varieties (Wynne *et al.*, 1973; Emery *et al.*, 1981; and Stalker and Wynne, 1983) compared with long days (LD) imposed by night breaks. By contrast, field studies involving extension of natural photoperiods by 3 to 4 h indicated greater pod yields under long days for some varieties (Witzenberger *et al.*, 1985 and 1988).

However, compared with the large effects in the experiments of Wynne and co-workers (e.g. five-fold difference in pod numbers: Emery *et al.*, 1973) the yield differences in the field were variable and generally small, with a doubling in SD in one variety (M13) and an increase of 15% in LD in another (Robut 33-1).

In this chapter, the experiments have been designed to examine how the timing and duration of photoperiodic treatments affect late stages of flowering and pod yield in a range of peanut varieties under controlled environment conditions. These photoperiodic treatments involved low intensity photoperiodic extension by incandescent lamps rather than night interruptions. The effects of irradiance and temperature on peg and fruit development are also examined.

Materials and Methods:

Germination and growth conditions:

Conditions for germination and early growth were as described in Chapter 2. Watering and nutrient application are described in Appendix 2. At first flower formation the plants were selected for uniformity, and then transferred from 1 litre red plastic pots (125 mm diameter) to 10 litre black plastic pots (250 mm diamter) of 1:1 perlite:vermiculite to allow for peg development, except for the PFD experiment where seedlings were planted directly into 10 litre pots. The repotting of

plants at first flowering was found to be a better method for selecting uniform plants than initial overplanting and subsequent selection. The transplanted, centrally placed peanut plants were less likely to have pegs overhanging the rim of the pot than randomly located plants. Repotting therefore maximized the probability that pegs would form pods in the growth medium. The choice of "bunch" rather than "runner" cultivars also reduced the potential for pegs to overhang the pots. The roots were not disturbed during transplanting. The cultivars examined included Early Bunch, Robut 33-1 (Virginia types), TMV2 and White Spanish (Spanish types). Top dry weights, when reported, are for oven-dried ($80^{O}C$ for 24h) shoots plus pegs and pods . Pods were counted when more than 10mm in length. Experiments were conducted in either phytotron glasshouse cabinets, open glasshouse space or in artificially lit LB cabinets (Morse and Evans, 1962). Harvests were conducted 60 to 70 days after germination or earlier, so that very few pegs grew outside the pots, and more than ninety per cent of pegs had sufficient time to start forming pods.

Glasshouse conditions:

Phytotron glasshouse experiments were conducted at $30/25^{O}C$ day/night temperature (8h/16h thermoperiod equals a $26.7^{O}C$ average) except when dealing specifically with the temperature response (Figures 3.4, 3.5, 3.6 and 3.7). The photoperiod in the open glasshouse was extended by

low intensity (60 µmol $m^{-2}s^{-1}$) incandescent lamps to 16 hours (0400 to 2000h Eastern Standard Time). In the glasshouse C-units (Morse and Evans, 1962) photoperiod was either 12 hours (natural light from 0600 to 1800h) or 16 hours made up of 12 hours natural extended by low intensity incandescent lamps (60 µmol $m^{-2}s^{-1}$ photon flux density) for 2h before and 2h after the 12 h day. In the glasshouses in the phytotron a fresh air exchange rate of 0.24 m^3s^{-1} during the day, and 0.024 m^3s^{-1} at night, maintains CO_2 concentration within 30 µmol l^{-1} when the glasshouses contain a full complement of plants.

Artificially lit conditions:

All experiments in the artificially lit LB cabinets were conducted at a constant 30°C. Illumination was provided by eight Wotan Powerstar HQI-T 400W/DH metalarc lamps (Siemens, Munich, FRG) supplemented by two 500W quartz iodide lamps. The photon flux density (PFD) at canopy level was 500 µmol $m^{-2}s^{-1}$, except in experiment 30.7.87 where it was varied after first flower formation. Where appropriate, the LD photoperiod extension with incandescent lamps (PFD of 10 µmol $m^{-2}s^{-1}$) was split into 2h extensions before and after the main light period. Appendix 5 provides further information about cabinet light quality.

Experimental:

The various experimental conditions and cultivars used
are listed below in Table 3.1. In all experiments
seedlings were selected for uniformity when 5d old.
Replicate numbers were 8 to 12 plants per treatment.

Table 3.1: Experimental conditions and cultivars.
"C" units are naturally lit glasshouse units in which
photoperiod can be controlled, while LB cabinets are
artificially lit cabinets, in which both photoperiod and
PFD are controlled. The cultivars were Early Bunch (EB),
Robut 33-1 (Ro), TMV2 (TMV) and White Spanish (WS). Expts
7.12.87, 7.2.88 and 5.2.88 involved the effect of
photoperiod on either flower, peg or pod numbers, whereas
Expt 30.7.87 concerned changes in growth, flowering and
fruiting due to different PFDs and Expt 9.8.86 examined
growth and fruiting response after different temperature
regimes from emergence. Expt 10.1.90 was a photoperiod/
temperature interaction study.

Exper-iment number		Facility	Temperature ($^\text{O}$C)	Photoperiod (h)	Cv.
7.12.87	Table 3.2 Figure 3.1	LB Cabinet	30	12 and 16	EB Ro
7.2.88	Table 3.3	Glasshouse then "C" unit	30/25	12 and 16	TMV Ro WS
5.2.88	Table 3.4 Figure 3.2	LB Cabinet	30	12 and 16	EB
30.7.87	Table 3.5 Figure 3.3	LB Cabinet	30	12	WS
9.8.86	Figs 3.4, 3.5 and 3.6	Glasshouse	24/19 to 33/28	16	EB
10.1.90	Table 3.6	Glasshouse "c" units	30/25 and 24/19	12 and 16	EB Ro WS TMV

Where feasible these replicates were split into two C
units or LB cabinets set at the same operating conditions
(Experiments 7.12.87, 7.2.88 and 5.2.88). In the absence
of differences between replicate cabinets within a
treatment, analyses of variance were carried out on
combined data. Plant densities were 7.6 plants m^{-2} in
the glasshouse (Expt 9.8.86), and 12.2, 14.1, 10.8, 5.4
or 2.9 plants m^{-2} in Expts 7.12.87, 7.2.88, 5.2.88,
2.4.88 and 10.1.90. Average radiation above the plants in
the glasshouse experiments was 16 MJ m^{-2} in Expt 7.2.88,
14.5 MJ m^{-2} in Expt 9.8.86 and 14.1 MJ m^{-2} in Expt
10.1.90.

Photoperiod Experiments:

The screening of cultivars for photoperiod response
(Expt 7.2.88) was conducted in glasshouse photoperiod
controlled cabinets (see previous Chapter for a
description of the rationale behind the use of the
different types of phytotron controlled environments).
The size of the various cabinets, the number of
replications of treatment plants, the size of the plants
and the number of treatments in particular experiments
severely limited the number of cultivars that could be
included in this chapter. The cultivars screened in the
glasshouse cabinets were TMV2 and White Spanish (Spanish
types), and Robut 33-1 (a Virginia type). The controlled
PFD/photoperiod experiments were conducted in the
artificially-lit cabinets on the Virginia cultivars Early

Bunch and Robut 33-1 (Expt 7.12.87). These experiments
were designed to show how pre-flowering photoperiodic
treatments compared with photoperiodic treatments during
flowering on the numbers of flowers and fruit that
subsequently formed. The pre-flowering treatment period
was from emergence to first flower appearance at 20d. The
subsequent treatment periods were from first flower
appearance to 20d later, and then from 20d after first
flower to 40d after first flowering i.e. three 20d
treatment periods between emergence and harvest. A later
experiment (Expt 5.2.88) on the cultivar Early Bunch
similarly involved transfers after differing numbers of
days at the two photoperiods.

Photon Flux Density Experiment:

The effect of PFD on growth, flowering and fruiting
(Expt 30.7.87) was studied on the cultivar White Spanish,
which is reputedly sensitive to small changes in light
intensity (M. Bell, pers. comm.). The competing
requirements for plant replicate numbers, treatment
numbers and cabinet space, precluded the inclusion of
other cultivars. All the plants were grown in 10 litre
pots after germination under the same PFD until flowering
(500 μmol m^{-2}s^{-1}), when they were selected for uniformity
and transferred to cabinets with PFDs of either 400, 550,
700 or 800 μmol m^{-2}s^{-1} for 24d until harvest. Photoperiod
was 12h in all treatments.

Temperature Experiment:

The temperature versus growth, flowering and fruit formation experiment (Expt 9.8.86) was conducted in the naturally lit glasshouses, all of which have the photoperiod extended to 16h by 150W "Comptalux" reflector lamps producing a PFD of 60 μmol m^{-2}s^{-1} at plant level. Temperature treatments commenced at emergence and continued till 70d later. The choice of glasshouse rather than cabinet space for this experiment was due to the large numbers of replicate plants, the requirement for relatively widely spaced pots and the number of treatments that were involved. The wide spacing of plants (7.6 plants m^{-2}) minimized the possibility of light limited conditions. The individual plants were randomized daily after flower counts. Individual flowers were marked with water-soluble non-toxic paint (a different colour for each week) and the time of the transition to pegs noted. Pot temperature was measured in the 33/28°C glasshouse and was within 1°C of air temperature under those conditions when maximal pot warming would have been expected i.e. at noon prior to watering the black pots on a clear sunny day. At the time of transfer from the 1l red pots to the 10l black pots the plant foliage effectively shaded the pots and reduced pot heating (Gibson, 1980). In analysing Experiment 9.8.86, a multivariate statistical package was used (Genstat V, Lawes Agricultural Trust, Rothamsted). This analysis generated a correlation matrix between the measured plant

variables including weekly flower and peg totals and harvest dry weights. Where applicable, errors presented are ±SE.

Photoperiod/temperature Interaction Experiment:

Seed of the cvs TMV2 and White Spanish (Spanish types) and Early Bunch and Robut 33-1 (Virginia types) were pre-germinated and selected for uniformity, then grown from emergence to harvest in phytotron controlled photoperiod glasshouse cabinets with temperatures of either 30/25°C or 24/19°C and photoperiods of 12 or 16 hours. At harvest pegs were counted and the tops oven-dried for weighing. Harvests for plants at 30/25°C was 35d after emergence for the Spanish and 40d for the Virginia cvs, while the harvests at 24/19°C were delayed until significant numbers of pegs formed in the majority of plants: 48d for Spanish cvs, 62d for Virginia cvs.

Results:

Does photoperiod affect flower and fruit production in peanut?

The effect of photoperiod from emergence on rates of flower production in peanut was examined in long (LD) or short (SD) days. Flower counts were made daily because newly opened flowers (yellow corolla) were only visible for 2 to 3 days. Short days clearly promoted flowering in
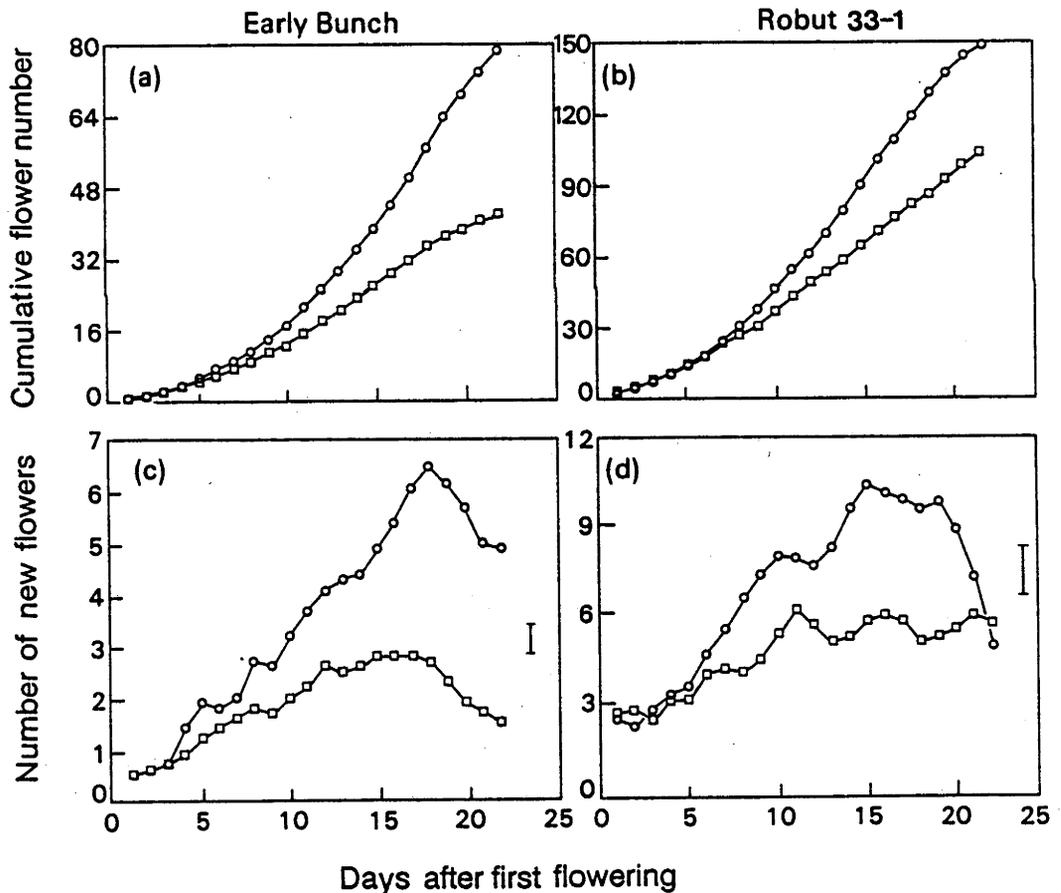
Figure 3.1: Flower numbers of the varieties Early Bunch and Robut 33-1 under either short (12h o) or long (16h □) days: a) Early Bunch cumulative flower number b) Robut 33-1 cumulative flower number c) Early Bunch daily flower number d) Robut 33-1 daily flower number. Eight plants per treatment. The transfer between photoperiods was on Day 0. Error bars equal mean SE. Expt 7.12.87.

both cultivars, compared with plants in continuous long days (Figure 3.1). Cumulative flower numbers after 24 d were 70% (Robut 33-1) and 88% greater (Early Bunch) than in LD. Differences in cumulative flower numbers were observed within 10 days of the transfer between daylengths (Figure 3.1a and 3.1b).

The two varieties had similar patterns of response to short days: daily flower numbers reached a peak after about 15 to 20 days of flowering and subsequently the rate of new flower formation dropped dramatically (Figure 3.1c and 3.1d). Conversely, the two varieties differed in long days. For Early Bunch (Figure 3.1c) in long days there was a peak in flower production at a similar time although it was less pronounced than in short days, whereas for Robut 33-1 in LD but not SD there was no apparent peak in rate of flowering (Figure 3.1d). As a consequence, the daily flower production in Robut 33-1 after 24 d was higher in long days than in short days (Figure 3.1d).

The two daylength treatments in Figure 3.1 were two treatments from an experiment in which plants were subjected to either short or long day treatments during three periods. Figure 3.1 shows data from the first two treatment periods. The transfers were at first flower formation (approximately 20d after emergence) and at 20d after first flower appearance. Flower numbers were only counted in the 22d after first flower appearance.

Thereafter the plants could not be moved to be counted because of their interlocking canopies. Conclusions drawn about flower to peg conversions are therefore qualified by the assumption that later formed flowers are less important to peg and pod numbers. This assumption has some support from other studies (Ono and Ozaki, 1971; Klepper, 1973; Hudgens and McCloud, 1975). In addition to the continuous long days and continuous short days (L-L-L and S-S-S treatments) illustrated in Figure 3.1, alternate treatments were either 20 short days before flowering, then 40 long days (S-L-L) or 20 long days, then 20 short days, and finally 40 long days (L-S-L). In those treatments which included 20 SD flower numbers were not significantly different from plants in continuous short days (Table 3.2). The exception to this observation was Robut 33-1, S-L-L, which produced slightly smaller plants than in its other treatments (see dry weights in Table 3.2). Thus, fewer flowers could be expected as plant size has large effects on peanut flowering (see later).

The conversion of flowers to pegs appears to be mildly sensitive to photoperiod. In all photoperiod treatments that included long days, the reduction in peg numbers compared to the S-S-S treatment was consistently greater than the equivalent percentage reduction of flower numbers (Table 3.2). Thus under continuous long days (L-L-L), Early Bunch produced 53% of the flowers and 37% of the pegs that the S-S-S treated plants formed,

Table 3.2: Effect on flower and fruit numbers plant after exposure to short (12h) or long (16h) photoperiods before or after first flower appearance. Harvest was 60d after emergence. The combinations of short and long days imposed over successive 20d periods were SSS, SLL, LSL and LLL. Flowers were counted in the second period only. The reduction in flowers, pegs or pods is reported as a percentage of the continuous SD treatment. Treatments marked n.s. are not significantly different from the SSS treatment (p=0.05). Values are means ±SE on a per plant basis in all tables. In this table n=8 for all treatments. This is experiment 7.12.87.

Variety: Early Bunch

Treat-ment	Flower No.	Per cent of SSS	Peg No.	Per cent of SSS	Pod No.	Per cent of SSS	Top dry weight (g)
SSS	78±9		79±20		11.8±3.7		42.3±6.4
LLL	41±5	53	29±14	37	1.0±0.5	9	41.2±6.0
SLL	71±9	n.s.	52±21	n.s.	3.6±2.3	31	41.6±6.5
LSL	57±9	n.s.	57±14	n.s.	1.2±0.5	10	40.0±4.4

Variety: Robut 33-1

SSS	152±23		102±19		12.9±4.6		51.0±5.3
LLL	90±15	59	56±19	55	2.6±1.8	20	49.0±5.8
SLL	117±12	n.s.	61±11	60	3.7±2.4	29	44.0±7.1
LSL	142±28	n.s.	66±15	65	3.2±1.6	25	49.2±9.8

while Robut 33-1 plants produced 59% of the flowers and 55% of the pegs. Flowers were not counted in the last 18 d of this experiment and late flowers must have formed pegs in the Early Bunch S-S-S treatment because the flower number was lower than the peg number in this

treatment. Pegs appeared within 3 to 4 d of flowering at this temperature (30°C).

The conversion of flowers and pegs to pods was also depressed by long days. Compared with the SD treatment (S-S-S), the late LD treatments L-L-L, S-L-L and L-S-L all depressed pod production (Table 3.2) and this was in some cases for treatments with similar flower production during the first 22d of flowering. A second experiment (Experiment 7.2.88) carried out in glasshouse conditions confirmed this repression by L-L-L for Robut 33-1 and extended the findings to another two cultivars (Table 3.3). Greatest responses to daylength were consistently

Table 3.3: The effect of photoperiod after first flower appearance on peg and pod formation in glasshouse cabinets. Plants were grown until first flower appearance in long days (16h) in the glasshouse and then transferred to either 12 or 16h photoperiods in glasshouse cabinets until harvest. Each photoperiod treatment was replicated 3 times. There were no significant differences between replicates, so the nine plants from each treatment were bulked for statistical analysis. Plant dry weights are of shoots, pegs and pods (but not roots). The plants were harvested 35d after first flower appearance. Expt 7.2.88.

Variety	Photoperiod (hours)	Peg number	Pod number	Dry Weight (g)
TMV2	12	31.4±2.8	5.6±1.5	19.9±1.6
	16	13.8±3.3	0.7±0.4	20.4±2.6
Robut 33-1	12	79.8±11.3	20.0±3.5	42.3±2.3
	16	55.5±10.9	4.1±1.4	45.1±3.2
White Spanish				
	12	73.2±7.6	19.7±2.3	30.8±2.6
	16	47.9±5.8	5.4±1.4	33.8±5.0

seen in the conversion of pegs to pods. There were 3 to 10-fold fewer pods produced in LD than in SD, whereas there was generally less than a two fold difference for flower and peg formation (Tables 3.2, 3.3). Short day exposures given prior to flowering (S-L-L above) or for 20 d after flowering (L-S-L above) could not prevent the poor pod set of plants experiencing long days late in their development.

Comparisons between ratios of pod numbers after SD and LD treatments for the cultivar Robut 33-1 were identical (5:1) in both glasshouse cabinets (Table 3.3) and artificially lit cabinets (Table 3.2) despite differences in light regime, plant density and night temperature. Peg numbers in LD were 55 per cent of those in SD treated plants in the glasshouse cabinets, and 70 per cent in the artificially lit cabinets and these proportions are not significantly different given the range of individual plant variation.

Further studies of timing of photoperiod response involved reciprocal transfers between long and short days for plants grown in replicated artificially-lit cabinets (Experiment 5.2.88). Space limitations and the large size of plants precluded transfers to short days late in development and restricted the number of cultivars to one (Early Bunch). However, the evidence presented earlier showed that all cultivars responded in a similar manner to photoperiod.

Table 3.4: Effect of transferring Early Bunch peanut plants between short (12h) and long (16 h) days. Treatments continued from emergence until the plants were harvested 60 d later. Each treatment had 5 sample plants. The experiment was replicated in different cabinets and with similar results. There are no significant differences between peg numbers or pod numbers, except for the All Long Day treatment. This is Experiment 5.2.88 and these are the same plants as in Figure 3.2.

Treatment	Peg Number	Pod Number	Top Dry Weight (g)
All Short	109.8±17.6	21.8±2.8	49.1±4.3
20 LD then 40 SD	132.8±34.1	19.0±7.0	56.8±9.3
30 LD then 30 SD	113.0±15.2	20.2±3.8	55.2±3.0
40 LD then 20 SD	94.5±26.5	16.0±8.0	57.9±6.6
All Long	51.4±10.6	5.2±2.3	56.2±3.8
20 SD then 40 LD	112.0±13.8	12.3±4.5	63.2±6.2
30 SD then 30 LD	123.0± 9.2	13.0±4.6	59.9±1.7
40 SD then 20 LD	123.8±15.3	19.0±2.6	59.4±3.2

When measured at 60 d from emergence, peg number was not significantly different between plants continually under SD and those exposed to short days at any stage of their life cycle (Table 3.4). Dry weights of tops were not significantly different except between continuous SD and the 30 SD followed by 30 LD treatment. By contrast pod numbers and pod filling (Figure 3.2) were very sensitive to photoperiod. Late LD treatments (30 or 40d before harvest) depressed pod number relative to all treatments with late SD. Timing of the SD treatment was

important. Thirty SD after emergence did not increase

yield as much as 30 SD before harvest (Figure 3.2). The

differences in plant dry weight of Early Bunch plants of

similar ages in Tables 3.2 and 3.4 were due to different

plant densities and provide evidence of the effect of

light intensity on plant growth. Notwithstanding the

differences in growth rate, the effects of continuous SD

versus continuous LD on peg numbers were similar i.e. LD

treated plants had one third to one half the pegs of
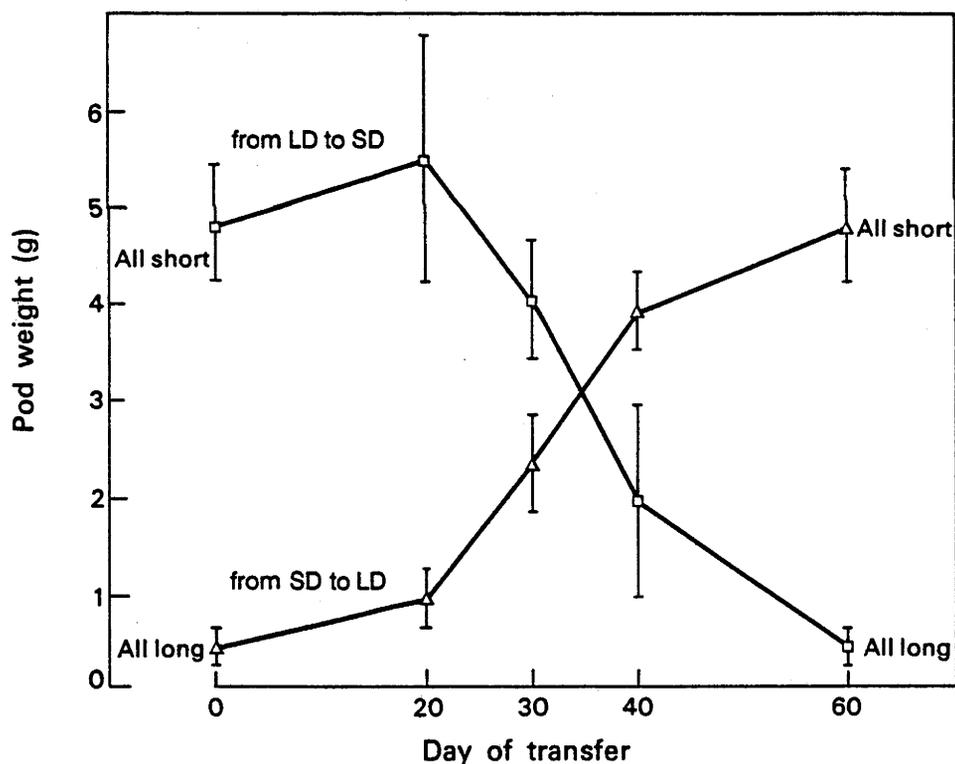
Figure 3.2: Pod weight per plant of Early Bunch peanut plants subjected to differing numbers of short and long days prior to transfer to the other daylength (S to L △ , L to S □). Five plants per treatment. Expt 5.2.88.

those plants in SD. The comparison of pod numbers under continuous short versus long days were not consistent between these two experiments. The smaller plants in Table 3.2 had formed relatively few pods in all treatments and the 12:1 ratio between short and long day treatments is exaggerated by the absence of pods in some LD treated plants (Table 3.2), whereas in the later experiment (Table 3.4), the ratio was 4:1. This delay in pod formation under LD appears to be one of the mechanisms by which photoperiod affects yield in peanut.

In these experiments pod dry weight at harvest was a very small proportion of total dry weight (up to 10 per cent in the most mature plants: Table 3.2). At harvest it was not possible to detect differences in plant morphology due to photoperiod. Although plant dry weights (excluding pod weights) of LD treated plants were, on average, larger than SD treated plants, these differences were not statistically significant.

In summary, the quantitative requirement for SD exposure differs between flowers, pegs and pods reflecting different development times for these organs to form. Peg numbers were significantly reduced only by the All Long Day treatment compared to all treatments with 20 or more SD (Table 3.4). This contrasts with the peg to pod conversion where pre-flowering SD were less effective than SD after first flowering (Table 3.4). Similarly pod yields of plants in the 30 LD then 30 SD were greater than those under 30 SD then 30 LD.

Photon flux density and flower and peg numbers:

Flower numbers were strongly influenced by PFD

imposed after first flower appearance (Figure 3.3).
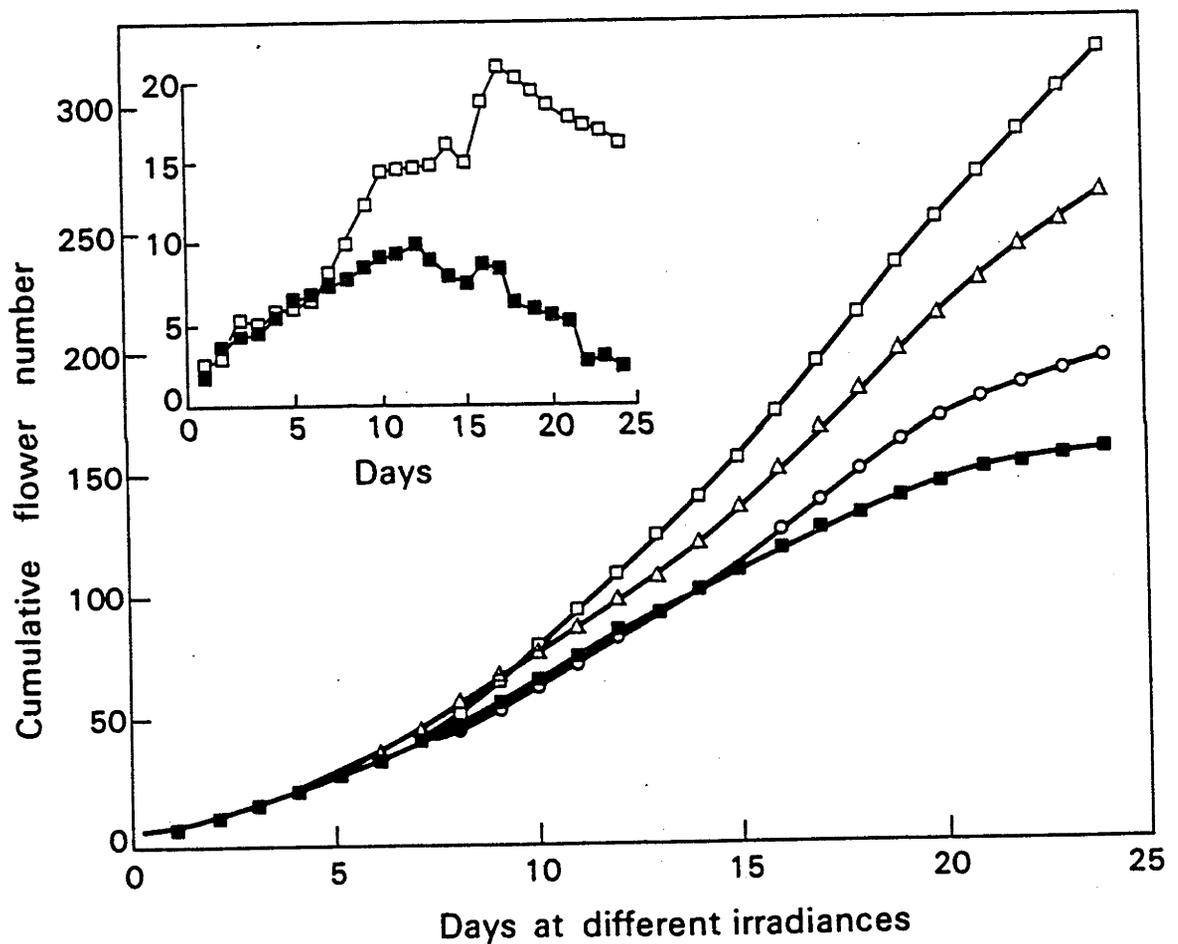
Figure 3.3: Cumulative flower numbers per plant (inset daily fresh flower numbers) of the variety White Spanish under different photon irradiances after first flower appearance 1000 ($\square$), 700 ($\triangle$), 550 ($\bigcirc$) and 400 ($\blacksquare$) μmol m^{-2}s^{-1}. These plants are the same as those in Table 3.5.

Differences were not evident over the first week but over the next 17 days flower numbers at 1000 μmol m^{-2}s^{-1} were double those of plants held at 400 μmol m^{-2}s^{-1}. The slowdown in flower production at about 12 to 17 days (Figure 3.3 inset) coincided with pod formation.

Table 3.5: Effect of differing photon flux densities on flower and peg numbers in the peanut variety White Spanish. All plants were grown under the same PFD until flowering (500 μmol m^{-2}s^{-1}) then transferred to cabinets with PFDs of either 400, 550, 700 or 1000 μmol m^{-2}s^{-1} for 24d until harvest. Root weights were not included in plant dry weight measurements. Photoperiod was 12h. Expt 30.7.87.

PFD (μmol m^{-2}s^{-1})	Plant dry weight (g)	Plant Height (mm)	Total Flower Production	Flowers: Dry Wt. Ratio	Total Pegs
400	19.7±1.8	205±5	157±14	7.9	60±8
550	22.8±5.4	179±22	194±25	8.7	82±15
700	28.6±4.2	192±21	261±19	9.4	98±12
1000	29.9±7.0	144±11	319±35	10.7	110±19

The effects of PFD on flower numbers was paralleled by changes in plant dry weight (Table 3.5). However, the ratio of flower numbers to dry weight suggests that at higher light intensities, there are proportionally more flowers (35% more) than at the lowest intensity. The ratio of flower number to pegs was similar across all four irradiances (in the range 0.34 to 0.41) with no statistically significant differences between these ratios. Plant height was markedly affected by irradiance in the 24 days between first flower and harvest. Plants

grown at high irradiance were shorter and heavier than those grown at low irradiance.

Temperature effects on flowers, fruiting and plant growth:

All of the plant growth variables including final dry weight, leaf number, flower number and peg number were positively correlated with temperature in the range from 24/19°C to 33/28°C (Figure 3.4). At 24/19°C (20.7°C
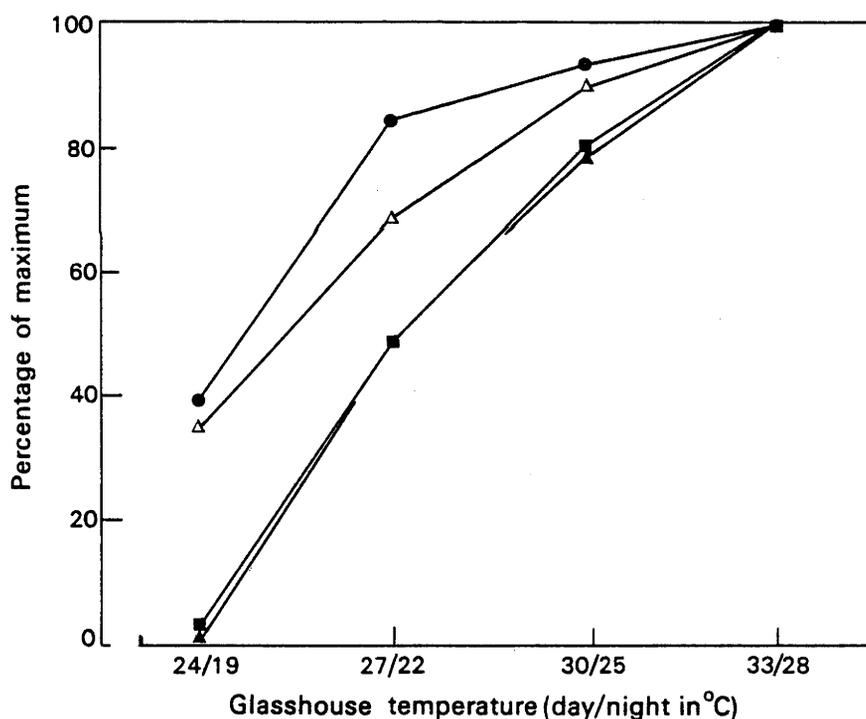
Figure 3.4: Effect of day/night temperature on shoot, peg and pod dry weight (●), leaf area (△), total flower number (■) and peg number (▲) of Early Bunch peanut plants harvested 70 d after sowing. Treatments commenced at emergence and continued to harvest. The maximum values (100%) at 33/28°C were: dry weight 44.4±10.8; leaf area 5851±1224 cm^2; total flower number 144±25; and peg number 102±19 (all ±LSD, p=0.05, n=12). These data, and Figures 3.5 and 3.6 refer to Expt 9.8.86.

average) rates of growth and flowering were markedly reduced relative to the warmer temperature treatments. The apparently greater depression of total flower and peg numbers at the lower temperatures was in part due to the timing of the harvest. Relative to the 33/28°C treatment at lower temperatures a larger proportion of potential flowers had yet to be formed. For individual plants in this experiment (data not shown) there were highly significant correlations between dry weight and leaf number (r=0.86), leaf area (r=0.96), and days to first flower (r=-0.62).

When days to first flower is converted to a development rate (i.e. the inverse of time to first flower- Roberts and Summerfield, 1987) there is a strong positive correlation with both dry weight and temperature. The lowest temperature regime (24/19°C) considerably slowed first flower appearance, and hence subsequent flower and peg production rates were also strongly depressed by low temperature (Figures 3.4 and 3.5). During the period from first flowering to peak flower production, the average flower production rate was 11 flowers per week at 33/28°C, 7.4 flowers per week at 30/25°C, 6.6 flowers per week at 27/22°C and 1.8 flowers per week at 24/19°C. Higher rates would have resulted had the plants been exposed to SD rather than a photoperiod of 16 hours, but the experimental design was constrained by the requirement for well-spaced plants which therefore were grown in the standard phytotron
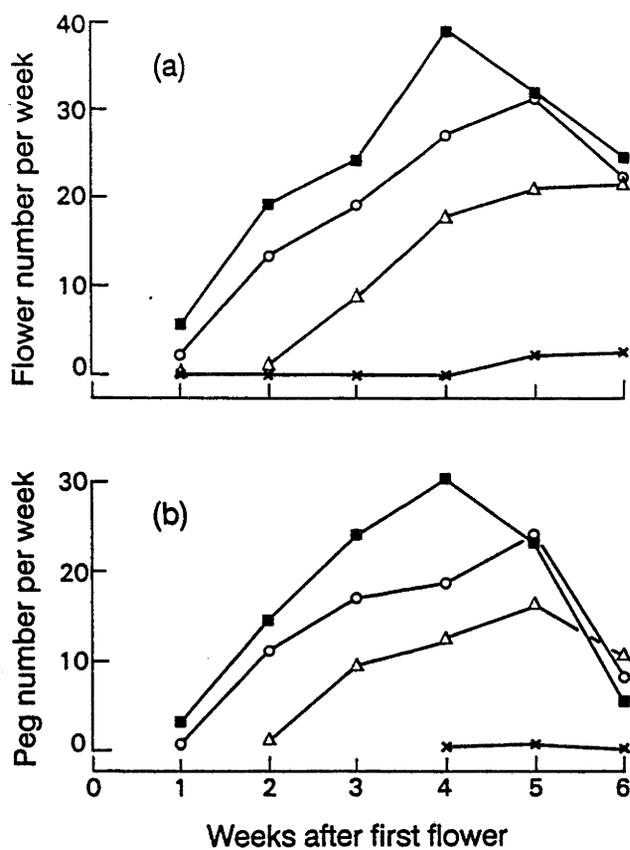
Figure 3.5: Weekly flower (a) and weekly peg (b) numbers of the variety Early Bunch grown at four temperature regimes. The time scale is based on the appearance of first flowers at 28d after emergence at 33/28°C (n=12 for each temperature). Treatment temperatures are 33/28°C (■), 30/25°C (O), 27/22°C (Δ) and 24/19°C (x).

glasshouse conditions (i.e. 16h photoperiod). Over a five week period peg numbers followed flower numbers fairly closely (Figure 3.5). The peak in flower numbers occured sooner in the high temperature treatments and it is possible that differences in total flower and peg numbers between high (33/28°C) and lower temperatures (27/22°C) would be smaller if the experiment had continued, i.e. the rate of peg formation was declining at 33/28°C faster than in the other treatments. Very high correlations

existed between flower numbers and total peg numbers in

the high flowering weeks 3 to 5 (r=0.93, 0.92 and 0.94)

and this implies a tight coupling between numbers of

flowers and numbers of pegs which set and a low rate of

flower abortion. Vegetative growth, as reflected by main

stem leaf number, was strongly correlated with
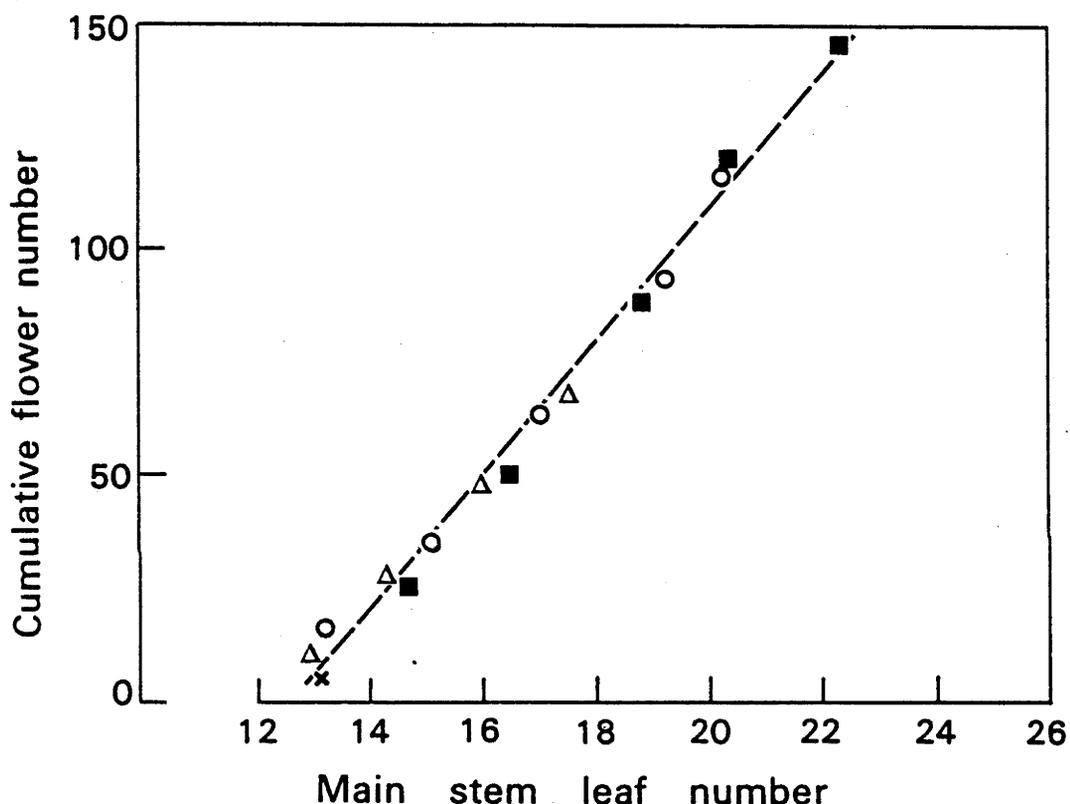
Figure 3.6: Cumulative flower numbers as a function of number of leaves on the main stem of Early Bunch peanut plants grown at four temperature regimes. The increases in flower and leaf number resulted over time from the temperature treatments. Temperatures same symbols as Figure 3.5.

cumulative flower numbers when these were recorded on a

weekly basis (Figure 3.6, r=0.99). Disregarding the

initial vegetative phase (approximately 12.5 leaves),

there were 14.7 flowers formed for every new leaf on the

main stem. Dry weight, although only sampled at final

harvest, was also strongly correlated with total flower numbers (r=0.89) and total numbers of pegs (r=0.76).

Photoperiod/temperature Interactions and peg numbers:

The results from the photoperiod/temperature interaction experiment (Table 3.6) confirm the results of the earlier photoperiod experiments in high temperature treatments (30/25°C). At the time of the 30/25°C harvest, pegs had not formed in some LD treated plants (TMV2 and Early Bunch), although the plants were the same size as SD plants. It appears that LDs delay peg formation at 30/25°C. In the low temperature treatment pegs were slow to appear for all cvs, particularly the two Virginia cvs Early Bunch and Robut 33-1. The important result from this experiment was that there were no differences between photoperiod treatment peg numbers for any of the four cvs at 24/19°C. This absence of photoperiodic sensitivity at low temperatures is similar to that observed in time to first flower experiments under winter light conditions (Table 2.7).

Table 3.6 Peg numbers of plants of four peanut cvs that were subjected to short (12h) or long (16h) days in glasshouse cabinets running at 24/19°C or 30/25°C. Time from emergence to harvest 35d for the Spanish cvs at 30/25°C; 40d for the Virginia cvs at 30/25°C; 48d for Spanish cvs at 24/19°C and 75d for Virginia cvs at 24/19°C. N=12 for all treatments. DW refers to oven dry top weight, ns refers to no significant difference between treatment pairs, * indicates a statistically significant (p<0.05) difference between treatment samples. Expt 10.1.90.

Cultivar	Temperature					
	24/19°C			30/25°C		
	Photoperiod			Photoperiod		
	12h		16h	12h		16h
TMV2						
Peg no.	7.1±1.3	ns	8.1±1.4	4.6±0.9	*	0.7±0.3
DW	8.3±0.7	ns	9.1±0.6	6.2±0.7	ns	5.8±0.2
White Spanish						
Peg no.	2.0±0.8	ns	3.0±1.0	3.8±0.8	*	1.2±0.4
DW	7.2±0.6	ns	7.1±0.8	5.0±0.4	ns	5.2±0.5
Early Bunch						
Peg no.	5.4±0.6	ns	7.6±2.2	2.2±0.4	*	0.0
DW	19.6±0.9	ns	20.8±1.9	6.7±0.3	ns	7.2±0.4
Robut 33-1						
Peg no.	2.5±0.7	ns	3.3±0.7	7.3±1.5	*	1.3±0.8
DW	20.4±1.3	ns	24.6±1.7	8.6±0.6	ns	7.9±0.5

Discussion:

Short days (SD) consistently promoted flower and pod production in the four peanut cultivars grown at optimal temperatures in these experiments. For the cultivar Early Bunch up to 12 times more pods were set in SD than in LD

(Table 3.2). Other workers have suggested that peanuts are indifferent to daylength in their control of flowering and yield (see summaries in Evans and King, 1975; Summerfield and Roberts, 1985; and Bunting *et al.* 1985). Some authors imply that some peanut varieties are long day plants (e.g. Wynne and Emery (1974), Witzenberger *et al.* (1985)) or that peanut has an optimum daylength for flowering at 10h with shorter or longer daylengths reducing flowering rates (Sengupta *et al.*, 1977). This study of yield response highlights a number of aspects which can confound interpretation of photoperiod response. The varieties TMV-2 and Robut 33-1 have been included here as they are common to a number of the earlier studies. Difficulties of interpretation have arisen in the past because of a number of factors:

- differences between flower initiation and flower development in their sensitivity to daylength.

- onset of a monocarpic development/senescence pattern stopping further flower initiation as a consequence of effective pod set.

- indirect, growth related changes, reflecting techniques for controlling daylength and of conditions for growing plant.

These three aspects of flowering control are considered below.

Floral stage and photoperiod:

In this chapter and the preceding chapter, it has been shown that daylength does not usually affect time to first flower of peanut, although SDs enhance subsequent flower production (Figure 3.1, Table 3.2) and pod set (Tables 3.2 and 3.3). The sensitivity of flower production to photoperiod was such that changes were seen within 10d of the commencement of flowering (Figure 3.1). The conversion of pegs to pods appears to be even more sensitive (Table 3.2). This was evident when peg numbers remained high, especially for the early transfers from SD to LD (Table 3.4). Wynne and co-workers (1973) have reported comparable promotive effects of SDs on peg and pod production of peanut and the present transfer experiments confirm the results of Emery *et al.* (1981). Greater dependence on daylength later in the life cycle is not unique to peanut, but is found in several grain legumes, including *Glycine max* (Nielson, 1942; Fisher, 1963; Schweitzer and Harper, 1985; Morandi *et al.*, 1988), *Vigna unguiculata* (Lush and Evans, 1980) and *Pisum sativum* (Haupt, 1969).

The similar numbers of flowers and pegs that formed under an adverse 16h photoperiod (Figure 3.4) suggests that formation of pollen and fertilization of the ovary are apparently insensitive to daylength. For peanut a high proportion of flowers (>70% - Table 3.2) converted to pegs in all photoperiods. Perhaps the change from flower to peg formation occurred too rapidly (within a

week, Figure 3.5) to allow expression of daylength
effects. In *Phaseolus vulgaris*, for instance, continuous
LD at the pollen stage dramatically increases flower
senescence and abortion (Ojehomon *et al.*, 1973).

These studies with peanut and those summarized above
for other legumes highlight the danger of extrapolating
the findings on daylength response for first flowering to
response over the full life cycle of a plant. Daylength
influences peanut fruit development but principally at
later stages of reproduction.

Plant development and photoperiodic effects:

In an earlier study, Emery *et al.* (1981) showed that
SD enhanced flower numbers in the first 30d of flowering
and that subsequent peg and pod numbers were also
enhanced by SD. In this study, flower numbers were
strongly correlated with peg and pod numbers. A majority
of the flowers were actually converted to pegs (>70%) and
flowers formed in any week correlated most with pegs
formed in the same week (Figure 3.5). However, where
flower counts are prolonged over a season (e.g. Sastry *et
al.*, 1985) the correlation between numbers of flowers,
pegs and pods will become less precise.

Although flower production is initially correlated
with pod production, subsequently the converse is true.
Flower production declines at pod formation (see Figures
3.1, 3.3 and 3.5 and Smith, 1954; Emery *et al.*, 1981).
The control of this switch to reduced flower production

appears to involve developing pegs or pods since flower removal allows continued flowering (Smith, 1954). Similarly, when pod formation is inhibited in LD then flower production continues (Emery et al., 1981 and see Figure 3.1). Effective pod set also reduces the rate of leaf production so that for recently released varieties like Early Bunch, vegetative growth ceases as soon as pod growth commences (Duncan et al., 1978).

The relationship between reproduction and growth:

Given that leaf number (Figure 3.6) and final dry weight (Figure 3.4) were highly correlated with flower production and final pod yield in peanut, then conditions such as low temperature (Figures 3.4, 3.5) or low PFD (Figure 3.3), which depress growth, also reduce flowering. For this reason, the data on daylength effects in this study have always been presented with data on final plant top dry weight and conclusions have only been reached where there were no significant dry matter differences associated with daylength treatments. There are several reports in the literature where the photoperiod treatments have affected dry matter accumulation and plant size must therefore have confounded photoperiodic comparisons. This is true for the positive LD response in the papers of Witzenberger et al. (1985 and 1988), and Ketring (1979) and presumably when Fortanier (1957) used higher irradiance daylength extensions to obtain his LD conditions. The SD treatment

of Sengupta *et al.* (1977) similarly reflected shorter
number of hours of photosynthetic light. Even a small
difference in plant temperature imposed with a daylength
treatment could dramatically alter growth (Figure 3.4)
and hence flowering, and this may explain the finding of
faster flowering of bigger plants in LD as reported by
Wynne and Emery (1974). Thus, for peanut, inferences
about environmental factors changing partitioning require
great care and the simultaneous measurement of dry matter
production. Moreover, even if total dry matter production
is no different during flower and peg formation in SD, an
unavoidable consequence is the reallocation of assimilate
to the developing pod with consequent cessation of shoot
growth and of flower production (Duncan *et al.*, 1978;
Emery *et al.*, 1981). Thus the value of high photoperiodic
sensitivity for pod yield has to be weighed against the
possible slower or later development of pods in non-
photoperiodic cultivars, or in non-optimal photoperiods.
In some conditions, slower pod development could benefit
yield, as was shown recently for soybean (Morandi *et al.*,
1988). Given the strong correlations between plant dry
weight when either temperature or PFD were varied
(Figures 3.3, 3.4 and 3.6), an increased vegetative phase
may provide more sites for fruit if a long growing season
is not disadvantageous. Conversely, inappropriate LD
sensitivity could depress yield when temperature was
optimal in only one season of the year. Another
possibility remains that season of sowing (daylength)

could be very important where temperature changes are small and its effects on growth are minimal.

Photoperiod/temperature interaction:

The absence of any photoperiod effect on peg number when peanut plants are grown at 24/19°C might be regarded as an anomolous result except that there is evidence from other phytotron and field studies that low temperature over-rides the effect of photoperiod in peanut. In the previous chapter, the time to first flower was reduced in SD compared to LD in warm (33/28 and 27/22°C) winter glasshouse cabinets, while at lower temperatures, photoperiod had no effect on time to flower (Table 2.7). The only other published phytotron study in which the photoperiod/ temperature effects on peg and pod numbers were examined (Wynne *et al.*, 1973) involved different growth temperatures to those in these experiments. At 26/22°C, peg numbers were doubled in SD compared with LD for each of three cvs, although these differences were *not* statistically significant (Wynne *et al.*, 1973). The peg numbers in 22/18°C were similar for the two photoperiod treatments for all three cvs, although peg numbers were low for mature plants. Notwithstanding the experimental shortcomings of the Wynne *et al.* study, their results confirm that photoperiodic effects (SD enhancement of pegs and pods) were most apparent at their warmest temperature (30/26°C) and least apparent at cool temperatures.

A speculative explanation of the absence of a photoperiodic effect on peg number at low temperature, and on time to first flower in winter light intensities at low temperature (Table 2.7) is provided by the similar absence of photoperiodic response at low temperatures in other SDP including *Xanthium strumarium* L. and soybean (Vince-Prue, 1975; Hamner, 1969). This response in SDP is due to low night temperature (i.e. during darkness) decreasing the inductive effect of a long dark period, and provides evidence of the important role of the dark period in the control of flowering in SDP (Vince-Prue, 1975). If the response in peanut is like that in other SDP then low *night* temperature, rather than low *average* temperature, might inhibit photoperiodic control of peg development.

This temperature/photoperiod interaction helps explain the absence of photoperiodic limitation of yield in the subtropics where the world's highest peanut yields have been reported under LDs (Hildebrand, 1975). Similarly, the absence of photoperiodic response to daylength extension in field experiments at Kingaroy, Australia (M. Bell, pers. comm.) could be due to low temperature inhibiting or over-riding photoperiod effects. By way of contrast, the daylength extension experiments in the field in India (Witzenberger *et al.*, 1985, 1988) were conducted partly at temperatures above 21°C and differences due to photoperiod were observed for some cvs. The apparent differences in DL sensitivity

observed by Witzenberger *et al.* (1985) may have reflected different rates of development of cultivars with exposure of different critical stages of the life cycle i.e. "windows of sensitivity" to photoperiod, when the cultivar was above 21°C.

In conclusion, this Chapter and the previous Chapter highlight the problems of assuming that daylength response for first flower appearance will be similar to the response over the full life cycle of a plant. Daylength influences peanut fruit development, but principally at later stages of reproduction. Examples of this response are the sensitivity to photoperiod at the peg to pod transition, and the requirement for continuous SD after first flower formation for maximum pod filling (Figure 3.2). Such continued sensitivity to daylength after first flowering severly limits models predicting agronomic success of crops on the basis of time to first flower.

A major breeding objective for most tropical grain legumes has been to develop photoperiod-insensitive genotypes with a determinate habit and synchrony of maturity (Lawn, 1989). However, responsiveness to photoperiod can be beneficial in adapting a variety to a particular location, as happens with soybean (Whigham and Minor, 1978), and it is important to know the extent to which photoperiod controls yield in peanut. To assess such a control, day neutral lines would provide a reference in varietal trials that could differentiate the

influence of photoperiod from that of other environmental variables. All of the peanut cultivars in this study responded to photoperiod with regard to peg and pod formation, and the identification of insensitive cultivars should be an important priority for peanut researchers.

Chapter 4

Temperature-dependent feedback inhibition

of photosynthesis in peanut

Introduction:

Peanut is a tropical crop which does not grow at

temperatures below 13°C (Angus et al., 1981) and severe

biochemical mulfunctioning has been observed below this

limit (Smillie, 1979). At temperatures from 15-20°C

growth is very slow when compared with growth at 25 and

30°C (Ono et al., 1974), although there are no obvious

signs of chilling injury (e.g. of wilting or chlorosis).

In parallel with this reduced growth rate there is a

reduction in both the formation of new leaf area and in

photosynthetic rate. What is not clear from such studies

is whether the reduction in photosynthesis is a direct

effect of temperature on leaf photosynthesis, or whether

there is some form of end-product inhibition of

photosynthesis. Inhibition refers to the accumulation of photosynthate in the leaves of peanut growing at low temperature conditions reducing CO_2 assimilation.

To investigate the possible direct or secondary effects of temperature on assimilation a number of approaches were tried, including: independent control of temperature of the assimilating leaf (the source) and of the remainder of the plant (the sink), and; exposure of plants to sub-optimal temperatures but under low atmospheric CO_2 concentrations to limit carbohydrate formation without reducing irradiance.

Materials and methods:

Growth conditions:

Seed of *Arachis hypogaea* L. cv. Chico (courtesy of M. Bell, Queensland Department of Primary Industries, Kingaroy, Qld. Aust.) were soaked for 2 h in tap water and then planted in vermiculite (medium grade supplied by Neuchatel, West Melbourne, V., Aust) in a seed tray. Plants were grown in a glasshouse of the Canberra phytotron (Morse and Evans, 1962) running at 30/25°C (8h/16h-day/night temperature). The 16h photoperiod is standard for all glasshouses in the facility (Morse and Evans, 1962). The decision to grow in the glasshouse rather than glasshouse cabinets maximized the radiant energy received by the plants; the open glasshouse has one less glass layer above the plants and glasshouse space provided greater flexibility with regard to spacing

of plants. All of the plants in this chapter and the next
chapter were vegetative or had just commenced flowering
at the time of the experimental treatments, and pegs and
pods had not developed. The LD growth conditions probably
helped slow the development of pegs (see Chapter 3). The
natural photoperiod was extended to 16 h by incandescent
lamps providing 60 μmol m^{-2} s^{-1} (PAR). After one week,
seedlings were selected for uniformity and transferred to
1 litre red pots containing a 1:1 (v/v) mixture of
vermiculite:perlite (perlite from Australian Perlite Ltd.
Banksmeadow NSW, Aust.). Watering was three times a day,
once with a modified Hoagland's No. 2 nutrient solution
(Hewitt, 1966; Appendix 2) and twice with tap water.

Whole-plant-growth experiment:

Seedlings grown at 30/25°C were selected for
uniformity one week after imbibition of the seed. Plants
were transferred to seven glasshouses running at 15/10,
18/13, 21/16, 24/19, 27/22, 30/25 and 33/28°C day/night
temperature. At this time and 12 d later, groups of 10
plants were harvested, leaf numbers recorded, and dry
weights determined after oven-drying at 80°C for 48 h.
Expt 20.1.86.

Changes in leaf dry weight in whole plants at sub-optimal
temperatures:

Ten-day-old plants were grown in the 30/25°C
glasshouse. An initial-sample was harvested and the

remaining plants were transferred to artificially lit
humidity-controlled cabinets running either at 20 or
30°C. A PFD of 800 μmol m^{-2} s^{-1} at leaf level was
provided for 12 h per day by 28 Phillips TLMF 140 W/33 RS
cool white fluorescent tubes and four 150 W incandescent
lamps (see Appendix 5 for spectral distribution). After
7 d the plants were harvested, dried for 48 h at 80°C,
and weighed (Expt 6.10.85). In a second experiment (Expt
6.11.85), 25d old plants were transferred to a 20°C
artificially-lit cabinet for five days then returned to a
30°C cabinet. At noon on each day CO_2 assimilation rate
was measured on a tagged, mature leaflet, and on one of
the adjacent three leaflets a 10mm diameter leaf disc was
punched out, oven dried at 80°C for 24h and weighed.
Assimilation rate was measured at a leaf temperature of
20°C with an ADC portable LCA-2 infra-red gas analyzer
(see section on "Response of A to sub-optimal temperature
over several days" for a description of the gas analysis
system.)

The immediate response of CO_2 assimilation rate to
changing temperatures:

Five-week-old plants were transferred from the
glasshouse to an artificially lit cabinet. Relative
humidity was maintained between 60 and 80%. A fully
expanded fifth leaf was enclosed in a water-cooled
plastic (Perspex) chamber of dimensions 2 x 5 x 25 cm.
Leaf temperature was monitored with specially constructed

0.28-mm copper-constantan thermocouples in contact with the underside of leaves. Leaf temperature inside the chamber was lowered from 30 to 15°C over about 90 min while the rest of the plant was held at 30°C. Illumination was from 28 Phillips (Netherlands) TL 140 W/33RS fluorescent tubes supplemented by a Phillips 1000 W HPLR mercury-vapour lamp (see Appendix 5 for a description of the spectral quality for this lighting system). The photon flux density (PFD) in the leaf chamber was 1000 μmol $m^{-2}s^{-1}$ (400-700 nm) which was close to saturation for photosynthesis of peanuts (Pallas and Samish, 1974). The supplementary illumination of the mercury vapour lamp was provided to maintain the plant in a light environment similar to the growth conditions. The bulky equipment associated with the cooling of the assimilation chamber precluded measuring A in the growth cabinets and it was essential that the rest of the plant was not in relative shade (acting as a sink for assimilates). The rest of the plant was under PFDs varying from 900 to 1200 μmol $m^{-2}s^{-1}$ depending on leaf position. CO_2 exchange was measured with an ADC model 225 Mark III Infra-red Gas Analyzer (Analytical Development Co., Hoddesdon, Herts, UK). Air-flow rates through the chamber were in excess of three $l.min^{-1}$ and the differential in the concentration of CO_2 did not exceed 30 $μl.l^{-1}$. Air entering the leaf chamber was humidified by bubbling it through water, but before passing through the infra-red gas analyzer it was redried

using a column of fused granular calcium chloride. Expt
3.11.85.

Response of CO_2 assimilation rate to sub-optimal
temperature over several days:

Plants were grown in the 30/25°C glasshouse for
five weeks and then transferred to a cabinet lit by eight
Wotan Powerstar HQI-T 400-W/DH metalarc lamps (Siemens,
Germany), supplemented by two 500 W quartz iodide lamps,
which together provided 1000 µmol $m^{-2}s^{-1}$ PAR at the leaf
surface. The photoperiod was 12 h. Cabinet air
temperature was continuously adjusted to maintain leaf
temperature at 30°C or 19°C, day and night. Relative
humidity was maintained at over 80%. On successive days
A was measured at midday at 20°C leaf temperature. This
measurement took 2-5 min per plant and was the only time
plants were not at their specified leaf temperatures of
19 or 30°C. The ADC Parkinson leaf cuvette includes a
precision thermistor which monitors the air temperature
(±0.2°C) and the leaf temperature is calculated from the
energy balance of the leaf within the cuvette. The small
volume of the cuvette (12 cm^3) and the vigorous mixing of
air in the cuvette result in a rate of air circulation
many times the rate of air flow through the cuvette. Leaf
temperature equilibrated within 10 seconds of the leaf
being clamped in the chamber. Assimilation rates usually
stabilize within 45 seconds (Anon, 1985), although in
these experiments, the stomates sometimes responded to

the transfer to 19°C with fluctuations in stomatal opening for two to three minutes. For each of the 5 replicate plants CO_2 exchange was measured on two tagged mature leaves of each plant using an ADC Parkinson leaf cuvette connected to an ADC portable LCA-2 infra-red CO_2 analyzer (Analytical Development Co.). During the measurement, the CO_2 concentration of the air entering the leaf cuvette was 333 $\mu l.l^{-1}$ and the PFD was 800 μmol $m^{-2}s^{-1}$. Assimilation rate (A), leaf conductance (g) and intercellular CO_2 concentration (c_i) were calculated using the equations of von Caemmerer and Farquhar (1981). After the initial day's measurements, one third of the plants were returned to the 30°C leaf condition while the rest were kept at 19°C. Expt 6.3.87.

This experiment was repeated (Expt 17.3.87) and A measured at both ambient (199 $ml.l^{-1}$) and low (20 $ml.l^{-1}$) partial pressures of oxygen, utilizing the portable ADC system. The CO_2 concentration of the air entering the leaf cuvette was 298 $\mu l.l^{-1}$. Experimental plants were held at 20°C and control plants at 30°C leaf temperature.

Source and sink cooling:

Five- to six-week-old plants were transferred to an artificially lit humidity-controlled phytotron cabinet and temperatures of a recently fully expanded leaf and of the rest of the plant were independently controlled at 30 or 19°C. The manipulation of the source leaf temperature was by enclosing the leaf in the temperature controlled

photosynthetic chamber (see earlier section "Immediate response of assimilation to temperature"). Leaf temperature was monitored with calibrated copper-constantan thermocouples in contact with the underside of leaves either in the assimilation chamber or in the growth chamber. The rate of CO_2 assimilation and the temperature of the chosen leaf were continuously monitored for 3-4 d. The PFD in the measurement chamber was 1000 μmol m^{-2} s^{-1}; other conditions were as for measuring the immediate response of assimilation to temperature. These experiments were conducted from 3.11.86 to 1.3.87. The data presented are for Expts 1.2.87, 5.2.87 and 11.2.87.

The effect of low CO_2 concentration on low-temperature depression of photosynthesis:

Six-week-old plants were grown in the 30/25°C glasshouse. Then, after measurement of A, one third were transferred to two air-tight glass boxes measuring 41 x 21 x 25cm, which had three plants per box. Calibrated copper-constantan thermocouples were in contact with the underside of leaves and the boxes were placed in an artificially lit phytotron cabinet. The temperature of the cabinet was adjusted to maintain leaf temperature at 19°C for the experimental plants. The CO_2 concentration was allowed to equilibrate at the CO_2 compensation point in the enclosed boxes. These conditions limit A whilst the plants were maintained under a high irradiance. The

remaining plants were divided among two phytotron artificially-lit cabinets (fluorescent and incandescent lamps as described earlier) where leaf temperatures were 19 or 30°C and ambient CO_2 concentration was in the range 310-360 $\mu l.l^{-1}$. For all treatments there was a 16 hour light period with a PFD of 800 $\mu mol\ m^{-2}s^{-1}$. Assimilation rates of individual, tagged leaves were measured prior to treatment and after 3 d. Conditions for measurement of A were as for the measurement of the immediate temperature response (above) except that leaf temperature was held at 25°C. Expt 18.12.86.

Results:

Growth of whole plants:

Over 12 d temperature had a strong influence on both the number of leaves ($r^2 = 0.98$) and the plant dry weight (DW) ($r^2 = 0.92$; Figure 4.1). Predictably, the DW change was also highly correlated with leaf number ($r^2 = 0.94$). At the lowest temperature regime (15/10°C, corresponding to a 11.7°C average temperature), no leaf tissue was formed and there was a loss of dry matter. Dry matter was not gained by the plants growing at 18/13°C (14.7°C average) although at this temperature new leaf tissue was formed. New leaf tissue failed to form chlorophyll at 18/13°C (data not shown).
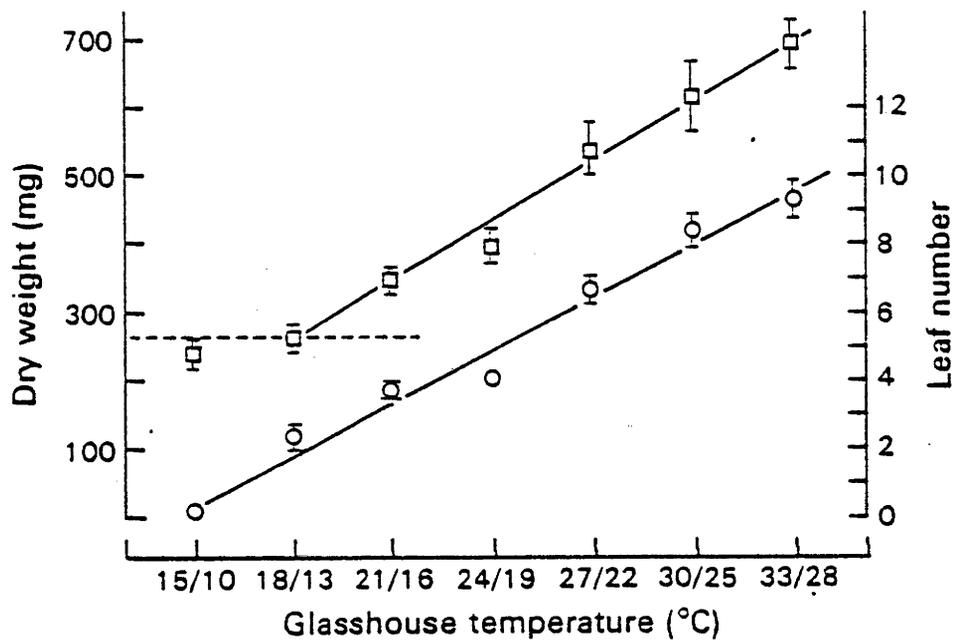
Figure 4.1: Plant dry weight including roots (□) and leaf number (O) of *Arachis hypogaea* L. cv. Chico plants grown at a range of glasshouse temperatures from 15/10 to 33/28°C for 12 d. The dotted line is the initial harvest DW. Error bars = 2 x SE. Each point represents 10 replicate plants. Expt 20.1.86. Experimental dates in this chapter and Chapters 5 and 6 refer to the dates of commencement of experimental treatments.

Changes in accumulation of dry weight in leaves of whole plants transferred to sub-optimal temperatures:

The effect of sub-optimal temperature on specific leaf weight (SLW) is given in Table 4.1. Plants held at 20°C for 7 d accumulated more DW to their leaves than plants held at 30°C. The weight of leaf per unit area (specific leaf weight) was nearly 50% higher at the sub-optimal temperature. Thus, accumulation of carbohydrate, judged by the increase in SLW of the leaves, occurred despite a restriction of A for plants held at 20°C. In a repeat experiment the increase in SLW was matched by a decline in A on each day of cooling (Figure 4.2). SLW increased by 65% over the 5d at 20°C while A was reduced by 50%. Returning these plants to 30°C reduced SLW and increased A. SLW of tagged leaves increased from 46 to 76 g m^{-2} at 20°C, and then decreased to 62 g m^{-2} after 2d at 30°C.

Table 4.1. Changes in whole plant growth and assimilation rates in ten day old Chico plants growth at 30/25°C and transferred to 20 or 30°C for 7 days. Total DW refers to oven-dried plant weight including roots. Photon flux density was 450 μmol m^{-2} s^{-1} for 12 h daily. Ten replicate plants were harvested initially and after each treatment. Expt 6.10.85.

	Initially	After 7 days	
		at 20°C	at 30°C
Total DW (g)	0.20±0.02	0.38±0.02	0.67±0.08
SLW (g m^{-2})	29.1±2.3	44.1±3.4	29.6±1.9
RGR (day^{-1})	–	0.095	0.176
NAR (μmol m^{-2} s^{-1})	–	7.7	11.1
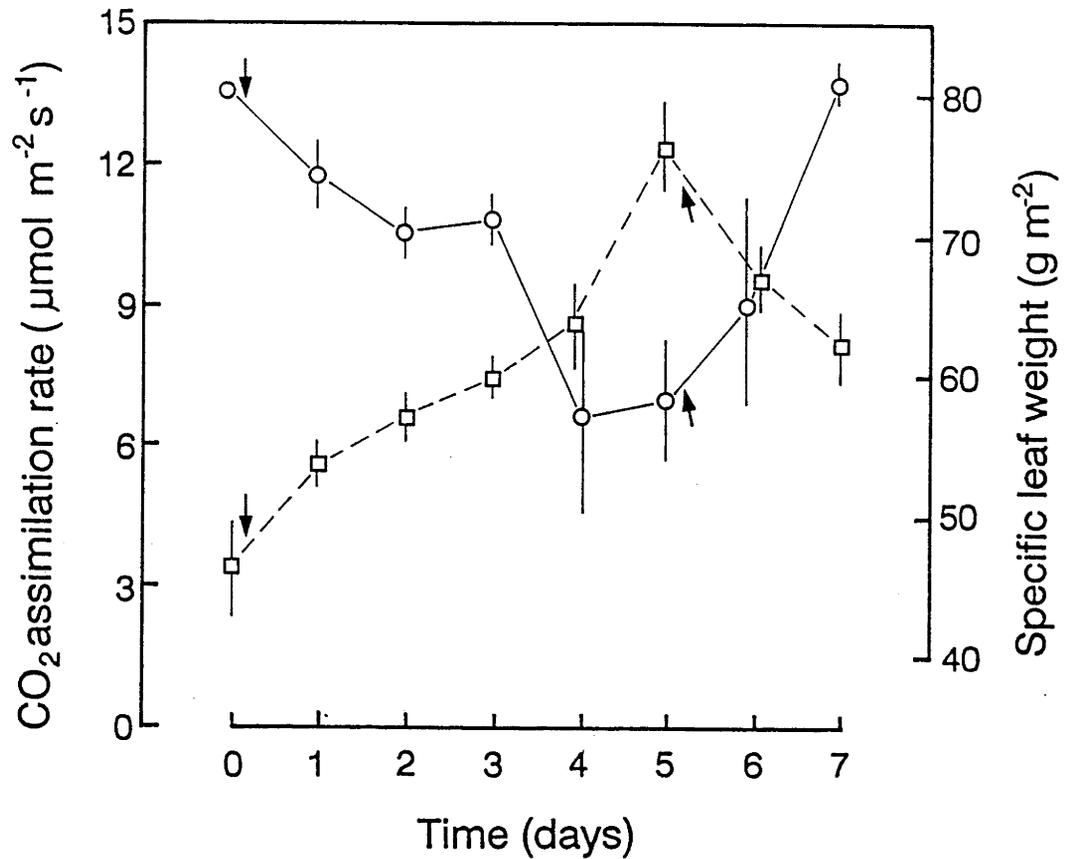
Figure 4.2: Changes in CO_2 assimilation rate (O) and specific leaf weight (□) of 25d old peanut plants (cv. Chico) during five days of cooling to 20°C and two days of rewarming. Arrows indicate beginning and end of cool treatment. N=8. Expt 6.11.85.

The immediate response of CO_2 assimilation rate to changing temperatures:

There was little immediate response of A to temperature over the range 15 to 30°C (Figure 4.3). The 17% decline in A below 22°C was reversible, complete recovery occurring after 20 min at 30°C. There did not appear to be a direct link between the short-term

temperature response of A (Figure 4.3) and the longer-term response of growth (Figure 4.1).
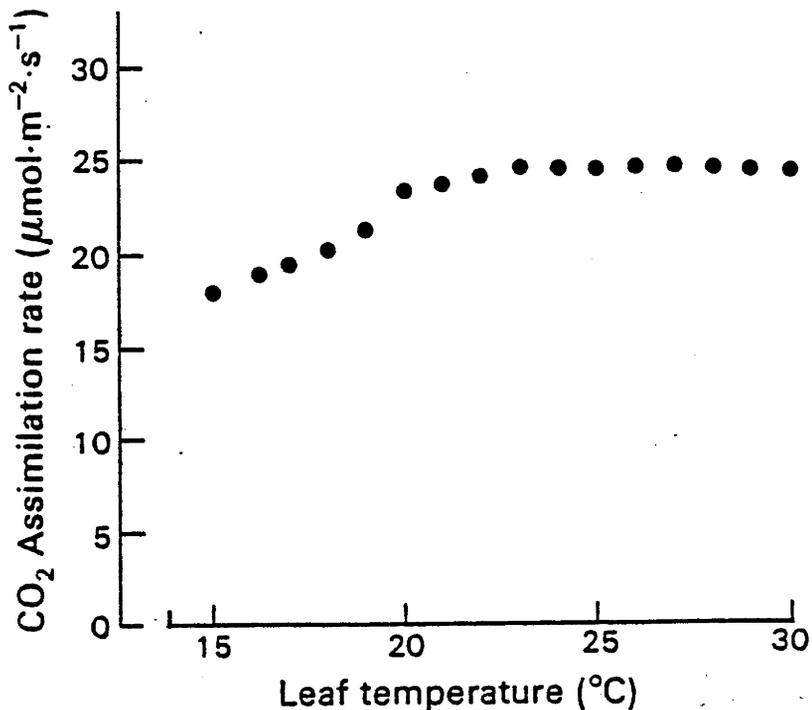
Figure 4.3: Effect of temperature on the immediate, "unadapted" rate of CO_2 assimilation of the fifth leaf of six-week-old Chico peanut plants that had been grown in the phytotron glasshouse at 30/25°C. Leaf temperature was varied in the photosynthetic chamber while the rest of the plant was held at 30°C. Each point is the mean of four separate experimental runs. Average standard errors smaller than symbols. Expt 3.11.85.

Leaf photosynthetic response of whole plants subjected to 3 d of sub-optimal temperature:

The changes in A, c_i and g during 3 d at 19°C are shown in Figure 4.4. All of the photosynthetic measurements were made at 20°C on the same recently fully expanded leaves.
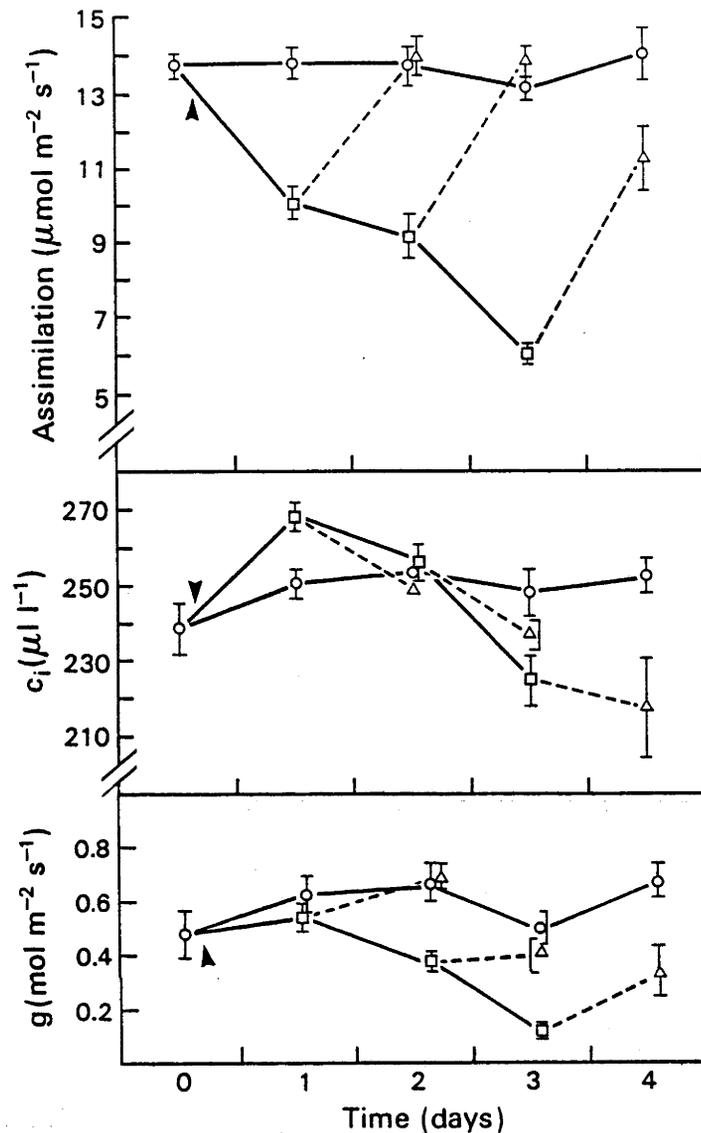
Figure 4.4: Effect of 3 d at 19°C on CO_2 assimilation
rate (A), intercellular CO_2 concentration (c_i) and
stomatal conductance (g). Low-temperature treated plants
(□) were transferred from 30°C to 19°C at 12:00 h on day
0 (indicated by arrow). All measurement were made at
12:00 h in the 19°C cabinet. Those plants that were
returned to 30°C (△) are shown by dotted lines and they
were moved after the mid-day measurement. Control plants
(o) were continuously at 30°C, except during
photosynthesis measurement. There were 5 replicate plants
for each measurement. Error bars - 2 x SE. Expt 6.3.87.

On day 0 all plants were at 30°C, then at midday the

experimental plants were cooled. One day later the

assimilation rate (at 20°C) of the experimental plants

had dropped to about two thirds of the initial value.

There were further decreases on the second and third days of cool temperature treatment. There is a small (15%) change in A associated with an immediate response to suboptimal temperature (Figure 4.3) but this is miniscule compared with changes occurring over 3 d.

Despite the drop in assimilation rate of the 19°C treatment there was initially no associated stomatal closure; g of cooled and control plants was not significantly different on days 0 and 1. The c_i of cooled plants increased significantly on the first day, and was similar to controls on the second day. The elevation in c_i was consistently observed in similar experiments (e.g. Figure 4.4) and two others. The concurrent increase in c_i but depression of A indicates that because of reduced photosynthetic capacity the intercellular CO_2 was not being utilised. Subsequently the conductance (g) of 19°C plants declined on days 2 and 3.

Plants maintained at 19°C for 2 d or less and then transferred back to 30°C for 24 h had A, g and c_i values similar to those of plants held continuously at 30°C. On the third day of cooling c_i dropped significantly below that of control plants and subsequent rewarming did not lead to complete recovery (Figure 4.4).
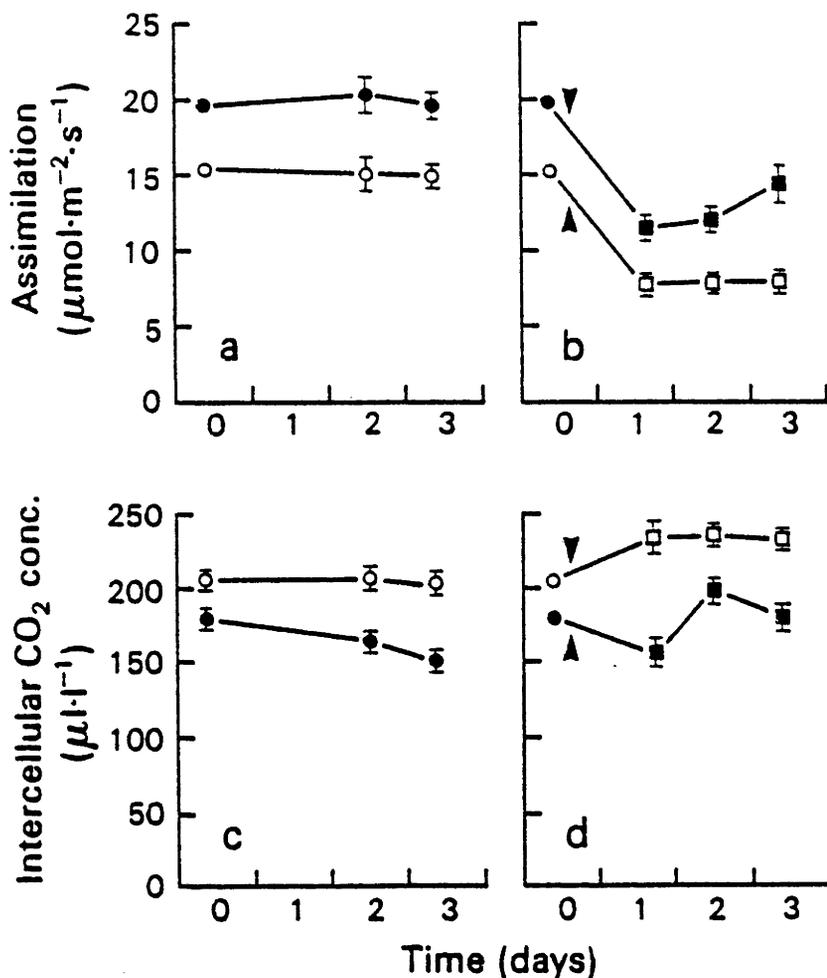
Figure 4.5 a-d: Effect of differing oxygen partial pressures on CO_2 assimilation rate and intercellular CO_2 concentration of peanut plants subjected to 30°C (Fig. a,c) or 19°C treatments (Fig. b,d). All plants were transferred from the glasshouse to the 30°C artificial light cabinet at 10:00 h on day 0. Cool-treated plants (□,■) were transferred from 30°C to 19°C at 16:00 h on day 0. A and c_i were measured at 15:00-16:00 h on days 0 and 1, at 12:00 on day 2, and at 10:00 on day 3. Closed symbols (■,●) represent low-oxygen treatments (20 ml. 1^{-1}), open symbols (□,○) represented ambient-oxygen treatment (199 ml.1^{-1}). Each point represents 6 measurements, error bars = 2 x s.e. Expt 17.3.87.

In a further experiment changes in O_2 sensitivity of A were followed with time after transfer of the plant to low temperature. The absence of O_2 sensitivity of photosynthesis under some environmental conditions has been explained by suggesting that A was limited by the capacity to regenerate inorganic phosphate for photophosphorylation (Sharkey 1985a,b). In this study

assimilation was promoted by about 4-5 $\mu mol.m^{-2}s^{-1}$ (30%) when the O_2 content of the air passing over the leaf was reduced from 199 $ml.1^{-1}$ to 20 $ml.1^{-1}$ (Figure 4.5). An equivalent promotion of A was obtained in leaves from plants held at either 19° or 30°C (Figure 4.5). This promotion was evident for both treatments over 3 d although total A was declining in the colder treatment. Assimilation remained stimulated for 30 min at least after transfer to low O_2 and there was no evidence of this being a transient phenomenon (data not shown). In all treatments c_i was lower than that in Figure 4.4 because the ambient CO_2 concentration was lower (298 $\mu l.1^{-1}$ vs 334 $\mu l.1^{-1}$). When expressed as a fraction of ambient CO_2 concentration, c_i was comparable to that in the earlier data (Figure 4.4).

Source- and sink-cooling:

To help establish if the effect of low temperature was directly on photosynthesis, the temperature of a leaf in a photosynthetic chamber (the source) was controlled independently of the temperature of the rest of the plant (the sink). When both the source and the sink were held at 30°C (day and night), the rate of photosynthesis was high and constant over several days (Figure 4.6a). After cooling the sink zone of the plant, there was no change in source-leaf photosynthesis rate for about 5 h, although subsequently there was a slight decline over the rest of the light period (Figure 4.6b). The following

morning A recovered nearly to the initial rate but

subsequently decreased throughout the day. (Similar

experiments in which the light period was extended from
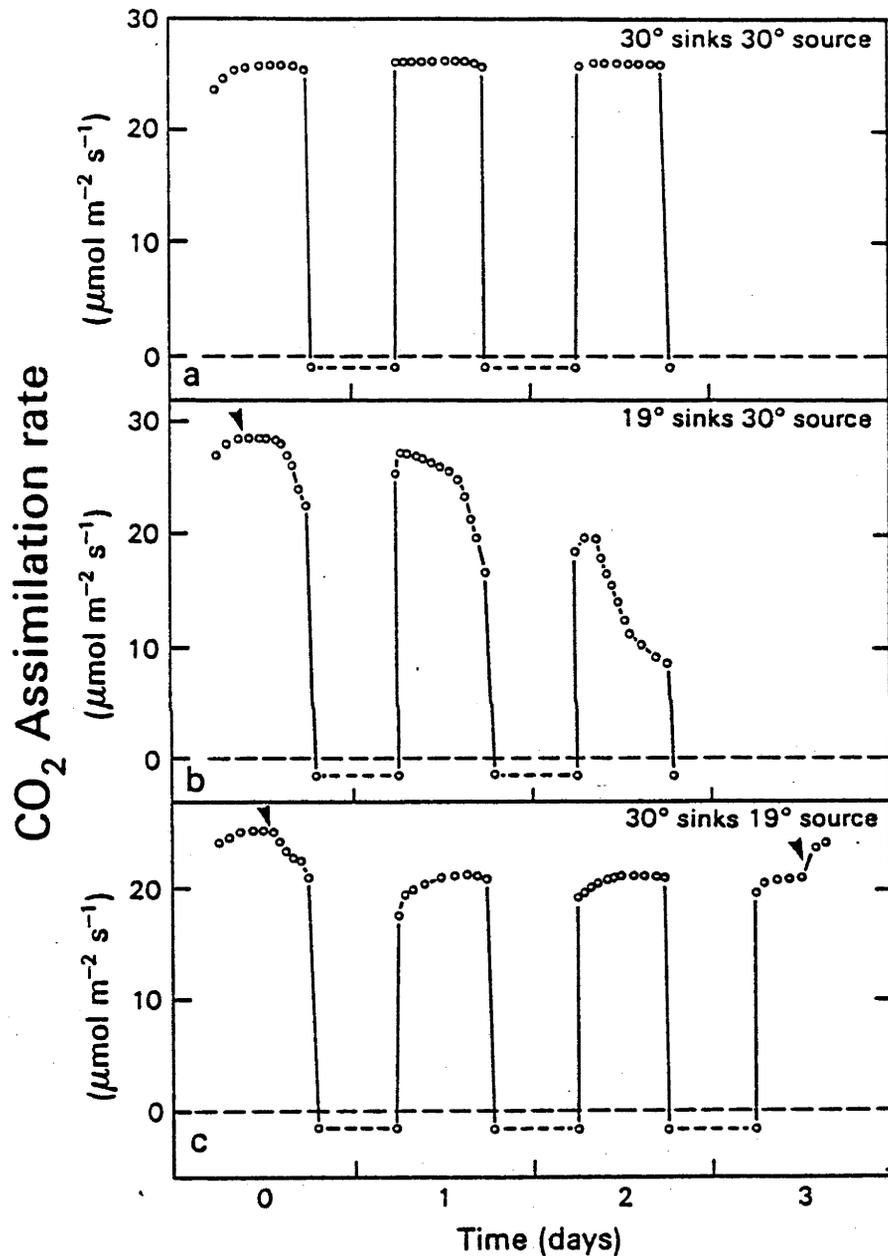
Figure 4.6 a-c: Effect of varying source and sink zone temperature on source CO_2 assimilation rate of peanut over 3 or 4 d. The source zone, the fifth mature leaf, was in the assimilation chamber and the sink zone was the rest of the plant. (a) source and sink both at 30°C, (b) source at 30°C, sink at 19°C, (c) sink at 30°C, source at 19°C. The arrows indicate when the cooling treatment was commenced or terminated. Comparable changes were observed for all 3 replications of each treatment. Expts 1.2.87, 5.2.87 and 11.2.87.

12 to 16 h resulted in further declines in rate of photosynthesis in the additional 4 h of light). On the morning of the third day A initially rose to a higher level than that at the end of the previous day, but after 2 h it again started to decline, falling to a much lower value than on the previous day. The pattern of fall in A resembled that which occurred when the whole plant was cooled (Figures 4.2 and 4.4). After 3 cold days, rewarming the sinks did not result in complete recovery. Over two subsequent days there was only a 5-15% recovery (data not shown).

Only small changes in respiration were observed at night when plants were subjected to low temperature treatment (i.e. below the zero Assimilation line in Figure 4.6). Thus the changes in assimilation rate for the 19°C treatment cannot be attributed to increased dark respiration.

The small decline in A when the source was chilled and the sinks were maintained at 30°C (Figure 4.6c), was equivalent to the immediate response shown in Figure 4.3. The rate of photosynthesis did not decline further over three more days when the source was held at 19°C and the sinks were at 30°C. The change in A during the day was markedly different from that of the "cooled-sink" treatment. Under the cooled source treatment, on each day A rose over 3-4 h to a plateau at which it remained for the rest of the day. After three complete days with the source leaf at 19°C, it was rewarmed and the rate of

photosynthesis returned nearly to the initial values within 20 min.

The effect of low CO_2 concentration on low-temperature depression of photosynthesis:

As explained in Materials and Methods, the treated plants in this experiment were placed in a glass box for 3 d and leaf temperature was controlled at 19°C. CO_2 concentration equilibrated to the CO_2 compensation point (about 50 $\mu l.l^{-1}$) in this box. The results are set out in Table 4.2. Under conditions where photosynthesis was restricted by CO_2 supply so that carbohydrate would not accumulate in the leaf, low temperature had no affect on photosynthesis subsequently measured at ambient CO_2 concentrations and a 25°C leaf temperature.

Table 4.2. Effect of differing CO_2 concentrations on photosynthesis in peanut, A was measured before and after 4 d of the various treatments. Expt 18.12.86.

Treatment	CO_2 assimilation rate ($\mu mol \ m^{-2} \ s^{-1}$)
Initially	22.1 ± 1.0
After 4 days of	
Ambient CO_2 and 30°C	21.8 ± 2.0
Ambient CO_2 and 19°C	4.4 ± 1.1
Low CO_2 and 19°C	21.6 ± 2.5

Discussion:

Direct inhibition of photosynthesis:

The results of these experiments have confirmed that growth of peanut, a tropical crop plant, is inhibited at temperatures below 25°C and quite severely at 20°C or lower (Figure 4.1), an observation also noted in earlier studies with peanut by Ono *et al.* (1974). Leaf number and dry-matter increases had parallel responses to temperature over a range of temperatures from 15/10 to 33/28°C (Figure 4.1). However there was no immediate affect of these temperatures on assimilation (Figure 4.3) and studies on other species, such as those by Evans and Bush (1985) on *Echinochloa* sp. and rice, and by Duncan and Hesketh (1968) on 22 maize cultivars showed no correlation between short term assimilation rate and either relative growth rate or relative leaf-growth rate. Similarly in *Atriplex* the A-versus-temperature response curves were flatter and broader (i.e. independent of temperature) than was the relative growth rate from 15-40°C (Björkman *et al.* 1974, 1975). In the current experiment there was also little immediate effect of temperature in the range from 15-25°C on A. However, over longer times (1-2 d), A of peanut was strongly depressed (Figure 4.4) by sub-optimal temperatures, an observation also made by Evans and Bush (1985) for *Echinochloa* and rice. Furthermore, the long term depression of A at low temperature in peanut was related to sink temperature, and not the temperature of the leaf

per se (Figure 4.6).

Sink-controlled photosynthesis:

The most likely explanation of the reduction in A in these experiments is that under low temperature conditions, in association with reduced growth, assimilates accumulate in the leaves, and reduce the rate of leaf photosynthesis. This proposal fits the classical sink regulated photosynthesis discussed by Neales and Incoll (1968) and Wardlaw (1985) and is based on several observations. Firstly, there is a rise in leaf dry weight that parallels the drop in photosynthesis with time at low temperature (Table 4.1). Secondly, cooling the sink tissues independently of the source leaf depressed A, but not the reverse, which shows that it is the sink which regulates assimilation rate. Thirdly, reducing the current rate of photosynthesis by placing the leaf in a CO_2-free atmosphere prevented the drop in photosynthesis due to low temperature (Table 4.2).

Stomatal conductance may provide the basic control of assimilation rate in either a source or sink limited situation and a decrease of stomatal conductance after low temperature treatments has been observed in several studies (e.g. Pasternak and Wilson, 1972; Crookston *et al.*, 1974). Certainly conductance decreased in the 19°C plants in the present experiments, but only after the initial decline in A. Conductance was not significantly different between 19- and 30°C-plants in this study on

days 0 and 1, although A decreased and c_i increased. Over several such experimental runs there was no evidence that stomatal behaviour was responsible for the initial decline in A.

Mechanisms of reduction of photosynthesis:

The reduction in A in these experiments occurred well above the "critical temperature" of 10-13°C at which peanut growth stops - Figure 4.1 (Angus et al., 1981; Leong and Ong, 1983). This critical temperature is usually the temperature at which chilling injury occurs (Lyons, 1973). The decline in A over 3 d did not lead to the typical symptoms of chilling injury that have been observed in a range of plants at 10°C, i.e. wilting or loss of chlorophyll, (Lyons, 1973, Taylor and Rowley, 1971). There is evidence for a light dependence for chilling injury and Taylor and Rowley for example found that damage at 10°C did not occur in the dark. The decline in A of a peanut leaf during days when sinks were at 19°C is similar to a light-dependent injury of the photosynthetic system with recovery overnight to near-initial values (Figure 4.6b). However, the experiment using low CO_2 and cool temperature (Table 4.2) shows that the reduction in A was not a result of photoinhibition. Further, during the first 2 d of sub-optimal temperature and normal CO_2 in the source leaves, subsequent rewarming led to complete recovery within 1 d (Figure 4.4). Assimilation rate also increased to pre-cooling rates

within 1 h of rewarming the source leaf to 30°C (Figure
4.6c). Overnight and fast recovery of the assimilation
rate in the peanut indicates some non-damaging
inhibition, possibly involving deactivation and
reactivation of ribulose-bisphosphate carboxylase-
oxygenase (Rubisco; Mächler and Nösberger, 1980) or
changes in the concentration of endogenous inhibitors of
Rubisco (e.g. 2-carboxyarabinitol 1-phosphate: Berry *et
al.,* 1987).

Respiration at night under the low-temperature sink
treatment increased by no more than 0.5 $\mu mol.m^{-2}.s^{-1}$
compared with 30°C plants (Figure 4.6). This represents
a 2% change compared with a change in A of 50% or more.
The slightly higher rate of respiration at night in the
cooler plants (Figure 4.6) may be associated with
increased dry matter accumulation (Azcón-Bieto and Osmond
1983, and Table 4.1). Clearly, then, changes in dark
respiration cannot account for the change in A.
Similarly, increases in photorespiration are unlikely to
account for the changes in A (Figure 4.5). The oxygen
sensitivity of photosynthesis remained unaltered after
cooling (Figure 4.5b) although the degree of oxygen
sensitivity depends on the species and on the
pretreatment temperatures (Cornic and Louason, 1980;
Sage and Sharkey, 1987). Also, in leaves limited by
inorganic phosphate, Farquhar and von Caemmerer (1982)
postulated that photosynthesis will become independent of
the ratio of oxygenation to carboxylation. Conditions of

feedback-limited photosynthesis may then lead to oxygen insensitivity (Sage and Sharkey, 1987) but this was not the case in the present experiment. Azcón-Bieto (1983) and Wardlaw and Eckhardt (1987) similarly observed under conditions where A was depressed by carbohydrate accumulation, that the greater the depression then the more low oxygen enhanced A.

Alternative explanations of the reduction of assimilation rate after cooling to $19^{\circ}C$ do not accomodate the various experimental results in this chapter as satisfactorily as does the sink-limited hypothesis. Some form of signal (e.g water flow or hormone like cytokinins; Leopold and Kriedemann, 1975) passing between the source and sink zones might explain the results in Figure 4.6, but the buffering effect of low CO_2 concentration during cooling on the subsequent assimilation rate (Table 4.2) is most easily explained by carbohydrate accumulation at ambient CO_2 concentrations limiting A. The specific mechanism proposed by Leopold and Kriedemann involved plant growth regulators controlling A via changes in stomatal aperture, and this was not the cause of the decline in A during the first two days of cooling (Figure 4.4). Similarly, mineral diversion (Sinclair and de Wit, 1976) does not provide a satisfactory explanation of the results reported in this chapter. The buffering effect of low CO_2 during low temperature treatment (Table 4.2) cannot be explained by mineral diversion and the time scale of the cooling

response (within 1d) and its rapid reversibility (Figure 4.4) would similarly exclude this as a satisfactory explanation.

Although these experiments have not provided evidence of the mechanism, it is clear that sink tissue feedback inhibition of source leaf photosynthesis may be the major cause of the reduced growth of peanut at low but not critical temperatures.

Chapter 5

Biochemical aspects of inhibition

of photosynthesis at cool temperatures

Introduction

The reduction of photosynthesis in peanut plants at cool temperatures (ca. $20^{\circ}C$) was shown in the previous chapter to be due principally to sink-tissue feedback inhibition of source leaf photosynthesis rather than to a direct effect of temperature on the leaf *per se*. Despite extensive evidence of feedback inhibition from whole plant experiments when sink demand has been changed (e.g. by shading, defoliation or depodding - see Wardlaw, 1985 for a review) a biochemical basis for end-product inhibition has until recently remained unclear. Sharkey (1985a) commented that only after imposing "drastic" measures and "extreme" treatments were plants susceptible to feedback inhibition and that there was no readily

apparent mechanism for short term feedback inhibition of
photosynthesis. An early difficulty with the biochemical
explanation of feedback inhibition was that the probable
regulatory enzyme for sucrose synthesis, sucrose-
phosphate synthase (SPS EC 2.4.1.14), was not always
inhibited by excess sucrose (Huber, 1981) and there was
no mechanism that had been observed, by which excess
sucrose manufacture could be stopped and photosynthate
diverted to starch production. A potential regulator of
photosynthesis was discovered by Stitt *et al.* (1983) who
found that fructose 2,6-bisphosphate (F2,6BP)
concentrations rose in spinach (*Spinacia oleracea* L.)
leaves after prolonged illumination accompanying the
onset of starch accumulation, and that an inverse
relationship existed between photosynthetic rate and leaf
F2,6BP concentration. F2,6BP is present in micromolar
concentrations in the cytosol and is a potent inhibitor
of fructose-1,6-bisphosphatase (EC 3.1.3.11) which
catalyzes the first irreversible step to sucrose
synthesis. Stitt (1987) suggests that *in vivo* both
Fructose-1,6-bisphosphatase activity (modulated by
F2,6BP) and SPS activation/inactivation are important
controls of sucrose synthesis and that at cool
temperatures sucrose synthesis is selectively restricted
because Fructose-1,6-phosphosphatase is more sensitive to
F2,6BP and the threshold for activating SPS is raised.
Foyer (1987) has proposed a different system for
biochemical regulation of feedback inhibition. She

observed that SPS activity was not inhibited by sucrose except at very high concentrations (Hawker and Smith, 1984) and that glucose and fructose had similar effects to sucrose on the stimulation of F2,6BP (Stitt *et al.*, 1984) and she proposed that excess sucrose accumulates in the apoplastic space where cell wall invertase breaks the sucrose down to glucose and fructose and these metabolites re-enter the cytosol and stimulate production of F2,6BP. In this chapter the role that F2,6BP may play in regulating assimilation at cool temperatures is investigated and how the starch, sucrose, fructose and glucose concentrations are related to F2,6BP levels in three varieties of peanut with reported differences in tolerance to cool temperatures.

Materials and methods:

Seed of varieties Tifton-8, Chico and Makulu Red (kindly supplied by Mike Bell of Kingaroy, Queensland) were germinated and grown for 25d under standard conditions (see Chapter 2: Germination and growth conditions) in a glasshouse of the Canberra phytotron (Morse and Evans, 1962) running at 30/25°C (8h/16h-day/night temperatures). The cultivars Tifton-8 and Chico were chosen for this study because of the contrast in their growth in cool conditions in the field; Tifton-8 was observed to be cool-tolerant and Chico cool-sensitive (R. Shorter, pers. comm.). Makulu Red is reputedly cool-tolerant (Williams *et al.*, 1975). Mean radiant energy

input above the plant canopy was 17.8 MJ day^{-1}. The plants were transferred to an artificially lit LBH cabinet running at 30/25°C for 2d (Days 0 and 1). A photon flux density (PFD) of 900 μmol m^{-2}s^{-1} (PAR 400-700nm) at leaf height was provided by 28 Phillips TMLF 140/33 RS fluorescent tubes with four 150 W incandescent lamps for 16h per day (0700 to 2300h). Temperature was lowered to 20°C at 0700 on Day 2 in the cabinet and maintained at 20°C for 4d then returned to 30/25° at 0100 on Day 6. At 1100h on Days 1-7 CO_2 assimilation rate was measured on fully expanded, tagged leaflets of the last matured leaf. At the same times leaflets adjacent or opposite to the photosynthetic leaflet were immediately plunged into liquid N_2 and subsequently stored at -80°C for later determination of metabolite concentrations.

Gas Exchange:

Gas exchange was measured in an ADC Parkinson leaf cuvette connected to an ADC portable LCA-2 absolute infra-red gas analyzer (Analytical Development Co.). Measurements were made *in situ* in the artificially lit cabinet set at 20°C and leaf temperature was in the range 20±1°C and PFD was 900 μmol m^{-2}s^{-1}. This system was portable and rugged, and had a quick response time for individual measurements of CO_2 assimilation. Changes in the mesophyll conductance (response of assimilation to intercellular CO_2) were measured with a Li-Cor LI 6200

portable photosynthesis system with a 1 litre chamber (Lambda Instruments, Lincoln, Nebraska). The LI-6200 was operated in the closed mode and the leaf reduced the CO_2 in the chamber down to the CO_2 compensation point. The leaf photosynthetic rate was computed every 10ppm CO_2. The bulkier Li-Cor chamber required a greater working space for its operation than the ADC portable system, and therefore a larger separation between the lights and the plants. PFD was 780 μmol $m^{-2}s^{-1}$ and leaf temperature was maintained at 20±0.5°C. Published studies comparing $A:C_i$ curves measured with this system and with a steady state system gave virtually identical results (Davis *et al.*, 1987). The particular advantages of this system compared to the steady state system was that it was portable and had a relatively quick response time. Longer term changes were measured on a steady state system described in Kirschbaum and Farquhar (1984) in which air flow rates were measured with a mass flow meter (Brooks model 5810) and changes in CO_2 concentration of air passing through the leaf chamber were measured with an absolute infra-red gas analyzer (Beckman model 315B). The difference in CO_2 and H_2O partial pressures entering and leaving the chambers were measured with a differential infra-red gas analyzer (Beckman model 865) and a Vaisala Humicap humidity sensor. Gas exchange parameters were calculated according to the equations of von Caemmerer and Farquhar (1981). PFD in the leaf chamber was 900 μmol $m^{-2}s^{-1}$, and leaf temperature was maintained at 20±1°C. The advantage

of this system, compared to the LI 6200, was that CO_2 content in the chamber could be increased to above ambient concentration, although the comparative disadvantage was that the system required more time for equilibration.

Measurement of metabolites:

The method of extraction of metabolites revolved around the requirement to measure fructose 2,6-bisphosphate, which is soluble in slightly alkaline methanol, with chloroform to precipitate the protein (Stitt *et al.*, 1982). After grinding in a liquid N_2-cooled mortar and pestle, subsamples of the leaf material from each harvest were added to microfuge tubes containing 0.3 ml of 50 mM Bicine KOH (pH-8.2), 0.3 ml of 5mM EGTA, 30 µl of 50 mM NaF, 1.5 ml of $CHCl_3$ and 3.5 ml of CH_3OH, and kept at 0^oC for 20 minutes. The samples were resuspended in 3 ml H_2O and centrifuged at 4^oC for 10 minutes at 3000rpm.

The aqueous upper phase (H_2O-CH_3OH) was freeze dried for metabolite determination, while the chloroform phase was diluted with 10ml ethanol and centrifuged for 10 minutes at 3,000 rpm and the absorbance of this supernatant measured at 652 nm for chlorophyll determination. Separate subsamples of the methanol-water (metabolite) supernatant were analyzed for soluble sugars, starch and fructose 2,6-bisphosphate.

Soluble Sugars:

Glucose, fructose and sucrose were determined enzymatically from NADP reduction rates monitored at 340 nm using a Shimadzu UV-240 Graphicord Spectrophotometer with a Shimadzu CPS-240 cell positioner linked to a Shimadzu PR1 graphic printer. To a cuvette of 800 µl of buffer solution (120 mM Imidazol-HCl adjusted to pH 6.9 by addition of 2mM MgCl) with 10 µl of 50mM NADP and 50 µl H_2O was added 10 µl of extract and 1.3 EU of glucose-6-phosphate dehydrogenase (Sigma - EC 1.1.1.49) in 5 µl buffer. This mixture was stirred and when the optical density (OD) at 340 nm was steady the value was recorded (OD_1). Subsequent steps involved addition of 5µl of 110mM ATP and 6 EU Hexokinase (Sigma - EC 2.7.1.1) in 5 µl buffer to give a stable OD reading (OD_2). Addition of 7 EU Phosphoglucose Isomerase (Boehringer - EC 5.3.1.9) in 5 µl buffer resulted in OD_3 and 10 EU of Invertase (Sigma - EC3.2.1.26) in 5µl buffer gave a reading of OD_4. From these readings glucose ($OD_2 - OD_1$), fructose and fructose 6-phosphate ($OD_3 - OD_2$) and sucrose ($OD_4 - OD_3$) concentrations were calculated.

Starch:

Sub-samples of supernatant were suspended in 400 µl dialysed Clarase 900 (Miles Laboratories, Springvale, Australia) in acetate buffer at pH 4.8 which was then shaken and incubated for 24 h at 37°C. After

centrifugation, 10 µl aliquots of supernatant were analyzed as above for glucose.

Fructose 2,6-bisphosphate:

Fructose 2,6-bisphosphate was assayed by monitoring its activation of pyrophosphate:fructose 6 phosphate phosphotransferase (Sigma EC 2.7.1.90). Fructose 2,6-bisphosphate activates this enzyme which then converts fructose 6-phosphate to fructose 1,6-bisphosphate, which via two further conversion steps is subsequently converted to dihydroxyacetone phosphate and to glycerol-3-phosphate. This conversion was monitored by the coupled conversion of NADH to NAD^+ in the spectrophotometer.

To each of 6 cuvettes was added 20 µl 0.25M HCl and to 4 of these cuvettes (a to d) was added 20 µl of extract. These were shaken together and allowed to stand for 10 minutes. A mixture of 6 ml of buffer solution (100mM Tris-HCl buffer adjusted to pH 8.1 by addition of 5 mM $MgCl_2$) with 80 µl 100mM fructose 6 phosphate, 150 µl 15mM sodium pyrophosphate, 70 µl 15mM NADH, 10EU of Coupling Enzyme in 70 µl Buffer (glycerol 3 phosphate dehydrogenase: Sigma EC 1.1.1.8/ triosephosphate isomerase Sigma EC 5.3.1.1) and 2 EU of Aldolase (Boehringer- EC 4.1.2.13) in 360 µl of Buffer and 5 EU fructose 6 phosphate phosphotransferase in 150 µl Buffer was prepared and 1 ml of the mixture added to all cuvettes. Cuvette (a) remained as a background and cuvettes (b), (c) and (d) were standards to which were

added, respectively 20 µl of 0.05 µM, 0.1 µM or 0.2 µM
fructose 2,6-bisphosphate. Twenty µl of extract was added
to cuvettes (e) and (f). Concentrations of Fructose 2,6-
bisphosphate were determined spectophotometrically at a
wavelength of 340 nm.

Results:

Changes in A in response to cooling:

Carbon dioxide assimilation rate (A) decreased in
all 3 varieties of peanut in response to transfer from
30°C to 20°C, but the cultivar Tifton was less affected
and recovered more quickly than either of the cultivars
Chico or Makulu Red (Figure 5.1). The photosynthetic
rates of all three varieties were similar on Days 1 and
2, although at the time of measurement on Day 2, the
plants had only been at 20°C for four hours. By Day 3, A
of both Chico and Makulu Red had dropped to between half
and one third of initial values, while that of Tifton was
similar to that before cooling. On the subsequent 2d at
20°C, and on the first day of rewarming, the temperature
sensitive varieties continued to photosynthesise at about
one third of the initial rate. The cultivar Tifton,
however, was significantly less affected by low
temperature and recovered to its initial A while still at
20°C on Day 5. The assimilation rate of Chico and Makulu
Red plants had not fully recovered 36 h after being
rewarmed.
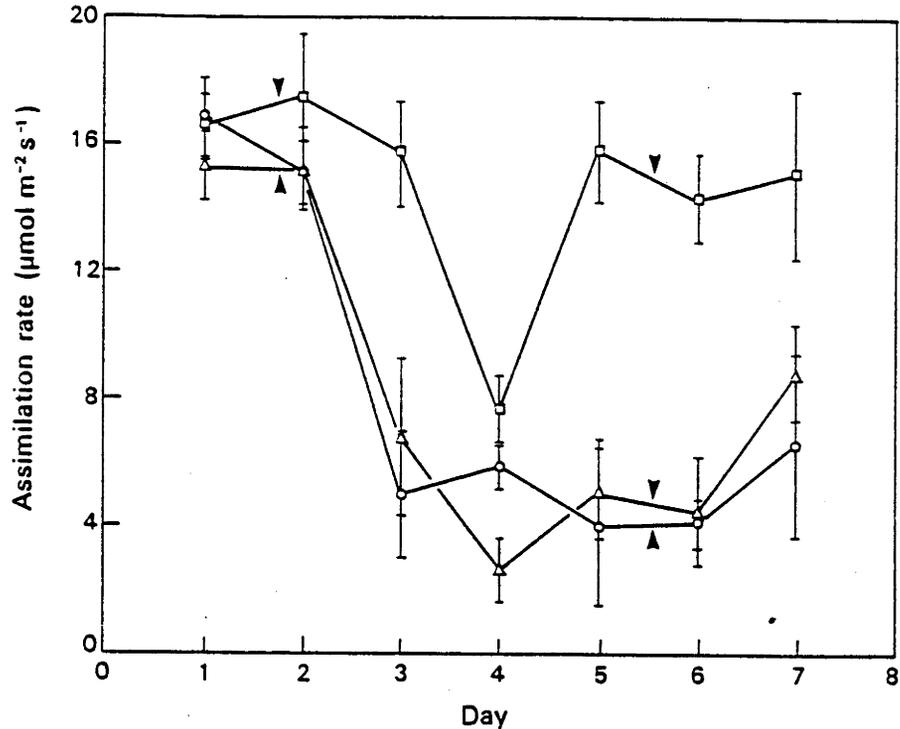
Figure 5.1: Changes in CO_2 Assimilation rate for 3 peanut cvs. during cooling to 20°C: Tifton (□), Makulu Red (△) and Chico (o). All plants were cooled at 0700 on Day 2 and rewarmed to 30/25°C at 0100 on Day 6 as indicated by the arrows. Assimilation rate measured on the ADC Parkinson leaf cuvette connected to the ADC LCA-2 portable gas analyzer. Expt 1.11.86.

The conditions imposed during cooling in this experiment are notably more severe than those imposed in Chapter 4: photoperiod has been extended from 12 to 16 hours and photosynthetic photon flux density (PFD) increased from 800 to 900 μmol $m^{-2}s^{-1}$. Overall the response to cooling was qualitatively the same. However, assimilation rate declined at a much faster rate than in the previous experiment for the variety Chico which is common to both experiments. For plants maintained under a 12h photoperiod, A declined approximately 43% after 3d at 20°C and recovered to 81% of initial A 24h after rewarming (Figure 4.4), whereas after 3d of 16h

photoperiods A declined to 34% and recovered to only 58% after 36h (Figure 5.1).

The response of assimilation rate to intercellular CO_2 concentration (c_i) has been modelled by von Caemmerer and Farquhar (1981) as two different processes limiting A at high and low CO_2 concentration. At low c_i, A is limited by the ribulose bisphosphate (RuBP) saturated rate of catalysis by ribulose 1,5-bisphosphate carboxylase (RuBP carboxylase: *syn*. Rubisco: EC 4.1.1.39), and at high c_i by the rate of RuBP regeneration. This model integrates the kinetic properties of Rubisco with rates of photosynthetic electron transport capacity and allows prediction of rates of carboxylation and regeneration of RuBP *in vivo*.

The CO_2 response curve for the sensitive variety Chico held at 21/16°C for seven days (Figure 5.2) showed a marked decline in both the initial Rubisco limited rate, as well as in the RuBP-regeneration limited rate where there was flat response to increasing CO_2 concentrations above 200 $\mu l.l^{-1}$. The reduction in assimilation after maintaining the plants at 21/16°C was approximately 64% of the pre-treatment assimilation rate at a c_a of 330 $\mu l.l^{-1}$, and is similar to that for both sensitive varieties, Chico and Makulu Red after 2-4 days at 20°C (Figure 5.1).
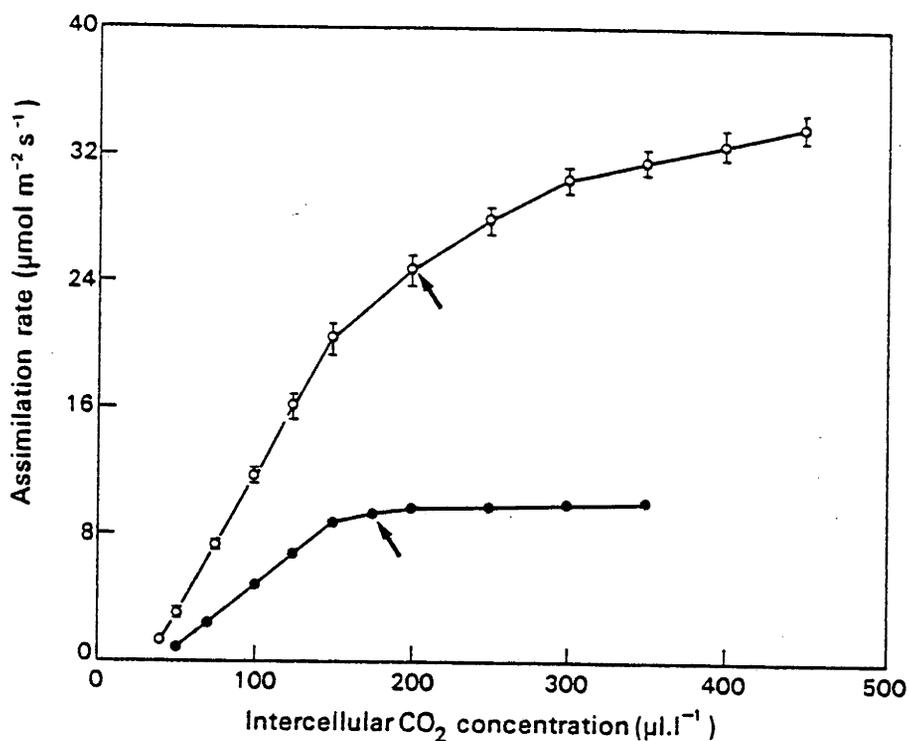
Figure 5.2: CO_2-assimilation rate versus intercellular CO_2 concentration (C_i) of peanut plants of the cv. Chico, before (o) and after (●) 7d at $21/16°C$ measured on the Kirschbaum and Farquhar (1984) system. The arrows indicate the rate at an external CO_2 concentration of 330 $\mu l.l^{-1}$. Expt 4.11.85.

Increasing the CO_2 content above ambient concentrations resulted in no increase in assimilation rate of the cool-treated plants, in contrast to the untreated plants which exhibited marked CO_2 sensitivity. The arrows in Figure 5.2 indicate the "operating" c_i at ambient CO_2 concentration of 330 $\mu l.l^{-1}$. Stomatal limitation of photosynthesis can be calculated according to the formula of Farquhar and Sharkey (1982) as (A_o-A)/A_o where A_o is the assimilation rate at a c_i of 330

$\mu l/l$ and A is the assimilation rate at the operational c_i. On this basis stomatal limitation is reduced from about 25% before cooling to about 8% after cooling at $21/16^{\circ}C$ for seven days. Thus, the major restriction to photosynthesis with cooling appears to be biochemical (mesophyll) rather than stomatal.

The initial slope of A versus c_i is independent of stomatal conductance and the changes in slope (Figures 5.2 and 5.3) are due to the effect of cooling on the mesophyll photosynthetic reactions. The data presented in Figure 5.3 provide confirmation of the result obtained on the steady state gas exchange system (Figure 5.2). The fast-response Li-Cor LI 6200 system has detected changes in mesophyll resistance after only two days of cooling. Both sets of readings were taken at approximately midday but the experiments were conducted at different times of the year and the plant assimilation rates measured on two different gas exchange systems after slightly different combinations of PFD and temperature. The experiments were conducted at different times of the year because of the constraints imposed by the availability of equipment, although there were similar responses notwithstanding the seasonal differences in growth conditions. The reduction in slope after 2d at $20^{\circ}C$ in an artificially lit cabinet was approximately 52 percent while after one week in a $21/16^{\circ}C$ glasshouse it was 55 percent. The numbers of the experiments in this chapter refer to the date of the commencement of the experimental treatment.
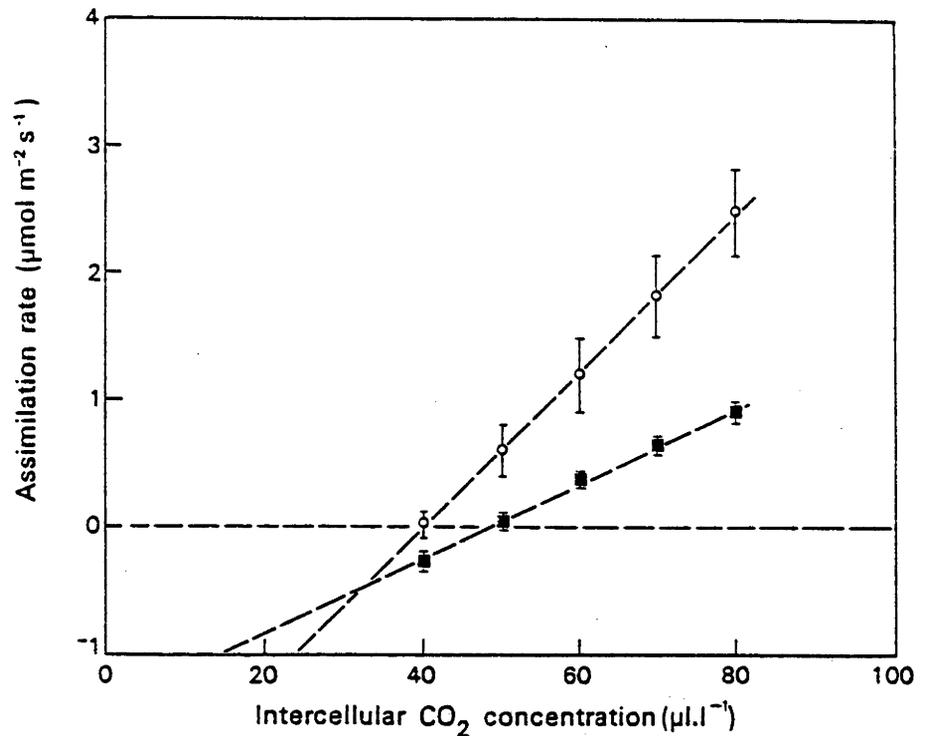
Figure 5.3: CO_2-assimilation rate versus intercellular CO_2 concentration (C_i) of peanut plants of the cv. Chico, before (o) and after (■) 2d at 20°C. Assimilation rate measured on the LI-Cor Li 6200 portable system. Expt 25.5.86.

Changes in metabolites in response to cooling:

The changes in metabolites for the two varieties Chico and Tifton are presented in Figure 5.4. The results for the variety Makulu Red were qualitatively similar to those of Chico and have been omitted to simplify presentation. In Figures 5.4(a) net assimilation rate of the Chico plants is markedly reduced on Day 3, whereas the pattern of F2,6BP concentration during the days of cooling was similar to that of the total soluble carbohydrate concentration, ie. rising on days 2 and 3 to a plateau on day 4 and decreasing on day 5. In the

cultivar Tifton, assimilation rate was reduced on Day 4
only, and only on that day was the F2,6BP concentration
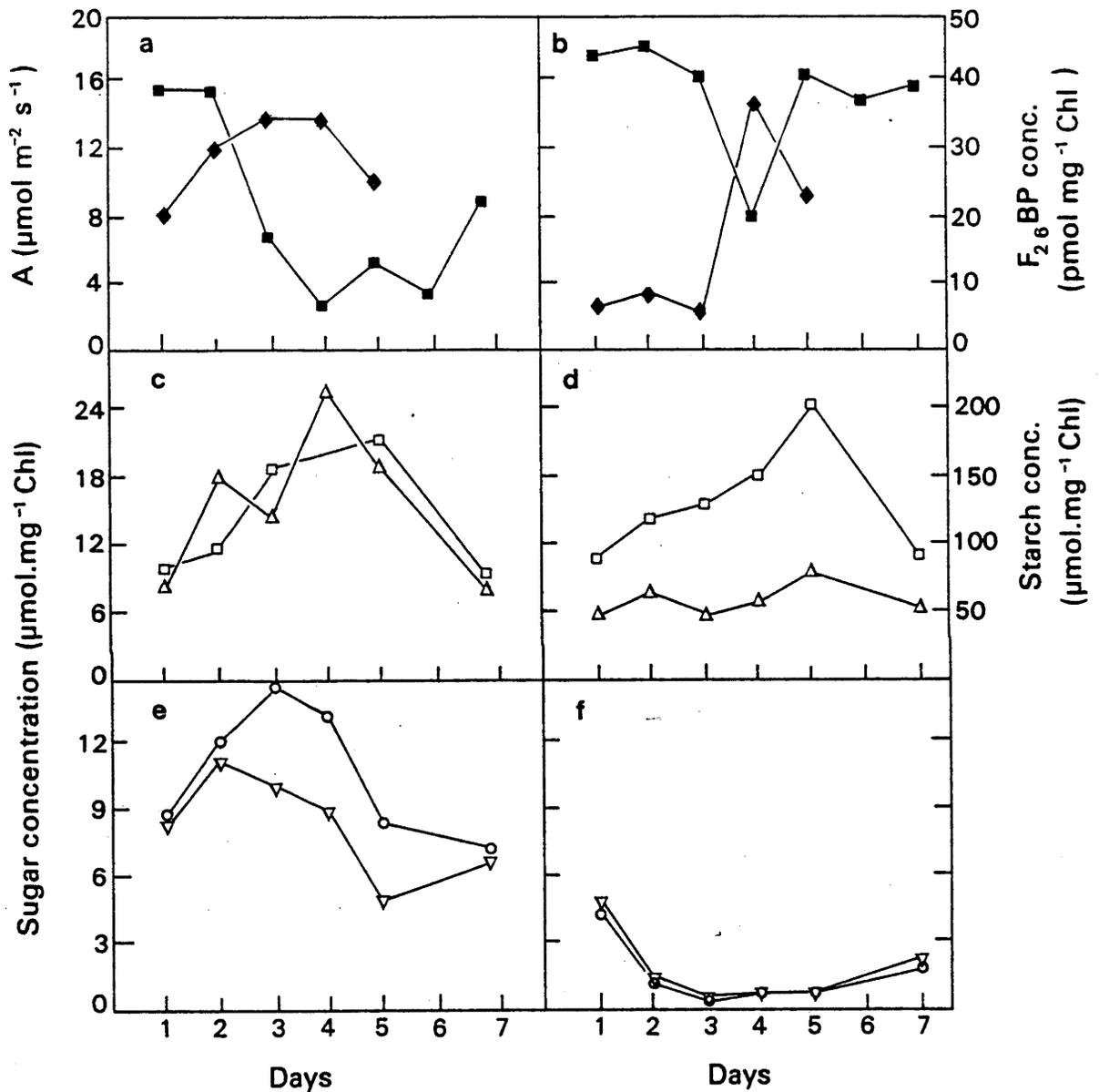
Figure 5.4: Changes in CO_2 assimilation and metabolite
concentrations of two peanut varieties, Chico, on the
left, and Tifton. Transfer to 20°C occurred at 0700 on
Day 2 and continued to 0100 on Day 6. In Fig. 5.1a) and
b) Assimilation (■) and F2,6BP (◆), in Fig. 5.1c) and
d), sucrose (Δ) and starch (□), and in Fig. 5.1 e)
and f) glucose (∇) and fructose (o) concentrations.
Starch concentrations in glucose equivalents. All
measurements made at 1100 h. Assimilation rate measured
on the ADC LCA-2 portable system. Expt 1.11.86.

above 30 pmol mg^{-1} (Figure 5.4b). Although F2,6BP is thought to be a potent inhibitor of photosynthesis (Stitt *et al.*, 1982), an explanation of the obvious differences in cool temperature sensitivity between these cultivars can not be explained solely by differences in F2,6BP concentration.

In all three varieties starch accumulated with increasing time at 20°C, up to and including Day 5, the 4th and last day at 20°C (Figure 5.4c and d). The biggest differences between the cultivars was in the soluble sugars concentration. In both sensitive cvs, soluble sugars increased, whereas in Tifton, the soluble sugars were maintained at close to pre-cooling concentrations (Figure 5.4d and f).

The response of sensitive and tolerant cultivars to cooling was associated with markedly different soluble sugar concentrations. The sensitive varieties, Makulu Red and Chico had increased sugar contents on each of days 2, 3 and 4 , while on Day 5 sucrose concentrations were high but declining (Figure 5.4c) and fructose and glucose concentrations had returned to below initial values in Chico (Figure 5.4e) and were still high in Makulu Red. After rewarming for 1.5 days all soluble sugars returned to pre-cooling concentrations in both sensitive varieties. By contrast, total soluble sugar concentrations in the variety Tifton did not change greatly throughout cooling with the slight increase

in sucrose concentration being offset by decreases in the concentration of fructose plus glucose.

Discussion:

In the previous chapter the reduction in CO_2 assimilation rate at cool temperatures was shown to be due principally to sink-tissue feedback inhibition, although small direct effects on photosynthetic rate were also observed (Figure 4.6c). There are a number of possible mechanisms to explain this inhibition and in the previous chapter it was shown that it was not due to changes in respiration or photoinhibition. In this chapter it has been shown that the dry weight changes that occurred during cooling involved carbohydrate accumulation and that the changes in A were principally biochemical and not stomatal. Although the experiments in Chapter 4 principally provided evidence that the effects of feedback inhibition were responsible for changes in assimilation rate, in this Chapter, when whole plants were cooled, both direct and feedback regulatory mechanisms may have been involved in controlling assimilation rate. However, the similar response of cooled sinks and whole plants (Figure 4.6b versus Figures 4.2,4.4 and 5.1) can be interpreted as evidence that feedback inhibition is the principal regulatory mechanism in whole plants.

The change in mesophyll conductance after cooling:

Farquhar and von Caemmerer (1982) have suggested that under saturating irradiance, the initial slope of the A versus c_i curve is directly proportional to the maximal Rubisco activity in the leaf, whereas at high c_i, photosynthesis is limited by the capacity for ribulose 1,5-bisphosphate (RuBP) regeneration. The decrease in slope in both glasshouse plants at 21/16°C for 7d (55% reduction) and cabinet plants at 20°C for 2d (52% reduction), suggest deactivation of RuBP carboxylase. Labate and Leegood (1988) similarly found changes in the initial slope of A/c_i curves after rapid cooling and they attributed the changes to the temperature dependence of Rubisco and to the relative solubility of O_2 and CO_2 although the changes they reported are smaller than those reported here. Sage and Sharkey (1987) compared summer and winter grown desert and horticultural plants some of which showed a significant reduction in mesophyll resistance although the authors claimed that "leaf temperature had little effect on the initial slope of the CO_2 response curve". Under "stress"" conditions it has recently been shown that patchy stomatal response can confound interpretation of A versus c_i relationships (Terashima et al., 1988), although in these studies the absence of change in stomatal conductance in the first days of cooling (Figure 4.3) suggests this was not relevant in this study. The changes in mesophyll resistance over several days at cool temperature

conditions were probably due to Rubisco being deactivated. The diversion of triose phosphate into starch (Figure 5.5) from the photosynthetic carbon reduction (PCR) cycle would reduce the regeneration of ribulose bisphospate (RUBP in Figure 5.5). Stitt (1987) comments that "sucrose synthesis can be adjusted to the performance of the chloroplast via allosteric control, time dependent adjustment of F2,6BP and SPS activation, and temperature-dependent adjustment of the enzyme activity. Nevertheless, it is possible to impose conditions under which these regulatory mechanisms are no longer able to maintain the balance between the cytosolic and chloroplastic reactions e.g. if conditions are particularly favourable for CO_2 fixation". These experiments were an excellent example of favourable conditions for CO_2 fixation in conjunction with low rates of carbohydrate utilization. According to Stitt (1987) an indication of the imbalance between cytosolic and chloroplastic reactions is provided by deactivation of Rubisco as apparently occurred in cooled Chico plants (Figures 5.2. and 5.3). The deactivation was not immediately reversible after one week at 21/16°C (Figure 5.2) since there was only a limited recovery of A on rewarming the plants (data not shown), although over shorter term treatments (2 or 3 days in Figure 4.4), recovery was almost complete within 24 hours. Similar changes in A were shown by von Caemmerer and Farquhar

LIGHT

CHLOROPLAST

ATP/NADPH

O_2

OXYGENASE

glycolate → glycine

P-glycolate (2C) → Pi

PEROXISOME

glycerate

Rubisco

CARBOXYLASE

serine (3C) → CO_2 / NH_4^+

CO_2

MITOCHONDRION

RU BP (5C)

ATP

P-glycerate (3C)

ADP

ATP

PCR CYCLE

ATP

ADP

NADPH

NADP

Pi

CYTOSOL

G 6 P ⇌ G 1 P

F 6 P

UTP

PPi

F2,6BP

F 1,6 P$_2$ (6C)

UDPG

Triose-P (3C)

ADP

ATP

Pi

Pi

Triose-P (3C)

Pi

SPS

Fructose 6 P (6C)

Sucrose-P

Glucose 6 P

Pi

Sucrose ⇌ SUCROSE (12C)

STARCH

VACUOLE

VASCULAR TISSUE →

Figure 5.5: Diagramatic representation of the possible metabolic pathways of carbon transfer from the photosynthetic fixation of CO_2 to the synthesis of sucrose in mesophyll cells of C_3 plants. Values in parentheses indicate length of carbon chains. Rubisco, ribulose bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5 bisphosphate; PCR cycle, photosynthetic carbon reduction cycle; $F1,6P_2$, fructose 1,6 bisphosphate; F6P, fructose 6 phosphate; G6P, glucose 6 phosphate; G1P, glucose 1 phosphate; UDPG, uridine 5-diphosphate glucose; F2,6BP, fructose 2,6 bisphosphate regulating fructose-1, 6-bisphosphatase; SPS, sucrose phosphate synthase. (With permission from Wardlaw, 1990).

(1984) to be due to Rubisco deactivation, although the adaptation was over months rather than weeks.

Changes in metabolites with cooling:

Cooling each of the 3 peanut varieties led to starch concentrations 10- to 20-fold higher than total soluble sugars over 4d at 20°C (Figure 5.4). Peanut, along with soybean and tobacco, is known to be a starch storer in contrast to the sucrose storers wheat, barley and spinach (Preiss, 1984). Conversion of triose phosphate to either sucrose or starch is thought to to depend on sucrose phosphate synthase (SPS) activity, which is inhibited by phosphate (Harbron *et al.*, 1981) and in most species stimulated by glucose 6-phosphate (Doehlert and Huber, 1983). In another study, Huber (1983) found that of 9 peanut varieties, addition of 10 mM sucrose to leaf cell-free supernatants in 4 varieties stimulated SPS activity, while in the other 5 varieties it inhibited SPS activity. An explanation of the different varietal responses to cool temperature i.e. the increase in concentration of soluble sugars in Chico and Makulu Red and no change in the cultivar Tifton, might involve different SPS activities. Transferring Chico and Makulu Red to 20°C increased sucrose within 4 hours which would stimulate additional SPS activity producing more sucrose which is converted to glucose and fructose (Foyer, 1987). Transferring Tifton to 20°C, on the other hand, led to slight changes in sucrose which may have inhibited SPS activity, repressing any subsequent increases in soluble sugar concentration.

The role of F2,6BP in regulating Assimilation Rate:

In the varieties Chico and Makulu Red sucrose plus fructose and glucose (the soluble sugars) concentration appears to be paralleled by the F2,6BP concentration: in both varieties the peak in sucrose concentration on Day 4 coincides with the maximum F2,6BP concentration. On Day 5 both sucrose and F2,6BP concentrations decreased while starch concentration continued to increase. It is possible that the increase in F2,6BP concentration contributes to the switching of triose phosphate from sucrose to starch formation.

Although fructose 2,6-bisphosphate appears to be involved in regulating assimilation rate, these data fail to reveal whether F2,6BP concentration responds to sucrose, fructose and glucose, or all three sugars (Foyer, 1987). The variety Tifton had low sugar concentrations each day at 01100 h, although it had low A and a large increase in the amount of F2,6BP on Day 4. It is likely that metabolite concentrations will be highest and A lowest late in the day, and the lack of a relationship between F2,6BP and soluble sugar concentrations might reflect differing rates of dissipation of sugars and F2,6BP overnight.

A possible explanation of the low concentrations of soluble sugars in the variety Tifton may involve deactivation of SPS, an enzyme that is not activated at cool temperatures (Stitt, 1987) or due to a larger

capacity for the vacuole to absorb sucrose in this cultivar. Apart from changing F2,6BP concentration, other consequences of cool temperature treatment might involve deactivation of Rubisco (Figures 5.2 and 5.3) and SPS (Stitt, 1987).

Measurement of assimilation rates at $20^{\circ}C$ suggest that there are significant differences in cool tolerance between peanut varieties, although these differences do not correspond with Botanical types. The Virginia variety Tifton was less inhibited than either the Spanish variety, Chico, or the Virginia cv. Makulu Red. Makulu Red has earned a reputation for cool-tolerance because of its high yields at cool temperatures (Williams *et al.*, 1975; Hildebrand and Smartt, 1980) but these data suggest that its photosynthetic response is indistinguishable from that of sensitive varieties. The biochemical differences between varieties that were observed in this chapter were probably not the underlying cause of different tolerances to cool temperature, but they are indicative of the regulatory mechanism that exists in peanut when temperature changes.

Chapter 6

Influence of high humidity on flower and fruit production

Introduction:

Much of the variation in yield of rainfed peanuts is due to limiting soil water and high atmospheric vapour pressure deficits (VPDs). Even with irrigation high evaporative demand in the middle of the day closes stomata (Black and Squire, 1979), which reduces assimilation and, subsequently, growth (Ong et al., 1985 and 1987). Further experiments by Leong and Ong (1983) established that soil water deficit reduced leaf and branch production, while the rates of flowering, pegging and podding were insensitive to soil water deficit in the ranges studied (up to 2.7 kPa). Surprisingly, low VPD (high humidity) may also sometimes be responsible for reducing yield in peanut (Dart et al., 1983). Although high humidity in the field is a pre-condition for the spread of foliar diseases (Smith, 1986) which in turn can be responsible for yield reduction, Dart

et al. (1983) claimed that high humidity resulted in "excess vegetative growth".

Field studies in the monsoonal tropics have indicated relatively low yields for peanut varieties of the Virginia bunch Botanical type (Dart *et al.*, 1983) under conditions that appear optimal for growth, i.e. saturating VPDs with short day photoperiods and near optimal temperatures. Dart *et al.* (1983) suggested that high humidities increased stem growth which placed the pegs of bunch- and erect-type cultivars too high off the ground for pod formation, whereas runner-type cultivars yielded well in the tropics. Reduced irradiance (under monsoonal cloud) might also increase plant height (see Table 3.5) but it is possible that other factors may be contributing to reduced yield in these varieties. Firstly, these erect and bunch-type varieties are more synchronous in their flowering than are the more "primitive" runner-type varieties (Duncan *et al.*, 1978) and perhaps they are therefore less suited to the tropics (see Chapter 3, Discussion). Secondly, it is possible that high humidity (near saturation) reduces flowering (Smith, 1954) and subsequent fruit development. This study examines this possibility.

Materials and methods:

Seed of the variety Early Bunch were germinated and grown under standard conditions (see Chapter 2) in a glasshouse of the Canberra phytotron (Morse and Evans, 1962) running at 30/25°C until emergence when they were

selected for uniformity and potted into 10 litre black pots of 1:1 perlite:vermiculite, and transferred to a humidified LBH cabinet (VPD~1.25 kPa) running at 30°C constant. The choice of the cultivar Early Bunch for these experiments was based on the advice of P. Dart (pers. comm.) who suggested that Virginia type bunch plants yielded particularly poorly in the tropics because of high humidity. A photon flux density of 500 μmol $m^{-2}s^{-1}$ (PAR 400-700 nm) at leaf level was provided by six Wotan Powerstar HQI-T 400W/DH metalarc lamps supplemented by two 500w quartz iodide lamps. Photoperiod was 12 hours. The plants were watered three times daily, with modified Hoaglands number 2 solution in the morning (Hewitt, 1966) and demineralized water at noon and in the afternoon. Before the noon watering, VPD was measured with a Solomat model MPM 500 thermo-hygro-tacho-anemometer (Solomat, Stamford, Ct., U.S.A.). The humidity sensor was shaded and held within the canopy at the level where flowers were forming. Mean values were recorded over 10 minutes. There was no foliar disease or insect damage to any of the plants in these experiments.

Effect of atmospheric VPD on peg and pod numbers:

Plants were grown under standard conditions until emergence and then transferred to cabinets running at "low" (0 to 0.8 kPa) or "high" (1.0 to 1.5 kPa) atmospheric VPD in two cabinets per treatment (20 plants per treatment). Plants were harvested 64 d after emergence, when pegs and

pods were counted and oven dry weights of tops measured. Top weights included pegs and pods.

This experiment was repeated in different cabinets and the plants were grown under standard conditions until first flower appearance when they were transferred to either "low" (0.4 to 0.8 kPa) or "high" atmospheric VPD (0.7 to 1.25 kPa). The variation in relative humidity between these two experimental runs was due to differences in the capacity of the humidifying equipment to humidify the different cabinets.

Effect of atmospheric VPD on cumulative flower number:

On the day following first flower appearance uniform plants were transferred into cabinets with either high (1.2-1.5 kPa) or low (0.5-0.8 kPa) atmospheric VPD and flower numbers were recorded over the next 8 days.

Effect of atmospheric VPD on transpiration rate in two peanut varieties:

Seedlings of the varieties Early Bunch and Chico were grown under standard conditions for 25 days after emergence and then transpiration was measured over 5 consecutive days as atmospheric VPD was varied in the cabinet. Transpiration measurement involved wrapping the pot in a plastic bag, sealing the neck of the bag around the base of the plant and weighing the plant before and after exposure to the various VPDs. Prior to each initial weighing, plants were watered, i.e. in the morning and rewatered at noon. The

interval between weighings was 3 hours and at each VPD transpiration was measured twice (am and pm). Relative water content (RWC) was measured (Slatyer, 1967) at the end of the first three hour period. A new VPD was established overnight prior to the next day's weighings. Leaf number was counted daily to account for changes in leaf area over the 5 d of measurement. Leaf area was measured after the last transpiration measurement.

Results:

Atmospheric VPD and peg and pod number:

High atmospheric humidity (low VPD) reduced both peg and pod number when it was applied either from emergence (Table 6.1) or from first flower appearance (Table 6.2) for the variety Early Bunch. In both experiments, plant top

Table 6.1: Effect of different atmospheric VPDs on peg and pod number on the variety Early Bunch from emergence. Peg and pod numbers are per plant. Expt 7.2.88.

	Peg number	Pod number	Plant top dry weight (g)
"High" VPD 1.0-1.5 kPa	121.9±12.7	17.9±2.1	54.3±3.3
"Low" VPD 0-0.8 kPa	60.0±9.9	10.2±2.7	58.0±5.7

Table 6.2: Effect of two different atmospheric VPDs on peg and pod number on the variety Early Bunch from first flowering. Peg and pod numbers are per plant. Expt 2.5.88.

	Peg number	Pod number	Plant top dry weight (g)
"High" VPD 0.7-1.25 kPa	138.7±15.9	8.7±1.8	50.5±4.9
"Low" VPD 0.4-0.8 kPa	107.2±12.7	2.3±0.8	54.7±7.3

weights (which includes peg and pod weights) were not significantly different and, on average, plants in the low VPD treatment were slightly bigger, thereby eliminating the possibility of any reduction in fruit numbers due to a limitation of growth (see Chapter 3).

Atmospheric VPD varied within the cabinets both temporally and spatially. The VPDs reported in Tables 6.1 and 6.2 ranged from a high value of about 1.5 kPa at emergence (Table 6.1) to a lower VPD when the canopy formed (0.4 kPa). Saturated conditions prevailed in some treatments.

The most humid pair of cabinets ("Low" VPD treatment in Table 6.1) remained near saturation once the canopy was interlocking, and the plants developed only half the pegs of the "High" VPD treatment. When humidity differences were imposed post flowering (Table 6.2), peg numbers were not as markedly different, i.e. 23% less pegs in the "Low" atmospheric VPD. The reduction was not as great as in Table 6.1, possibly because the relative humidity never reached 100% in the "Low" VPD treatment.

Pod numbers were significantly different between High and Low treatments in both experiments; high humidity reduced pod numbers by either 43% (Experiment 7.2.88) or 74% (Experiment 2.5.88). The differences in pod numbers between the experiments might reflect the different sized plants in the two experiments. The slightly larger plants in Expt 7.2.88 produced more pods at harvest than the equivalent plants in Expt 2.5.88.

Atmospheric VPD and flower numbers:

Plants were transferred to the different humidities after first flower appearance. Cumulative flower numbers were higher under high VPD (1.2 to 1.5 kPa) than low VPD (0.5 to 0.8 kPa) and within the first three days of them being subjected to differing humidities (Figure 6.1). Plant dry weights at the final harvest did not differ significantly between the two treatments.
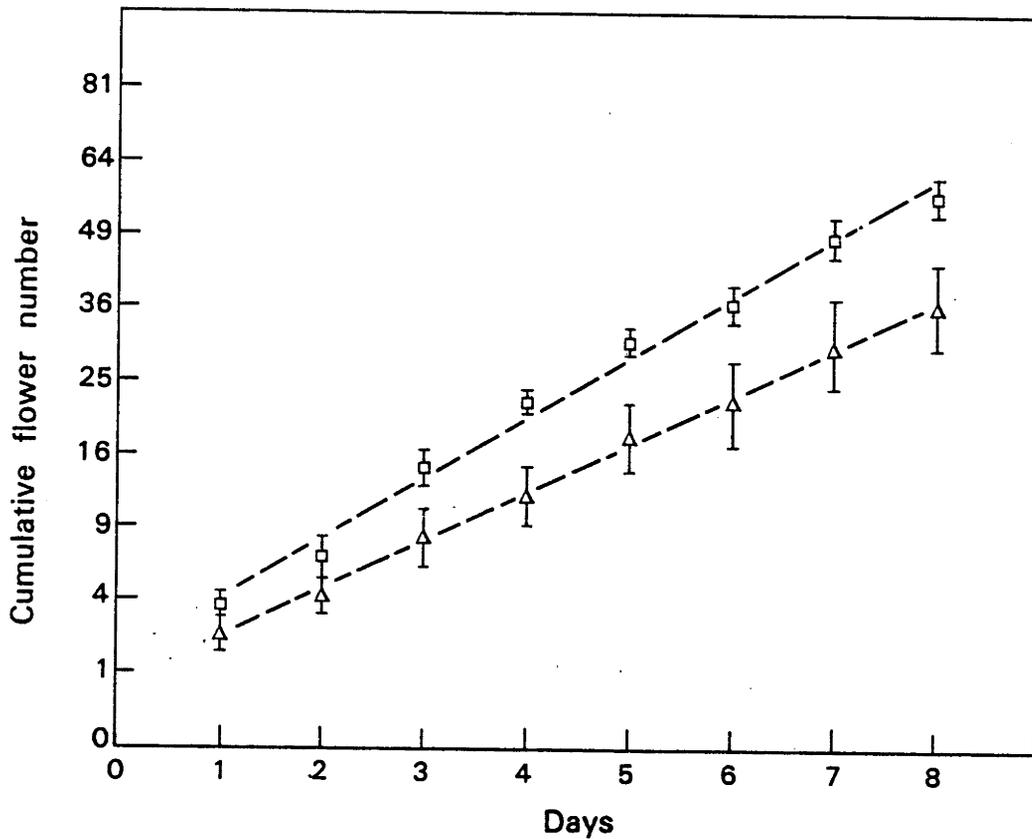
Figure 6.1: Cumulative flower numbers for Early Bunch
peanut plants at high VPD (□) or low VPD (△) over the 8d
after first flower appearance. Cumulative flowers are
presented on a logarithmic scale. This transformation was
made so that straight lines could be fitted to the data.
The fitted regressions had significantly different slopes
(p<0.05). Error bars = 2 x SE. Expt 27.11.88.

Atmospheric VPD and transpiration rate:

Transpiration rate responded linearly to evaporative

demand up to a VPD of at least 1.5 kPa for the variety

Early Bunch and up to a VPD of 1.3 kPa for Chico (Figure

6.2). At all VPDs Chico was more profligate with water than

Early Bunch and at VPDs in excess of 1.5kPa this variety

began to close its stomates. Both varieties maintained high relative water contents across this range of atmospheric VPDs. Leaf relative water contents after 3h at a VPD of 1.5 kPa were 95.1±1.2% for Chico and 96.2±1.6% for Early Bunch.
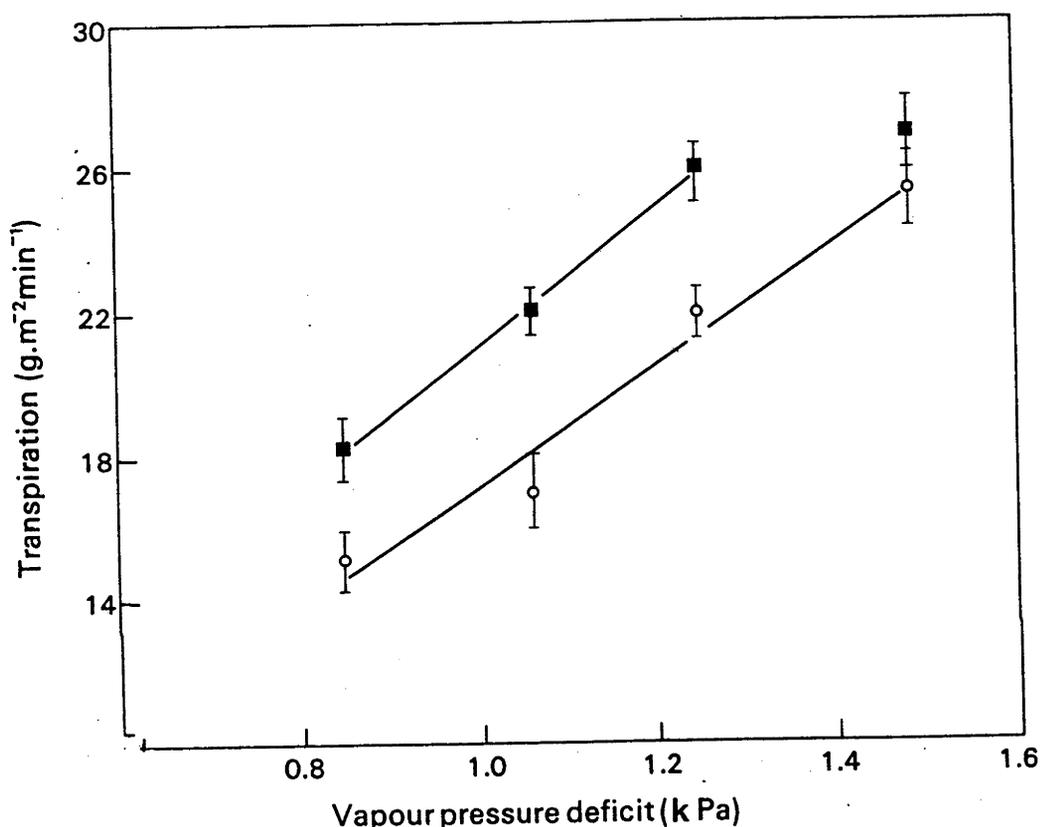
Figure 6.2: Transpiration rates for Early Bunch (o) or Chico (□) peanut plants at a range of VPDs. Error bars equal to 2x S.E. Expt 5.12.88.

Discussion:

Poor yield of bunch type peanut varieties in the tropics (Dart et al., 1983) will be an obvious result of reduction in flower and fruit numbers at high humidities as observed here. The reduction in flower numbers (Figure 6.1) probably generates the reduction in subsequent peg and pod numbers. In Table 6.1 for example, there were approximately

double the number of pegs and pods at high VPD than at low

VPD. However, in Table 6.2 the comparable ratios diverged

(pegs, 1.3 and pods, 3.8) although pod numbers were

relatively low and therefore liable to greater variability,

and the replication numbers were smaller (N=9 in Table 6.2

versus N=20 in Table 6.1).

In another controlled environment study of peanuts Ong

et al. (1987) found high humidity reduced flower and peg

numbers although they did not comment on this finding.

Their irrigated, well-watered treatment grew at almost

twice the rate of the "dry" treatment (19.7 versus 8.3 g

m^{-2} d^{-1}). However, flower numbers were significantly lower

in the wet treatment (47.7±3.1 in the "wet" versus 60.3±4.2

in the "dry") and peg numbers differed ("wet", 18.5±1.1

versus 22.7±4.4 in the "dry"). Given the larger size of the

plants in the "wet" treatment these are exceptional changes

in dry matter partitioning to flowers and pegs.

What is the possible mechanism by which humidity might lead

to reduced flowering and peg and pod formation?

It is possible that growth might be reduced at high

humidity and this could lead to reduced flowering in

peanut. Winneberger (1958), for instance, reported that

high humidity stopped pear (*Pyrus communis* L.) flower bud

development and halved growth in sunflower (*Helianthus*

annuus L.) seedlings. However, whilst flower formation in

peanut is very sensitive to assimilate supply (see Chapter

7) growth was not altered with the reduction in flowering
at low VPD.

An alternative explanation is that reduced
transpiration (Figure 6.2) leads to a reduction in the
uptake of an ion or plant growth regulator in the xylem.
Hylmö (1953) showed that varying transpiration
correspondingly changed calcium uptake in the xylem of pea
(*Pisum sativum* L.) plants and subsequent studies have shown
that reduced transpiration can lead to various calcium
deficiency symptoms in several species. For example,
reduced transpiration and diminished calcium uptake have
been associated with blossom end-rot in tomato
(*Lycopersicon esculentum* Mill.)and bitter-pit in apples
(*Malus pumila* Mill.; Wiersum, 1966). In tomatoes grown at
95% relative humidity, growth was slowed and new leaves
were calcium deficient (Armstrong and Kirkby, 1979). Such
an explanation is attractive for peanut since pod
development is heavily dependent on calcium uptake directly
from the soil-pod zone. When calcium was deficient in the
fruiting zone, pods developed from less than 1% of pegs
(Harris, 1949) but such calcium uptake to the pod is not
via the xylem (Colwell and Brady, 1945; Bledsoe *et al.*,
1949; Harris, 1949; Wiersum, 1951). However, this cannot be
the explanation in these experiments because the peg to pod
conversion rates were near to 15% in Table 6.1. Even so,
although it is unlikely that high humidity reduced calcium
availability in the fruiting zone, reduced uptake of
calcium in the xylem may still have been responsible for

the reduced flower numbers as both calcium and boron deficiences have been shown to reduce peanut flower numbers (Harris and Brolman, 1966).

The approaches advocated by Dart *et al.* (1983) to overcome the apparent problem of high humidity include "using spreading runner types, cutting the haulms for fodder at the start of flowering and spraying with a growth retardant....after the start of flowering". All of these approaches assume that the restriction to yield under near saturating conditions is "excess vegetative growth" in *both* bunch type Virginia cultivars and erect, Spanish cultivars, whereas runner Virginia types may not be limited because of the proximity of the pegs to the ground. The speculation of Dart *et al.* awaits experimental verification although the suggested differences between the responses of the botanical and morphological types might reflect differences in transpiration efficiency (dry weight gained/water lost) that have been observed between cultivars (Hubick *et al.*, 1988). If transpiration is inhibited and calcium or plant growth regulator transport from the roots is reduced in near saturation humidities in cultivars like Early Bunch, then perhaps a variety that profligately uses water like Chico (Figure 6.2) might not have reduced yields in these conditions. Although bunch type Virginia cultivars are often more conservative with water use than other cultivars (Hubick *et al.*, 1988), the overlap in response of cultivars from the Botanical types suggests that plant morphology is not related to differences in transpiration efficiency.

Chapter 7

Discussion and Conclusions

Through the plant's life cycle the environmental
factors temperature, photoperiod, irradiance and humidity
change progressively in their importance for peanut growth
and development. The evidence presented in this thesis
shows that temperature is of overriding importance up until
the start of pod-filling, whereas photoperiod and
irradiance increase in importance as the plant develops.
Once the canopy has been formed, high humidity may limit
flowering and this in turn may result in reduced pod
numbers.

Although each of these environmental variables can
affect the yield of peanut cultivars, it has generally not
been possible to explain genotype x environment
interactions in the field because of the way in which
temperature, photoperiod, light intensity and humidity co-
vary. In most studies the relative importance of these
environmental factors can not be separated. In the

following paragraphs the role these environmental factors play at various stages of the crop's life cycle in peanut fruit formation will be reviewed. The unifying themes in this assessment are production of photosynthate, the control of its allocation and the synchrony of reproduction. Photoperiod effects were largely on assimilate allocation since dry matter production was similar in treatments with large photoperiod-induced differences in fruiting. Temperature and irradiance, by contrast, affected photosynthate production and as a consequence its allocation. Synchrony of reproduction is related to environment in a more complex way. The degree of synchrony of flowering can be changed directly by photoperiod or indirectly by light and temperature via growth effects.

One of the clearest demonstrations of the role of synchrony of flowering and its interaction with partitioning of assimilate was reported by Smith (1954). He showed a depression of flower production with time which could be relieved by removing existing flowers. It must be questioned, however, whether the mass of flowers and pegs present or the demands of filling pods can act as a sink which restrict the formation and development of later flowers and pegs. For instance, very few pods were present at harvest for any of the treatments in Figures 3.4 and 3.5. Although flower numbers had peaked pod-filling was not restricting the assimilate available for flowering. An alternative explanation is therefore possible, i.e. that

growth regulators produced by the flowers themselves control this synchronous pattern of flowering and peg formation as has been argued by Pate and Farrington (1981) from their studies with lupin.

Evidence of reduction in fruiting due to limited availability of assimilate is more widespread as seen for example with shading (Hudgens and McCloud, 1975; Hang et al., 1984), varying temperature (Wood, 1968) or defoliation (Williams et al., 1976). A limitation of photosynthate for pod fill might also be argued from the report of Duncan et al.(1978) who found that the cultivar Early Bunch produced no more leaves once pod fill commenced. The commencement of pod filling thus establishes the size of the photosynthetic apparatus (leaf number) which in turn sets a limit to further flower, peg and pod production. Hence, the correlation could be expected between flower and peg number and plant dry weight (Figure 3.4) as was also evident in the earlier observations of Wood (1968).

Overall, although there are these simple unifying themes, their interactions with each other and their complex responses to environment preclude any but the most broad conclusions from being made. Subsequently, therefore, discussion is directed to flowering and fruiting responses of peanut to each different environmental variable.

Temperature:

Temperature controls peanut yield by influencing both growth and development (progress through phenological

stages). Considering firstly growth, prior to pod-formation higher temperatures (up to about 30°C) resulted in bigger plants with more flowers and pegs (Figure 3.4). These responses were the result of temperature dependence of both leaf appearance (leaf number in Figure 4.1) and leaf area (Figure 3.4). These changes in leaf area and growth occurred independently of photosynthetic rate which was not particularly temperature dependent (Figure 4.3). Both Spanish and Virginia Botanical types have distinct flowering patterns in which specific branches and nodes are floral or vegetative (Bunting and Elston, 1980). It is therefore inevitable that growth, which necessarily involves node and branch production (Figure 4.1) will directly control flower number. These distinctive patterns of flowers, leaves and branches effectively constrained the particular cultivar to an established relationship between flowers and leaves that did not vary under different temperatures, notwithstanding differences in growth rate (Figure 3.7).

The period of early growth prior to pod filling has been described as being dominant with regard to final seed yield (e.g. Ono and Ozaki, 1971; Klepper, 1973; Hudgens and McCloud, 1975; Williams *et al.*, 1978). However, whether early growth does limit yield will depend on the subsequent environmental conditions and may vary with season, location and variety. Also, temperature affects the rate of

progression through phenological stages and therefore the
duration of those phases, which in turn affects yield. Time
to first flower and time to first peg appearance are
controlled by temperature (Tables 2.2 and 3.6) and time to
maturity will be similarly temperature-dependent.

One implication of the various responses to
temperature relates to comparisons of yield at different
localities. In Indonesia, in the monsoon season, yield of
well-managed crops of peanut is substantially less than for
the same cultivars in cooler, subtropical Australia because
earlier maturity and overcast skies in Indonesia limit,
respectively, life cycle duration and growth (Bell, pers.
comm.). Conversely, slow development rates at low
temperatures can be associated with high yields in peanut
through increased duration of pre-pod-fill plant size
(Table 3.6) or pod-filling duration (Dreyer *et al.*, 1981)
particularly if the growing season coincides with high
irradiance (Williams *et al.*, 1975).

From studies by other workers, peanut pod growth has a
lower optimum temperature than vegetative growth i.e. in
the range 20 to 25°C (Williams *et al.*, 1975; Cox, 1979). It
appears likely that the signal for stopping the initiation
of new fruit comes from mature fruit and at 20°C this can
take 90d after pegs enter the ground, compared with less
than 20d at 37°C (Dreyer *et al.*, 1981). The optimum
temperature for yield for particular peanut varieties will
depend on the timing of harvest, with yields as high as 9.6
t/ha being reported for crops grown at altitude under mean

temperatures near 20°C (Hildebrand, 1980), but taking 200d to mature. Lower temperatures delay the cessation of pod filling and therefore increase the ability of the plant to continue accumulating assimilate.

Irradiance:

Light intensity is a major determinant of peanut yield after flowering commences because it influences both flower numbers (Figure 3.3) and therefore fruit numbers, as well as plant dry weight (Table 3.5). Although time to first flower in peanut is unaffected by photon flux densities (PFDs) normally experienced in the tropics and sub-tropics (i.e. above 500 μmol m^{-2} s^{-1}) at PFDs less than 500 μmol m^{-2} s^{-1}, slower first flower appearance is linked with slow growth.

If alternate sinks are present (e.g. developing leaves under very low PFDs, or many flowers and pegs at higher PFDs), then further flower formation ceases or is severely reduced. Once flowering commenced, it was shown that flower number was principally dependent on light intensity with a doubling in flowers and a 50% increase in pegs over 3 weeks when PFD was increased from 400 to 1000 μmol m^{-2} s^{-1} (Table 3.5). Both the relationship of rate to first flower versus PFD (Figure 2.3) and the flower number versus PFD (Table 3.5) were similar to that of assimilation rate versus PFD (Pallas and Samish, 1974) providing further correlative evidence for flower number being dependent on assimilate availability.

Prior to pod set, high PFD and warmer temperatures have similar effects. Both increase the size of the plant (photosynthetic source) and the potential sinks for assimilate (flower number). However, in contrast to warm temperature (which hastens the arrival of the flowering peak: Figure 3.5), high PFD maintains flowering over a longer period and delays the flowering peak, presumably because of the greater availability of assimilate.

After pods are initiated, final seed yield is linearly related to the radiant energy received by the crop between this time and maturity and is also correlated with leaf area duration of the crop (Waggoner and Berger, 1987). This latter study summarized yield data from 78 crops of Florunner peanuts over 14 seasons by five growers in Florida with various defoliation treatments and reported an excellent correlation between yield and absorbed solar radiation ($r^2 = 0.84$). This relationship described the behavior of one variety at one location, but it suggests that yield of all varieties is controlled by leaf area duration and consequently by absorbed insolation, although the exact relationship may change under different photoperiods, or with different varieties which partition less dry weight into seed. Leaf area duration is itself dependent on a range of environmental variables, particularly temperature, nutrition and water status, and this plant parameter integrates these factors.

Photoperiod:

Photoperiod has little effect on time to first flowering (Tables 2.1 and 2.2) but controls the subsequent partitioning of dry weight into flowers and fruit. Under optimal temperatures for growth, flower numbers were almost double in 12h SDs relative to 16h LDs (Figures 2.1 and 3.1, Table 3.2) and the differences between fruit numbers were even greater: up to ten-fold more fruit under continuous SD (Tables 3.2 and 3.3). However these responses to photoperiod contrast markedly with those to PFD or temperature. The photoperiodically controlled changes in flower numbers were not due to plant size as dry matter was no different across all photoperiodic treatments. Furthermore, there was no evidence in any of the experiments where photoperiod was varied that branching, leaf size, or plant dry weights were photoperiodically controlled. Thus photoperiod affected partitioning only and this response was distinct from the response to either irradiance or temperature.

Photoperiod, irradiance interactions:

The effects of varying photoperiod and irradiance simultaneously on flower and fruit number were not directly assessed in this thesis. However, a comparison of the effects of varying PFD at a 12h photoperiod in the artificially lit cabinets (Table 3.5), with the seasonal irradiance changes in 16h glasshouses (Table 7.1) can be interpreted as evidence that the flower to plant dry weight

ratio was dramatically changed by photoperiod. Although different cultivars were involved in the two experiments, the response in Table 7.1 was consistent between the Spanish and Virginia cultivars, and throughout the thesis there was no evidence that cultivars responded differently to photoperiod.

Table 7.1. Cumulative flower number and plant top dry weights at harvest for two cultivars grown at four temperatures in naturally lit 16h glasshouses in spring (Spr.) and summer (Sum.) experiments. Ratio refers to the ratio of cumulative flower number to plant oven-dried top weight. Spring growth conditions as in Figures 3.5 and 3.6. Summer growth conditions were in the same controlled temperature glasshouses for the same number of days. Spring grown plants received 14.5 MJ d^{-1} and summer grown plants 21.0 MJ d^{-1}. Each value is the mean$\pm$SE, n=12. Expts 9.8.86 and 17.10.86.

Cv.	Temp ($^{\circ}$C)	Cumulative flowers		Dry Weight (g)		Ratio (flowers/g)	
		Spr.	Sum.	Spr.	Sum.	Spr.	Sum.
Early Bunch	24/19	3±1	11±1	18±1	22±1	0.2	0.5
	27/22	48±4	60±3	38±5	60±4	1.3	1.0
	30/25	93±6	60±3	42±3	61±2	2.2	1.5
	33/28	120±11	121±7	44±5	65±2	2.7	1.8
Chico	24/19	52±4	50±6	12±1	22±1	4.4	2.3
	27/22	181±9	188±14	31±3	45±4	5.9	4.1
	30/25	308±36	264±10	53±3	58±1	5.9	4.6
	33/28	249±39	398±27	29±4	70±2	8.7	5.7

Under short days (12h) in the artificially-lit cabinets, the ratio of total flower numbers to plant dry weight consistently increased with increasing PFD i.e. the bigger plants that grew under higher PFDs produced disproportionately more flowers (Table 3.5). By way of contrast, the plants of the two cultivars grown in long day glasshouses (16h), had proportionately fewer flowers in summer than in spring although all the plants were larger in the summer experiment in all the pairs of identical temperature treatments (Table 7.1).

The underlying change in the flowers to dry weight ratio of glasshouse-grown plants in the spring compared to those grown in the summer (Table 7.1) probably reflected differences in the flower to leaf ratio given that the leaf number was highly correlated with plant dry weight (r^2=0.89 for Early Bunch and 0.85 for Chico). When plant leaf number is plotted against cumulative flower number for the two cultivars in summer and spring glasshouse experiments (Figure 7.1) the reduction in slope at the higher summer irradiance indicates that the ratio of flowers to leaves is changing for both cultivars in LD. Although the cultivars are from different Botanical types with differing floral/vegetative sequences, they respond similarly to increased radiation. The reduced slope of the flower to leaf number relationship in summer in the glasshouse was due to a relative increase of leaf production over flower production in LD. The balance between leaf and flower production can apparently be manipulated by photoperiod and

this observation has implications for peanut yield. In the subtropical summer, vegetative growth would be favoured while days were lengthening, whereas later in the life cycle, pod filling might be favoured, as happens in soybean (Cure *et al.*, 1982). Presumably the transition would be triggered when the critical daylength was passed.
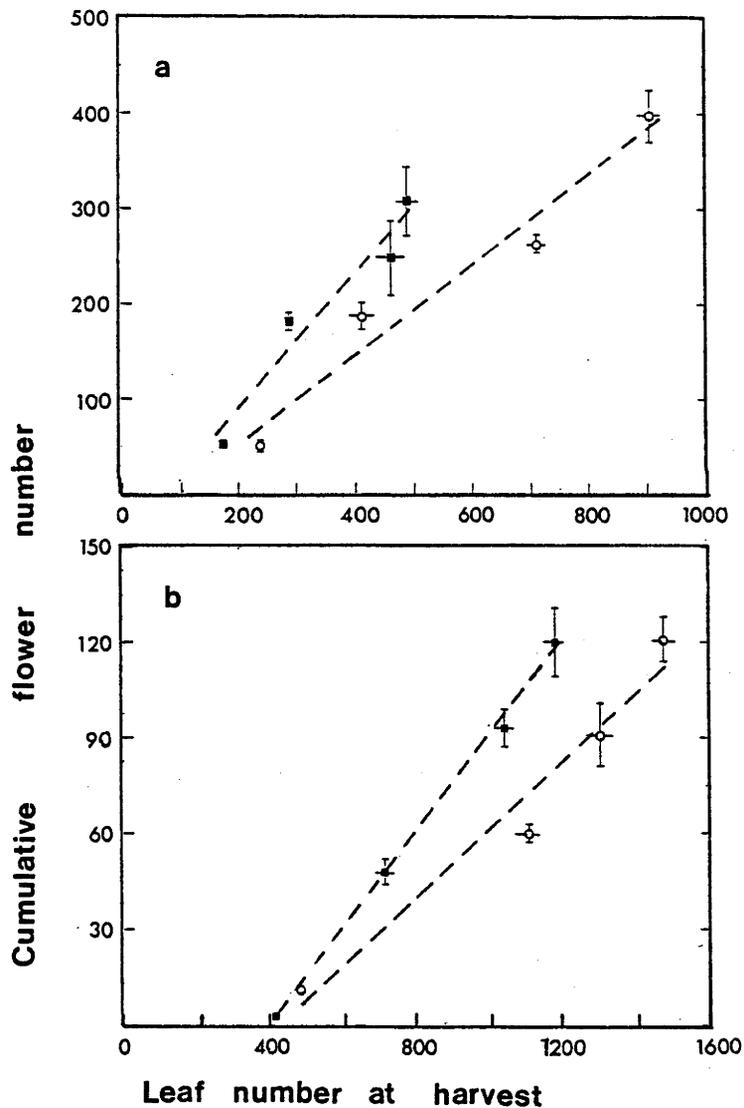
Figure 7.1. Cumulative flower numbers of Chico (a) and Early Bunch (b) plants as a function of leaf number after being grown at four temperature regimes under spring (■) and summer (O) irradiances. Growth conditions as in Table 7.1. Expts 9.8.86 and 17.10.86.

Humidity:

After a canopy has formed, high humidity may affect partitioning by reducing flower numbers (Figure 6.1). It appears that the flower numbers were reduced at low VPD in response to reduced transpiration (Figure 6.2). This reduction in flower numbers is like the photoperiodic response in that growth was unaffected i.e. it was a direct affect on partitioning. The greater sensitivity of flower number of Virginia Bunch type cultivars (like Early Bunch) to high humidity may be related to their lower transpiration rate than cultivars like Chico (Figure 6.2).

Synchrony and yield:

According to McWilliam and Dillon (1987) high yielding cultivars from the U.S.A., like Early Bunch are not particularly suited to tropical regions because of their lack of adaptation to low latitudes although they could not explain why this was so. The relatively poor performance of these varieties may be due to low light increasing plant height or reduced flowering under high humidity, but it may also reflect the highly synchronous flowering pattern in some of these varieties. Here synchrony of flowering refers to continuous flowering through pod filling (i.e. non-synchronous) or, alternatively, flowering is which is markedly reduced after 3-4 weeks (i.e. synchronous) as seen in a bell-shaped distribution of flower appearance with time (e.g. Figures 3.1c, 3.3 and 3.5d).

The differences in synchrony between peanut varieties is most noticeable in long days. Cultivars which have been selected for sub-tropical cultivation had a synchronous pattern of flowering in both SD and LD; for example, the cultivars Early Bunch (origin: USA) and Chico (from the USSR). The cultivar Robut 33-1 (origin: Israel) is not particularly synchronous in its flowering pattern when held in LD (Figure 3.1d). This pattern of flowering was similar to the Spanish-type land race variety C2 of Emery *et al.* (1981) which was selected in Paraguay. Flowering in LD continued at a steady rate over 24d (Figure 3.1d) or for more than 50d (Emery *et al.*, 1981) after first flower appearance, although pegs and pods had formed. Fruit numbers in SD were two to five times greater than in LD depending on the experiment (Table 3.2; Emery *et al.*, 1981). Emery *et al.* reported that in the 50d after first flower appearance, long day flower numbers were double the total flowers in short days because SD flower numbers conformed to a bell-shaped distribution. The increased total flower number in LD compared to SD in this non-synchronous variety is at variance with expectations of a short-day plant, although in the period before fruit were formed, more flowers appeared on plants in the SD treatment.

The large genotype x environment interactions that have been reported for peanut yield (Wynne and Gregory, 1981) may be explained in part by differences in synchrony of flowering pattern. At the ICRISAT Research Station at

Patancheru, India (latitude 18°N), the non-synchronous cultivar Robut 33-1 is the benchmark high-yielding cultivar for selection trials and the source of high yielding germplasm in their breeding program (ICRISAT, 1984). There would therefore appear to be advantages in non-synchronous flowering in varieties selected for the tropics.

Synchronous flowering is probably indicative of the degree of "determinateness" (Lawn, 1989) of cultivars, and at high temperatures, crop duration and leaf area will be restricted in a similar manner to flower numbers in the more determinate cultivars (Lawn and Williams, 1987). A non-synchronous, indeterminate cultivar in high temperature conditions will therefore have more vegetative tissue and extra photosynthetic machinery and sites for fruit. The less synchronous cultivars might also benefit from the earliest maturing pods not restricting the duration of pod-filling (Dreyer *et al.*, 1981). The advantage of synchronous flowering in the sub-tropics is that the seed maturity is more uniform at harvest and therefore there is a reduction in the loss of older seed with fragile pegs and fewer immature fruit are harvested. The greater potential for switching of peanut from vegetative to reproductive growth by photoperiod might also contribute to high yields of synchronous cultivars in the sub-tropics. Thus, highly synchronous modern varieties may be well adapted to particular sub-tropical locations, but may be limited closer to the Equator by inadequate or excess leaf or flower production in unsuitable photoperiods or due to a

lack of flexibility with regard to progress through
phenological stages restricting the duration of either
vegetative growth or pod filling.

Comparisons of Environmental Responses of Botanical types:

Comparisons between Spanish and Virginia Botanical
types are frequently made throughout this thesis, and
differences were sometimes apparent between these types.
The photoperiodic response of the Virginia and Spanish
Botanical types was very similar in the various experiments
in this thesis. Time to first flower appearance was
unaffected by photoperiod in any of the cultivars. (Table
2.2). At later stages of the life cycle SD increased peg
and pod numbers for both Virginia and Spanish type
cultivars (Table 3.3) and there was no evidence to support
the hypothesis of Wynne *et al.* (1973) that Botanical types
differ in their response to photoperiod.

The thermal times to first flower (Θ_f) of Spanish
cultivars were consistently smaller than the Virginia cvs
in these experiments (Table 2.3) and in the field (Bell *et
al.*, 1990a) so that at a given temperature, Spanish cvs
should flower before Virginia cvs. Also, the apparent base
temperatures (T_b) extrapolated from this linear temperature
model indicate that Virginia cultivars flower at lower
temperatures (by 1 to 3°C) than Spanish type cultivars,
although the extrapolation required in this particular case
probably limits the accuracy of the predictions. Bell *et
al.* (1990a) reported similar T_b values to those in Table

2.3 but with the same shortcoming, i.e. extrapolation limiting accuracy.

As for time to first flower, the Virginia types also progressed more slowly to peg formation and had a larger thermal time to first peg than did the Spanish types (Table 7.2). The consequence of this slower development of pegs and pods, particularly at cool temperatures in the Virginia cultivars Early Bunch and Robut 33-1, was that at the time of peg development plant top dry weights were approximately two to three times the plant weights of TMV2 and White Spanish at the same phenological stage (Table 3.6).

Table 7.2. Time from emergence to first peg appearance at two temperature regimes (24/19 and 30/25°C) and thermal time to first peg appearance for the cultivars TMV2, White Spanish, Early Bunch and Robut 33-1. Same plants as in Table 3.6. The date of the appearance of the first peg in each sample of 12 plants was recorded and in each sample all plants had pegs within 5d of this date. LD plants from 30/25°C excluded due to photoperiod response. Thermal time calculated as in Table 2.3. Expt 10.1.90.

Cultivar	Days to first peg appearance		Thermal time to first peg ("day-degrees")
	24/19°C	30/25°C	
White Spanish	50	26	328
TMV2	47	25	321
Early Bunch	68	34	405
Robut 33-1	70	37	472

The limit to plant size in modern, determinate cultivars is set by the start of pod filling (Duncan *et al.*, 1978) and in cool environments Virginia cultivars will be comparatively larger because of the relative delay in first peg appearance. Bigger vegetative plants have more potential sites for flowers and fruit and more photosynthetic machinery. Thus, under high irradiance conditions it is not surprising that Virginia cultivars outyield Spanish cultivars in subtropical, cool conditions. However a definition of yield on a per unit time per unit area basis would not provide such a marked difference between Botanical types.

Overview of environmental regulation of yield:

Integration of the effects of all of the environmental variables into a coherent model that predicts growth or yield at all locations has not yet been achieved. The PNUTS model of McCloud *et al.* (1980) relies on a modified heat sum approach that includes a daily assimilation factor (dependent on energy received) and on a partitioning factor for individual varieties. A difficulty with the model is that the partitioning factors of varieties change with location (McCloud *et al.*, 1980). Notwithstanding this shortcoming, temperature and radiation were excellent predictors of plant weight up until pod filling began. The effects of photoperiod, which might explain the changes in partitioning with location, were not included in this model and are likely to be greatest after first flowering. Bell

and co-workers (1990a and 1990b) similarly found that growth and rate to first flower were both strongly correlated with temperature, but that subsequent partitioning into fruit was correlated with daylength. Daylength was a single measure of photoperiod and irradiance, and Bell *et al.* (1990b) suggest that "positive effects of increased daylength on incident PAR with associated dry matter increase may have been moderated by suppression of reproductive development in long photoperiods." Bell *et al.* imply that varieties differ in the degree to which photoperiod and PFD control yield. The problem of resolving the relative importance of photoperiod and PFD with different genotypes was avoided by Waggoner and Berger (1987) who clearly showed that energy received and leaf area duration are major determinants of yield for a single variety. However, given the temperature effects on growth of different varieties and the different flowering and therefore partitioning responses in spring and summer a simple model to predict yield in all varieties seems unlikely. At the experimental temperatures in this thesis, peanut fruit weight was generally positively correlated with temperature, although for a crop at maturity, the inverse relationship between temperature and duration of developmental stages (Tables 2.2 and 7.1) will counteract this trend. Any integrated model of yield will therefore have temperature separately affecting phasic development (with a photoperiod input) and growth (with a radiation input) (Angus and Zandstra, 1980).

Available soil water and nitrogen both affect growth and consequently peanut yield (Ong, 1984; Nambiar *et al.*, 1986) and any integrated model of yield must incorporate these inputs. Currently interactions between the various environmental factors and the plant-rhizobial symbiosis are however not fully understood (Sinclair, 1986) and these interactions can limit the predictions of legume yield. Two of the models which might be adapted to predict peanut yield (Angus and Zandstra, 1980; Sinclair, 1986) have nitrogen fixation and water-use sub-routines affecting vegetative growth, which then, in turn affects yield. In this study, plants were grown on mineral nitrogen and under well-watered conditions and the results might be incorporated into similar models.

The apparently complex responses of peanut cultivars to the different environments under which the crop is grown (see Chapter 1) can in part be explained by changes in assimilation (growth) and partitioning. The partitioning (allocation of photosynthate) is dependent on flowering to provide sinks (fruit) and this in turn is dependent on photoperiod and humidity. Temperature and irradiance also influence flowering indirectly through effects on growth. Regulation of growth by temperature has been shown to involve sink-controlled inhibition of photosynthesis. Growth, and subsequently yield, will therefore reflect a complex series of interactions between environmental and plant (cultivar) factors.

Appendix 1

Classification of the peanut cultivars in this thesis.

The cultivars used in this thesis are listed in
Table A1.1 according to their Botanical type, maturity
group, plant habit and origin. The determining
characteristics of the various Botanical types are
described in Chapter 1. The maturity group classification
is based on Bell *et al.* (1990a) who classified peanut
cultivars on the basis of the time between emergence and
harvest in Kingaroy, Australia. They suggested the
following classification: Very Early less than 90d, Early
90-120d, Medium 120-135d, Late 135-150d and Very Late
more than 150d. In tropical environments, all cultivars
mature earlier and many of the differences between
cultivars' time-to-maturity disappear (Bell, pers.
comm.). The plant habit classification is according to
IBPGR (1981). The origin of the cultivars refers to where
they were initially registered or selected as cultivars.

Table A1.1. Botanical type, maturity type, habit and origin of cultivars used in this thesis. Plant habit classification according to IBPGR (1981). D3 refers to IBPGR decumbent-3 class. Maturity group according to Bell *et al.* (1990a) and Bell (pers. comm.). NC refers to North Carolina, Ga to Georgia and Fl to Florida.

Cultivar	Botanical Type	Relative Maturity	Plant habit	Origin
Chico	Spanish	Very Early	Erect	USSR
Red Spanish	Spanish	Early	Erect	Aust
TMV2	Spanish	Early	Erect	India
White Spanish	Spanish	Early	Erect	India
Q18660	Valencia	Early	Erect	USA (NC)
Q18657	Valencia	Early	Erect	USA (NC)
Q18636	Valencia	Early	Erect	USA
Robut 33-1	Virginia	Medium	D3	Israel
Q18164	Virginia	Late to very late	D3	USA (Ga)
Early Bunch	Virginia	Medium	D3	USA (Fl)
VB223	Virginia	Medium to late	D3	Aust
NC17209	Virginia	Medium to late	D3	USA (NC)
VB187	Virginia	Late	D3	Aust
Mani Pintar	Virginia	Late to very late	D3	Bolivia
Makulu Red	Virginia	Late to very late	D3	Bolivia
Virginia Bunch	Virginia	Late	D3	Aust
Tifton-8	Virginia	Medium to late	D3	USA
Shulamit	Virginia	Medium	D3	USA-Israel

Appendix 2

Nutrient provision and watering in the Canberra

phytotron.

The standard phytotron watering schedule consists of nutrient solution (see Table A2.1) at 0900h, and tap water at noon and at 1600h. The nutrient solution is a modified Hoagland (number 2) solution (Hewitt, 1966).

Table A2.1. Composition of phytotron nutrient solution.

Composition	Concentration ($mg\ l^{-1}$)
$Ca(NO_3)_2.4H_2O$	950
$(NH_4)H_2PO_4$	120
KNO_3	610
$MgSO_4.7H_2O$	490
H_3BO_3	0.6
$MnCl_2.4H_2O$	0.4
$ZnSO_4.7H_2O$	0.09
$CuSO_4.5H_2O$	0.05
H_2MoO_4	0.02
$Co(NO_3)_2.6H_2O$	0.025
$FESO_4.7H_2O$	24.8
NaOH	6.6
$[CH_2.N(CH_2.COOH).CH_2.COONa]_2.2H_2O$	33.2

Leaf relative water contents were assessed (Slatyer, 1967) at noon before watering and were maintained above 90%. Additional humidification was provided in each glasshouse by two Defensor model 3001 water atomisers (Defensor Aktiengesellschaft, Zurich) which were individually capable of generating four litres of fine water mist per hour. In the glasshouses relative humidity is usually not controlled, although in these experiments the atomizers maintained relative humidity above 70%. In the case of one experiment in the open glasshouse (Exp. 9.8.86) an additional watering was applied at 1400h to maintain both plant RWC and glasshouse humidity. In the artificially lit cabinets relative humidity was maintained above 70% RH. The pots were watered to pot capacity i.e. until water or nutrient solution drained freely from the pot. Approximately 300-400 ml of water or nutrient was usually applied to 1 litre pots. As the plants grew, and were transferred to 10 litre pots, the volume of water required to bring the pots to capacity increased to approximately 1 litre depending on the plant's size which was in turn dependent on the growth temperature and light conditions. It was not possible to stand the pots in saucers because of the susceptibility of peanut to waterlogging.

Appendix 3

Rhizobial inhibition by nutrient solution

The experiments in this thesis examine plant
responses to various environmental factors rather than
the responses of the host-symbiont complex (see Chapter
1). Although nodules were not observed on the roots of
any of the plants in the experiments in the various
chapters of this thesis, and none of the plants were
inoculated, a preliminary experiment was conducted in
which the nitrogenase activity of plants with initially
healthy, established nodules was measured after these
plants received daily waterings of standard nutrient
solution. This experiment therefore provided information
about the consequences of accidental infection of these
plants by *Rhizobium* from other experiments in the
facility, and the activity of this symbiont after daily
doses of the standard phytotron nutrient solution.

Materials and methods:

Seed of *Arachis hypogaea* L. cv. Virginia Bunch were germinated in trays of vermiculite in a phytotron glasshouse running at 30/25°C and watered with tap water three times per day for three days. They were then repotted into 75mm diameter pots of 1:1 perlite: vermiculite and inoculated with pure peat cultures of *Rhizobium* strain CB756 (Agricultural Laboratories, Regents Park, N.S.W.). There were approximately 10^8 viable bacteria applied to each seed. They were grown in the glasshouse for three weeks (Expt 14.6.85) or five weeks (Expt 2.7.85) receiving nitrogen-free nutrient (Gibson, 1980) in the morning and tap water at noon and in the afternoon. At 1000h on Day 0, and on subsequent harvest days, nodule nitrogenase activity was determined by acetylene reduction (Turner and Gibson, 1980). From Day 0, one half of the plants were subjected to normal phytotron watering (Appendix 2) and the remainder were fed nitrogen-free nutrients in the morning and tap water at noon and in the afternoon. Each harvest was at noon and nodules were collected, oven dried at 80°C for 24h and weighed. Acetylene reduction assays and harvests were conducted either 10, 18 and 27d after Day 0 (three week old plants) or 3 and 7d after Day 0 (five week old plants).

Results and discussion:

The acetylene reduction assay can provide an estimate of nitrogen fixation in nodules because acetylene is reduced by nitrogenase to ethylene. A fundamental assumption of this method is that nitrogenase activity is not affected by the substitution of acetylene for nitrogen and this assumption is not always valid (Peoples and Herridge, 1990). The acetylene reduction assay can however be used to assess nodule nitrogenase activity in peanut because Minchin *et al.* (1983) reported that peanut was not one of the species to which this limitation applied.

The phytotron nutrient solution is a particularly effective inhibitor of nitrogenase activity in legumes (Figure A3.1). Within 3d of commencement of watering with this solution the nitrogen fixation rate had dropped to below 50% of plants maintained on nitrogen-free nutrient solution. The decline in relative nitrogen fixation of nitrate fed plants was due to both a decline in nitrate-fed plants' nitrogenase activity and the increase in nodule weight in the later harvests of rhizobially grown plants. In both experiments nitrate-fed plants' nodule weights were the same at the final harvest as initially, whereas nodule weight increased five-fold in three week old plants over 35d and increased 50 per cent in seven days in five week old plants. The marked inhibition of both nitrogenase activity and nodule growth following watering with phytotron nutrient solution were similar to

those for other legumes that have accidently received

phytotron nutrient solution (Gibson, pers. comm.).
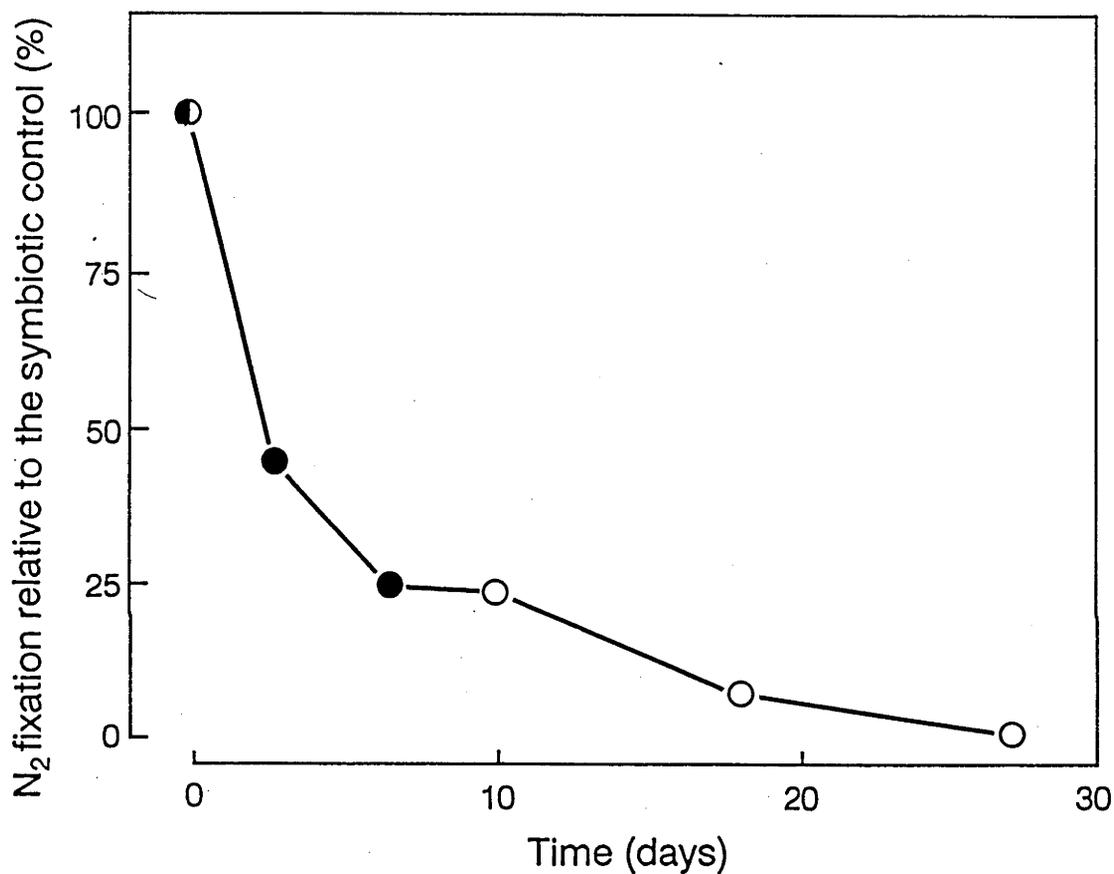

Figure A3.1. Acetylene reduction of plants watered with phytotron nutrient solution over four weeks as a proportion of symbiotic plants of the same age watered with nitrogen free nutrient. At the initial harvest acetylene reduction was 1.55 µmol plant^{-1}h^{-1} for the three week old plants (O) and 14.2 µmol plant^{-1}h^{-1} for the five week old plants (●).

Appendix 4

Time to first flower appearance and nutrition

The classification of plants into photoperiodic response groups is usually made on the basis of first flower appearance (Vince-Prue, 1975). This preliminary experiment was designed to find whether nitrate fed peanut plants flowered at the same time as rhizobially grown plants.

Materials and methods:

Seed of *Arachis hypogaea* L. cv. Virginia Bunch were germinated and grown in 10 l pots of 1:1 perlite: vermiculite in a 30/25°C phytotron glasshouse. One half of the seed were inoculated at sowing with pure peat cultures of *Rhizobium* strain CB 756 (Agricultural Laboratories, Regents Park, N.S.W.), and were subsequently watered with modified McKnight's nitrogen-free solution (Bergerson's solution in Gibson, 1980) in the morning and tap water at noon and in the afternoon. The remaining plants were fed mineral nitrogen in the form of standard phytotron nutrient solution (Appendix 2)

in the morning and tap water at noon and in the
afternoon. Dates of emergence and first flower appearance
were recorded for individual plants, as well as the
number of leaves at first flower appearance.

Results and discussion:

There was no evidence from these data that the
source of plant nitrogen (mineral or rhizobial) made any
difference to the time to first flower appearance or that
there were differences in leaf number at that time. The
rhizobially grown plants were more variable in appearance
and the rhizobially-grown sample exhibited larger
standard errors for both plant attributes (Table A4.1).
The increase in variation associated with rhizobially
grown plants and difficulties associated with isolating
rhizobially grown plants from accidental splashing of

Table A4.1: Days to first flower appearance and leaf
number of rhizobially grown and nitrate fed Virginia
Bunch plants. N=12 for both samples. All values mean±SE.

	Number of days between emergence and first flower appearance	Number of leaves at first flower appearance
Rhizobial	34.2±0.7	28.6±0.6
Nitrate-fed	34.5±0.4	29.0±0.3

nutrient solution from other experiments in the Canberra
phytotron glasshouses contributed to the decision to feed
with nutrient solution in subsequent experiments.

Appendix 5

Lighting in the Canberra phytotron.

Two artificial lighting systems were in use in the
phytotron cabinets during the period of the experiments. The
transition from the fluorescent plus incandescent to the
metal arc, quartz halogen and incandescent lamps was
commenced in 1987 by the phytotron management because of
increasing costs of the fluorescent tubes and the reported
cessation of their manufacture. Before the introduction of
the metal arc plus quartz halogen lighting system, trial
experiments compared growth rates and morphology of a range
of species (including peanut) with plants grown in the
glasshouse (R.W. King and I.A. Dawson, pers. comm.). The
combinations of light sources were modified following
measurements of spectral characteristics and the preliminary
growth comparison experiments. Growth of *Pinus radiata*
D.Don, sunflower, tomato, wheat and peanut were compared,
and growth rates and internode lengths of the plants in the
metal arc cabinets were similar to those in the glasshouse,
whereas under fluorescent lights internode lengths of the
conifer, sunflower and tomato were suppressed. The red/far
red ratio in the metal arc cabinets was observed to be

closer to that of sunlight than that of the fluorescent cabinets (R.W. King, pers. comm.). Some of the experiments reported in this thesis in Chapters 4 and 5 were completed before the transition to metal arc lighting had commenced. The metal arc plus quartz halogen lights provided routinely greater illumination than the fluorescent tubes, and lower drop in intensity with time (G. Dinnerville, pers. comm.)

Materials and methods:

The spectral charateristics of the respective cabinets were measured with a Macam SR 3000A spectroradiometer (Photometrics Ltd Livingstone, Scotland).

Results and discussion:

The combination of fluorescent and incandescent lamps that light the phytotron cabinets have provided the standard artificial light source in the phytotron since it opened in 1962. The spectral characteristics of that system are shown in Figure A5.1. The choice of combination of light sources for the phytotron system involved

"the design of a light source whose colour-rendering properties are very similar to those of daylight.. ...an appropriate combination of tungsten and fluorescent lamps is still regarded as the most practical artificial source approximating to daylight in spectral quality... To obtain a similar broad division of energies using a combination of fluorescent and tungsten lamps, the power consumption of the tungsten lamps should be about one third that of the fluorescent lamps" (Blevin, 1962).
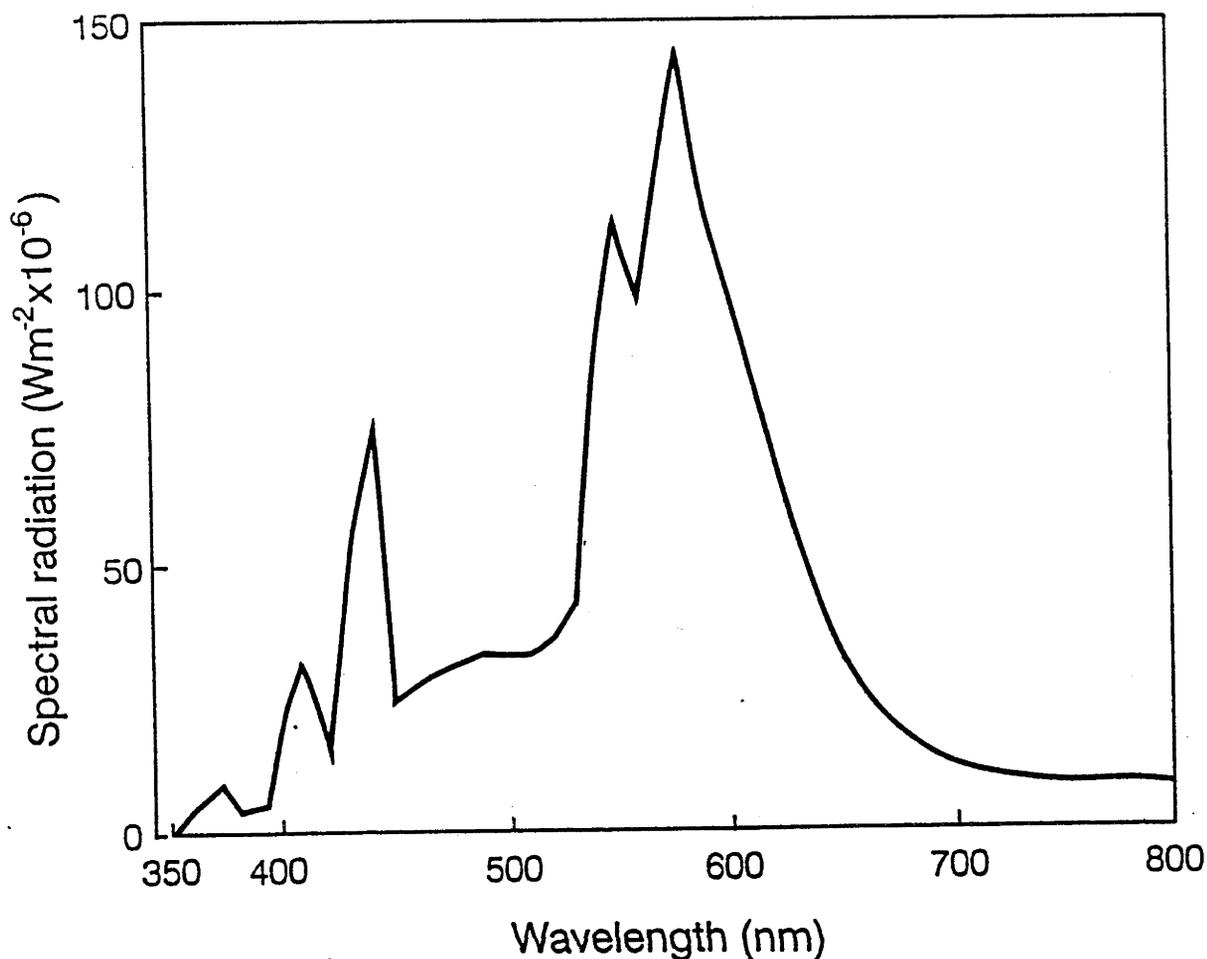
Figure A5.1. Spectral characteristics of growth cabinet lit by fluorescent tubes plus incandescent lamps. PFD 800 μmol m^{-2} s^{-1}.

As Bickford and Dunn (1972) observed, the spectral

characteristics of cool white fluorescent tubes supplemented

by incandescent lamps provides the ideal combination in

controlled environment rooms. The incandescent lamps

compliment the spectral radiation of fluorescent tubes in

the 700-800nm region producing a particularly favourable

distribution of energy for plant growth and development

(Bickford and Dunn, 1972). Apart from cabinets in the

Canberra phytotron, the majority of manufactured

artificially lit cabinets have used similar light

combinations such that Bickford (1979) commented that "most

investigators are familiar and comfortable with controlled environments in which the radiation sources are some combination of cool, white fluorescent and incandescent lamps. After all, such a combination is the old standard that has been used for years".

The spectral distribution of the new metal arc plus quartz halogen sytem is shown in Figure A5.2. Bickford and Dunn (1972) commented that the advantages of metal halide
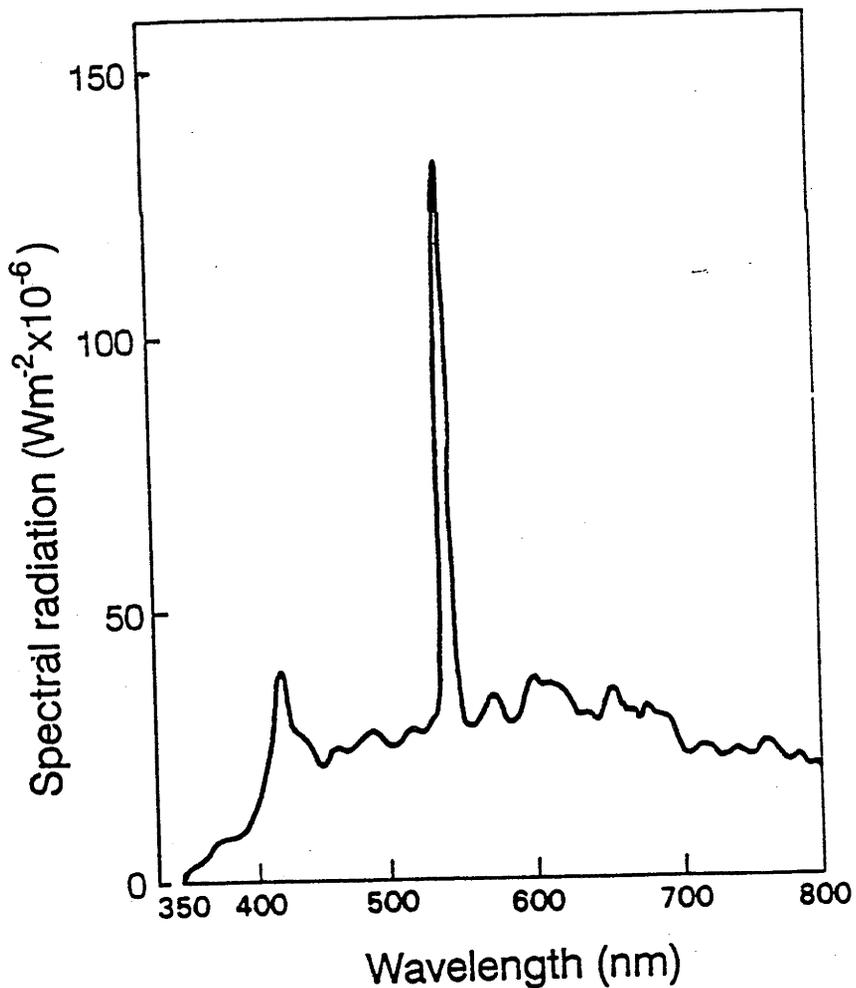

Figure A5.2. Spectral characteristics of a growth cabinet lit by metal arc plus quartz halogen lamps. PFD 700 μmol m^{-2} s^{-1}.

lamps include high intensity, a more uniform continuum of colour across the spectrum, greater energy conversion efficiency and a greater spectral emmission flexibility. The spectral quality in Figure A5.2 is more uniform across the spectrum than the fluorescent plus incandescent system and is very similar to the spectral distribution of sunlight.

The fluorescent plus mercury vapour lamp system that was used in Chapter 4 provided high intensity light in the cabinets prior to the introduction of the quartz halogen plus metal arc system. Bickford and Dunn (1972) comment that mercury vapour lamps are particularly suitable as a "supplemental light source that provides a high light output". This particular light source, the Phillips HPLR 1000w lamp, was of the mercury-vapour fluorescent reflector type and the spectral distribution (Figure A5.3) was similar to the fluorescent plus incandescent system (Figure A5.1) except for the marked peak at 578 nm, and increases corresponding to the other spectral lines of mercury at 405, 436 and 546nm. The reflector of the lamp is coated with yttrium vanadate phosphor to improve the spectral characteristics (Bickford and Dunn, 1972). This lighting system was used to measure the unadapted CO_2 assimilation rate (Figure 4.3) and in the source-sink experiment (Figure 4.6). There is a possibility that this lighting system might be detrimental to plant growth in long term experiments, but over 3d duration, significant changes would not be expected and were not observed (e.g. Figure 4.6a).
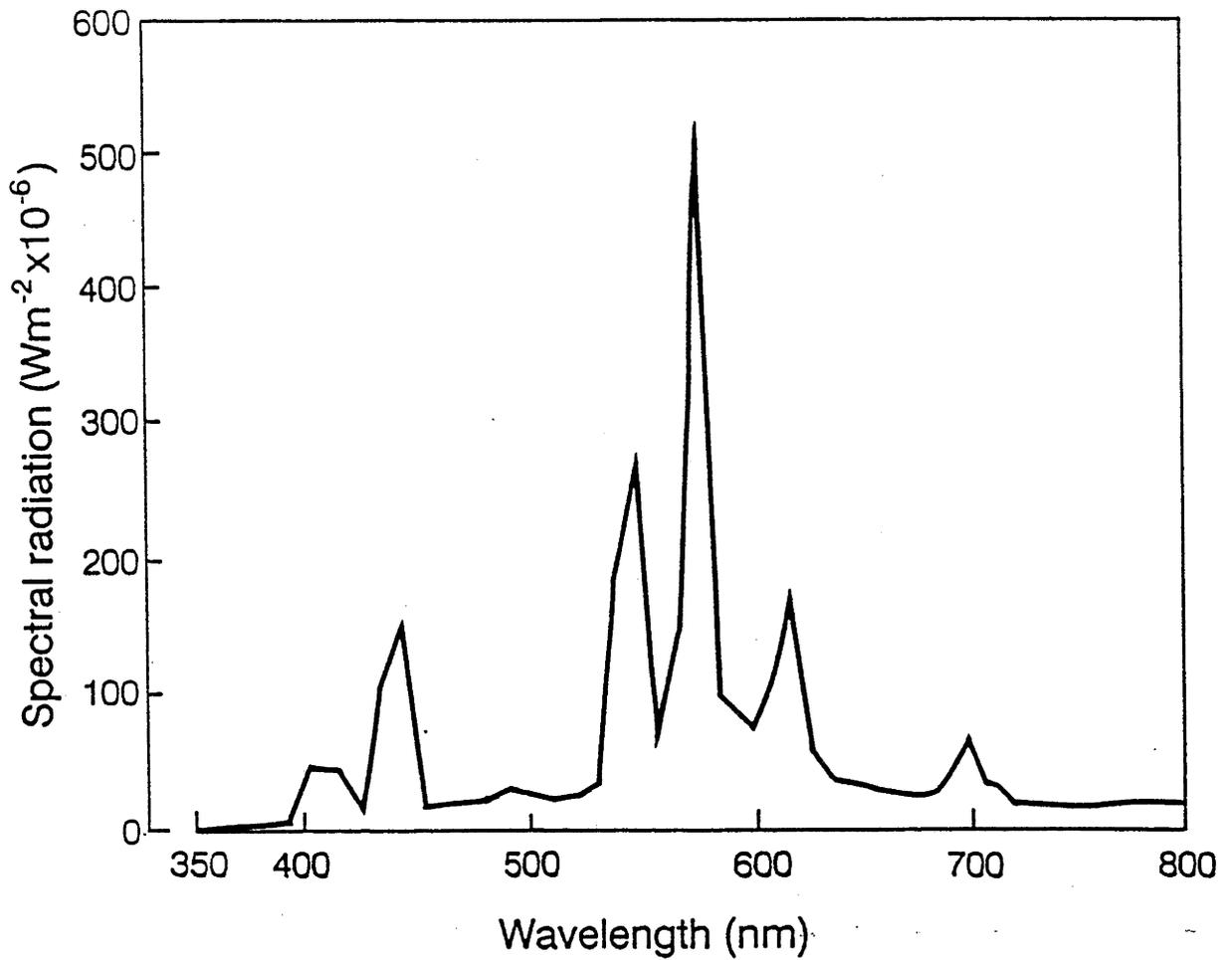
Figure A5.3. Spectral characteristics of a growth cabinet lit by fluorescent tubes plus a mercury vapour lamp. PFD 1000 μmol m^{-2} s^{-1}.

Appendix 6

Statistical comparisons of quadratic and straight line fits
of temperature versus rate to first flower data.

Table 2.4 sets out the complete analysis of variance
comparison for the straight line and quadratic fits for the
rate to first flower appearance as a function of temperature
data for the cv Early Bunch. Tables A6.1, A6.2 and A6.3 set
out the equivalent information for the cultivars TMV2, White
Spanish and Robut 33-1 respectively. The information in
these tables is summarized in Table 2.5.

Table A6.1. Analysis of variance of curved and straight-line
fit for the cultivar TMV2.

Model Y=-1.807+0.2343(T) which accounts for 91.2% of the
variance.

	d.f.	Sum of Squares	Mean Squares
Regression	1	8.7802	8.78016
Residual	14	0.7850	0.05607
Total	15	9.5652	0.63768

Model $Y=-13.06+1.16(T)-0.01875(T)^2$ which accounts for 96.4%
of the variance.

	d.f.	Sum of Squares	Mean Squares
Regression	2	9.2695	4.63475
Residual	13	0.2957	0.02275
Total	15	9.5652	0.63768
Reduction (due to quadratic term)	-1	-0.4893	0.48934

$$\text{Significance of fitting quadratic} = \frac{\text{Total change in SS}}{\text{Residual SS}}$$

$$= \frac{0.48934}{0.02275}$$

$$= 21.509$$
on 1,13 d.f.

which is a significant
improvement (p<0.001)

Table A6.2. Analysis of variance of curved and straight-line fit for the cultivar White Spanish.

Model $Y=-1.924+0.2435(T)$ which accounts for 79.3% of the variance.

	d.f.	Sum of Squares	Mean Squares
Regression	1	9.4843	9.4843
Residual	14	2.2780	0.1627
Total	15	11.762	0.7841

Model $Y=-17.96+1.563(T)-0.02672(T)^2$ which accounts for 87.4% of the variance.

	d.f.	Sum of Squares	Mean Squares
Regression	2	10.478	5.23904
Residual	13	1.284	0.09876
Total	15	11.762	0.78414
Reduction (due to quadratic term)	-1	-0.994	0.99376

$$\text{Significance of fitting quadratic} = \frac{\text{Total change in SS}}{\text{Residual SS}}$$

$$= \frac{0.99376}{0.09876}$$

$$= 10.624$$
on 1,13 d.f.

which is a significant improvement ($p<0.01$)

Table A6.3. Analysis of variance of curved and straight-line

fit for the cultivar Robut 33-1.

Model Y=-0.893+0.1758(T) which accounts for 82.0% of the
variance.

	d.f.	Sum of Squares	Mean Squares
Regression	1	4.9474	4.94736
Residual	14	0.9972	0.07123
Total	15	5.9446	0.39631

Model $Y=-12.76+1.153(T)-0.0198(T)^2$ which accounts for 91.2%
of the variance.

	d.f.	Sum of Squares	Mean Squares
Regression	2	5.4919	2.74594
Residual	13	0.4527	0.03482
Total	15	5.9446	0.39631
Reduction (due to quadratic term)	-1	-0.5445	0.54451

$$\text{Significance of fitting quadratic} = \frac{\text{Total change in SS}}{\text{Residual SS}}$$

$$= \frac{0.54451}{0.03482}$$

$$= 15.64$$
on 1,13 d.f.

which is a significant
improvement (p<0.01).

Appendix 7

The quadratic relationship between rate to first flower appearance as a function of temperature (as presented in Table 2.4) can be presented in an equation that includes the rate to first flower at the optimum temperature (Y_{opt}), the optimum temperature (T_{opt}) and the base temperature (T_b).

$$Y = Y_{opt}\left[1 - \left\{\frac{T_{opt} - T}{T_{opt} - T_b}\right\}^2\right]$$

$T_{opt} - T_b$ can be replaced by a constant k

$$= Y_{opt} - Y_{opt}\left\{\frac{T_{opt} - T}{k}\right\}^2$$

$$= Y_{opt} - \frac{Y_{opt}}{k^2}\left\{T_{opt}^2 - 2\ T_{opt}\ T + T^2\right\}$$

$$= \left(Y_{opt} - \frac{Y_{opt}}{k^2}\ T_{opt}^2\right) + \left(\frac{Y_{opt}}{k^2}\ 2\ T_{opt}\right)\ T - \left(\frac{Y_{opt}}{k^2}\right)\ T^2$$

$$= c \qquad\qquad + \quad bT \qquad\qquad - aT^2$$

where $c = Y_{opt}\ \left(1 - \frac{T_{opt}^2}{k^2}\right) = Y_{opt}\ \left(1 - \left\{\frac{T_{opt}}{T_{opt} - T_b}\right\}^2\right)$

and c is less than 1.

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