Growth and Quality of Green Tea

(*Camellia sinensis* var *sinensis*)

by

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

University of Tasmania / Dept of Agricultural Science

August, 1997

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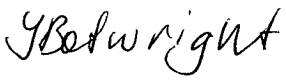

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July, 1997

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Abbreviations

ABA	abscisic acid
ADC	Analytic Development Co.
ANOVA	analysis of variance
DMS	dimethylsulphide
ESEM	environmental scanning electron microscope
GA	gibberellic acid
GC/MS	gas chromatograph and mass spectrometer
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HRC	horticultural research centre
IAA	indole acetic acid
IRGA	infra-red gas analyser
LCA	leaf chamber analyser
LD HN	long days, high night temperature
LD LN	long days, low night temperature
NDF	neutral detergent fibre
PAL	phenylalanine ammonia lyase
PFD	photon flux density
PLC	portable leaf chamber
Pc	compensation point
Pmax	maximum net photosynthesis
Pn	net photosynthesis
Qi	photosynthetic efficiency
PVPP	polyvinlypolypyrrolidone
Rd	dark respiration
RIA	radioimmunoassay
SD HN	short days, high night temperature
SD LN	short days, low night temperature
VPD	vapour pressure deficit
ZR	zeatin riboside

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Acknowledgments

I would like to thank my supervisors Professor Bob Menary of the University of Tasmania and Dr Gordon Brown of the Department of Primary Industry and Fisheries for their support and guidance over the duration of the project. A special thanks also to Dr Phillip Brown of the University of Tasmania for his time and advice. The funding for this project was provided by an Australian Postgraduate Award and the Department of Primary Industries and Fisheries, and is gratefully acknowledged. Special thanks are extended to the technical assistance provided by Phillip Andrews and David Wilson (Horticultural Research Centre); Lynne Dow, Laura Maddock, Bill Peterson and Darren Bradford (Department of Agricultural Science); Dr Noel Davies and Wieslaw Jablonski (Central Science Laboratory); and Dr David Ratkowsky (Department of Agricultural Science) and Bruce McCaulkey (DPIF). Thanks also to the staff within the Horticultural and Export Development of the DPIF and the Horticultural Research Group at the University for their assistance and encouragement. Special thanks to my fellow postgraduate students within the Department of Agricultural Science and to David Miles and my family for their endless support and encouragement.

Summary

Green tea (*Camellia sinensis* var *sinensis*) is a potential introduction to the range of crops produced in Tasmania. Information is required regarding growth and development of green tea in relation to harvested yield and quality, which is fundamental to the successful production of tea. In this study, shoot growth of green tea at four stages of development was used to examine changes in photosynthesis, assimilate partitioning and the concentration of endogenous plant hormones occurring during rhythmic growth. The effect of environment on rhythmic growth, harvested yield and quality of green tea was also examined under controlled conditions.

Shoot growth in green tea occurs in repeating cycles of growth and dormancy. Morphological and anatomical characteristics of vegetative shoots defined four key stages of development of rhythmic growth. These stages include stage I, bud burst, expansion of cataphylls and start of shoot growth; stage II, expansion of flush leaves and shoot extension; stage III, cessation of shoot extension and expansion of the final flush leaf to expose the dormant bud; and stage IV, completion of leaf expansion. Leaf primordia initiate during dormancy, and expand during shoot extension. The newly expanded leaves of the flush are subsequently harvested as the green tea crop.

Photosynthetic capacity, the pattern of assimilate partitioning and the concentration of endogenous plant hormones vary during shoot growth, as defined by the stages of development. Net photosynthesis of mature, fully expanded leaves is lowest ($13\ \mu mol\ CO_2\ m^{-2}\ s^{-1}$) during stage III, dormancy, and highest ($18\ \mu mol\ CO_2\ m^{-2}\ s^{-1}$) during stage I, at the beginning of flush growth. Starch reserves in leaves and internodes reach a maximum of 22 % (dw) and 5 % respectively at dormancy, during stage III, and decline significantly to 9 % and 0 % respectively at the start of flush growth, during stage I. At the start of flush growth, the majority of ^{14}C labelled assimilate is partitioned to the bud, with the developing leaf primordia and internodes assumed to be the major sink organs as leaf initiation has ceased. During shoot extension, in stage II, labelled ^{14}C assimilate is instead partitioned to expanding leaves and internodes. During stage III, dormancy, import of ^{14}C labelled assimilate declines as leaves became net exporters of assimilate.

The concentration of endogenous abscisic acid increases during stage III, and, as an inhibitor of growth, may be involved in the maintenance of bud dormancy during rhythmic growth in tea. Gibberellin reaches its highest concentration in buds during stage I, at the start of shoot growth. The concentration of zeatin riboside decreases during stage II, shoot extension, and may be involved in the cessation of leaf initiation

at the apex. Changes in sink activity and ontogeny are linked to changes in photosynthesis, partitioning of assimilates and endogenous plant hormone concentration during shoot growth and culminate in rhythmic growth of tea.

Environment affects the initiation of primordia, rate of growth and shoot production during rhythmic growth in tea. Environmental conditions of long days (16 hours) and a high night temperature of 15 °C increases the number of leaves initiated and subsequent rate of growth. Growth and yield in these conditions are then significantly greater than in other combinations of day length and night temperature. Achieving maximum yields by harvesting four, rather than two leaves and a bud, under conditions of long days and high night temperatures, was, however, at the expense of quality, which declined. Growth of tea following pruning, used to encourage growth of new shoots, is delayed by both long days and a night temperature of 7 °C, and short days of 9 hours and a night temperature of 7 °C, where production of shoots in the latter treatment was insufficient for harvesting. Pruning, under natural conditions, increases the time to harvest and reduces yield, but does not affect quality. A suitable compromise between yield and quality is to harvest three leaves and a bud, under conditions of long days and a low night temperature.

1. General Introduction

Tea is produced from the young shoots of *Camellia sinensis* (L.) O. Kuntz. Green tea, (*Camellia sinensis* var *sinensis*), the subject of this study, is traditionally produced in south east Asia, predominantly in China and Japan. The popularity of green tea is increasing in western countries, because of its refreshing taste and reported health benefits. Increased demand for green tea, both in Japan and western countries, has led to production of green tea in countries outside south east Asia, including New Zealand, and more recently, Tasmania, Australia.

Previously, Australia has produced only black tea (*Camellia sinensis* var *assamica*) in the tropical regions of Queensland and northern New South Wales. In 1989, three green tea varieties, including Sayamakaori, Okuhikari and Yabukita were imported into Tasmania from Japan, by the Department of Primary Industry and Fisheries. After a period in quarantine, plant numbers were increased through propagation, and trial sites established at several locations. The results of these trials indicated that inland areas in northern Tasmania, which experience slightly warmer conditions and higher rainfalls than southern Tasmania, were most suitable for tea production. Larger trial sites were established in northern Tasmania, following large scale plant propagation by private industry. Small scale tea processing machinery was purchased from Japan in 1990, for the production of Japanese-style green tea. Yield and quality of tea produced at these sites are currently being examined by the Department of Primary Industry and Fisheries to assess the profitability of tea produced in Tasmania. The Department of Primary Industry and Fisheries is also providing plant material to states on the mainland, in a combined effort to successfully produce green tea in Australia, if not Tasmania.

Yield of green tea is a function of the number and size of shoots harvested, but yield is limited by the number of leaves which are harvested to produce tea (Willson and Clifford, 1992). Yield is also influenced by the environment, which affects the rate of growth. Up to four harvests of green tea are possible in one season, but the decision to harvest the fourth flush depends on tea quality (Anon., 1990). Quality in tea is defined according to both physical attributes of the processed leaf, flavour and aroma (Yamanishi, 1995). Like yield, tea quality is influenced by the environment. Tea of the highest quality is produced from the first (spring) harvest, quality then declining with each subsequent harvest (Yamanishi, 1995). Yield and quality of tea are closely related; quality declines as more than three leaves and a bud are harvested. Returns are then a compromise between yield and quality.

In the natural state, shoot growth in tea occurs in repeating cycles of growth and dormancy (Bond, 1942, 1945; Barua, 1970). Such rhythmic cycles are proposed to be endogenous, and result from cycling between shoot growth and leaf initiation in the terminal bud (Crabbé and Barnola, 1996). The rhythmic cycle may be also associated with changes in physiological processes, such as photosynthesis, assimilate partitioning and plant hormones. The amplitude and frequency of the cycle may be altered as environmental conditions change (Koukkari *et al.*, 1974, cited Koukkari and Warde, 1985).

As the Department of Primary Industry and Fisheries is addressing yield and quality of tea produced under Tasmanian conditions, the next step is to develop an understanding of growth and development of green tea, which influence both quality and yield. This knowledge can then be used to make informed decisions regarding cultivation, harvesting, and in the manipulation of tea quality and yield. Morphological and physiological (photosynthesis, assimilate partitioning and plant hormones) changes occurring during rhythmic growth of tea were examined in context of four stages of development, as defined through quantitative measurements. The effect of environment on duration, shoot extension, leaf initiation and leaf production was examined under four different combinations of day length and night temperature. The same conditions were used to examine the effect of plucking standard and environment on harvested yield and quality of tea, which is otherwise complicated by other experimental variables in field conditions.

II. Literature review

1. Introduction

1.1 World tea production

Tea originated in south west China, with reports of tea consumption dating back to 2700 B.C. (Yamanishi, 1995). Tea was introduced to south east Asia from the second century B.C. to the sixth century A.D. by Chinese emigration and trade to the Middle East, Japan and Korea (Taylor and McDowell, 1993).

Tea trade to western countries from south east Asia began in the 16th century. Tea became very popular by the 17th century, especially in Britain where tea consumption has since become a daily habit. Tea was not successfully cultivated in India until the discovery of native 'assam' tea (*Camellia sinensis* var *assamica*) in the 19th century (Purseglove, 1974). Tea was introduced to Ceylon (now Sri Lanka), Georgia, Russia and Malawi in the 19th century, and Iran, Kenya, Turkey and Argentina in the early 20th century (Yamanishi, 1995).

The production of tea has increased at an average annual rate of 3 % from 1961 to 1988, and is projected to increase at a slower rate of 2.6 % annually until the year 2000 (Taylor and McDowell, 1993). In 1990, total production of tea was 2.5 million tonnes, of which 20 % was green tea (Yamanishi, 1995). Green tea is produced in Asia, where China is the greatest producer, followed by Japan. The total area cultivated is greater than 2.6 million hectares, with over 1 million hectares cultivated in China alone (Taylor and McDowell, 1993).

Production and consumption data for tea in 1990 are shown in Table 1. The largest exporters of black tea are India, Sri Lanka, and Kenya. Over 50 % of Indian black tea is exported to Russia, while 60 % of Kenyan black tea is exported to the UK. The largest exporter of green tea is China. Around 50 %, or most of the tea produced in countries such as Russia and Japan, is consumed internally. The UK, although not included in Table 1 as it is not a producer of tea, is by far the greatest importer of tea, at 162.7 kilotonnes in 1988. The main importers of green tea include Morocco, Russia, Algeria, USA, Afghanistan and Japan. In Japan, for example, 1941 tonnes of green tea were imported in 1990. This highlights the opportunity for production of green tea outside Asia.

Table 1. Production and export of tea during 1990 (x 1000 tonnes) (Annual Bulletin of Statistics, 1989, cited Taylor and McDowell, 1993).

Country	Production			Export		
	Black tea	Green tea	Total	Black tea	Green tea	Total
Asia						
Bangladesh	43	1	44	26	-	26
China	207	339	546	120	79	199
India	693	8	701	220	2	222
Indonesia	99	31	130	93	-	93
Iran	56	-	56	2	-	2
Japan	-	90	90	-	1	1
Malaysia	5	-	5	1	-	1
Sri Lanka	227	1	228	218	1	219
Taiwan	24	NA	24	6	1	7
Turkey	153	-	153	-	-	-
Vietnam	10	28	38	4	10	14
Total	1518	498	2016	690	94	784
Africa						
Burundi	4	-	4	4	-	4
Cameroon	3	-	3	-	-	-
Kenya	164	-	164	138	-	138
Malawi	40	-	40	37	-	37
Mauritius	7	-	7	5	-	5
Mozambique	2	-	2	1	-	1
Rwanda	12	-	12	9	-	9
South Africa	12	-	12	-	-	-
Tanzania	15	-	15	10	-	10
Uganda	3	-	3	3	-	3
Zaire	3	-	3	2	-	2
Zimbabwe	17	-	17	14	-	14
Total	282	-	282	223	-	223
USSR	96	24	120	-	-	-
S.America						
Argentina	35	-	35	34	-	34
Brazil	10	-	10	10	-	10
Ecuador	2	-	2	1	-	1
Peru	3	-	3	-	-	-
Total	50	-	50	45	-	45
Oceania						
Australia	1	-	1	-	-	-
PNG	8	-	8	6	-	6
Grand Total	1955	522	2477	964	94	1058

1.2 General morphology

Camellia sinensis (L.) Kuntz is the only economically important species of the family Theaceae, from which the young vegetative shoots are harvested to produce tea (Fig. 1). Tea is produced from two different varieties of *Camellia sinensis*, including 'assam' tea, (*Camellia sinensis* var *assamica*), and 'china' tea (*Camellia sinensis* var *sinensis*). Black tea is usually produced from the assam variety, green tea from the china variety. Related ornamental species, favoured for their flowers, include *Camellia japonica*, and *Camellia sasanqua* (Purseglove, 1974).

General morphological descriptions of tea are detailed in texts including Ukers (1935), Eden (1965), Purseglove (1974), Willson and Clifford (1992), and the review by Yamanishi (1995). Tea is a slow growing evergreen shrub. The main stem has lateral branches arising from buds in leaf axils, and both buds and internodes may be glabrous or hairy. Leaves are positioned alternately on the stem. *Camellia sinensis* var *sinensis* is a dwarf tree (4 to 6m in height when left unattended), with dark green leaves, which are small, erect, comparatively narrow, markedly serrated, smooth with a matt surface. *Camellia sinensis* var *sinensis* is relatively resistant to cold, and is produced in temperate regions. *Camellia sinensis* var *assamica*, in comparison, is faster growing and taller (12 to 15m when left unattended) than *Camellia sinensis* var *sinensis*, with larger, glossy elliptical leaves. Both varieties produce cataphylls, which are smaller than leaves and lack marginal serrations. Cataphylls enclose the bud during dormancy, and delineate successive flushes along stems. The vegetative apex is relatively flat, with two tunica layers (Nakayama, 1979). Leaf primordia are initiated in the second tunica layer by periclinal division. The auxiliary buds of the previous flush may develop into lateral shoots (Pethiyogoda, 1964; Barua, 1970).

Flowering occurs in the previous flush, during growth of current flush leaves (Bonner, 1947; Barua, 1970; Bezbaruah, 1975). Flowers, subtended by two bracts, form in leaf axils. Should the apical bud become dormant, flowers within the final flush also remain dormant, although flowers in previous flushes may reach maturity (Barua, 1970). Flower formation is undesirable in tea, representing a loss of energy that could otherwise be used in shoot growth, and is minimised by cultural practices to maintain the dominance of shoot production over flower formation (Barua, 1970).

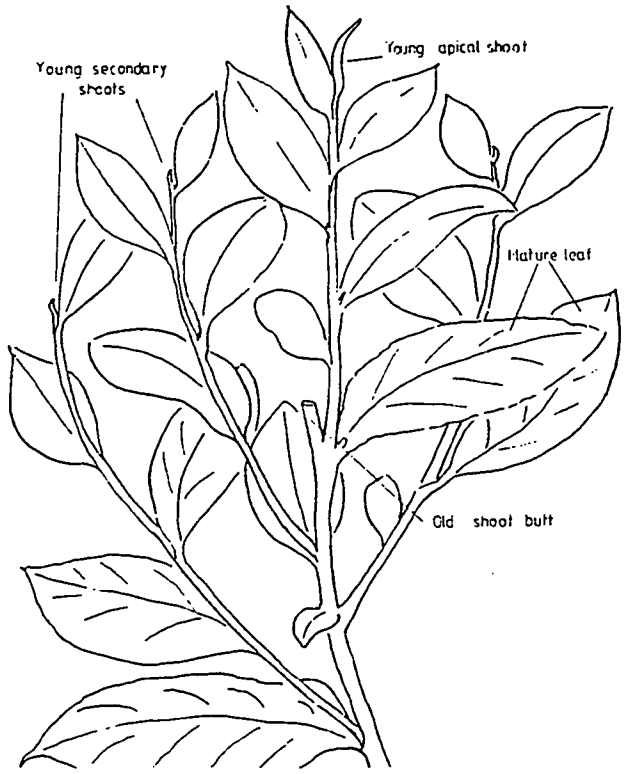

Figure 1. Tea shoots with large actively developing terminal bud on the apical shoot and well-developed secondary shoots with small dormant apical buds (R.T. Ellis, reproduced from Willson and Clifford, 1992)

1.3 Introduction to tea production

The existence of two distinct varieties of tea allows cultivation in both tropical and temperate climates. Tropical regions in which tea is grown include Sri Lanka, and regions of India and Taiwan, and are characterised by distinct wet and dry seasons. Tea can then potentially be harvested year round, although it is reported that yield declines during the dry season due to low atmospheric humidity and water stress (Eden, 1965). In comparison, temperate regions, including China and Japan, experience seasonal production of tea, as low temperatures and short days combine to induce winter dormancy (Willson and Clifford, 1992).

Plants are propagated from seeds, cuttings or tissue culture (Eden, 1965; Anon., 1990; Owuor and Robinson, 1993). Young plants are transplanted into the field after 3 years for seedlings, or 6 months for cuttings (Owuor and Robinson, 1993). Contour or terrace plantings are used in hilly areas (Eden, 1965). The plant canopy is formed by pruning young tea plants at yearly intervals. In Japan, this is achieved by pruning after two or three years to a height of 30 to 35 cm (Fig. 2), followed by skiffing (light pruning) in the third or fourth year, to form a domed canopy (Anon., 1990). Japanese experience has also shown tea to be susceptible to frost, which is controlled using

either anti-frost fans, or covers (Anon., 1990). The Japanese also produce high quality tea by shading plants, using either traditional methods of rice straw, or plastic covers, which are placed over the canopy two weeks before harvesting (Ukers, 1935; Eden, 1965; Willson and Clifford, 1992).

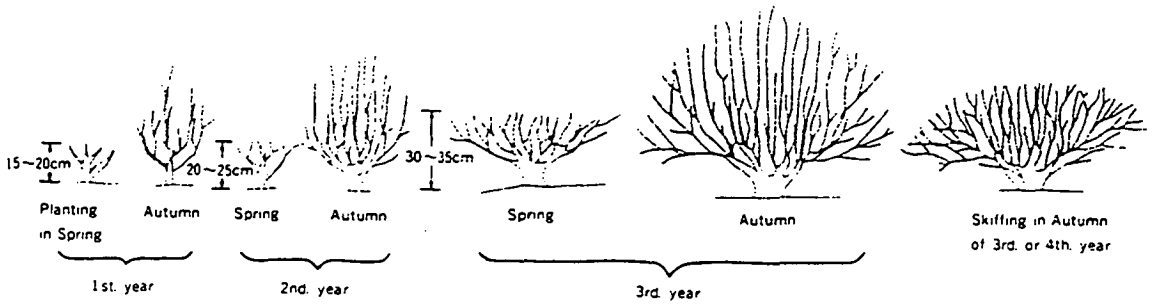

Figure 2. Skiffing for canopy formation (Anon., 1990)

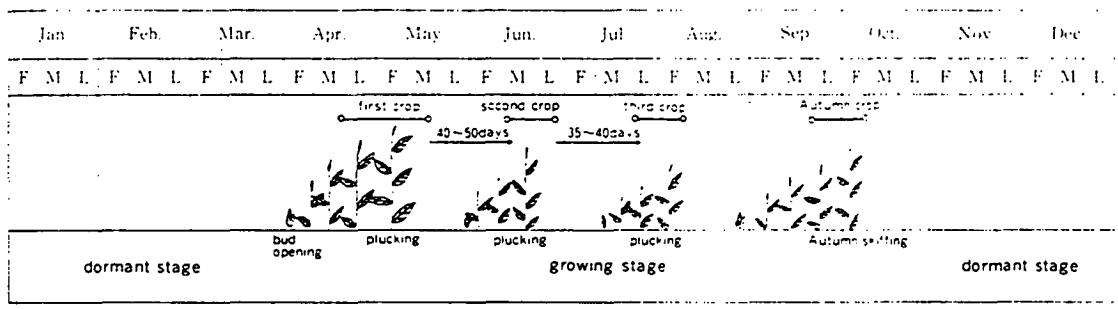

Figure 3. Harvest schedule, Japan (Anon., 1990)

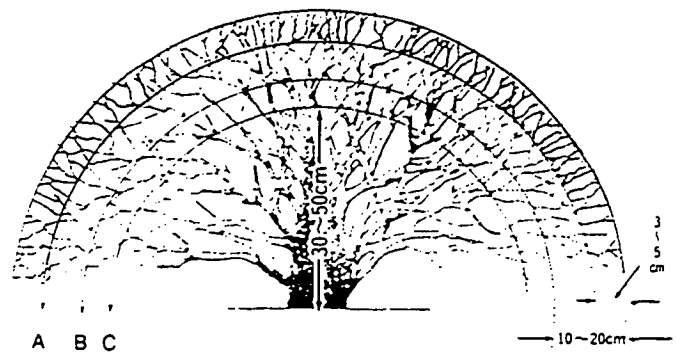

Figure 4. Methods of skiffing and pruning. A) light skiffing; B) deep skiffing; C) medium pruning (Anon., 1990)

Tea is produced from young, actively growing shoots of two to four leaves. The number of leaves harvested is referred to by industry as the plucking standard. Two leaves and a bud are referred to as fine plucking, while four leaves and a bud are

referred to as coarse plucking. Harvesting practices differ between black and green tea. In black tea, shoots are harvested at a plucking standard of two to three leaves and a bud on 7 to 21 day rounds, with about 8 harvests per year yielding 1 to 4 t ha^{-1} of processed tea annually (Carr and Stephens, 1992). In green tea (Fig. 3), a maximum of four harvests is possible per season, yielding around 18 t/ha of fresh leaf annually (Anon., 1990). Pruning and skiffing, apart from being used in canopy formation, are reported to induce growth of new shoots and maintain plant height to manageable levels (Eden, 1965). In Japan, plants are pruned every second year to a height of 30 to 50 cm (Fig. 4), with light or medium skiffing in most seasons (Anon., 1990).

Tea is harvested by hand or machine (Eden, 1965; Anon., 1990). Mechanical aids, such as hand shears, are run over the surface of the tea hedge, and sheared leaves collected in attached bags (Anon., 1990). Tea harvested by machine is more efficient, yet harvesting by hand persists for the production of high value speciality teas such as ceremony teas in Japan (Anon., 1990).

It is not only yield of harvested shoots that determines economic returns, but also quality. Desirable and undesirable attributes of Japanese green tea quality are outlined in Table 2. Organoleptic testing is the traditional method of quality assessment in tea, which considers colour, strength, briskness and flavour (Hilton and Ellis, 1972). However, organoleptic testing is criticised as subjective, and influenced by factors such as demand, supply and consumer preferences (Biswas and Biswas, 1971). Tea quality is increasingly assessed through instrumental analysis of individual chemical components. These include polyphenolics, caffeine, nitrogen, amino acids and fibre by methods including HPLC, GC/MS and NIR (Yamanishi, 1995).

Table 2. Quality of green tea.

Attribute	Desirable	Undesirable
Physical		
Leaf colour	Dark green	Light green, yellow
Colour of liquor	Bright green to clear	Brown to yellow
Particle size	Leaf shape maintained	Broken leaves, dust
Stalk		Yellow, hard and unwanted
Unrolling in infusion	Leaf completely unrolls	
Sensory		
Sweet	Monoterpenes, amino acids	
Bitter		Polyphenolics too high

The major difference between black and green tea is not in morphology, but the final product. Fermentation of black tea during processing (Table 3) results in the distinctive black leaf and associated flavour and aroma (Sanderson and Graham, 1973). During fermentation, the enzyme polyphenol oxidase (PPO), previously released during rolling, reacts with leaf phenolics forming tannins (Sanderson *et al.*, 1971). Enzymic activity ceases during final drying (Yamanishi, 1995). Processing of semi-fermented Oolong tea differs from black tea (Table 4), where fermentation is limited to withering. Fermentation by PPO is halted by high temperatures during subsequent pan firing (Yamanishi, 1995).

Table 3. Processing of black tea (Yamanishi, 1995)

1.	Fresh leaves
2.	Indoor withering for 16 to 18 hours; no shaking
3.	Rolling (45 minutes, 2 times)
4.	Fermentation (incubation, 20 to 26 °C, 2 to 3 hours)
5.	Drying (inhibitive process)
	- First drying (15 minutes, 80 to 90 °C)
	- Second drying (20 minutes, 75 °C)

Table 4. Processing of Oolong tea (Yamanishi, 1995)

1.	Fresh leaves
2.	Solar withering (90 minutes)
3.	Indoor withering and shaking (4 to 7 hours)
4.	Panning(inhibitive process, surface temp. 300 to 350 °C)
5.	Rolling (5 to 12 minutes)
6.	Mass breaking
7.	Drying

Green tea, unlike black or oolong tea, is not fermented (Table 5). Leaves need to be processed within 12 hours of harvesting, otherwise fermentation starts (Anon., 1990). In Japan, steaming inactivates the enzyme PPO and leaves will remain green in colour (Ukers, 1935; Eden, 1965). The distinctive rolled leaf of green tea is achieved by a series of tea processing machines which twist, roll and curl leaves at different temperatures (Table 5). Leaves are finally dried in the tea drier, to a moisture content of 6 %. Sencha is the main type of green tea produced in Japan, while the higher quality Gyokuro, Kabusecha and Tencha require specialised cultivation and processing (Anon., 1990). Tencha, for example, is ground to a powder after processing, and

used in the traditional tea ceremony of Japan. Processing of Chinese green tea differs in that leaves are pan fired to inactive PPO, rather than steamed (Yamanishi, 1995).

Table 5. Processing of (Japanese) Green tea (Yamanishi, 1995)

1.	Steaming (100 °C, 30 to 60 seconds)
2.	Primary rolling (90 to 110 °C, 35 to 45 minutes)
3.	Rolling (no heat, 15 minutes)
4.	Secondary rolling (50 to 60 °C, 30 to 40 minutes)
5.	Final rolling (80 to 90 °C, 30 to 40 minutes)
6.	Drying (70 to 90 °C)

The issue of central importance to the tea industry is to maximise yield and quality of harvested shoots. An important aspect of this is shoot growth and development, which is the main topic of this review. This subject has been approached by comparing and contrasting morphological and physiological differences, and the effect of environment on shoot growth and development in cultivated tea to that of the freely growing plant. This is an area that has received comparably little attention in the past, yet has the potential to advance our understanding of tea production.

2. Shoot growth and development

2.1 Pattern of shoot growth and development in tea

In a plant harvested only for its vegetative shoots, shoot growth and development becomes a primary concern. Surprisingly, there are few studies on the pattern of shoot growth in the tea plant, the most significant being those reported by Bond (1942, 1945). A typical flush was reported by Bond (1942) to originate from the terminal bud, and consisted of two cataphylls, a leaf of intermediate morphology between a cataphyll and foliage leaf (the so called 'fish' leaf) and around four leaves. During dormancy, buds are enclosed by the cataphylls. Internodes associated with the cataphylls were reported to be shorter than those associated with the foliage leaves (Bond, 1945; Pethiyogoda, 1964; Tanton 1981a). The cataphylls later senescence, leaving scars, which together with shortened internodes delineates successive flushes along the shoot.

Of particular interest, leaf production was reported by Bond (1945) to occur in repeating cycles of growth and dormancy of approximately 12 weeks in duration, although apparently the cycle is asynchronous (Wight and Barua, 1955).

Consequently, shoots within the one plant are in either the active or dormant phase of growth at any one time. Bond (1945) proposed that the origins of the flush cycle in tea resulted from alternating activity between the terminal bud and shoot. Leaves were reported to be initiated during dormancy, reaching a maximum number at bud burst. Subsequent expansion of these primordia from the apex produced a new flush. During this period, cataphylls were initiated, while the number of primordia in the bud declined, approaching a minimum of four at dormancy. Bond (1945) concluded that differences in the rate of growth between cataphylls and leaves culminated in rhythmic growth. Aperiodic growth of either leaves or cataphylls was reported by Bond (1942) to occur should leaf unfolding be matched by leaf initiation.

One might draw a comparison between the morphological description of shoot growth and development reported by Bond (1942, 1945) to that of cultivated tea. Harvesting removes not only the new leaves, but also the terminal bud. New shoots must therefore develop from lateral buds (Barua, 1970). Lateral buds are initiated in the axis of the third leaf primordium, below the apex in the corpus (Nakayama, 1979), and similar to the bud at dormancy, are reported to be enclosed by cataphylls (Goodchild, 1968). Harvesting is reported to release lateral buds from apical dominance (Barua, 1970) where the promotion of leaf initiation will culminate in bud burst after around seven leaf primordia are produced (Goodchild, 1968). The cataphyll previously enclosing the lateral bud will form the first leaf, followed by further foliage leaves. This is then similar to that observed in free-growing tea, although the harvest of shoots at two to four leaves prevents the completion of a full cycle of growth. A common morphological feature between free-growing and cultivated tea is the cataphyll. Upon harvesting, a new shoot develops from lateral buds on the remaining stub. Consequently, there is a hierarchy of shoots, and the branch system will then become increasingly complex with each additional harvest (Barua, 1961).

Buds are not only reported to become dormant at the completion of a flushing cycle in freely growing tea (Bond, 1942, 1945), but also with repeated harvesting (Wight, 1955; Barua, 1961). This is reported to contribute to a decline in yield, as fewer shoots are in the active phase of growth, but can be overcome by pruning to improve plant vigour (Wight and Barua, 1955). Understanding the morphological and physiological changes leading to bud dormancy in tea can provide some explanation of bud dormancy in relation to harvesting.

Shoot growth and development in both free growing and cultivated tea occur in flushes, although the mechanism regulating the former is endogenous and the latter a consequence of repeated harvesting. Similarities are observed in the pattern of shoot

growth in free growing and cultivated tea. Cataphylls were identified as a common morphological feature between free-growing and cultivated tea, and their significance to shoot growth will be considered in the following section.

2.2 Significance of the cataphylls

Cataphylls are characteristic of rhythmic growth. Loosely translated from German 'Neiderblatt', cataphyll means 'lower leaf' (Romberger, 1963). In tea, cataphylls are up to 5 mm in length, lack serrations, and enclose the bud during dormancy. Cataphylls were initially considered by Goebel (1880) (cited Foster, 1931a) to be reduced foliage leaves, which reflected 'arrested development'. Perhaps consistent with this proposal was the morphology of the cataphyll, which is typically less differentiated than leaves (Goebel, 1880, cited Foster, 1931a). Cataphylls of *Carya*, for example, lack palisade parenchyma, have poor vascular development and only a few stomata (Foster, 1931b).

Contradictory to this argument, the morphology of cataphylls and leaves are apparently determined shortly after initiation. Therefore cataphylls could not be considered as a product of 'arrested development'. Foster (1931b), for example, reported that the differences in morphology between cataphylls and foliage leaves in *Carya* were obvious shortly after their initiation; similar observations have been reported by Abbott (1970) on scale formation in *Malus*. The same applies for foliage leaves, although apparently the timing of leaf determination varies with plant species. For example, leaf form in *Osmunda* is reported not to be determined until the third plastochron (Steeves and Wetmore, 1953; Steeves *et al.*, 1993). When isolated, primordia younger than three plastochrons produced shoots, while older primordia produced leaves. In comparison, leaf form in the angiosperms *Helianthus* and *Nicotiana* are reported to be determined by their second plastochron and at initiation respectively (Cutter, 1954; Poethig and Sussex, 1985). There have been no reports concerning leaf determination in tea.

It has also been proposed that leaf form is influenced by growth rate, and time at the apex. For example, growth of cataphyll primordia was proposed by Fulford (1965) to be inhibited by the comparatively longer time spent at the apex compared to foliage leaves. Perhaps the relatively slow rate of growth of cataphyll initials as reported by Bond (1945) is consistent with this argument. However, contradictory to this, in *Pisum*, leaf complexity has been shown to be due to differences in the number of cells or size of the meristem, rather than rate of growth, and therefore leaf form was considered to be the consequence, not the cause of its future morphology (Gould *et al.*, 1989). Instead, as first proposed by Foster (1931a), the key to the control of

rhythmic growth lies in the physiological and ontogenetic changes occurring during shoot development that culminate in cataphyll formation. The following section compares and contrasts the various models used to describe shoot growth in tea.

2.3 Modelling of shoot growth in tea

Modelling permits quantitative comparisons in shoot growth between different conditions, such as environment or variety. Bond (1942) first reported that leaf production in free-growing tea could be described by the Logistic function (equation 1), which predicts that the rate of leaf production declines over the duration of the flush. Shoot extension is reported by Smith *et al.* (1990) to follow a similar trend, where the rate of shoot extension declines over the duration of the flush, as predicted by the Gompertz function (equation 2).

Logistic function: $y = c/[1 + e^{-b(t-m)}]$ (equation 1)

Gompertz function: $y = ce^{-e-b(t-m)}$ (equation 2)

where y is shoot length or number of leaves; t is time and c, b and m are parameters of the curve.

In contrast, Herd and Squire (1976) and Squire (1979) proposed that shoot extension in cultivated tea was exponential until shoots were 2 to 3 cm in length, and then linear until 10 to 15 cm at harvest. Linear growth implies that the rate of shoot extension is constant with time. In comparison, Tanton (1981b) proposed that shoot extension was exponential until harvestable size of 15 cm. The exponential model implies that the rate of shoot extension in cultivated tea continually increases until shoots reach a harvestable size. Linear and exponential models differ to the declining rate of shoot extension predicted by the Logistic (Bond, 1942) or Gompertz model (Smith *et al.*, 1990). However, linear and exponential models are reported by Smith *et al.* (1990) to respectively under- and over-estimate the true rate of shoot extension as predicted over a complete flush. This example highlights the importance of understanding rhythmic growth in free-growing tea.

Since Bond's original paper, there has been no further examination of leaf production during rhythmic shoot growth in tea. Current models describing shoot growth in tea are based only on the rate of shoot extension, while ignoring leaf production and apical activity. As the current level of understanding of this topic has been limited to only a few publications, it is necessary to compare and contrast shoot growth in tea to other species that exhibit a similar pattern of growth.

2.4 Pattern of shoot growth and development in other plant species

Rhythmic growth is defined by Koukkari *et al.* (1974, cited Koukkari and Warde, 1985) as a change or event that recurs systematically with a specifiable pattern and probability. A rhythm is further defined by its length, and includes ultradian (less than 20 hours), circadian (daily), and infradian (greater than 28 hours). Rhythmic (shoot) growth is then an infradian rhythm as the length of the cycle is greater than 28 hours. Rhythmic growth can be recognised by the regular alternation between a series of short internodes bearing relatively reduced leaves, and a series of long internodes bearing large leaves (Hallé *et al.*, 1978). Some plant species other than tea which exhibit rhythmic growth include *Hevea* (Hallé and Martin, 1968), *Theobroma cacao* (Greathouse *et al.*, 1971), *Quercus* (Borchert, 1975), *Pinus taeda* (Drew and Ledig, 1980), *Gnetum* (Mialoundama *et al.*, 1984), *Ficus* (Corlett, 1987), *Manigifera* (Parisot, 1988), *Guarea guidonia* (Miesch and Barnola, 1993), *Litchi chinensis* (Batten and Lahav, 1994) *Fraxinus excelsior* (Collin *et al.*, 1995) and *Citrus* (El-Morsy and Millet, 1996). Although these examples collectively exhibit rhythmic growth, there are some differences in flush duration, leaf production and morphology between the species.

During flush growth in *Quercus rubra*, shoot extension was reported by Hanson *et al.* (1986) to be complete before leaf expansion commenced, where there was a gradient in the completion of internode extension along the shoot. Leaves did not reach full expansion until after shoot extension was complete and the bud was dormant. The average duration of the flush was then approximately five weeks, during which nine leaves, including two cataphylls, were produced. In comparison, *Quercus palustris* seedlings were reported by Borchert (1975) to produce three flushes of growth over a two month period, each producing around eight leaves. Flushes were, however, asynchronous, and in some instances replaced by indeterminate growth where flushes were longer in duration (more than 30 days) and produced more leaves (24). Only three consecutive flushes were reported in field conditions, due to seasonal growth (Borchert (1975).

In *Theobroma*, leaf expansion and shoot extension occur simultaneously, as in tea, during which on average 11 leaves are produced over a 7 week period (Greathouse *et al.*, 1971). Leaf expansion was not complete until after shoot extension, with leaf size decreasing from the first to last leaf (Greathouse *et al.*, 1971). In *Hevea*, each flush is reported by Hallé and Martin (1968) to consist of cataphylls separated by long internodes followed by around 10 trifoliate foliage leaves with progressively shorter

internodes. There are around six flushes per year, but synchronised flush growth was reportedly lost as plants age (Hallé and Martin, 1968).

In each of these examples rhythmic growth is reported to be maintained in controlled conditions, which together with the observed lack of synchronised growth has led to the conclusion that the cycle is controlled endogenously (Barnola *et al.*, 1993). Although rhythmic growth in tea is similarly considered to be endogenous, the only studies to date have been undertaken in field conditions (Bond, 1942, 1945; Smith *et al.*, 1990), where variation in microclimate could potentially affect both the duration and frequency of the cycle.

Another common feature of rhythmic growth is alternation between shoot extension and bud activity, as reported in *Quercus* (Barnola *et al.*, 1993), *Hevea* (Hallé and Martin, 1968) and *Theobroma* (Greathouse *et al.*, 1971). The initiation of leaf primordia at the apex is reported by Hallé and Martin (1968) and Nougarède *et al.* (1996) to cease during shoot extension as mitosis is blocked at interphase. In contrast, Bond (1945) reported that leaf initiation in tea was continuous between shoot extension and dormancy, with variation only in the length of the plastochron. This then differs from that of other plants, and in the literature is reported as unique (Hallé *et al.*, 1978). However, a slight discontinuity in the rate of leaf initiation observed by Bond (1945) could be consistent with the observations of Barnola *et al.* (1993) and Hallé and Martin (1968) with more frequent sampling and measurement of mitotic activity. To achieve this aim first requires the development of a suitable sampling strategy, as discussed in the following section.

2.5 Defining shoot growth and development

To study physiological changes occurring in shoot growth and development in tea requires the development of a sampling strategy. The most common sampling strategy is one that is based on chronological age, as applied, for example, in studies concerning the growth and development of annual crops. However, chronological age cannot account for differences in ontogeny, which in perennial species with indeterminate growth can introduce sampling variation (Hanson *et al.*, 1986). Descriptions based on changes in morphology of tea during flush growth are reported by Bond (1942). As shown in Table 6, the morphological stages of development are based on qualitative features, such as stage C; 'lignification complete and dormant bud swollen'. Stages of growth based on such qualitative descriptions can introduce sample variation as stages may not be reproducibly identified (Hanson *et al.*, 1986).

An example of a quantitative index based on measurable changes in shoot morphology is the Plastochron Index (Erickson and Michelini, 1957; Lamoreaux *et al.*, 1978). The Plastochron Index has been successfully used in studies on *Poplar* species, but is dependent on a constant rate of initiation between successive leaves. The Plastochron Index is not applicable to species exhibiting rhythmic growth as the plastochron varies depending on the development of the flush. An alternative is to base the stages of growth on measurable changes in shoot and leaf initiation that occur during flush growth, as in the *Quercus* Morphological Index (Hanson *et al.*, 1986), which has been successfully applied in studying changes in net photosynthesis during shoot development (Hanson *et al.*, 1988). Similar stages of development could be defined for tea to assess physiological changes occurring during rhythmic growth, according to quantifiable changes not only in leaf expansion and internode extension, but also mitotic activity.

Table 6 Description of shoot growth (Bond, 1942)

Stage	Description
A	Exposure dormant bud, 5 mm in length, easily distinguished from initials of previous flush leaves
B	Shoot maximum length, uppermost leaf maximum size
C	Lignification complete, dormant bud swollen
D	Start of bud break
E	Outer cataphyll exposed, second cataphyll about to unfold
F	Outer cataphyll defoliated, inner cataphyll unfolded, fish leaf emerging
F+	Fish leaf fully unfolded, first flush leaf exposed
G, G+	Successive unfolding of flush leaves until dormancy

Understanding shoot growth and development is essential for maximising tea production, which can be improved through studying the morphological and physiological changes that culminate in flush growth in free growing tea. Areas identified as requiring further examination include; the pattern of leaf initiation and production and shoot extension, based on Bond's (1942, 1945) initial publications; an assessment of mitotic activity of the meristem to address the issue of apical activity; and the development of quantitative stages to define shoot growth in tea. The factors likely to regulate rhythmic growth in tea will be discussed in the following sections.

3. Yield, quality and environment

According to the definition of an infradian rhythm, the amplitude and frequency of the rhythm can altered by a change in environment (Koukkari and Warde, 1985). From the previous discussion, specific components of the rhythmic cycle in tea which could be affected by environment include the rate of leaf initiation, shoot extension and leaf production and the duration of dormancy. To date, the effect of environment on rhythmic growth has only been reported for a few species in field conditions (Borchert, 1975), and in tea is further limited only to that reported in the cultivated plant. This is discussed in the following section, and some conclusions will be drawn concerning the effect of environment on rhythmic growth in green tea.

3.1 Seasonal effects

The annual yield of green tea is around 18 t ha^{-1} of fresh leaf or about 4 t ha^{-1} processed leaf (Anon., 1990). Harvests of only the first few leaves in cultivated tea is reported by Tanton (1979) and Magambo and Cannell (1981) to limit yield, as only 11% of the total dry matter yield is harvested. In green tea there are usually three harvests per season, commencing in spring with a possible fourth harvest in autumn (Anon., 1990). Yield is reported to decline with successive harvests of green tea; from 8 t ha^{-1} (fw) in the first (spring) harvest, 6 t ha^{-1} in the second harvest and 4 t ha^{-1} in the final harvest. At the same time the plucking round or time between each harvest declines as the season progresses (Anon., 1990). These observations suggest that either environment or repeated harvests influence yield as the season progresses. Environmental factors likely to affect yield, and their relationship with rhythmic growth, are presented in the following sections.

3.1.1 Dormancy

Dormancy is defined as the lack of visible growth (Doorenbos, 1953). The wide variety of terms previously used to describe dormancy was recently unified by terminology proposed by Lang *et al.*, (1987). These include ecodormancy (regulated by environmental factors); paradormancy (regulated by physiological factors outside the affected structure); and endodormancy (regulated by physiological factors inside the affected structure). This classification has however been criticised by Junttila (1988) as in some circumstances dormancy can be described as a combination of two or more of the different physiological states. This is evidenced in tea, where both dormancy during rhythmic growth and winter dormancy could be either endodormancy or paradormancy, or a combination of the two. To alleviate confusion

it is far easier to use 'winter dormancy' or ' dormancy' in reference to rhythmic growth in this discussion.

Dormancy in woody perennials can be divided into three phases, namely the induction, maintenance and breaking of dormancy (Dennis, 1994). These phases are affected by changes in temperature, day length, light quality, mineral availability or water supply (Lavender and Salim, 1987), although this discussion is primarily concerned with dormancy resulting from change in temperature and day length. Growth of green tea, as a temperate species, ceases and plants become dormant from May to October (southern hemisphere) as environmental conditions are not suitable to maintain growth (Anon., 1990). Dormancy in tea was first proposed by Barua (1969) to be induced when day length was less than 11 hours and 15 minutes for six weeks. This was based on the observation that tea grown near the equator would continually flush year round, even at high elevations where temperatures were lower than in areas where tea was dormant for three months of the year. Consistent with this, exposure of tea plants to day lengths of greater than 13 hours was reported by Barua (1969) to increase yield, hasten bud break and inhibit flowering, in comparison to untreated control plants.

Barua's original proposal did not include the effect of low temperature, which together with a short day length is reported to induce dormancy in other perennial species, such as *Salix* (Junttila, 1988). In fact, recent reports have concluded that (winter) dormancy was more likely to occur in response to change in temperature than day length (Tanton, 1982b; Das and Barua, 1987). However, neither Tanton (1982b) nor Das and Barua (1987) assessed whether dormancy was in response to an interaction between short day length and low night temperature. Therefore, the evidence supporting that (winter) dormancy in tea is due to either short day length or low temperature is inconclusive. Both of these factors could then potentially affect the duration of dormancy occurring during rhythmic growth, although this has not been illustrated. The effect of temperature and day length on shoot growth will be discussed in the following sections.

3.1.2 Air temperature

Correlations between air temperature and bud break, time of first plucking and yield are reported for green tea in Japan (Yanase, 1980; Kuranuki, 1988). Shoot extension was reported to be at a maximum of 7 mm day^{-1} at a temperature of 25 °C (Yanase, 1980). Increasing air temperature from spring to summer then contributed to a reduction in the number of days from flushing to plucking, although the effect of day length was not considered (Yanase, 1980).

Shoot growth in black tea is reported to be restricted to air temperatures from 13 to 30 °C (Carr, 1972). A linear relationship reportedly exists between shoot extension to harvestable size in cultivated tea and temperatures from 17 to 25 °C, with a base temperature of 13 °C (Squire, 1979). Practical outcomes of such information are models based on relative shoot growth rate and thermal time which calculate the number of day degrees required for a shoot to reach harvestable size (Tanton, 1982a). This relationship has been extensively adopted in Malawi to predict harvest time, but has only been confirmed for the one cultivar at five sites of differing altitudes (Carr and Stephens, 1992). However, the validity of the base temperature has been questioned, as differences in clones, initial shoot length, pruning and day length were not accounted for in the original equation (Smith *et al.*, 1993a).

Common to both black and green tea is that the effect of air temperature on growth has been restricted to shoot extension. However, not only shoot extension but also the rate of leaf initiation (Bond, 1945) and leaf production to harvestable size could be affected by temperature, and thus affect yield. Temperature could also be expected to affect the frequency of the rhythmic cycle in free-growing tea, although this has not been reported.

3.3.3 Day length

The effect of day length on shoot growth has only been considered in tandem with air temperature by Tanton (1982b). Tanton reported that short day lengths of 11 hours combined with a high night temperature of 20 °C significantly reduced shoot extension compared to long day lengths of 13 hours. Observations that a low night temperature of 10 °C combined with the long day length further reduced shoot extension was reported by Tanton to indicate that the effect of air temperature on shoot extension was greater than that of day length. Tanton (1982b) however did not consider the possibility that shoot extension was in response to an interaction between day length and night temperature. The potential effect of day length on leaf initiation and leaf production has not been reported in the available literature.

3.1.4 Soil temperature

Soil temperature may be involved in the control of shoot extension, and as such, warrants brief consideration. Shoot extension is reported to cease below soil temperatures of 17 to 18 °C, and to increase linearly with increasing soil temperatures from 20 °C to 25 °C (Fordham, 1970, cited Carr and Stephens, 1992). The rate of shoot extension apparently declines at soil temperatures greater than 25 °C. Consistent with these reports is that artificial heating of soil using mulches, or black plastic,

increases dry matter yield (Othieno and Ahn, 1980). However, in contrast to previous reports the increase in yield was correlated with soil temperature over the range of 14 to 21 °C. Mulches such as rocks or grass, which reflect radiation, in contrast reduced soil temperature and yield. There is no further advantage in yield from mulching following closure of the canopy, due to less incident radiation reaching the soil (Othieno, 1982).

However, it was suggested by Carr (1969, cited Othieno, 1982) that the relative change in soil temperature to air temperature may contribute more to the observed effect on shoot growth, rather than the absolute soil temperature. Finally, although soil temperature does appear to influence yield, observations that artificial heating of soil during winter to 25 °C will not maintain shoot extension indicates that factors other than soil temperature influence shoot extension (Tanton, 1982b). It can then be concluded that temperature may affect the rate of shoot extension in tea, and thus the duration of rhythmic growth. Any potential effect of soil temperature is however likely to be minimised under controlled conditions.

3.1.5 Humidity

The effect of high temperature (greater than 30 to 35 °C) in reducing shoot growth is reported by Carr and Stephens (1992) to be closely linked to the effect of low atmospheric humidity. This is consistent with the origins of tea, where the climate was characterised by high humidity with frequent mists (Eden, 1965). Shoot extension was reported by Squire (1979) to decline as the vapour pressure deficit (VPD) increased from 1 to 3 kPa. The resultant reduction in shoot water potential from -0.6 to -1.4 MPa significantly reduced shoot extension through effects on cell turgor (Squire, 1979). High VPD of greater than 2.3 kPa is reported to override the effect of increasing air temperature on shoot extension (Tanton, 1982b). Therefore, studies on the effect of air temperature on shoot extension can potentially be influenced by secondary effects of low atmospheric humidity at high air temperatures.

3.2 Cultural effects

Yield of tea is not only influenced by season, but also cultural effects, such as the plucking standard and pruning. Firstly consider the plucking standard, which is the number of leaves harvested per shoot; and which is usually between two leaves for high quality tea, to four leaves and a bud for lower quality tea. Harvesting additional leaves will obviously increase yield (Palmer-Jones, 1977; Cloughley *et al.*, 1983; Mwakha, 1991; Owuor and Othieno, 1991), while also increasing the length of the

plucking round due to the extra time required to grow the additional leaves (Grice, 1982; Owuor and Odhiambo, 1990).

The plucking round could be considered as consisting of two components, including the time to bud burst and the time until the shoot is of harvestable size. The latter is a reflection of the plucking standard, while the time to bud burst will depend on the time required for initiation of primordia. As discussed previously, the time required for bud burst is influenced by the environment (Kuranuki, 1988), and potentially by lateral bud age. For example, Goodchild (1968) has reported that the number of primordia in lateral buds of free growing tea, from which the new shoot will be produced, increases as distance from the apex increases, as a result of apical dominance and increasing leaf age. Although not reported in the available literature, the plucking standard could then affect the regeneration time, depending on the age of the remaining lateral buds.

An apparent anomaly is that harvested yield of green tea declines by the time of the third (summer) harvest (Anon., 1990). During summer, from the above discussion temperature and day length should be optimal for shoot extension and hence maximise yield (Yanase, 1980). Such observations suggest that some factor other than environment affects yield. Repeated harvests are reported to increase the predominance of dormant buds, contributing to the decline in yield (Wight and Barua, 1955; Magambo, 1983, cited Magambo and Waithaka, 1985). This is also observed in black tea, as shown in the following example.

Yield of black tea varies within the growing season, occurring in three major peaks (Fordham and Palmer-Jones, 1975). Simultaneous growth of new shoots following the dry season culminates in a first peak in yield (Fordham and Palmer-Jones, 1975). This would be equivalent to the first flush of green tea in spring following winter dormancy. Harvest of these shoots releases lateral buds from apical dominance and growth of these buds will produce a further peak in yield, around 6 weeks after the first. This also reflects a green tea harvesting schedule. A further peak in yield may occur later in the season. However, not all shoots are synchronised with these peaks in growth, and will instead contribute to harvested yield between the peak yield periods. The distribution of these peaks may also be influenced by the environmental conditions during growth (Fordham and Palmer-Jones, 1975). Harvesting shoots more frequently, such as on a 7 day rather than 14 day round, can remove apical dominance and standardise yield of black tea during the season (Cloughley *et al.*, 1983). Dormancy can also be overcome by frequent pruning or skiffing, which, depending on the intensity, is reported to also affect yield.

Yield of tea in the first year after pruning is low in comparison to subsequent years. Recovery from pruning is reportedly affected by the severity of pruning, and seasonal timing. Although yield declines in the year following heavy pruning, light pruning can increase yield (Mwakha, 1989; Mwakha and Anyuka, 1990), which was attributed by Mwakha (1989) to a relatively earlier bud break. There is, however, no difference in yield after the first year following pruning when light and heavy pruning are compared (Mwakha, 1989). The height of pruning may also influence yield; pruning and maintaining plants at a height of 70 cm is reported to increase yield when compared to plants pruned and maintained at 25 cm (Magambo and Waithaka, 1985). Lung shoots, which are retained on the plant and not pruned, can increase the yield of the first crop as plants may recover from pruning more quickly, but limits the speed of harvesting (Mwakha and Anyuka, 1990). Autumn, rather than spring skiffing, similarly increases crop yield, as spring skiffing or pruning removes current growth.

Yield of green tea is then influenced by both the environment and cultural techniques. As most studies are conducted under field conditions, it is however difficult to separate the effect of environment from harvest, plucking standard or pruning on yield. This could be achieved by examining the effect and potential interactions between these factors on yield under controlled conditions. Although there have been no specific studies on the effect of environment on rhythmic growth, some likely effects can be drawn from the above discussion. It is postulated that environment may influence the rate of leaf initiation, shoot extension and leaf production and the duration of dormancy during rhythmic growth. For example, it is reported that bud burst is delayed by low temperatures which perhaps indicates that environment may alter leaf initiation and hence the duration of dormancy, although the effect of day length needs to be considered. Temperature and day length are similarly likely to be the dominant influence on shoot extension and leaf production, and the possibility of interactions between these two factors needs to be assessed. Secondary effects from soil temperature and VPD can otherwise be minimised under controlled conditions.

3.3 Quality

It is not only the yield of harvested tea that determines profitability, but also quality. Tea quality may be defined by physical and sensory properties, which are influenced by cultivation, environment and processing (Ukers, 1935; Eden, 1965; Willson and Clifford, 1992; Yamanishi, 1995). A high quality Japanese tea has dark green, rolled leaves with a balanced mixture of astringency, bitterness, brothiness, slight sweetness and 'green hay' aroma (Yamanishi, 1995). It is generally reported that the highest quality is achieved in the first spring crop that is harvested at a fine plucking standard (two leaves); in contrast the summer crop and coarser harvests result in a decline in tea

quality. To determine why tea quality differs in these situations has led to the quantification of the various components that contribute to tea quality, including phenolics, caffeine, amino acids and nitrogen, aroma and fibre.

3.3.1 Phenolics

Phenolics are chemically defined as phenylpropanoid compounds, which may be condensed to form polymers (Swanson, 1993). Tannins and phenolic compounds are widely distributed secondary metabolites in plants, and are involved in defence strategies, and contribute to food quality (Ukers, 1935; Swanson, 1993). Phenolics are water soluble, and contribute to the astringency and bitterness of green tea, which is regarded as a desirable attribute. This is reflected in the positive correlations reported by Price and Spitzer (1993) between total catechins and tea quality, as defined by price. Catechins present in tea include free catechin, esters of catechin and catechin gallates, which are an esterified form of gallic acid (Table 7 and Fig. 5) (Huang *et al.*, 1992; Taylor and McDowell, 1993). It is proposed that the relative bitterness and astringency of the catechins vary depending on their molecular weight; the smaller molecular weight catechin, epicatechin and epigallocatechin are more bitter than astringent, and possess a sweet aftertaste, while the higher molecular weight catechin gallates more astringent than bitter (Nakagawa, 1970).

Other polyphenolics present in tea, include flavonols, flavonol glycosides, phenolic acids and depsides (Taylor and McDowell, 1993). The three major flavonol aglycones in the fresh leaf are kaempferol, quercetin and myricetin. These occur as free flavonols and glycosides of glucose, rhamnose, galactose, arabinose or rutinose, and also contribute to the bitterness and astringency of tea (Taylor and McDowell, 1993). The major phenolic acids, which are derived from cinnamic acid include gallic, chlorogenic, neo-chlorogenic and p-coumarylquinic acids (Swanson, 1993) and depsides including quinic acid or theogallin (Taylor and McDowell, 1993).

Table 7. Polyphenols of green tea (Yamanishi, 1995)

(+) catechin	13.4%
(-) epigallocatechin	20.5%
(-) epicatechin gallate	10.5%
(-) epigallocatechin gallate	55.6%

Because of the wide variety of polyphenolics, various methods for their detection have been developed. Early quantification of polyphenolics used Lowenthal titration of total oxidisable material, but has been replaced thin layer chromatography, HPLC and GC-MS techniques (Pierce *et al.*, 1969; Hagerman and Butler, 1989; Makkar and Singh,

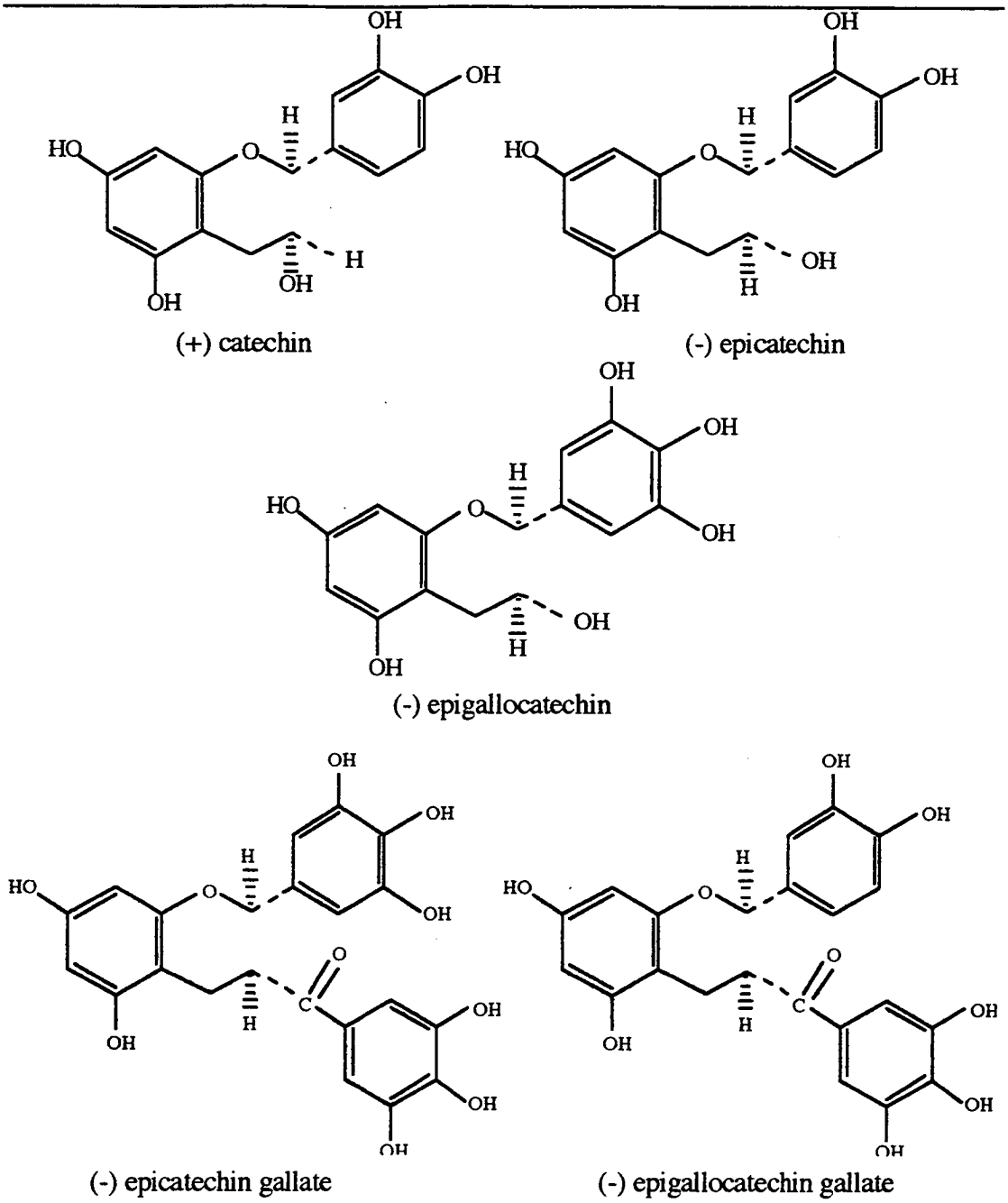

Figure 5. Catechins of green tea (Yamanishi 1995)

1995). Simple spectrophotometric analyses of total phenolics include Folin-Denis and Folin Ciocalteu assays (Singleton and Rossi, 1965; Booker *et al.*, 1996).

As catechins are desirable to tea quality, it then may be expected that these compounds would predominate in tea harvested in spring, and at fine plucking standards of two leaves and a bud. Consistent with this, Forrest and Bendall (1969) have reported that phenolics are present in highest concentration in young tissue, such as buds, young leaves and internodes, while wood, roots and flowers have less than 2 % catechin. As leaves mature the concentration of desirable catechin gallates decreases, while

undesirable epigallocatechins increase. This contributes to the decline in leaf quality associated with coarser harvests (Forrest, 1969; Forrest and Bendall, 1969; Nakagawa and Furuya, 1975). Similarly, in black tea, the concentration of oxidised catechins (theaflavin) declines from harvests of two to four leaves (Cloughley, 1983). New shoots produced following pruning reportedly have a higher concentration of catechins (Ukers, 1935). Consequently fine plucking standards of one or two leaves and a bud contain more catechins than coarse plucking standards of four or more leaves and a bud, thus contributing to improved tea quality.

The relationship between harvest and environment on phenolics in tea is however a complicated issue. Phenolics are synthesised by the shikimic acid pathway from D-erythrose-4-phosphate and phosphoenol pyruvate (PEP), a key step of which is regulated by the enzyme phenylalanine ammonia lyase (PAL) in the presence of light, which catalyses the reaction from phenylalanine to cinnamate (Iwasa, 1976). Consistent with this, shading, by decreasing the activity of the enzyme PAL (Saijo, 1980), is reported to reduce the amount of total catechins (Forrest, 1969; Iwasa, 1976; Owuor and Odhiambo, 1993). The overall effect on tea quality is positive, as the decline in (undesirable) epigallocatechin and epicatechin is greater relative to (desirable) epicatechin gallate and epigallocatechin gallate (Iwasa, 1976). Gallic acid can be synthesised from shikimic acid, or can bypass the light regulated PAL enzyme through ß-oxidation of 3,4,5-trihydroxy cinnamic acid - therefore explaining the increase in catechin gallates relative to catechin at low light intensities (Saijo, 1981).

Nakagawa *et al.*(1977) reported that although the first spring crop was of higher quality, the amount of desirable catechin gallates did not reach a maximum until the second (summer) harvest. This observation would appear to indicate that high quality of the first (spring) harvest was not associated with increased phenolics. However, the effect of day length and temperature on catechin concentration has not been well documented. Yanase (1980), has reported that there is no evidence to indicate that temperature alters catechin concentration. There are, however, no specific reports on the effect of day length, although it would seem logical that an increase in photoperiod may increase the total activity of PAL, and hence increase total catechin concentration.

3.3.2 Nitrogen and amino acids

The amino acids present in green tea include asparagine, threonine, glutamine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, tryptophan and theanine (Roberts and Sanderson, 1966). Theanine is unique to tea, and accounts for 50 % of the total amino acids (Feldheim, 1986; Ohtsuki *et al.*, 1987; Liang *et al.*, 1990). Theanine is the ethylamide of glutamic acid,

and synthesised in tea roots from glutamate and ethylamine, by L-glutamate: ethylamine ligase and transported to developing shoot tips (Takeo, 1974). Amino acids contribute to the brothy taste of green tea, and are reported to be highly desirable; but are detrimental to the taste of black tea (Taylor and McDowell, 1993). It is however reported that the concentration of theanine in brewed tea is close to its threshold level, and hence not necessarily detected during taste testing (Millin *et al.*, 1969). As theanine is otherwise considered important in the taste of green tea, it was proposed that theanine interacts with other components as a flavour potentiator (Millin *et al.*, 1969).

Positive correlations are reported between total nitrogen content and tea price (Mukai *et al.*, 1992). Total nitrogen is then considered to be a useful indicator of tea quality. Total nitrogen, which is inclusive of the amino acids, is also reported to have a positive correlation with tea quality, and is reported to vary from 3 to 5 % (Willson, 1975). Nitrogen, as a mobile element, tends to be in higher concentration in immature tissues (Salisbury and Ross, 1985), as reflected in the content of nitrogen in the first leaf (4.1 %) when compared to the third leaf (3.3 %) of tea shoots (Willson, 1975). Hence, the content of nitrogen in leaves and internodes decreases with leaf age (Barua and Deb, 1960). Coarser plucking standards tend to reduce nitrogen content (and amino acids) in harvested shoots, which is associated with the decline in tea quality (Morita *et al.*, 1996). There is also evidence of a seasonal effect, where total nitrogen is reported by Nakagawa and Furuya (1975) to decline from the first harvest in spring, to the final harvest in summer or autumn. This may reflect the pattern of seasonal uptake of nitrogen, which is highest during spring, and then declines until autumn (Okano and Matsuo, 1996). The effect of environment on nitrogen content of leaves has however not been assessed under controlled conditions, which would establish whether the difference was due to a change in day length or temperature.

3.3.3 Caffeine

Caffeine is the major purine alkaloid present in tea, at approximately 3-4 % (Taylor and McDowell, 1993). Caffeine is odourless and has a bitter taste (Millin *et al.*, 1969). In brewed tea, caffeine forms complexes with the polyphenols, reducing the bitterness associated with caffeine and the astringency of the phenolics, resulting in a brisk taste. Caffeine is rapidly absorbed by the gastrointestinal tract, and acts as a stimulant to the central nervous system (Arnaud, 1993). Caffeine is a xanthene, and is synthesised in leaves from xanthosine by four sequential reactions. Methyl groups are transferred from S-adenosyl-L-methionine (SAM) to xanthosine, yielding 7-methyl xanthosine, which undergoes further methylation through 7-methylxanthosine and theobromine to caffeine (Ashihara and Kubota, 1986; Fujimori *et al.*, 1991). Caffeine

content varies with clone, and is synthesised in young leaves, accumulating in leaves as they mature (Ashihara and Kubota, 1986; Owuor and Chavanji, 1988). However, it is reported that the amount of caffeine in leaves declines with age, such that caffeine content is maximised when only two leaves are harvested per shoot (Owuor and Chavanji, 1988; Owuor and Odhiambo, 1990; Owuor and Othieno, 1991). The caffeine content in leaves tends to remain fairly constant throughout the growing season, but is slightly greater in the first spring harvest, and following pruning (Ukers, 1935). Caffeine is greater in shaded tea (Anan and Nakagawa, 1974). Under conditions of low light, the metabolism of nucleic acids is reduced, and the precursor purine is instead used to synthesise caffeine (Suzuki and Waller, 1985).

3.3.4 Aroma

The formation of aroma of green tea is highly complex. Some 638 aroma compounds have been detected in tea, using various methods to isolate, concentrate and identify the volatiles. These volatiles include various hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones and phenolics, as well as oxygenated, nitrogenous and sulphur based compounds (Bondavich *et al.*, 1967; Yamanishi, 1978; Cloughley *et al.*, 1982; Kawakami and Yamanishi, 1983; Hazarika *et al.*, 1984; Kawakami *et al.*, 1993; Yamanishi, 1995).

The contribution and sensory impact of some of the volatiles to green tea quality are greater than others. Important components contributing to the aroma of green tea include (Z)-3-hexen-1-ol, (Z)-3-hexenyl acetate, plus linalool, geraniol, benzyl alcohol and dimethyl sulphide (Yamanishi *et al.*, 1970; Howard, 1978; Mahanta *et al.*, 1985; Kitamura *et al.*, 1992). Higher quality green teas, such as Gyokuro, have increased amounts of ionone and dimethyl sulphide.

Aroma compounds are frequently produced during processing, and are derived from a variety of precursors. Some aroma compounds, such as geraniol, linalool, benzyl alcohol and (Z)-3-hexenol are present as glucosides in fresh leaf (Co and Sanderson, 1970). Heating during processing activates ß-glucosidases, cleaving the glucoside moiety. Steaming and heating during processing also degrades carotenoids, forming the ionones (Co and Sanderson, 1970), while (E)-2-hexenal is derived from fatty acids, including linolenic, linoleic and palmitic acids (Saijo and Takeo, 1973).

Given the complexities of the aroma profile of green tea it is perhaps not surprising that reports linking aroma to change in quality associated with different harvesting practices are only for those compounds that are considered of greatest importance. The increased concentration of cis-3-hexenol and its esters, dimethylsulphide, indole,

alcohols, esters, and mono terpenes are for example reported to contribute to the higher quality of spring compared to summer green tea. In the summer crop, the concentration of these apparently desirable components declines and instead benzyl alcohol, Z-jasmone and nerolidol increase (Iwasa, 1987, cited Willson and Clifford, 1992; Choi, 1991). There are however few available reports on how the contribution of each of these aroma compounds to green tea quality varies with environment (day length and temperature), plucking standard or repeated harvesting. In black tea, the distinction between desirable and undesirable components of the aroma profile has been expanded forming quality indices based on relative terpene concentrations, to distinguish quality between different clones (Owuor and Takeo, 1987). Another example of quality determination in black tea based on aroma components is the volatile flavour compounds (VFC), which distinguishes undesirable components, thereby forming an index (Owuor *et al.*, 1990). There is presently no such index for green tea.

3.3.5 Fibre

The highest quality teas are produced from the first immature leaves of the new flush. As more leaves are harvested per shoot, the increase in leaf maturity and stalk makes processing more difficult, but also reduces tea quality (Selvendran *et al.*, 1972; Nakano *et al.*, 1996).

Measurement of the amount of fibre present in harvested shoots is a simple quantitative measurement of tissue maturity. Plant fibre is derived from the cell walls, and consists of cellulose, hemicellulose, pectins and lignin (Goodwin and Mercer, 1983). The primary wall consists of microfibrils of cellulose, the secondary wall hemicellulose. In dicotyledons, the hemicelluloses include xylan, and pectins, which are composed of polyruonic acids of galacturonic acid residues, arabinan and galactans. Lignin is composed of repeating units of phenylpropane, and is laid down following formation of the primary and secondary cell walls.

Selvendran *et al.*, (1972) reported an increase in the structural components from the first to fourth leaf; the eighth leaf containing significantly greater cellulose, hemicellulose and lignin. Transverse sections of leaves also showed differentiation of parenchyma to collenchyma, and the formation of sclerenchyma, also noted by Barua and Dutta (1959). Plant fibre has been quantified using the detergent system (Goering and Van Soest, 1970), both in tea (Nakano *et al.*, 1996), and more generally in feed analysis (Mongeau, 1993). Consistent with the observation of Selvendran *et al.* (1972), neutral detergent fibre was reported by Nakano *et al.* (1996) to increase from 8 to 15% in immature shoots, to 26% at a depth of 16 cm within the tea canopy,

reflecting shoot maturation. There are few reports on the effect of environment on fibre, with the exception of Yanase (1980); where it was reported that high temperatures increased stem and leaf lignification, contributing to increased hardness of these tissues.

Yield and quality of green tea are affected by several factors, including the plucking standard, environment, harvest and pruning. Quality of green tea can be defined by its chemical components; where high levels of phenolics, nitrogen and caffeine are desirable, while increased fibre is undesirable. Although the first spring flush harvested at a fine plucking standard is prized for its high yield and quality, there are few published reports on the effect of change in environment (not only temperature, but also day length) and plucking standard on yield and quality of tea undertaken in controlled conditions - which would allow for an assessment of each factor and the possibility of interactions between environment, plucking standard and harvest on yield and quality of tea.

4. Control of growth

In the previous section, changes in environment were presented as one mechanism that can potentially affect not only rhythmic growth in green tea, but as an extension of this, the yield and quality of the harvested shoots. Other mechanisms that are likely to influence rhythmic shoot growth in tea are changes in physiology, which will be discussed in the following sections.

4.1 Partitioning of assimilates

Partitioning of assimilates in plants is a function of sources and sinks. Sinks may be meristematic, elongation, or storage sinks (Gifford and Evans, 1981), and in tea potentially include new shoots and roots, flowers and fruit. Sinks are defined in several ways. *Actual sink strength* accounts for net carbon gain and respiratory losses. The *potential sink strength*, or *sink capacity*, is the maximum ability of a sink to obtain assimilates. Sink strength is quite difficult, if not impossible to measure (Ho, 1988; Farrar, 1996). Therefore most measures of sink strength usually concern *apparent sink strength.*

The competitive ability of a sink to attract assimilates is influenced by a number of factors; including phloem unloading; transfer efficiency between the phloem and the sink; and the relative distance between the source and the (competing) sinks (Wardlaw, 1990). Unloading of sucrose from the phloem is thought to occur passively through

the plasmadesmata in the symplast, down a concentration gradient driven by utilisation of sucrose by the sink (Turgeon, 1987). Hence, dominant sinks tend to be those with a high relative growth rate, rather than of larger size (Starck and Ubysz, 1974, cited Wardlaw, 1990). It is proposed that relative sink strength declines in order of flowers and fruit, buds, leaves, stems and finally roots (Kozlowski, 1992). In a plant cultivated only for shoot production, the shoot by default becomes the sink of primary importance, while the flowers and fruit are undesirable sinks. Harvesting and pruning practices maintain the dominance of (young) leaves as sinks for assimilate, therefore minimising the formation of flower and fruit, which would otherwise predominate (Wight and Barua, 1955).

In the mature tea plant, the main source of carbohydrate is current photosynthate (Sanderson and Sivipalan, 1966a; Manivel and Hussain, 1982a; Okano *et al.*, 1995), which is fixed predominantly in leaves, with a smaller proportion from other chlorophyll containing organs including branches (Sivipalan, 1975), flowers and fruits (Wardlaw, 1990; Kozlowski, 1992). Carbon incorporated into the leaf during photosynthesis can be directed through a variety of potential pathways. Fixed carbon can be further metabolised to starch, or transported from the chloroplast to the cytosol and incorporated into sucrose (Wardlaw, 1990). Sucrose is the main carbohydrate transported from leaves to sinks elsewhere within the tea plant (Sanderson and Perera, 1966). Temporary reserves of carbohydrates in tea are reported in leaves as starch (Selvendran and Selvendran, 1972), and in branches and roots again mainly as starch, but also as galactan, xylan, araban and rhamnan (Sanderson and Perera, 1966; Selvendran and Selvendran, 1972; Kandiah and Wimaladharma, 1978; Hakamata, 1983).

4.1.1 Assimilate partitioning and bud burst

Recently initiated leaf primordia require imported assimilate for growth and metabolism, as they are autotrophic (Fellows and Geiger, 1974; Turgeon and Webb, 1975).. Consistent with this, it is reported by Manivel and Hussain (1986) that ^{14}C label is partitioned from mature maintenance leaves to buds during bud burst. Similar observations are reported by Sleigh *et al.* (1984) in *Theobroma.* The relationship between bud sink strength and the rhythmic cycle has to date only been examined in *Quercus*, where there is evidence to suggest developing leaf primordia at bud burst replace the apex as the dominant sink for imported assimilate; a similar mechanism may be applicable to tea. In *Quercus*, ^{14}C DMO (5-5-dimethyloxazolidine-2,4-dione [2-^{14}C]) label was reported by Alatou *et al.* (1989) to accumulate in the apex during initiation, but declined as initiation at the apex ceased during shoot extension. It has been proposed by Alaoui-Sossé *et al.* (1994) and Parmentier (1993) (cited Crabbé and

Barnola, 1996) that reduced sink activity of the bud preceding the cessation of initiation leads to a shortage of sucrose, which is trapped in the axis below the bud. This region, in which the primordia for the new flush are rapidly expanding, is proposed to replace the apex as the dominant sink. Abbott (1970) has proposed a similar change in dominance leading to bud burst in apples, which is instead based on differences in hormone activity.

If current photosynthate is inadequate to meet demand, additional carbohydrate is mobilised from storage reserves, such as starch, or by a change in the partitioning of carbon between storage and translocation in leaves, or in the rate of photosynthesis (Wardlaw, 1990). Consistent with this the increase in demand for assimilate during spring bud burst and after pruning is reported to use both current photosynthate and carbohydrate reserves. Tea only becomes completely dependant on carbohydrate reserves to sustain bud burst and shoot growth after pruning, as all the maintenance leaves are removed. This is illustrated in the observations of Selvendran and Selvendran (1972), where it was reported that starch in the stem and root wood of tea declined after pruning, which indicated that these reserves were either depleted through root respiration, or alternatively translocated to the developing shoots. There is however some dispute in the literature concerning the use of starch reserves from stems and roots following pruning. In contrast to Selvendran and Selvendran (1972), Kandiah (1971) reported that starch in root wood could not be used to support shoot growth, as it was depleted immediately after pruning, presumably through respiration. In comparison, starch in the stem wood declined before bud burst, and was not replenished until the new shoots were established. The depletion of reserves in tea following pruning is comparable to that in deciduous perennials during spring growth (Kozlowski, 1992), where starch reserves decline in roots and branches, as reported in sweet cherry (Keller and Loescher, 1989) and apple (Yoshioka *et al.*, 1988). After leaves are fully expanded, reserves of starch again accumulate, until leaf fall in autumn and dormancy (Dickson and Nelson, 1982).

In contrast to pruning, bud burst during spring growth in tea is supported by both current photosynthate from leaves and carbohydrate reserves. Hakamata (1983) is one of the few to report that ^{14}C label that accumulated in soluble sugars and reserve carbohydrate in stems and roots of tea during autumn and winter was depleted before bud burst in spring, although there was also a potential loss of ^{14}C label due to respiration; while Okano *et al.* (1996) reported that current photosynthate generated by the overwintered leaves is likely to be used at bud burst, although this declined with mutual shading and the development of photosynthetic capacity of the new shoots. In contrast, in some perennial evergreen species, such as Scots pine (*Pinus sylvestris*),

assimilates for spring growth are mainly supplied from current photosynthate of the previous year's needles, and not reserve carbohydrate (Hansen and Beck, 1994). Leaves, which are a (temporary) site of starch storage, as shown by (Selvendran *et al.*, 1972) in tea, have however not been considered as potential contributors of carbohydrate reserves to shoot growth. The relationship between carbohydrate reserves and rhythmic growth in tea has not been examined, but given the evidence from cultivated tea it would appear that the assimilates required for bud burst are likely to be supplied as current photosynthate and mobilised carbohydrate reserves. The only reported evidence relating the utilisation of reserve carbohydrate during rhythmic growth is in *Quercus*, where Alaoui-Sossé *et al.* (1994) reported that starch reserves in stems declined during bud burst, and were replenished during dormancy.

Net photosynthesis has also been reported to increase in response to demand if carbohydrate reserves are inadequate. Increased rates of photosynthesis in maintenance leaves have been observed during spring growth and following harvesting in tea (Aoki, 1980, 1981); similar observations have been made following (artificial) defoliation in other species (Geiger, 1976; Herold, 1980). Photosynthesis is also reported to increase in leaves during fruit or seed formation, as observed in *Vitis* (Chaumont *et al.*, 1994). In *Quercus* (Hanson *et al.*, 1988) net photosynthesis of previous flush leaves increases during growth of the subsequent flush, apparently in response to increased demand. However, not all plants exhibiting rhythmic growth have associated increases in net photosynthesis at the start of shoot growth, as reported in *Hevea* where net photosynthesis is maintained at a constant level throughout the growth of new shoots (Samsuddin and Impens, 1979). Similarly, in *Theobroma*, photosynthesis in maintenance leaves remains constant during growth of new shoots (Baker and Hardwick, 1973; Sleigh *et al.*, 1984). Given that net photosynthesis in tea is reported to increase during harvesting and during rhythmic growth in *Quercus*, perhaps then net photosynthesis may also increase in response to increased demand during bud burst during rhythmic growth in tea. However, increased photosynthesis in response to increased demand for assimilate could also be affected by other factors, such as plant hormones (Herold, 1980). Given the reported literature from cultivated tea and other plant species exhibiting rhythmic growth, current photosynthate and carbohydrate reserves as starch, potentially from the stem, roots or leaves, may be utilised to provide a source of assimilate for bud burst during rhythmic growth in tea.

Changes in assimilate partitioning leading to increased net photosynthesis is thought to involve a feed-forward mechanism. An increase in photosynthesis is proposed by Stitt (1990) to increase the concentration of fructose 1,6 phosphate, which overcomes

inhibition of fructose 1,6 phosphate by fructose 2,6 phosphate, leading to, by the accumulation of substrate and increased sensitivity of sucrose phosphate synthetase, increased sucrose synthesis in the cytosol over starch synthesis in the stroma. For example, if leaf photosynthesis is inadequate to sustain demand for assimilate, leaf carbohydrate reserves will be mobilised, as observed in *Beta vulgaris* (Servaites *et al.*, 1989), and postulated during fruit growth in *Lycopersicon*(Walker and Ho, 1977). Conversely, partitioning of starch will be favoured over sucrose when sucrose accumulates in leaves, such as due to a fall in demand for assimilates,. This is apparently related to inactivation of sucrose phosphate synthetase (SPS), resulting in increased fructose 2,6 phosphate, which inhibits fructose 1,6 phosphatase and hence sucrose synthesis (Foyer, 1988; Stitt, 1990).

Assimilate partitioning may also be controlled at the source through phloem loading. Loading of sucrose from leaf mesophyll cells into the vascular system is thought to occur through the apoplast, followed by loading into the companion cells of the sieve cells (SC-CC complex) of the phloem, driven by a proton motive force (Giaquinta, 1983). The requirement for ATP, and the area of the SC-CC complex, are potential factors limiting apoplastic phloem loading. Sucrose could alternatively be loaded into the phloem through diffusion through the symplast to the SC-CC complex, although the apparent accumulation of sucrose in the SC-CC weakens this argument (Geiger *et al.*, 1973).

4.1.2 Assimilate partitioning and the developing shoot

The development of photosynthetic capacity by the newly expanding shoot will alter partitioning of assimilates, which can be shown to be dependant on the pattern of shoot development. In tea, leaves and their associated internodes unfold sequentially from the apex, resulting in a gradient in leaf age from the youngest leaf, at the apex, to the oldest leaf, at the start of the flush (Bond, 1942). Photosynthesis is generally reported to commence during early leaf development, but does not reach a maximum until later in shoot development. Consistent with this, Barua (1960) reported that net photosynthesis in recently unfolded leaves of tea, at around 3 μmol CO_2 m^{-2} s^{-1}, was around one-third of that of fully expanded leaves. There is evidence that older leaves incorporate greater quantities of ^{14}C label through photosynthesis than the younger leaves of a shoot (Sanderson and Sivipalan, 1966a), which is reflective of leaf maturation. Similarly, although in cultivated tea, Okano *et al.* (1996) reported that fixation of ^{13}C label was greater towards the centre of the layer of developing shoots, declining in the upper layers which had a greater proportion of immature leaves, while comparatively low fixation of ^{13}C label in the lower layers was attributed to mutual shading.

However, not only is there a gradient in leaf maturation along the shoot, but also within the leaf itself. Larson *et al.* (1972) for example reported that leaves of *Poplar* matured basipetally, with the result that the tips of the leaf blade exports assimilate, while the remainder of the leaf is still photosynthetically immature. Assimilate produced in leaf tips and imported assimilates are then used to complete leaf development (Dickson and Larson, 1975), with some export of assimilate occurring at 40 to 50 % of full leaf expansion (Dickson and Shive, 1982). However, developing leaves have an overall net requirement for imported assimilate to sustain leaf growth, until they become net exporters of assimilate when leaf expansion is almost complete (Dickson and Shive, 1982).

That newly unfolded leaves of tea require imported assimilate for growth has been shown using autoradiography by Sanderson and Sivipalan (1966b); young leaves were reported to import ^{14}C label, as current photosynthate, from fully expanded maintenance leaves further down the stem. Qualitative autoradiography, as used by Sanderson and Sivipalan (1966b), can not however account for potential differences in import of ^{14}C label between leaves of different stages of development. Similarly, Manivel and Hussain (1986) have reported that a greater percentage of ^{14}C label from maintenance leaves is imported to the bud compared to shoots of one to three leaves, which was interpreted to indicate that the sink capacity declined with shoot development; but again there was no account for differences in import of ^{14}C label due to differences in leaf maturation. An assessment of the partitioning of ^{14}C label from a mature source leaf to the individual components of the developing shoot (leaves and internodes) would clarify the relationship between shoot development and assimilate partitioning.

The continued demand for assimilate by the expanding shoot is a likely explanation of the low quantities of starch detected in stems in tea during shoot extension as reported by Kandiah (1971). There is also evidence in *Theobroma* (Sleigh *et al.*, 1984) and *Quercus* (Alaoui-Sossé *et al.*, 1994) for increased turnover of assimilate in mature leaves during shoot extension, due to increased export of ^{14}C label from mature source leaves; which in *Quercus* is also associated with continued high rates of net photosynthesis (Hanson *et al.*, 1988). Although it is presently not known whether shoot extension in tea is associated with increased turnover of assimilate in leaves, it is perhaps unlikely that net photosynthesis remains elevated in tea during rhythmic growth after initial bud burst, given that this does not occur in cultivated tea (Aoki, 1981).

At some point towards the completion of flush growth, the requirement for imported assimilate by the leaves will decline, as previously mentioned, as the leaves become

net exporters of assimilate. As a result, assimilate will instead be partitioned to other tissues. This is reflected in mature shoots of tea, where Sanderson and Sivipalan (1966b) reported that ^{14}C label was partitioned only to the bud and stem. The lack of imported label into the mature leaves of the flush is consistent with leaves being net exporters of assimilate. Similarly, in *Theobroma*, Sleigh *et al.* (1984) reported that as leaves approached full expansion during dormancy, ^{14}C label was instead partitioned to buds and roots, which are reported to alternate with shoot growth. The accumulation of ^{14}C label in buds during dormancy is significant as it is consistent with the recommencement of initiation of primordia, at least in *Quercus* (Crabbé and Barnola, 1996). In tea, partitioning of assimilates to roots is also likely to occur at the completion of shoot growth, given reports that root growth alternates with rhythmic growth (Kandiah, 1971) and that during dormancy in autumn and winter ^{14}C label fixed in leaves is either lost through respiration or partitioned to the roots and stems of the mature tea bush (Hakamata, 1983).

Associated with the decline in demand and the completion of flush growth in *Theobroma* is the reported reaccumulation of carbohydrate reserves in the stems and roots (Sleigh *et al.*, 1984), as is similarly reported in *Quercus* (Alaoui-Sossé *et al.*, 1994), but not to date in tea. In tea, the only comparable situation is after harvesting or pruning, where carbohydrate reserves are reported to reaccumulate in the roots and wood at the completion of flush growth (Kandiah, 1971; Selvendran and Selvendran, 1972).

Maximum reported rates of net photosynthesis of fully expanded leaves of tea start at 4 μmol CO_2 m^{-2} s^{-1}, as reported by Smith *et al.* (1993b, 1994), with the majority otherwise reported at around 10 to 14 μmol CO_2 m^{-2} s^{-1} (Barua, 1960, 1964; Sakai, 1975; Squire, 1977; Roberts and Keys, 1978). Some of the variation in reported photosynthetic rates for tea could be attributed to using detached leaves to measure photosynthetic rates (Barua, 1960; 1964; Sakai, 1975), which may be underestimated should stomates close (Lakso, 1982). The comparably low rate of photosynthesis reported by Smith *et al.* (1993b, 1994), using infra red gas analysis under field conditions, is not easily explained, but could similarly be due to closure of stomates (Barua, 1960) as a result of decreased air humidity in the leaf chamber. Net photosynthesis also varies between different species of *Camellia*, for example, photosynthetic rates of ornamental species *C. japonica* and *C. rusticana* are 6.0 μmol CO_2 m^{-2} s^{-1} and 3.9 μmol CO_2 m^{-2} s^{-1} respectively (Kume and Tanaka, 1996). Photosynthesis in tea is considerably lower than other C3 crop species, which typically vary from 10 to 20 μmol CO_2 m^{-2} s^{-1} (Larcher, 1995).

Net photosynthesis in tea is also reported to vary with light intensity and temperature; which is a common feature to all plant species (Salisbury and Ross, 1985). Photosynthesis in tea becomes saturated at light intensities of around 400 μmol photons m^{-2} s^{-1} (Okano *et al.*, 1995); below this light intensity becomes limiting and photosynthetic rate declines. Shade culture is used to produce high quality green tea (Saijo, 1980; Owuor and Othieno, 1988), but is associated with declining yields when compared to tea grown under normal light conditions as light intensities limit photosynthesis (Willson and Clifford, 1992). Optimum temperatures for photosynthesis are reportedly between 20 to 25 °C (Sakai, 1975; Smith *et al.*, 1993b). Photosynthesis is reported by Sakai (1975) to decline at temperatures greater than 30 °C, which was only attributed to increased dark respiration. Photorespiration, due to inhibition of photosynthesis by increased concentrations of oxygen at high temperatures, is likely to contribute more to the decline in net photosynthesis at high temperatures (Salisbury and Ross, 1985), but was not considered by Sakai (1975).

Newly developing leaves were identified as the desired sink for assimilate in tea production; yet the relationship between assimilate partitioning and rhythmic growth in free growing tea has not been previously examined. Based on reports from cultivated tea and other species exhibiting rhythmic growth, imported assimilates required for bud burst during rhythmic growth in tea are provided by current photosynthate and carbohydrate reserves, and could potentially also involve increased rates of net photosynthesis in the mature leaves. The subsequent growth of new shoots is likely to reduce the demand for assimilate as the photosynthetic capacity of the shoot increases; but there is currently a lack of data relating the partitioning of fixed carbon and net photosynthesis to leaf and internode expansion during shoot growth. This information would not only provide a basis for understanding rhythmic growth in tea, but also further clarify the relationship between assimilate partitioning and shoot growth and development in cultivated tea.

4.2 Plant hormones

The discovery that the control of rhythmic shoot growth in perennial species was endogenous led many to believe that hormones, as growth inhibitors and promoters, controlled rhythmic growth. Early experiments showed that grafting a bud in the active phase of development onto a similarly active scion increased plant height and length of leaves and internodes (Kulasegaram, 1969b). In contrast, less growth occurred in grafts between dormant buds and scions - leading to the proposal that plant hormones, or some transportable substance, controlled dormancy in tea. This is

discussed in the following sections based on reports linking shoot growth and extension to applied and endogenous hormone concentration.

4.2.1 Plant hormones and dormancy

Abscisic acid (ABA), as an inhibitor of growth, on first appearances, is perhaps the most likely candidate to regulate dormancy in tea. In *Gnetum*, for example, prolonged application of exogenous ABA was reported by Mialoundama *et al.*(1984) to reestablish bud dormancy during rhythmic growth by inhibiting initiation of primordia at the apex. Similarly, Abo-Hamed *et al.* (1981) reported in *Theobroma* that applied ABA extended the length of the dormant period. Perhaps consistent with this was the observation that the removal of cataphylls, which enclosed the bud during dormancy, promoted shoot extension (Abo-Hamed *et al.*, 1981; Mialoundama *et al.*, 1984). There is, however, the possibility that removal of the cataphylls modified the sink-source balance which instead promoted growth.

However, it has been difficult to prove that the observed response is caused by the applied hormone, due to limitations in the technique. In many cases it is unknown how much hormone is absorbed, due to either poor penetration into the target tissue or degradation prior to uptake. Metabolism of a plant hormone following uptake can also vary depending on both the stage of development and environmental conditions at the time and following application (Lavender and Salim, 1987). Also, little is known about the distribution of hormones following application, and whether hormone distribution reflects compartmentation (Lavender and Salim, 1987). Finally, the response may not be a true reflection of the physiological state in question; where, for example, dormancy induced in buds by the application of ABA is frequently transient (Trewavas and Jones, 1991), and the inhibition of growth by ABA in some instances could be due to closure of stomates, which inhibits photosynthesis (Cummins *et al.*, 1971). Such problems can be overcome by quantifying endogenous hormone concentration in the tissue in question; using techniques such as immunoassays, HPLC and GC/MS. The use of immunoassays is valid so long as there is no competitive or non-competitive inhibition between the antibody and substances other than the plant hormone being quantified (Pengelly, 1985).

In *Theobroma*, high concentrations of ABA in leaves in the last phase of dormancy were reported by Orchard *et al.* (1980, 1981) and Abo-Hamed *et al.* (1981) to maintain, rather than induce bud dormancy occurring during rhythmic growth. ABA has also been implicated in the regulation of the first two of three physiological stages of dormancy, which include the induction, maintenance and release from dormancy (Dennis, 1994); although evidence supporting an increase in ABA during winter is

conflicting. There is a general lack of support in the literature that the increase in ABA is due to change in photoperiod. For example, there was no reported correlation between ABA and bud dormancy induced under short day lengths in either *Acer* or *Betula* (Lenton *et al.*, 1972; Loveys *et al.*, 1974; Phillips *et al.*, 1980; Barros and Neill, 1986). The lack of any clear relationship between ABA and photoperiod has led to the conclusion that ABA is not the sole cause of dormancy (Powell, 1987).

The potential effect of temperature on ABA has not been discounted, due to reported correlations between winter conditions and ABA in tea, where it was proposed a decline in free to bound ABA in shoots reported by Nagar (1996) both induced and maintained bud dormancy. ABA has also been reported to increase over winter in other perennial species including *Acer* (Phillips and Wareing, 1958), *Corylus* (Rodríguez *et al.*, 1991), and *Betula* (Rinne *et al.*, 1994). The lack of agreement between these examples may not necessarily be related to the conditions, but rather the method used to quantify hormone concentration. Although the development of immunoassay and GC/MS techniques has significantly improved hormone detection and quantification, bulk tissue was previously required to quantify endogenous hormones because of their very low concentration in plant tissue (usually in nanograms). As a result, there is a considerable dilution effect when entire shoots are used to quantify hormones, rather than the bud. For example, Barros and Neill (1986), Johansen *et al.* (1986) both quantified ABA in entire shoots, rather than the bud, in which ABA was later shown to significantly increase during dormancy (Rinne *et al.*, 1994). However, even the quantification of ABA in the entire bud may bear little relationship to the concentration of hormone in meristems, or cell, cell compartment or hormone receptor site (Davies, 1995).

It has been suggested that the (sometime) lack of correlation between observed response and hormone concentration could be attributed to changes in tissue sensitivity to the particular hormone (Trewavas, 1982). There is still, however, enough evidence to warrant an examination into whether or not endogenous ABA is correlated with dormancy occurring during rhythmic growth in tea.

4.2.2 Plant hormones and shoot growth

Although ABA is thought to be in some way involved in the induction and maintenance of dormancy, few reports support a role of ABA in the release from dormancy; which is instead thought to involve other factors which override ABA (Powell, 1987). The possibilities include gibberellin (GA), auxin and cytokinins, which may promote internode extension and leaf expansion (Davies, 1995). Reports supporting this proposal in relation to rhythmic growth usually cite that applied GA

and cytokinin promotes bud burst both in tea (Kulasegaram, 1969a, 1969b; Kulasegaram and Kathiravetipillai, 1972; Manivel *et al.*, 1981) and in other species exhibiting rhythmic growth (Champagnat and Come, 1986; Parmentier *et al.*, 1991; cited Crabbé and Barnola, 1996). For example, GA, and zeatin, when used individually, or in combination, will reduce the length of dormancy by two weeks (Kulasegaram, 1969a, 1969b, Kulasegaram and Kathiravetpillai, 1972; Manivel *et al.*, 1981); and are also reported to produce a transient increase in yield and quality, although this is apparently dependant on variety (Barman and Manivel, 1989; Liang *et al.*, 1996). However, as discussed previously, bud burst promoted by applied hormones may not necessarily reflect endogenous activity.

Of the various GA's detected in plants, GA 1 is proposed to be the bioactive form regulating shoot elongation, in both annual (Sponsel, 1995), and perennial woody species. Examples of perennial species in which GA1 is reported to be the active form include *Silex* (Junttila and Jensen, 1988), *Pseudotsuga* (Graham *et al.*, 1994), *Alnus*, *Betula* and *Populus* (Zanewich and Rood, 1994), and *Citrus* (Plummer *et al.*, 1991). However, the active GA in tea is not known.

A primary role of GA is in the regulation of internode elongation, which has been well documented (Davies, 1995). It would then seem logical that endogenous GA may be involved in the regulation of bud burst, which in tea is the culmination of a shortened plastochron and, more significantly, rapidly expanding internodes (Bond, 1942). The only reports to date which link bud burst in tea to GA concentration is by Kulasegaram and Kathiravetpillai (1979), where a compound exhibiting GA-like activity was shown using paper chromatography, to be in higher concentration in actively growing terminal buds compared to dormant lateral buds. Although there has been a significant improvement in techniques for hormone quantification since the report of Kulasegaram and Kathiravetpillai (1979), there has been no confirmation that GA is associated with bud burst in tea.

GA has been correlated with spring bud burst in perennial species, although reports are contradictory and vary with species. For example, bud burst in *Salix* was reported to be correlated with an increase in GA (Lenton *et al.*, 1972), but in *Populus* there was no reported correlation between GA concentration and bud burst (Bachelard and Wightman, 1974). There is, however, evidence that in some instances the effect of GA may be related to a change in turnover, rather than concentration, as demonstrated in spinach. Zeevart (1974), for example, reported that increased shoot extension resulting from a change in photoperiod was correlated with an increase in turnover of GA. An examination of GA concentration in buds and leaves in relation to bud burst during rhythmic growth is then warranted.

Increased concentration of endogenous cytokinin has also been implicated in the promotion of bud burst. For example, in *Theobroma*, Orchard *et al.* (1981) reported that the concentration of cytokinin increased in mature leaves at the time of bud burst during rhythmic growth. It was proposed that increased activity of roots during dormancy, as the site of cytokinin synthesis, led to increased cytokinins in the shoot. Consistent with this is the reported increase in concentration of cytokinin in roots and stems in *Malus* (Luckwill, 1970) and buds of *Populus* (Hewett and Waering, 1973) before bud burst. In contrast, Dumbroff and Brown (1986) reported an increase in cytokinin activity in roots during spring, but as this occurred after bud burst it was concluded that cytokinins were unlikely to be the stimulus for dormancy release.

In tea, cytokinin-like activity has previously been reported in lateral buds by Kulasegaram and Kathiravetpillai (1979), but to date cytokinin has not been examined in relation to bud burst during rhythmic growth. Observations that root growth alternates with shoot growth of tea (Kandiah, 1971) may be consistent with that reported in *Theobroma* (Orchard *et al.*, 1981), and could potentially lead to increased cytokinin in the shoots and bud burst.

It has also been proposed that a decline in cytokinin may promote bud dormancy. Abbott (1970), for example, proposed that a decline in supply of cytokinin from the roots of apple led to the abortion of leaf primordia at the apex, such that scales became the dominant sink. This was proposed not to be overcome until senescence of the scales, which modified partitioning in favour of the apex. It can then be concluded that a change in concentration of cytokinin in leaves and buds of tea may be involved in the regulation of rhythmic growth in tea.

There is however little evidence supporting a role for auxin in promoting shoot extension. Auxin does not stimulate bud burst in tea, nor other perennial species, and is therefore considered not to control dormancy (Kulasegaram and Kathiravetpillai, 1972; and Lavender and Salim, 1987; Powell, 1987 respectively). Auxin is instead likely to be of greater importance to shoot growth in cultivated tea, through apical dominance. Auxin, produced in the apex and transported basipetally down the stem, is thought to inhibit the production of auxin in lateral buds, hence inhibiting lateral bud growth (Cline, 1991). This assumes that the export of auxin from the lateral bud is required for growth (Bangerth, 1989).

4.2.3 Plant hormones and assimilate partitioning

As previously discussed, assimilate partitioning reflects the balance between sources and sinks within plants, and thus contributes to the control of plant growth. It is proposed that endogenous plant hormones tend to accumulate in areas of metabolic activity (sinks), are transported along the same pathways, and consequently influence assimilate partitioning through altering leaf photosynthesis, sucrose synthesis, and the transport and accumulation of assimilates (Brenner and Cheikh, 1995). This is illustrated in the following examples.

Increased rates of photosynthesis, for example, which are reported to occur in response for increased demand for assimilate, could potentially be mediated by plant hormones (Brenner and Cheikh, 1995). Caers and Vendrig (1986) for example, have reported that net photosynthesis in *Zea* is increased by applied cytokinins, by increasing leaf chlorophyll and associated enzyme activity. Similarly, net photosynthesis is reported by Tamas *et al.* (1973) (cited Brenner and Cheikh, 1995) to increase following the application of IAA, by increasing phosphorylation and net fixation of carbon dioxide; although Robinson *et al.* (1978) failed to observe a similar response. In contrast, applied ABA has a well documented effect on decreasing photosynthesis, which is due to stomatal closure and reduced gas exchange rather than any direct effect on photosynthesis (Cummins *et al.*, 1971; Hein *et al.*, 1984). Plant hormones have not only been implicated in increasing the rate of photosynthesis, but also in the subsequent partitioning of assimilates through the control of sucrose synthesis (Brenner and Cheikh, 1995). For example, applied GA to soybean leaves is reported by Cheikh and Brenner (1992) to increase SPS activity, which is one of the enzymes thought to control the pool size of sucrose in leaves (Stitt, 1990). In contrast, applied ABA is reported by Cheikh and Brenner (1992) to inhibit the activity of fructose biphosphatase.

There is also some evidence to suggest that IAA and GA may affect the transport of assimilate in the phloem. Phloem loading is apparently increased by applied IAA (Baker *et al.*, 1985); and Patrick (1979) has proposed that applied IAA to decapitated bean plants directs assimilates to the cut stump, which regulates the concentration of ions in the phloem and translocation of assimilates. Similarly, applied GA is reported by Aloni *et al.* (1986) to increase phloem loading in broadbean; while in contrast applied ABA may apparently inhibit phloem loading (Baker *et al.*, 1985). However, applied BA to capsicum leaves increases the rate of leaf expansion, and hence competitive ability, but does not change sucrose uptake, which suggests that cytokinin is not involved in phloem loading (Nielson and Ulvskov, 1992). The majority of

research into control of phloem unloading by plant hormones has involved reproductive tissues (Brenner and Cheikh, 1995), and is thought to be promoted by cytokinins and ABA. For example, uptake of sucrose into *Glycine* cotyledons is reported to increase following the application of ABA; however this apparent trend does not appear to occur in all species (Brenner and Cheikh, 1995).

The mechanism by which hormones may influence assimilate partitioning still remains unclear, and most of the evidence is from the effect of applied hormones, which as previously discussed may not be a true reflection of the endogenous process. Although there have been no specific studies on hormone directed transport in relation to assimilate partitioning in tea, the possibility cannot be discounted, given the presented evidence from other species.

5. Summary and scope of thesis

South east Asia is the greatest producer of green tea, but demand currently exceeds supply. In Japan, for example, the amount of land available for cultivation is declining as the population increases, while the popularity of green tea in western countries is increasing. The opportunity therefore exists for countries outside of south east Asia, such as Australia and New Zealand, to produce and export green tea.

Green tea is produced from only the young, vegetative shoots, which are harvested up to a maximum of four times per year, and, unlike black tea, is an unfermented product. This retains the green colour of the leaf and associated flavour and aroma compounds. Increasing yield through harvesting more leaves per shoot reduces tea quality by increasing fibre, which is undesirable, and decreasing the concentration of desirable compounds such as catechin, nitrogen and amino acids. Consequently, the production of green tea is a balance between yield and quality.

Yield of green tea is a function of shoot growth, which under natural conditions, occurs in repeating cycles of growth and dormancy. In cultivated tea, shoot growth is promoted by releasing lateral buds from apical dominance. The rate of shoot growth in tea may be affected by environmental conditions, such as air and soil temperature, humidity and day length, which then affect yield. The control of shoot growth in tea is ultimately due to endogenous factors, such as assimilate partitioning and hormones.

This study has concentrated on developing an understanding of the factors which control shoot growth in tea. This is considered essential to the successful production of tea, as the manipulation of yield and quality in tea can then be based on scientific knowledge. It is logical to begin by considering shoot growth in tea under natural conditions, that is, without harvesting. Apart from early research concerning rhythmic growth, for example, Bond (1942, 1945), little advances have occurred in our understanding of this fundamental process in tea. This study commences by quantitatively describing shoot growth in tea, considering both measurable changes in morphology of the shoot and terminal bud. This data was subsequently used to develop specific quantitative, and hence reproducible, stages of growth to describe shoot development in tea.

In other species, rhythmic growth is considered to be endogenous, as the environment modifies, rather than controls the rhythmic growth cycle. Although rhythmic growth in tea is considered endogenous, there is currently a lack of evidence to support this claim. Quantification of (rhythmic) shoot growth under a range of environmental conditions of temperature and day length would not only provide evidence that the

rhythmic growth cycle in tea is endogenous, but also further elucidate the role of these factors in tea dormancy. Such an experimental design would also provide an opportunity to assess the effect of temperature and day length on harvested yield and quality, which is currently poorly documented in the literature and as a consequence not well understood.

Rhythmic growth can also be described in terms of changes in physiology, which together with changes in morphology, culminates in rhythmic growth. Of particular interest, are changes occurring in photosynthesis, the partitioning of assimilates, and hormones. Although maintenance rates of photosynthesis in tea are well documented, it is currently not known if photosynthesis varies during rhythmic shoot growth, as in other similar perennial species, although photosynthesis is known to increase in response to demand after harvesting and during spring growth. Changes may similarly occur in the partitioning of assimilates during shoot growth, which shall be assessed using quantitative ^{14}C labelling. Finally, there has been no research into the potential role of (endogenous) hormones in the inhibition or promotion of rhythmic growth in tea.

III. General materials and methods

1. Plant material, media and nutrition

Three varieties of green tea, Sayamakaori, Okuhikari and Yabukita, were used in these experiments.

Plants were maintained in pots under controlled glasshouse conditions (temperature differential of 15 to 25 °C, day length of 16 hours maintained using artificial lighting) and a shade tunnel at the Horticultural Research Centre (HRC) at the University of Tasmania. The potting medium was a 7:2:1 mixture (total volume of 50 L per mix) of pine bark:sand:peat, and included ferrous sulphate (25 g), slow release Micromax (20 g) and Osmocote (300 g), at a pH of 5.5.

Plants were watered daily with either overhead watering or drip irrigation. Plants were fertilised weekly using Normal Hoaglands solution (Anon., 1950). Malathion and Ampol DC white oil were applied when necessary to control scale insect, as was Pyrethum for light brown apple moth and Pyranica for mites. Pot sizes were increased from 8" to 12" pots during the project, to maintain plant vigour.

2. Solvents and reagents

Reagents were reagent grade and obtained from either Sigma-Aldrich Australia Pty. Ltd, or Ajax Chemicals Pty. Ltd. Australia, unless otherwise stated.

Technical grade acetone and ethanol were double distilled before use.

HPLC grade methanol was obtained from Mallinckrodt.

3. Apparatus

Balances:	2 decimal places, Mettler PM 4600
	4 decimal places, Mettler AE 200
Distilled water:	Still from Labglass, Qld, Aust.
Centrifuges:	Easy-Spin, Sorvall Instruments, Du Pont, max. 6000 rpm.

	Beckman J2-21ME; Beckman Instruments, Aust. Pty. Ltd.
Freeze drier:	Dynavac High Vacuum Pty. Ltd. Aust., Model FD IG.
Hammer mill:	Glen-Creston, Stanmore, 1.5 mm mesh screen.
Hot plate:	Woodson Technolab Marketing Pty. Ltd. Aust.
IRGA:	Analytic Development Co. Ltd. fitted with a Portable Leaf Chamber analyser (PLC) and Type 3 Parkinson Leaf Chamber (PLC).
Light meter:	Lambda L1-185 meter fitted with a quantum flux sensor; range 400 to 700 nm.
pH Meter:	Radiometer, Copenhagen.
Scintillation counter:	Beckman
Spectrophotometer:	Shimadzu UV-160.
Speed Vac concentrator:	Savant, SVC 200H.
Stereo microscope:	Olympus SZ40, Japan.
Vials:	6 mL glass scintillation vials, Canberra-Packard. 20 mL glass scintillation vials, Kimble. 12x55 mm Greiner round bottom polypropylene test tubes, Interpath Services, Aust.
Vortex:	Scientific Industries Australasia Pty. Ltd., Model K-550-GE.
Water baths:	FSE Scientific Mixer, Hakke D1 Orbital shaking water bath, Paton Industries Pty. Ltd., Model OW 1412

4. Environment

4.1 Glasshouse

Glasshouse temperatures were maintained at 15 °C (night) to 25 °C (day). Incident light in the glasshouse was reduced with a heavy application of glasshouse whitewash, as otherwise plants were susceptible to leaf scorch. Whitewash was renewed in spring, and maintained throughout summer. Light intensity varied between 200 to 500 μmol photons m^{-2} s^{-1}. Daylength naturally varies between 9.04 (winter) to 15.30 hours (summer) annually. Artificial lights (400 W mercury vapour lamps) were used to maintain a 16 hour daylength.

4.2 Shade tunnel

The shade tunnel was constructed of Sarlon shade cloth, which reduced light intensity to two-thirds of natural sunlight. Plants were otherwise subject to fluctuations in temperature. Plants were maintained in pots on a dolerite gravel bed, with overhead watering as required.

4.3 Growth cabinets

Three controlled growth cabinets were housed in the main glasshouse complex at the HRC. The cabinets were each 1.5 x 4 m in area, light proof and lined with polystyrene blocks 50 mm thick. Constant night temperatures were maintained using a thermostatically controlled, refrigerated cooling system. Daylength was controlled using electronically operated trolleys. The trolleys could be programmed to move in and out of the cabinets, thereby manipulating daylength. Where necessary, daylength was extended using 400 W mercury vapour lamps suspended within the cabinets. This gave a light intensity of 100 μmol photons m^{-2} s^{-1} at plant level.

Four treatments of all possible combinations of long (16 hour) and short (9 hour) daylengths with high (15 °C) and low (5 °C) night temperatures were maintained using the three cabinets and main glasshouse (Table 1).

Table 1. Environment conditions

Day length	Temperature (°C)	
(hours)	15	5
16	LD HN	LD LN
9	SD HN	SD LN

5. Growth measurements

Growth measurements, either at 2 to 3 day intervals or at the completion of growth, included the following (Fig. 1):

A. Total shoot length, from base of shoot to bottom of bud (mm).
B. Length leaf blade, excluding petiole (mm).
C. Length internodes, between leaves from abaxial insertion of petiole (mm).
D. Number and length (mm) of bud primordia, using stereo microscope.

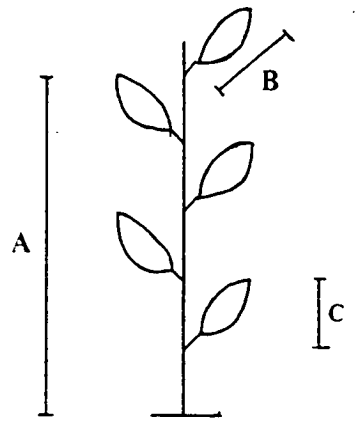

Figure 1. Growth measurements A) shoot length; B) leaf length; and C) internode length

6. Plant sampling

Plants were sampled according to the stages of development (Fig. 2):

I. Bud burst, expansion of cataphylls

II. Shoot extension, involving expansion of leaves and internodes

III. Dormancy, completion of shoot extension

IV. Completion of leaf expansion.

Stages of growth within each flush are identified as 1 and 2.

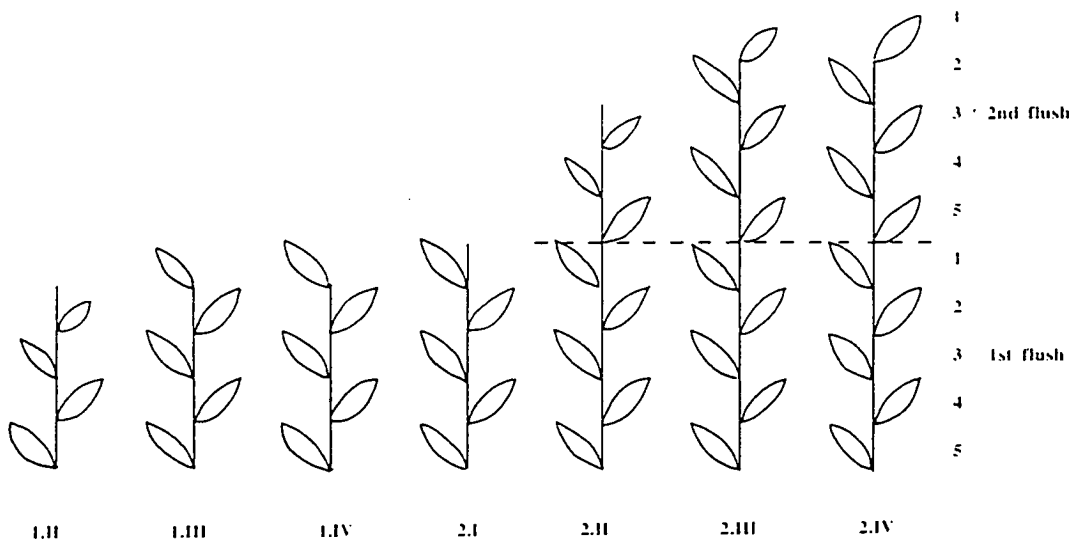

Figure 2 Plant sampling strategy for I) start of shoot growth; II) shoot extension; III) dormancy; and IV) completion of shoot growth; for 1) first and 2) second flushes. Numbers 1-5 denote leaf and internode position.

7. The logistic model

The maximum shoot length and number of leaves per shoot, the rate of shoot extension and rate of leaf unfolding was estimating by fitting data to the logistic model using Systat (version 5.2 for the Mackintosh):

$$y = c\,[1 + e^{-b(t-m)}]$$

where y is shoot length; t is time and c, b and m are parameters of the curve. The parameter c gives the upper asymptote of the curve (the maximum attainable shoot length), m gives the time at the point of inflection (ie. the time of maximum absolute growth rate) which occurs when the shoot has length ce^{-1}, and b, often referred to as the slope parameter, gives the relative growth rate at the point of inflection and hence the maximum absolute growth rate bce^{-1}.

8. Tea processing

Following harvest, shoots were semi-processed using tea processing machinery (Terada Seisakusho Co. Ltd. Kanaya, Japan) at the Department of Primary Industries and Fisheries, New Town Research Laboratories. Only the first and last (of six) machines were used, including the tea steamer, shown in Plate 1 (Terada Tea Steaming Machine, ED2000) and tea drier, shown in Plate 2 (Terada Tea Drier, ND4-60Sp). Semi-processing the harvested tea was chosen as it provided a reliable indication of quality characteristics (pers. comm., G.Brown) and could be used with the small sample sizes (1 to 14 g, dw) used in this study. During steaming, fresh leaves were fed on a wire mesh conveyor belt into the steaming machine, and steamed at 100 °C for 60 seconds. Steam was produced by heating water in a boiler (Simons VS300 electric boiler, 60 kW by Simons and Sons, NSW, Australia), from which steam was carried to the tea steamer through insulated copper piping at a rate of 25 kg $hour^{-1}$. Steamed leaves were deposited into a metal hopper through which air (room temperature) was circulated. Leaves were then spread into stainless steel trays with a fine mesh base, which fit into drawers within the tea drier. The gas powered tea drier heated air to 80 °C, which was passed through the drier using forced air ventilation. Leaves were dried to a constant weight in approximately 4 to 6 hours.

Plate 1. Boiler, and tea steaming machine

Plate 2. Tea drying machine

9. Quality assessment

Following processing, the dry weight was recorded per sample. Samples were then ground using a hammer mill with a 1.5 mm mesh screen. Four components of quality were assessed, including neutral detergent fibre, total phenolics, total nitrogen and aroma.

9.1 Total phenolics

The procedure for determining total phenolics was modified from Singleton and Rossi (1965) and Booker *et al.* (1996).

Dried (100 mg), ground sample was added to a 20 mL glass scintillation vial, to which 15 mL of distilled water was added, and then heated at 60 °C for one hour in a shaking water bath. The sample was then centrifuged at 16 000 rpm for 6.5 minutes to separate the extract from the solid fraction. An aliquot of 50 µL was added to a 6 mL glass scintillation vial, plus 1.5 mL of 0.25 N Folin-Ciocalteu reagent, and after 3 minutes, 1.5 mL of 1 M sodium carbonate. Absorbance at 724 nm was measured after 60 minutes using a spectrophotometer. A standard curve was prepared using (-) catechin, the concentration of which ranged from 0.1 mg ml^{-1} to 2.0 mg ml^{-1}. Assay results were expressed as percentage catechin equivalents.

9.2 Total nitrogen

The procedure for determining total nitrogen was modified from The Ministry of Agriculture, Fisheries and Food (1986), and Reddy (1987). Nitrogen was determined though conversion of ammonium-nitrogen by digestion with sulfuric acid and sodium sulphate with a sodium-selenium catalyst. The ammonia liberated with sodium hydroxide was removed by steam distillation and determined titrimetrically.

Dry (100 mg) ground samples were weighed into a Tecator Kjeltec digestion tube, to which a Kjeldahl catalyst tablet and 5 mL of concentrated H_2SO_4 (containing 1 g per 30 mL salicyclic acid) were added. A blank, containing 50 mg of glucose, was also included. The solution was refluxed at maximum heat until the solution cleared to a straw yellow colour, then cooled and diluted with 20 mL of distilled water to prevent sulphate precipitation. The solution was distilled in a Kjeltec system 1002 unit, after adding 25 mL of 50 % NaOH. The distillate was collected in a flask containing 10 mL of 4% H_3BO_3 with bromocresol green-methyl red indicator, and titrated against 0.1 N HCl. Results were expressed as % nitrogen in tissue.

9.3 Neutral detergent fibre (NDF)

The NDF procedure was modified from Goering and Van Soest (1970). The NDF solution (1L) contained 1 L distilled water, 30 g sodium lauryl sulphate, 18.61 g disodium ethylene diamine tetra acetate (EDTA), 6.81 g sodium borate decahydrate, 4.56 g disodium hydrogen phosphate and 10 mL 2-ethoxyethanol.

Dried (300 mg), ground sample was placed in a 1 L round bottomed flask, to which 100 mL of NDF solution, 2 mL decahydronaphthalene and 0.3 g sodium sulphite were added. The flask, fitted with a condenser, was refluxed for 1 hour in a sand bed heated by a metal hot plate.

The solution was immediately filtered through a ceramic crucible, leaving the fibre residue. Two 20 mL washes of hot water were used to rinse the flask, which were also filtered. Two rinses of 20 mL of acetone were used to wash the fibre residue. The residue was then dried at 70 °C overnight, and weighed. NDF was reported as a percentage of total dry weight.

9.4 Aroma

Two methods were developed to assess the aroma profile of green tea, for volatile compounds.

In the first method, volatiles were extracted from 2 g of semi processed green tea (from Japan) heated for 15 minutes at 90 °C in a 10 mL headspace vial, sealed with a teflon septum. A 10 mL sample of the headspace was removed with a Hamilton syringe, and injected onto a 25 m HP1 GC column of which the first 10 cm was immersed in a beaker of liquid nitrogen/acetone slurry at approximately -70 °C. The cryotrap was then removed, the oven closed and the GC analysis program started. A Hewlett Packard 5890 GC, and a HP 5970 mass spectometer (MS) were set for split-less gas flow, with an injection temperature of 100 °C. The detector was set at 260 °C, with a 70 eV ion source. The oven was set for an initial temperature of 30 °C for 2 minutes, with a gradient of 6 °C per minute until 110 °C.

In the second method, volatile compounds were extracted from 50 g of ground, semi processed tea, in 1 L of distilled water at 60 °C in a 2 L erlenmyer flask, shaken at 125 rpm. After 20 minutes, 5 mL of double distilled hexane and 25 µL of C11 standard at a concentration of 1.06 mg mL^{-1} were added, and shaken for a further 40 minutes. An aliquot of the hexane extract was concentrated to approximately 0.1 g in a 50 mL round bottom flask using a rotary evaporator. The sample was then analysed

using GC/MS, where 1 μL of sample was injected onto a 25m HP1 column. The head pressure was 10 psi with a flow rate of 2 mL minute^{-1} and a 50 mL minute^{-1} spit vent flow. The oven temperature was held at 30 °C for 2 minutes, then increased by 6 °C minute^{-1} until it reached 250 °C.

IV. Results

IV.1 Shoot growth and morphology

1.1 Introduction

Shoot growth in free-growing tea in field conditions occurs in repeating cycles of growth and dormancy, as described by Bond (1942). The aim of this chapter is to examine the pattern of shoot growth and development in both field and controlled conditions. The former, to provide some indication of the suitability of green tea to Tasmanian conditions, and the latter, to determine if rhythmic growth of free-growing tea is maintained in a constant environment.

In other species exhibiting rhythmic growth, such as *Quercus* (Barnola *et al.*, 1993) and *Hevea* (Hallé and Martin, 1968), leaf primordia for the new flush are initiated during dormancy, while during shoot extension the initiation of primordia is reported to cease as mitotic activity at the apex declines. Yet, in tea, which exhibits a similar pattern in growth, Bond (1942, 1945) reported that leaf initiation was continuous with only a slight change in the plastochron. This apparent discrepancy was addressed by assessing leaf initiation and mitotic activity - which has not previously been assessed - of the apical bud in tea at different stages of the rhythmic cycle.

Finally, there is a need for the development of a sampling strategy which identifies specific stages of development during rhythmic growth of tea, for use in subsequent experiments. A previous description of shoot growth by Bond (1942) is inadequate as it is based on qualitative characteristics. Stages of growth will instead be described according to quantitative measurements, including rate of shoot extension, leaf initiation and leaf production.

1.2 Materials and methods

1.2.1 Field trials

Field trials were conducted from November 1994 to March 1995, at two sites including the Grove Research Station (Department of Primary Industry and Fisheries), 25 km south of Hobart in the Huon Valley (43° 01'S, 147° 01'E); and Westerway, 50 km north west of Hobart in the Derwent Valley (42° 48'S, 146° 30'E). Day length and air temperature for the two sites are included in Appendix I.

The experimental design at each site was a completely randomised block design consisting of three blocks, containing plots of thirty to forty plants of each of three cultivars (Okuhikari, Yabukita and Sayamakaori) of green tea. One shoot of every second to third plant was tagged; shoot extension and the number of leaves per shoot were assessed weekly, and the position of the fish leaf was recorded at the Westerway site at the completion of the trial. The average rate of shoot extension and average total shoot length per cultivar at each site was estimated by fitting data to the logistic model (chapter III.7 of the general materials and methods).

1.2.2 Glasshouse trial

Plants were grown as described in Chapters III.1 and III.4 of the general materials and methods. Growth was assessed for one shoot in each of twelve replicate plants. Total shoot, internode and leaf length were measured three times per week, as described in Chapter III.5 of the general materials and methods. Maximum shoot length and number of leaves per shoot, and the rate of shoot extension and rate of leaf unfolding was estimated by fitting data to the logistic model using Systat (version 5.2 for the Macintosh), as in Chapter III.7 of the general materials and methods (see also Appendix II). In a separate experiment, the number and length of primordia within the apical bud of ten replicate shoots were measured at the expansion of each new leaf.

An environmental SEM (2020) was used to illustrate morphology of the apical dome. The ESEM is operated at lower pressure than the traditional SEM, and consequently sample preparation is minimal. Apices were dissected under water using a stereo microscope, and positioned on an electron microscope stub with double sided adhesive tape. Electron micrographs were recorded on a Rolex 120 camera, using Ilford FP4 film and developed on Ilford ID-11.

Mitotic activity of apices was qualitatively assessed. The apex was dissected from the apical bud, heated in a drop of 1 M HCl for a few seconds, and stained with aceto-

orcein (Searle Scientific). Apices were gently squashed beneath a coverslip, and the presence or absence of mitotic activity (prophase, metaphase, anaphase, telophase) in the squashed cells was detected using a Carl Zeiss microscope (West Germany), using the Nomarski differential interference contrast method. This required a polarising filter (473600), differential interference-contrast condenser (465279) and type 3 interference-contrast slide (Inco slide, 474433).

1.3 Results

1.3.1 Field trials

The rate of shoot extension, total shoot length and duration of flush growth varied both between the three cultivars and two sites. At Westerway, shoots of the cultivar Sayamakaori attained the greatest average total shoot length, at about 230 mm, at a rate of 3.1 mm day^{-1} (Fig. 1.1, Table 1.1). In comparison, the cultivar Okuhikari possessed the fastest average rate of shoot extension at 4.4 mm day^{-1}, but attained a slightly shorter average shoot length of 220 mm (Fig. 1.1, Table 1.1). The cultivar Yabukita had the slowest rate of growth, at 2.3 mm day^{-1} (Table 1.1). The average shoot length of this cultivar was limited to only 150 mm by the comparably earlier onset of dormancy during early March, 1995 (Fig. 1.1, Table 1.1). The cultivars Sayamakaori and Okuhikari did not become dormant for a further two weeks, at the end of March (Fig. 1.1).

Table 1.1 Predicted average shoot length and average shoot extension for cultivars Okuhikari, Yabukita and Sayamakaori at sites Westerway and Grove (1994/95)

Site	Cultivar	Shoot length (mm)	Shoot extension (mm day^{-1})
Westerway	Okuhikari	218.1	4.4
	Yabukita	150.1	2.3
	Sayamakaori	232.5	3.1
Grove	Okuhikari	258.5	5.2
	Yabukita	235.5	3.6
	Sayamakaori	199.4	4.1

At the Grove site, the cultivar Okuhikari attained around a 15 % greater average total shoot length, at about 260 mm compared to the Westerway site (Table 1.1). This could be attributed to both the increased duration of the flush, by 25 days, and an average faster rate of shoot extension at 5.2 mm day^{-1}. Shoot extension of Okuhikari

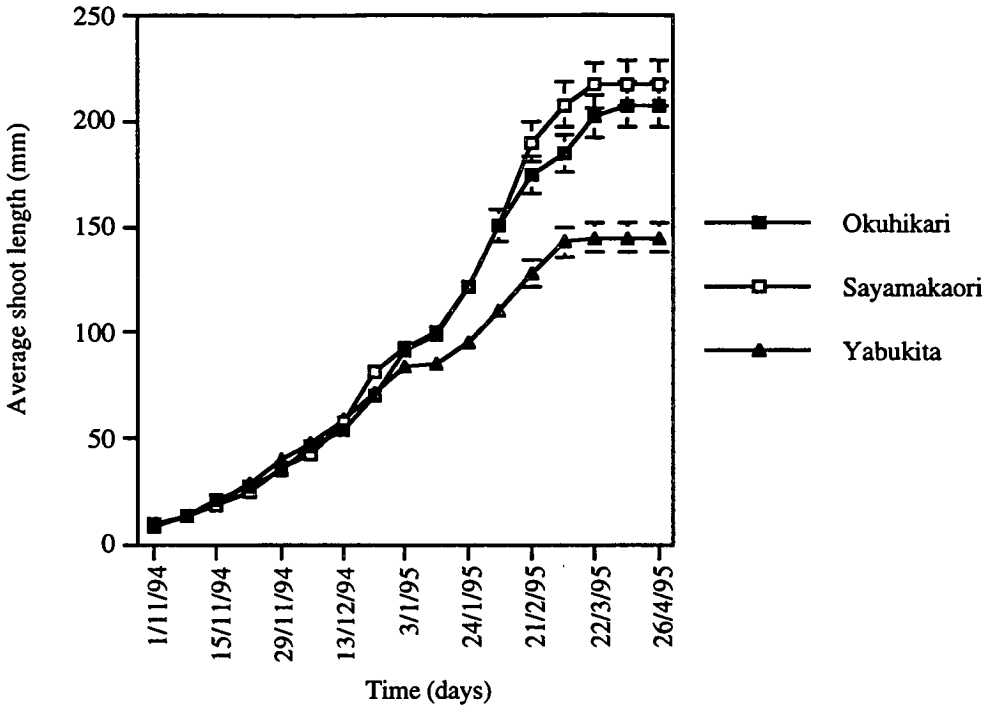

Figure 1.1 Shoot extension for three cultivars of green tea at Westerway from 1/11/94 to 26/4/95. Bars SE of 45 replicates.

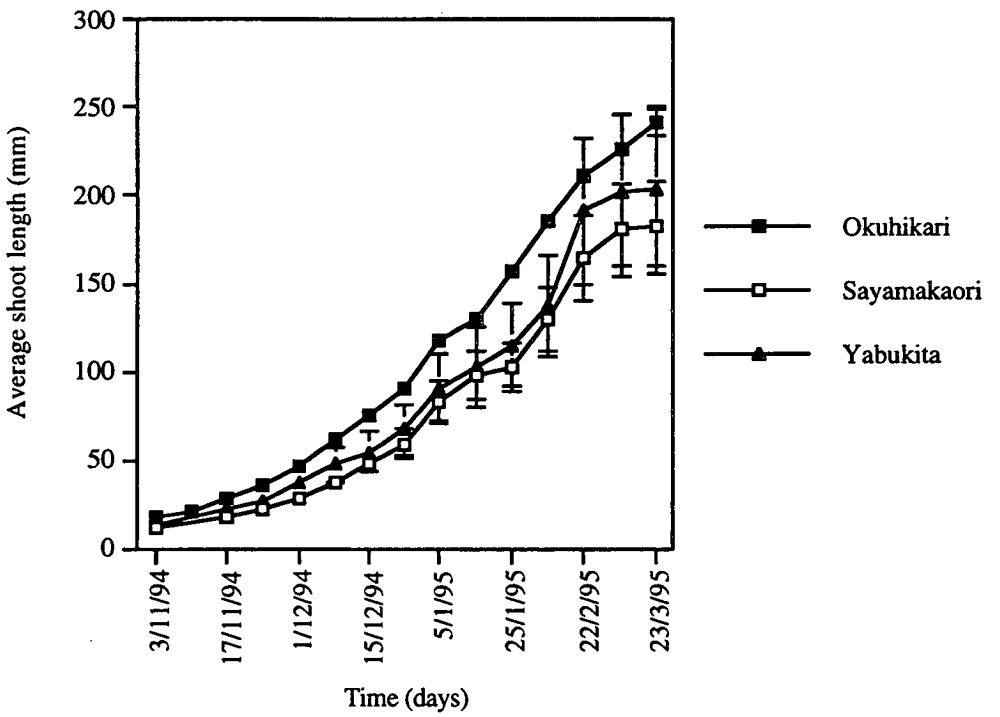

Figure 1.2 Shoot extension for three cultivars of green tea at Grove from 3/11/94 to 23/3/95. Bars SE of 45 replicates.

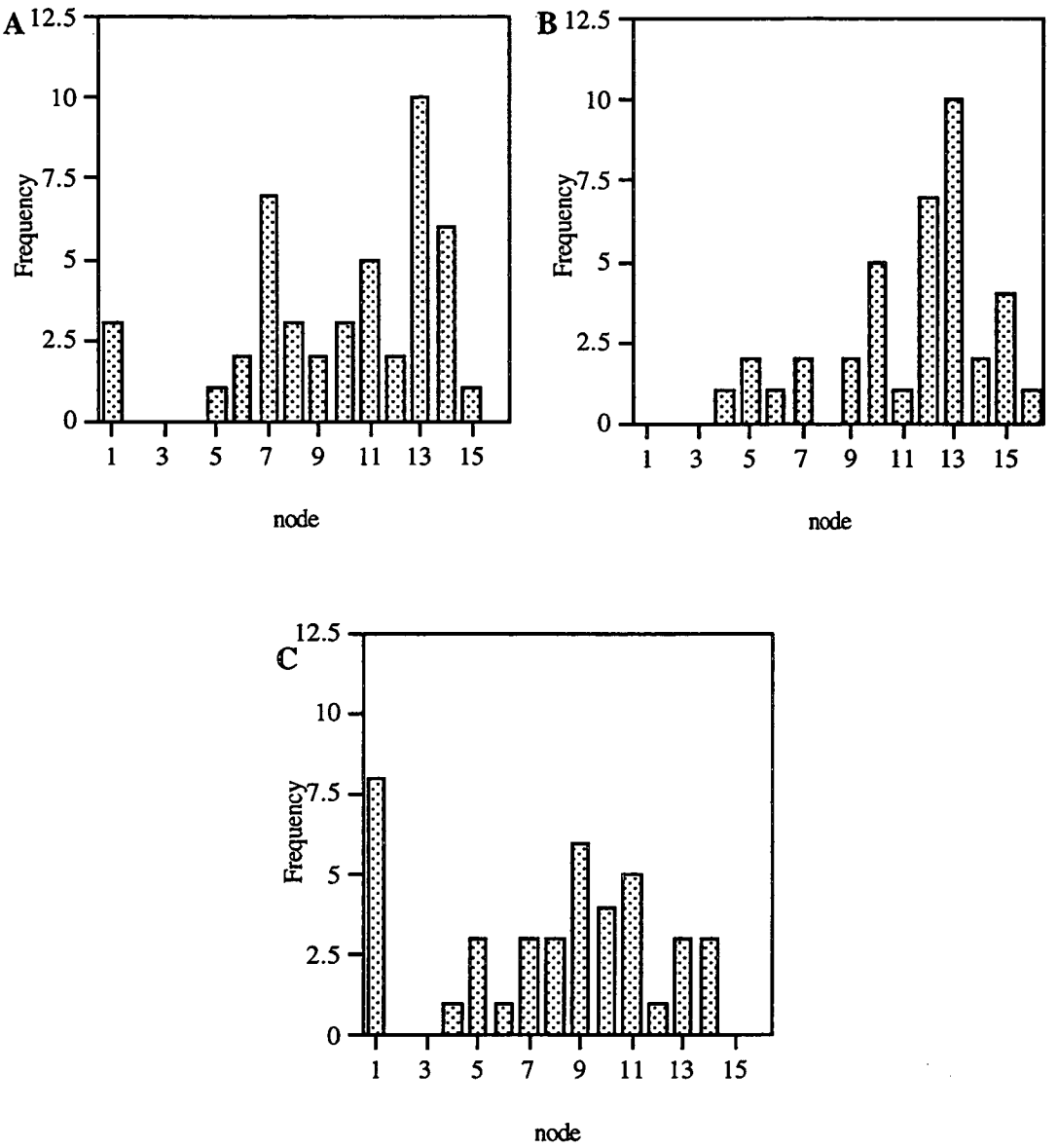

Figure 1.3 Frequency of position of fish leaf along shoots at Westerway for cultivars A) Sayamakaori; B) Okuhikari; C) Yabukita

at Grove had the potential to continue beyond the 23rd of March (Fig. 1.2), but was curtailed by an early frost. The cultivar Sayamakaori at Grove attained an average total shoot length of 200 mm, which was 13 % less when compared to Westerway. This could be attributed to the shorter duration of the flush, where growth ceased 20 days earlier compared to Westerway. The rate of shoot extension of Sayamakaori was otherwise similar at both Grove and Westerway. Average total shoot length of the cultivar Yabukita was 35 % greater at Grove, at 235 mm, as a result of a faster average rate of shoot extension at 3.6 mm day^{-1} compared to 2.3 mm day^{-1} at Westerway. Differences in shoot growth between the two sites could be attributed to different microclimate, which is therefore likely to be an important factor in the choice of site location for future plantations.

The slight discontinuity in the rate of shoot extension at Westerway around the 1st of January may be consistent with a synchronised flush at this site (Fig. 1.1). Further evidence to support this is the presence of 'fish' leaves on shoots as observed upon the completion of flush growth (Fig. 1.3). These leaves are intermediate between cataphylls and foliage leaves, and delineate the start of a new flush along a shoot. Fish leaves were in higher frequencies at nodes seven and thirteen in the cultivar Sayamakaori (Fig. 1.3 A); at the thirteenth node in the cultivar Okuhikari (Fig. 1.3 B); and at nodes one and nine in the cultivar Yabukita (Fig. 1.3 C). It then appears likely that most shoots experienced at least two flushes of growth during the 1994-95 season. There is little evidence from Fig. 1.2 to indicate that shoot extension at Grove occurred in synchronised flushes. Fish leaves were observed to be present on shoots, but the overall pattern of shoot extension was approximately linear with respect to time and declined at the onset of winter dormancy. Variation in the frequency of fish leaves both along the shoot and between cultivars, indicated that flush growth of plants at both sites was asynchronous. This would culminate in the overall sigmoidal pattern of shoot extension as observed in Figs. 1.1 and 1.2.

1.3.2 General description of shoot growth

The pattern of shoot growth and development in green tea was then described in controlled conditions where shoot growth was synchronised by pruning. Shoot growth in tea can be described by the following four stages:

I. Bud burst, expansion of cataphylls
II. Shoot extension, expansion of leaves and internodes
III. Dormancy, completion of shoot extension
IV. Completion of leaf expansion.

During stage I, bud burst is observed in the expansion of two cataphylls (plate 3) and increased length of the apical bud to about 10 mm (plate 7A). The cataphylls, at approximately 10 - 20 mm in final length, are smaller than the leaves of the previous flush and lack marginal serrations. The apical bud, internodes and the abaxial surface of the cataphylls are hirsute. Leaves of the previous flush are approximately 10 cm in length, are dark green and glossy in appearance with distinct venation and marginal serrations. Immature flowers are present in leaf axils of both mature leaves and the first cataphyll. Wood formation is observed to be progressing along the stem of the previous flush, replacing the soft, green and hirsute stem.

Stage II is characterised by the expansion of leaves and internodes from the apical bud, preformed in the bud during dormancy (plate 4). During stage II the apical bud has increased in length from about 10 to 20 mm and is distinctly hirsute (plate 7B). Leaves are a lighter shade of green and thinner than the mature leaves of the previous flush. Leaves and internodes expand sequentially, such that leaves first to expand will be chronologically and physiologically older than the final leaves.

During stage III, expansion of the final flush leaf (plate 5) shows the dormant bud (plate 7C), which is hirsute, approximately 2 mm in length and is one-quarter of the bud length during stages I and II. The bud is enclosed by cataphylls, which later expand to form the first two leaves of the new flush as observed in stage I. Shoot extension has ceased, and it is generally observed that the length of internodes at the start and end of the flush is shorter than median internodes. Expansion of the final leaves is still incomplete.

During stage IV, expansion of the final leaves is complete (plate 6). The leaves are now dark green in colour, thicker and leathery. The bud remains dormant, although some expansion of the enclosing cataphylls has increased the size of the bud to approximately 5 mm (plate 7D). The bud is less hirsute than during stage III. Cataphylls senescenced either during stage IV, or later during growth of the following flush.

The number of leaf primordia within the apical bud varies depending on the stage of development. Leaf primordia first appear as 'triangular' projections to one side of the apex of the meristem, and are approximately 180 μm wide and 130 μm in height (plate 8). By the second plastochron, primordia widen laterally and extend vertically to enclose the youngest leaf primordium, now positioned on the opposing side of the meristem. The primordium forms a distinct, cone shaped tip, approximately 300 μm in length (plate 9). Older primordia possess a similar morphology and arrangement.

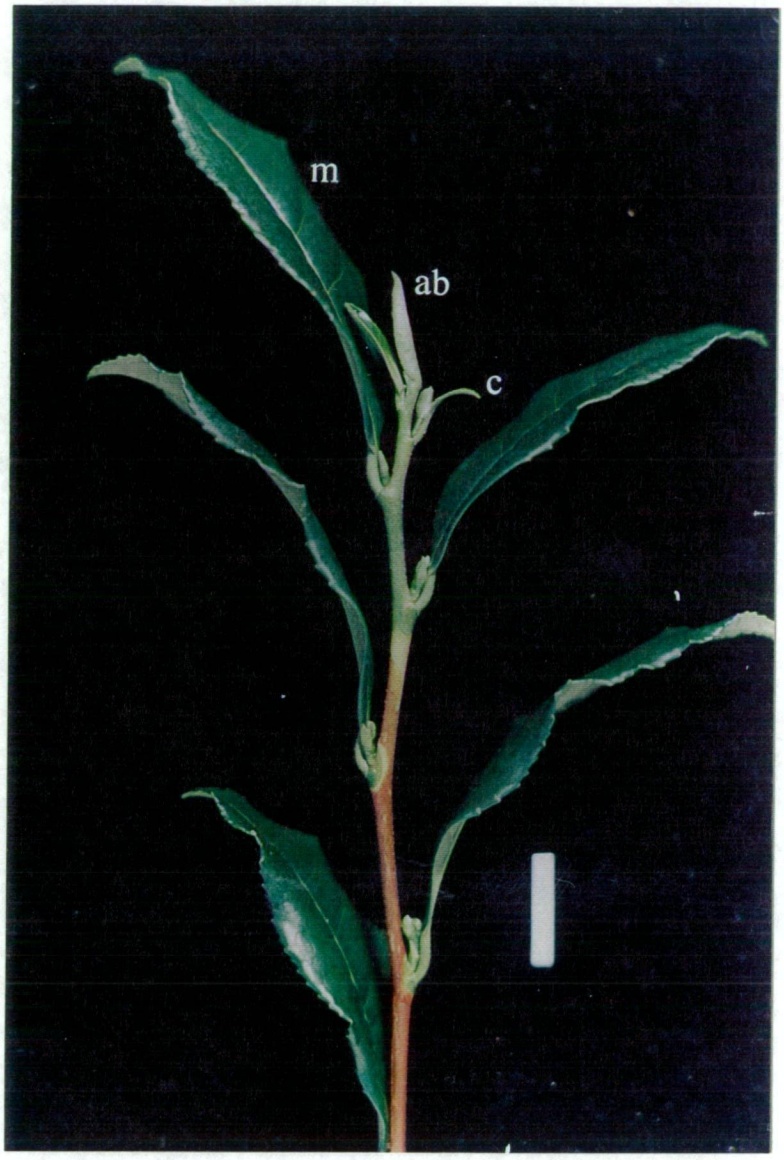

Plate 3. Stage I. Cataphylls expanded while previous flush leaves are mature and formation of wood is occurring in the stem. c) cataphylls; ab) active bud; m) mature leaf. Bar = 25 mm.

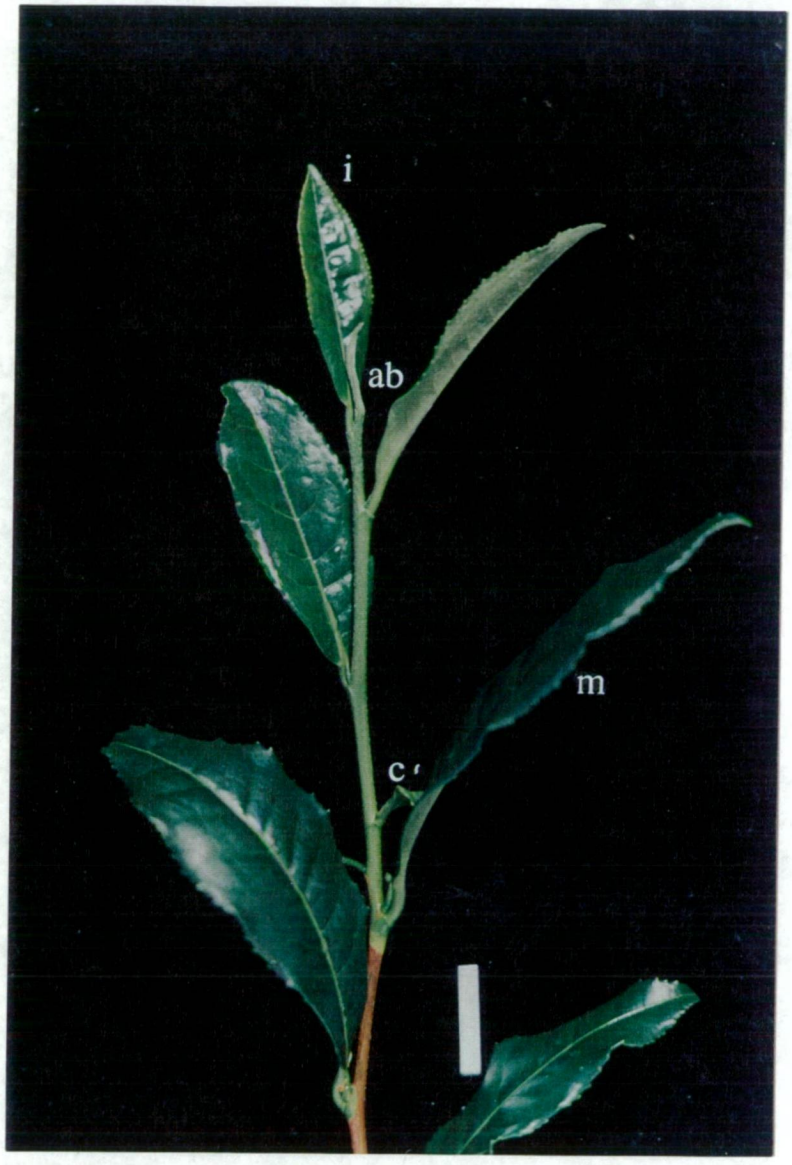

Plate 4. Stage II. Extension of leaves and internodes. c) cataphylls; ab) active bud; m) mature leaf; i) immature leaf. Bar = 25 mm.

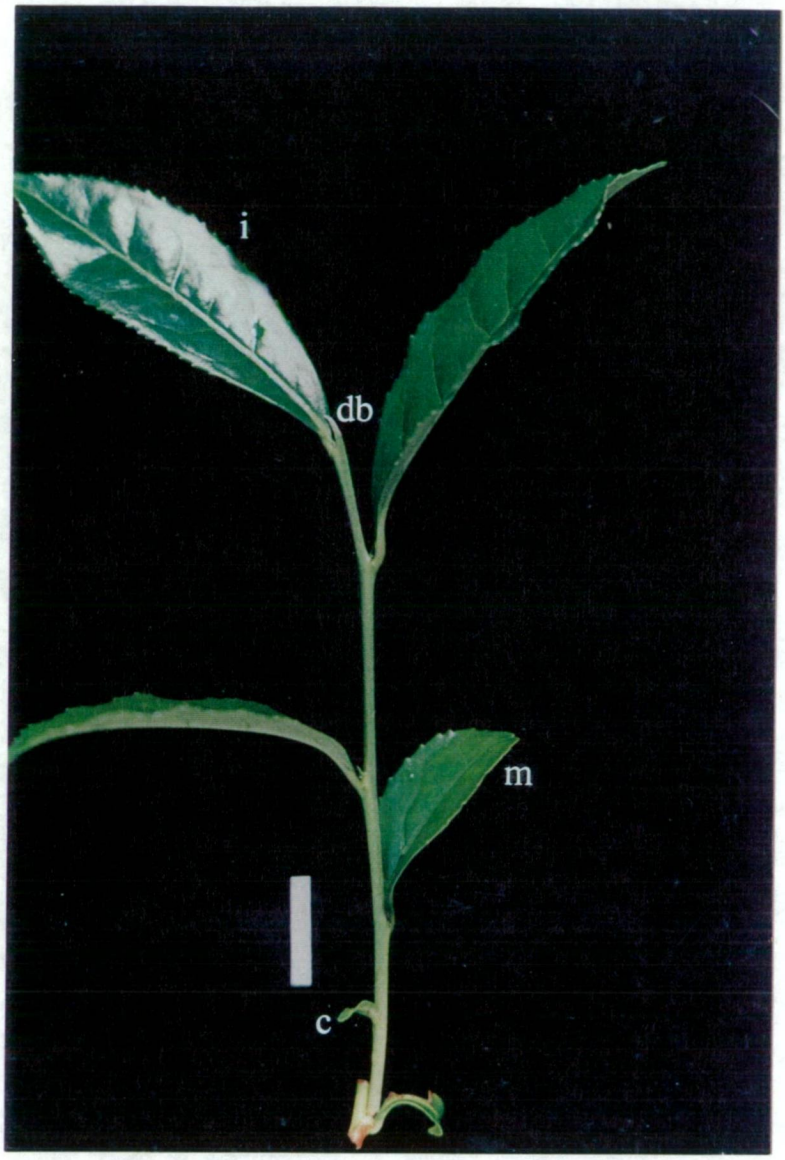

Plate 5. Stage III. Exposure of dormant bud and the completion of shoot extension, although final leaves have not reached full expansion. c) cataphyll; db) dormant bud; m) mature leaf; i) immature leaf. Bar = 25 mm.

Plate 6. Stage IV. Leaves mature, bud dormant. c) cataphylls; db) dormant bud; m) mature leaf. Bar = 25 mm.

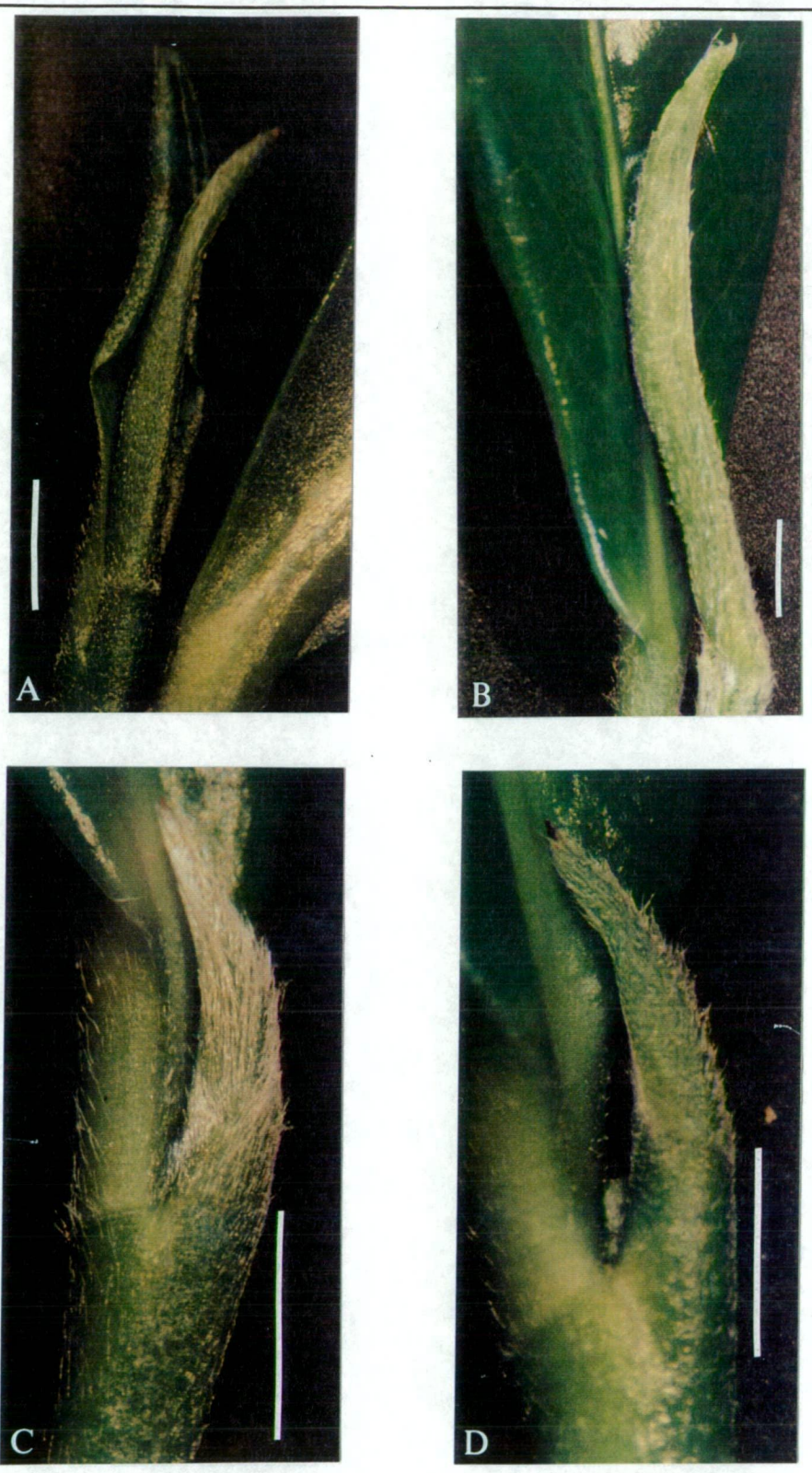

Plate 7. Bud morphology according to stage of development. A) stage I; B) stage II; C) stage III; D) stage IV. Bar = 2.5 mm.

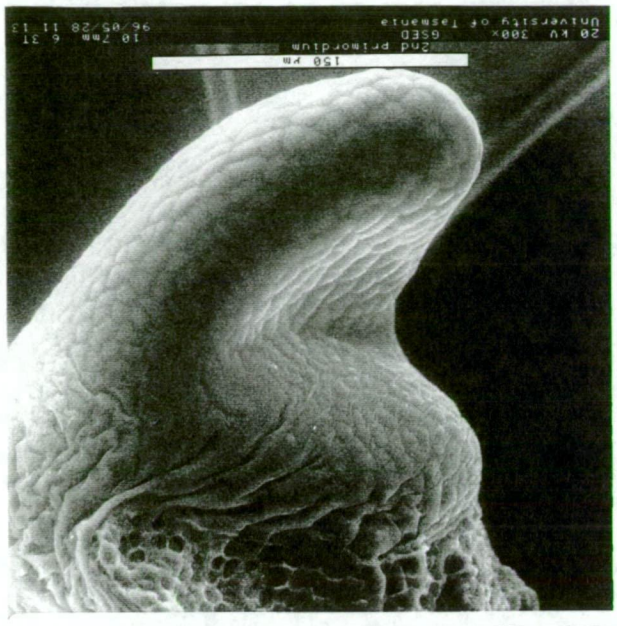

Plate 8. Youngest primordia of apical bud. Bar = 150 µm.

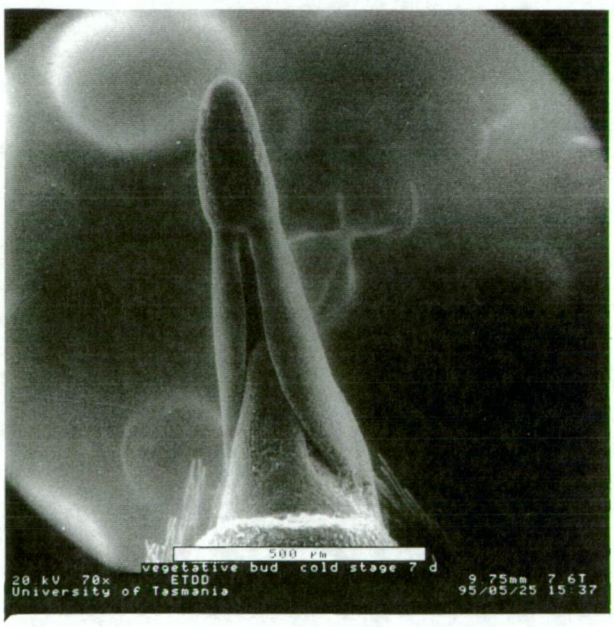

Plate 9. Youngest primordium of apical bud, enclosed by 2nd primordium. Bar = 500 µm

1.3.3 Shoot extension and leaf production

The four stages of growth can be further defined by quantitative measurement.

Total shoot extension and leaf production reflected a cyclic pattern of shoot growth, where the duration of the first flush at 25 days was half as long as the second flush, at 50 days, and separated by a period of dormancy for 25 days (Fig. 1.4). Using the logistic model, the rate of shoot extension in the first flush was estimated at 10.6 mm day^{-1}, attaining a maximum length of 120 mm (Figs. 1.4, 1.5). In comparison, the estimated rate of shoot extension in the second flush was slower, at 6.4 mm day^{-1}, but reached a similar total length of 128 mm (Fig. 1.4, 1.5). Total shoot length of the combined flushes was then 245 mm.

The final leaf to unfold from the apex continued to expand after shoot extension had ceased (Fig. 1.5). An average of 5.9 leaves was produced (flushes one and two) at a rate of 0.38 (flush 1) and 0.33 (flush 2) leaves per day respectively. A combined total of 11.8 leaves was produced for both flushes.

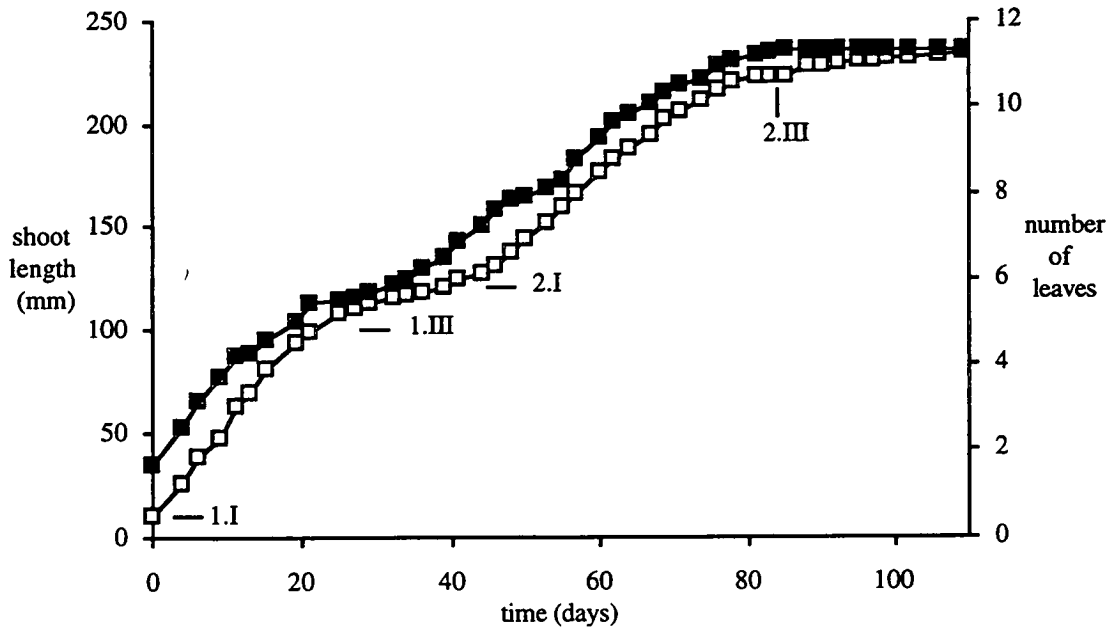

Figure 1.4 Increase in shoot length and number of leaves over time. □) Shoot length; and ■) number of leaves. I) bud burst; III) dormancy for flushes one and two respectively.

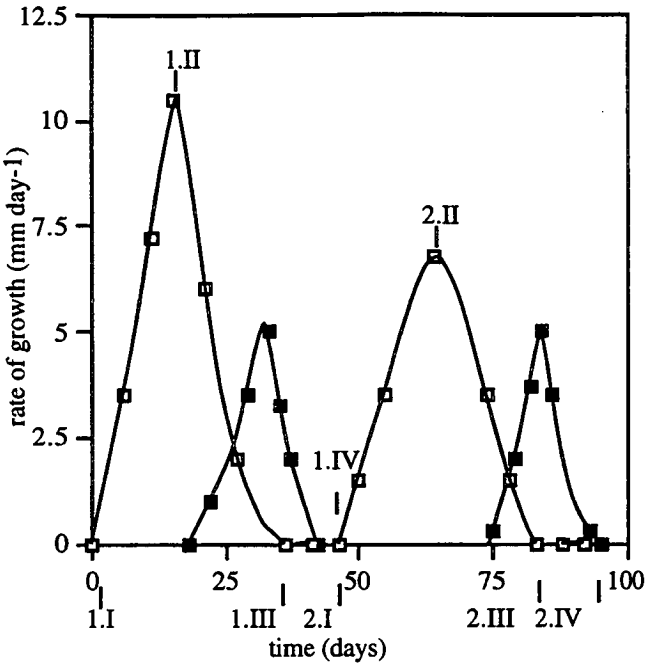

Figure 1.5 Rate of shoot extension and rate of growth of final leaf over two flushes. □) Shoot extension; and ■) final leaf. I) bud burst; II) shoot extension; III) dormancy; IV) completion of shoot growth for flushes one and two respectively.

1.3.4 Leaf and internode expansion

Leaf and internode extension was dependent on nodal position. Leaf extension increased from a minimum of about 1 mm day^{-1} for cataphylls, to a maximum of about 4 mm day^{-1} for the last leaf of the flush (Fig. 1.6 A). Internode extension increased from a minimum of almost 1 mm day^{-1} at the start of the flush, to a maximum of about 2 mm day^{-1} during the middle of the flush, declining again to 1 mm day^{-1} at the end of the flush (Fig. 1.6 B).

Leaves unfolded sequentially from the apex during shoot extension (Fig. 1.7). The rate of leaf expansion (Fig. 1.6 A) reflected final leaf size, where cataphylls, at 20 to 40 mm in length (leaves labelled 1.1, 1.2, 2.1 and 2.2 in Fig. 1.7) were considerably shorter than final leaves from 80 to 120 mm in length (leaves labelled 1.3 to 1.6 and 2.3 to 2.6 in Fig. 1.7).

Internode expansion accompanied leaf unfolding from the apex (Fig. 1.8). Internode expansion (Fig. 1.6 B) reflected their final length, being 10 to 25 mm at the start and end of the flush (internodes labelled 1.2, 1.6, 2.1 and 2.6 in Fig. 1.8), to 20 to 50 mm mid flush (internodes labelled 1.4 and 2.5 in Fig. 1.8).

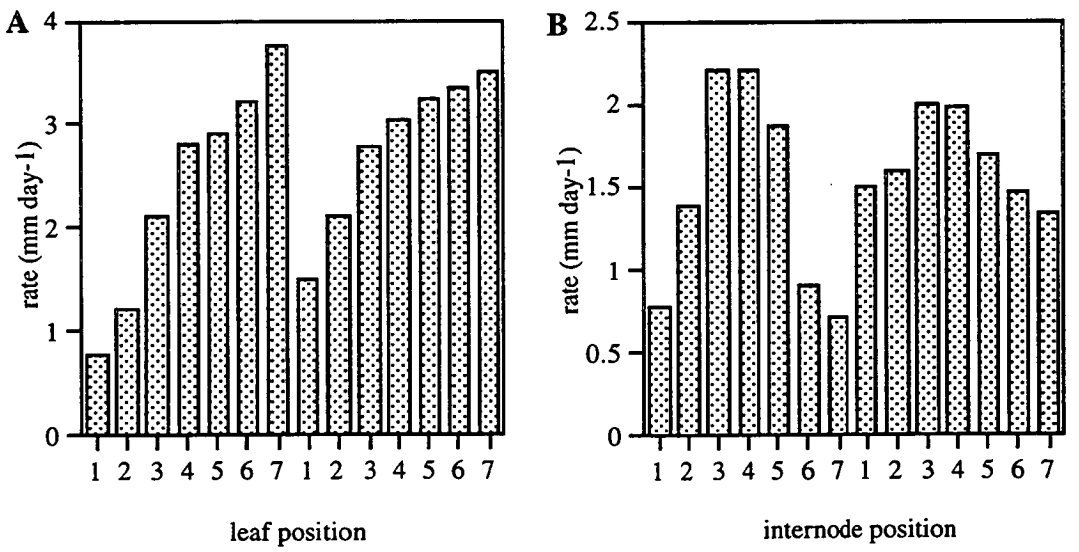

Figure 1.6 Rate of extension for A) leaves; and B) internodes according to position and flush.

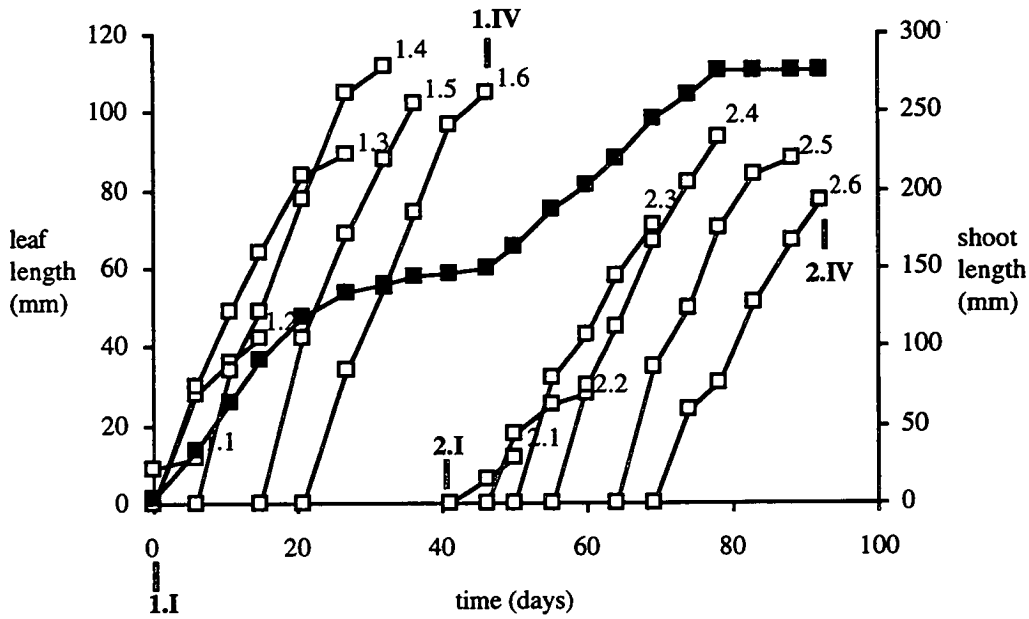

Figure 1.7 Leaf expansion with respect to shoot extension over two flushes of growth. □) Leaf; and ■) shoot extension. First flush leaves labelled as 1.1 to 1.6; second flush leaves 2.1 to 2.6. I) bud burst; IV) completion of shoot growth for flushes one and two respectively.

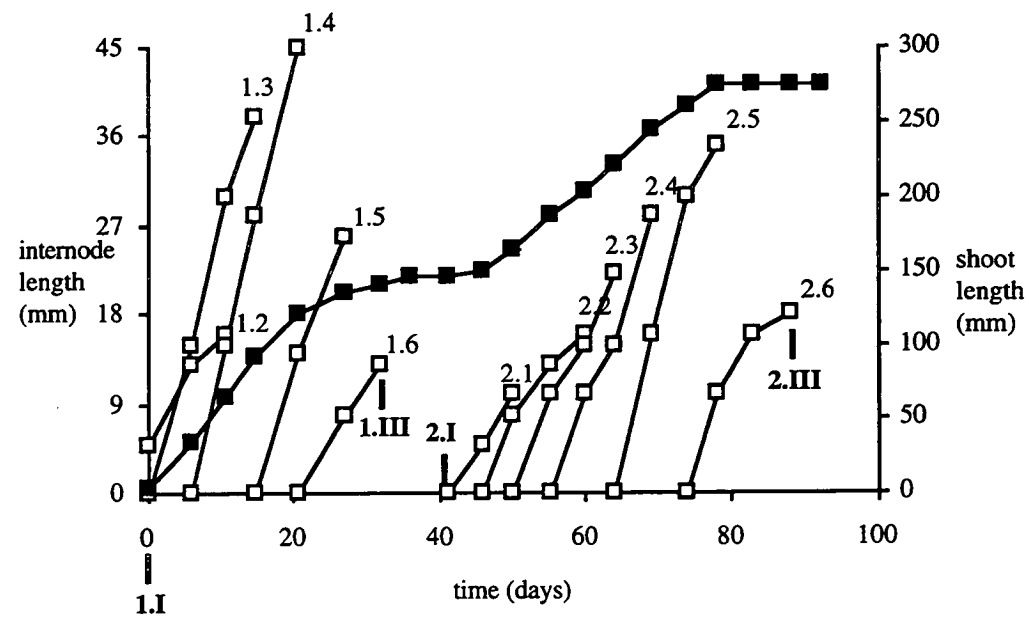

Figure 1.8 Internode extension with respect to shoot extension over two flushes of growth. □) Internodes; and ■) shoot extension. First flush internodes labelled as 1.1 to 1.6; second flush internodes 2.1 to 2.6. I) bud burst; III) dormancy; for flushes one and two respectively.

1.3.5 Leaf initiation

The initiation of leaf primordia at the apex was also cyclic. Leaf primordia were initiated during dormancy, that is, before stage 1.I of the first flush (Fig. 1.9 A). Hence there was a total of seven primordia in the bud at bud burst, during stage 1.I (Fig. 1.9 A). At this time, the first two leaves (cataphylls) had unfolded from the apex. Continued expansion of leaf primordia to form leaves resulted in the number of primordia in the apical bud declining to a minimum of four at dormancy, during stage 1.III. No further leaves were produced until stage 2.I, after the initiation of leaf primordia during stage 1.III, dormancy.

In the bud, cataphyll primordia could be identified by their reduced size compared to leaf primordia. Observation revealed that the last three of the seven primordia in the bud at stage 1.I included two cataphylls, and one further primordia. These cataphylls would enclose the dormant bud during stage 1.III. Thus, primordia older than the cataphyll primordia would constitute the first flush leaves, while those initiated after the cataphyll primordia would constitute the second flush leaves. Initiation of leaf primordia for the second flush ceased during shoot extension, at stage 1.II (Fig. 1.9 B). Leaf initiation did not recommence until dormancy, reaching a maximum of five primordia in the apical bud at stage 1.III (Fig. 1.9 B). The number of primordia then declined as these expanded into leaves (Fig. 1.9 B).

The expansion of cataphyll primordia initiated at or just before stage 1.I, bud burst, is negligible until dormancy at stage 1.III (Fig. 1.10). Expansion of leaf primordia is similar to that of the cataphylls until the completion of shoot growth at stage 1.IV (Fig. 1.10). Expansion of leaf primordia then increases rapidly at bud burst at stage 2.I (Fig. 1.10). At this stage, the cataphylls for the next flush are initiated.

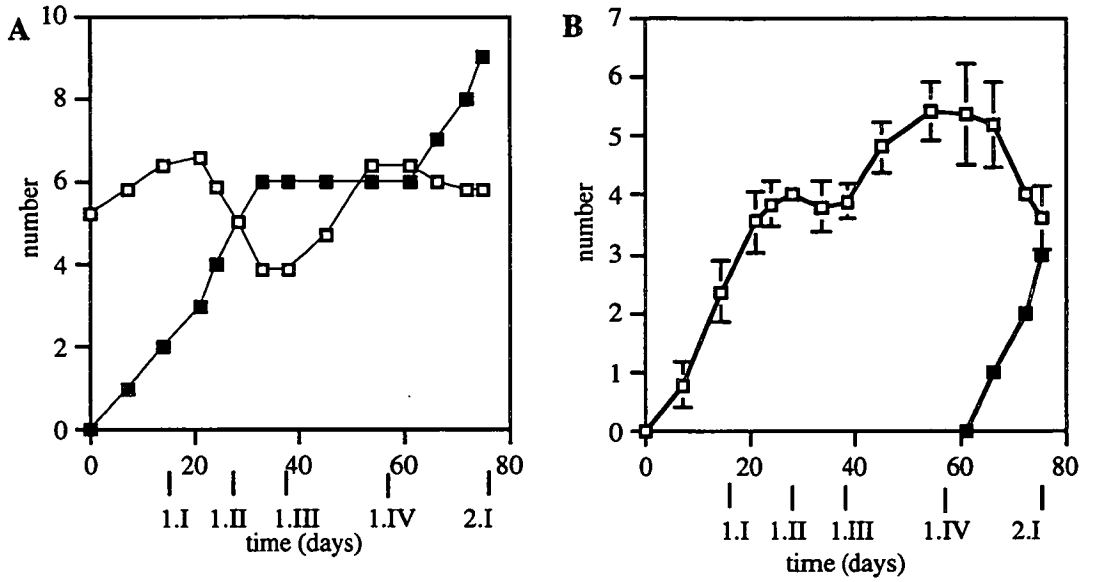

Figure 1.9 Leaf production and initiation of primordia during flush growth for A) total primordia; and B) second flush primordia.. ■) Number of expanded leaves; and □) primordia. I) bud burst; II) shoot extension; II) dormancy; IV) completion of shoot growth for flushes one and two respectively.

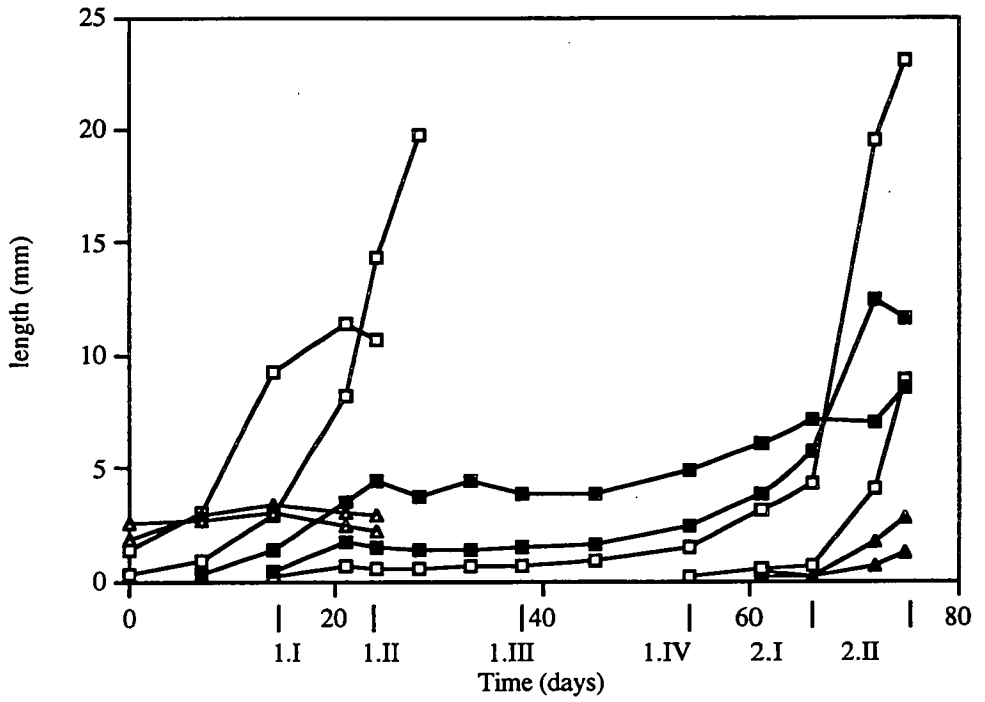

Figure 1.10 Length of primordia from initiation. Δ) cataphylls, first flush; ■) cataphylls, second flush; ▲)cataphylls, third flush; □) leaves. I) bud burst; II) shoot extension; III) dormancy; IV) completion of shoot growth for flushes one and two respectively.

1.3.6 Definition of stages of shoot growth

The stages of development can then be quantitatively described using these data. During stage I, foliage leaves and their associated internodes have not yet commenced to unfold (Fig. 1.4), such that the rate of shoot extension is 0 mm day^{-1} (Fig. 1.5). Consequently there is a maximum of seven primordia within the apical bud (Fig. 1.9 A), which is from 10 to 20 mm in length. These primordia include recently initiated leaf primordia from 5 to 10 mm in length, plus a further two cataphyll and one foliage primordium initiated during stage 1, all less than 2 mm in length (Fig. 1.10).

Stage II is characterised by a period of linear shoot extension, which is increasing at a maximum rate of 6 - 10.5 mm day^{-1} (Fig. 1.5). Internodes which were first to expand have attained a maximum of 15 to 20 mm (Fig. 1.8). The number of leaves per shoot is increasing linearly with time (Fig. 1.4), at a maximum rate of 2.5 to 3.0 mm day^{-1}. The first foliage leaf following the cataphylls has fully expanded to 30 to 60 mm, which is one to two thirds the final leaf length (Fig. 1.7). The bud is 15 to 20 mm in length and contains the remaining 1 to 3 leaf primordia for the first flush (Fig. 1.9 A), and a further two cataphyll and two leaf primordia for the second flush (Fig. 1.9 B). No further primordia are initiated until stage III (Fig. 1.9 B).

During stage III, stem elongation ceases (Fig. 1.5), and a total length of around 250 mm is attained (Fig. 1.7). The number of leaves reaches a maximum of seven at the unfolding of the final leaf, which includes two cataphylls with the remainder as foliage leaves (Fig. 1.7). However, the final leaves to unfold continue to expand at the maximum rate of 4 mm day^{-1} (Fig. 1.6 A), until a final length of 80 to 120 mm is attained (Fig. 1.7). The bud is 2 to 3 mm in length, and contains a minimum of four primordia (Fig. 1.9 A).

During stage IV, the rate of extension of the final leaves to unfold ceases (Fig. 1.5), and they are at their final length of 80 - 120 mm (Fig. 1.7). The bud has increased in length to 2.5 to 5.0 mm, which reflects the increase in length of cataphylls to 2.5 to 5 mm (Fig 1.10). The number of leaf primordia has increased to a maximum of six to seven (Fig. 1.9 A), including foliage primordia which are 1 to 2.5 mm in length (Fig 1.10).

1.3.7 Mitotic activity

The presence or absence of mitotic figures in the youngest primordium was qualitatively assessed for each stage of development. Evidence of mitosis was detected in all stages of development. This included cells in the various stages of mitosis (prophase, metaphase, anaphase and telophase), plus cells in interphase (Plates 10 to 13).

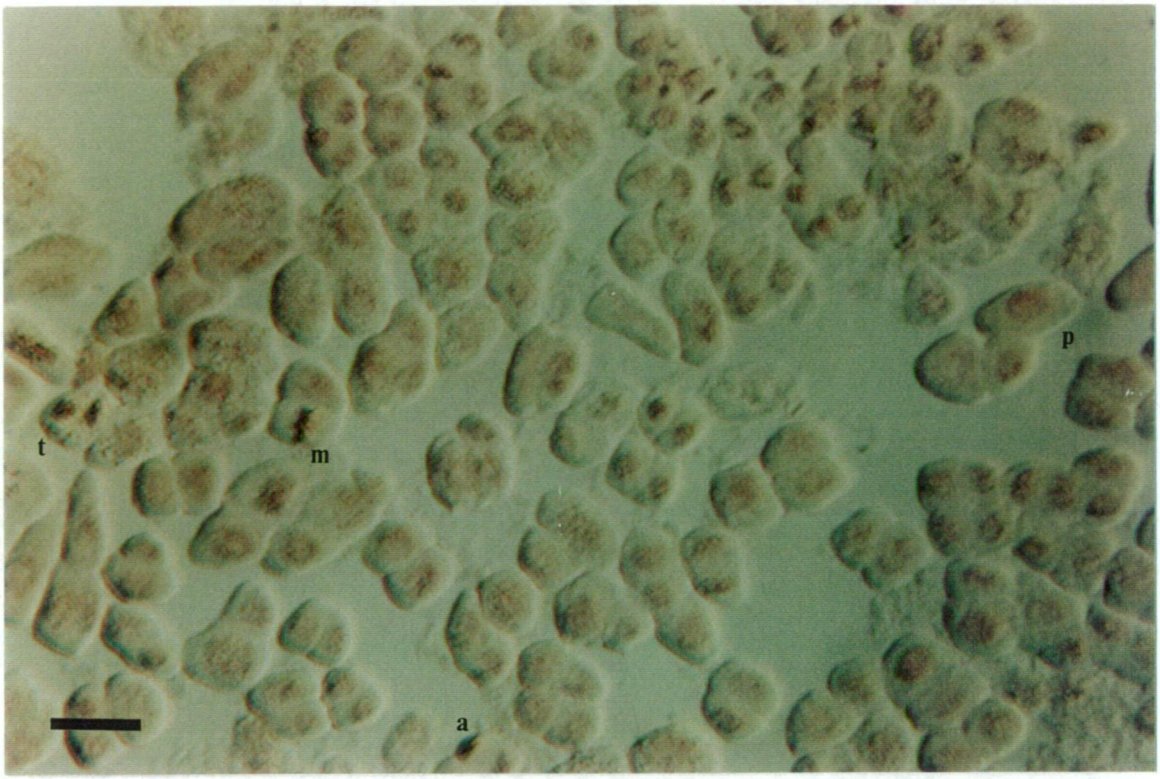

Plate 10. Apical squash, showing cell mitosis during Stage I. P) prophase; M) metaphase; A) anaphase; T) telophase. Objective 16x. Bar = 10 µm

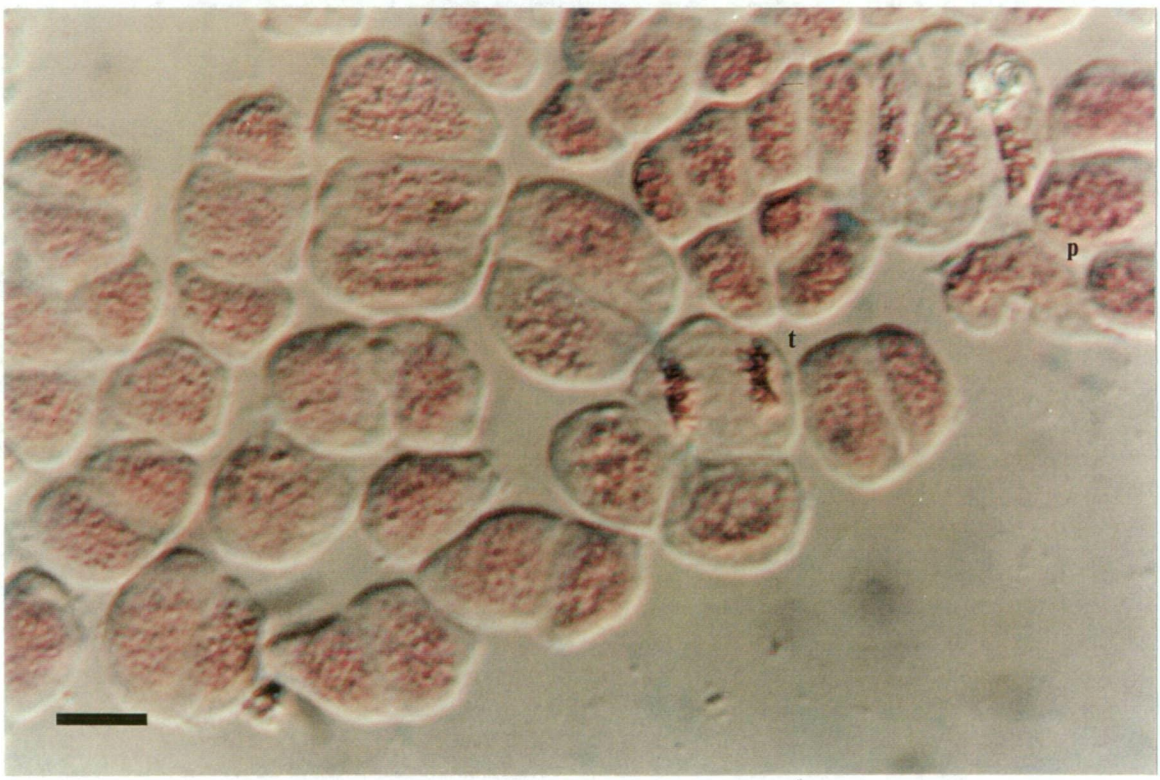

Plate 11. Apical squash, showing cell mitosis during Stage II. P) prophase; M) metaphase; A) anaphase; T) telophase. Objective 40x. Bar = 4 µm

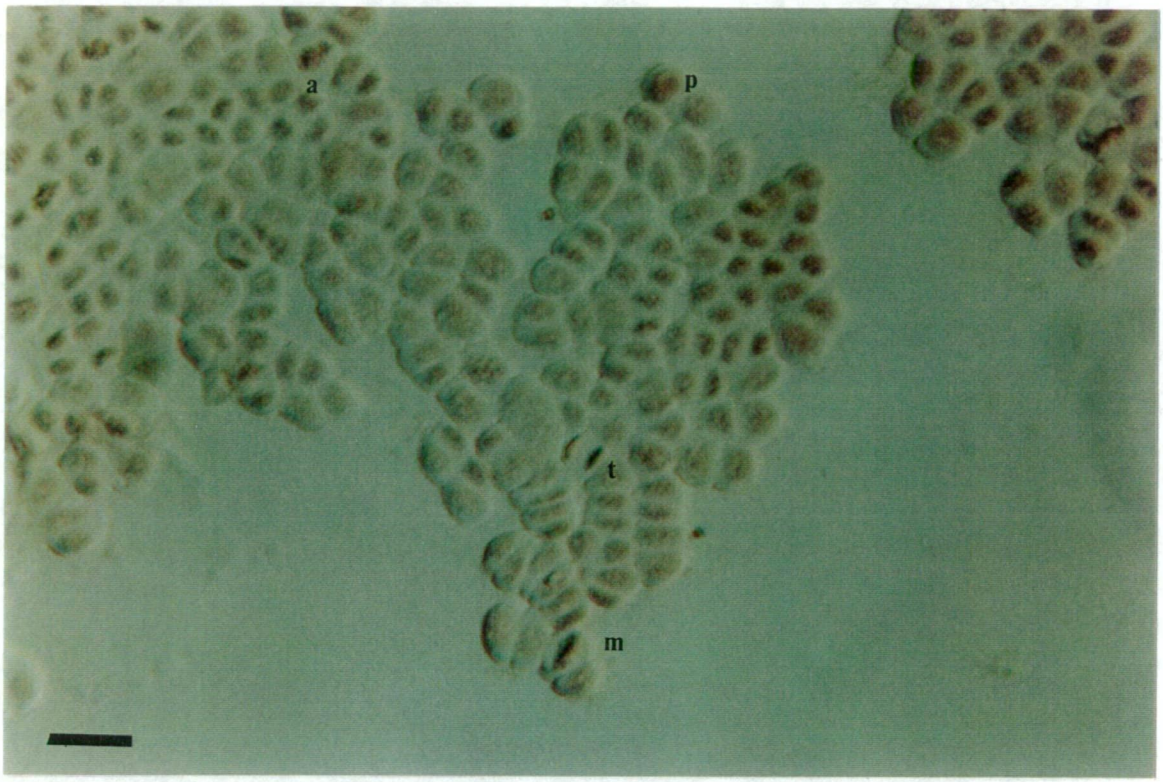

Plate 12. Apical squash, showing cell mitosis during Stage III. P) prophase; M) metaphase; A) anaphase; T) telophase. Objective 16x. Bar = 10 µm

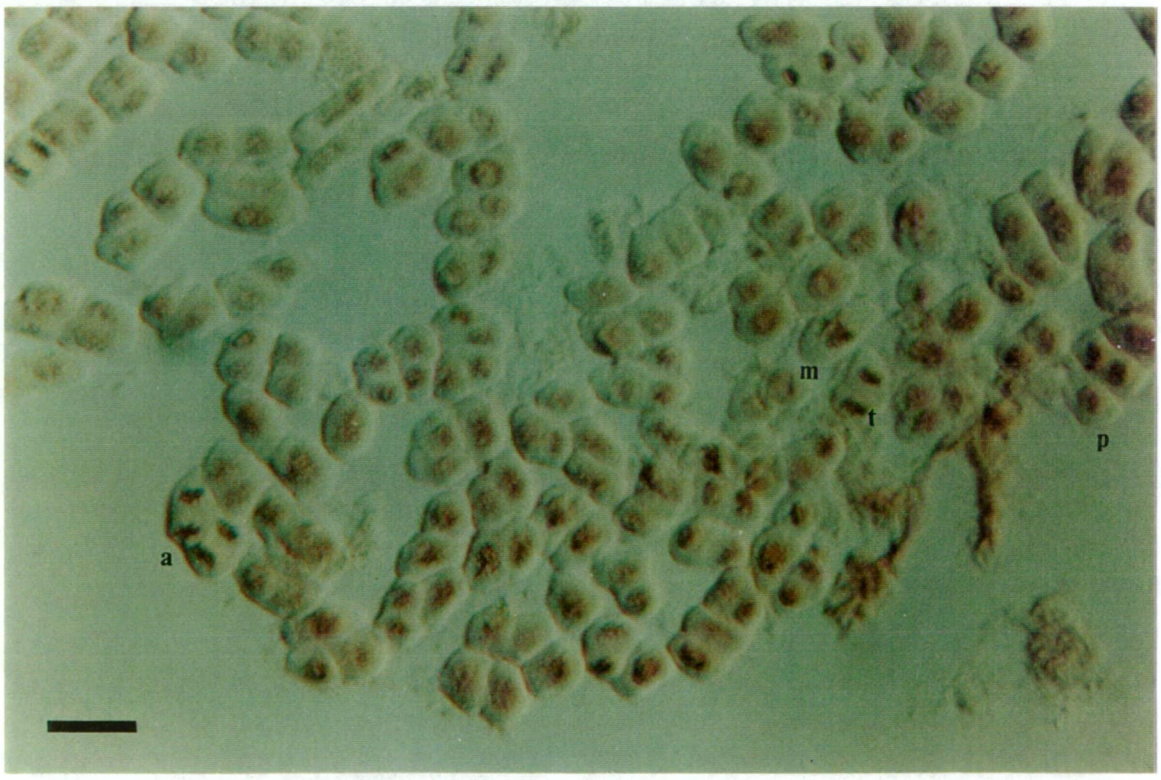

Plate 13. Apical squash, showing cell mitosis during Stage IV. P) prophase; M) metaphase; A) anaphase; T) telophase. Objective 16x. Bar = 10 µm

1.4 Discussion

Shoot growth of the three green tea cultivars, Okuhikari, Sayamakaori and Yabukita was assessed at two sites, Grove and Westerway. The cultivar Okuhikari had the fastest rate of growth at both sites, but an early frost at Grove limited total shoot length in the 1994-95 season to around 200 mm. Variability in the average total shoot length both between varieties and sites can perhaps be attributed to differences in the microclimate, and as such, highlights the importance of site selection in plant productivity.

Shoot extension in field conditions at Grove did not reflect the cyclic pattern of shoot growth reported by Bond (1945), as the pattern of shoot extension was approximately linear with respect to time, the rate of shoot extension declining only at the onset of winter dormancy. However, at Westerway, a slight discontinuity in the rate of shoot extension at the start of January could perhaps be consistent with a flush. The presence of fish leaves along the new shoots at both sites indicated that growth did occur in flushes (Bond, 1942), but the scattered distribution of these leaves along shoots perhaps indicated that flush growth became asynchronous as the season progressed. According to the literature, this is not unusual, as at any one time shoots on the one plant are reported to be in either the active or dormant phase of the growth cycle (Wight, 1955; Wight and Barua, 1955; Pethiyogoda, 1964). Asynchronous growth in the free-growing plant is unlikely to affect flush growth during harvesting, which in itself is reported to synchronise flush growth (Fordham and Palmer-Jones, 1975).

In contrast, in controlled conditions, the finding that shoot growth in free growing tea cycles in a synchronised manner between shoot extension and dormancy is then similar to growth reported by Bond (1942) in field conditions. Then, according to the definition of rhythmic growth as proposed by Koukkari and Warde (1985) the cycle is endogenous. Shoot extension ranged from 25 to 35 days, during which six leaves were produced, followed by a period of dormancy for 25 days. The total duration of the cycle at eight weeks was three weeks shorter than the average of 12.1 weeks reported by Bond (1942). This was perhaps due to differences in environment, such as day length or temperature. The general morphological features of flush growth in free growing tea were consistent with the observations of Bond (1942), Pethiyogoda (1964) and Barua (1970). To elaborate, the rate of expansion of cataphylls was half that of foliage leaves, and there was a gradient in the rate of expansion of leaves, such that the final leaf experienced the fastest rate of growth and attained the greatest final length. In comparison, internodes at the start and end of a flush expanded more slowly and were shorter than median internodes.

During dormancy, up to seven leaf primordia were initiated in the apical bud, while a further two cataphyll and leaf primordia were initiated at bud burst. Similar trends were observed by Bond (1945), yet Goodchild (1968) reported that apical buds contained on average six primordia, failing to recognise that the number of primordia varies depending on the stage of the growth cycle. Further initiation of primordia ceased until dormancy, as has been observed in other plant species exhibiting rhythmic growth, including *Hevea* (Hallé and Martin, 1968), *Quercus* (Barnola *et al.*, 1993) and *Fraxinus* (Cottignies, 1993). This contradicts previous findings of Bond (1945), who stated that the initiation of primordia in tea was continuous, although the rate of initiation of primordia varied depending on the stage of growth. However, qualitative measurement of mitotic activity in the apex, which included the youngest primordium, showed the presence of dividing cells at all stages of shoot development. Although not quantitative, such information indicates that although leaf initiation ceases during shoot elongation, the remaining primordia are still undergoing mitosis.

Data including the rate of shoot extension, leaf extension, leaf production, internode length, leaf length, bud length, length and number of primordia were used to define stages of shoot growth and development in tea. In summary, these criteria included: stage I, bud swelling, expansion of cataphylls and start of shoot growth; stage II, shoot extension and leaf expansion; stage III, completion of shoot extension, unfolding of final flush leaf revealing dormant bud; and stage IV, completion of leaf expansion. Stages of growth that are defined according to quantitative measurement are therefore reproducible and minimise sample variation in physiological studies, unlike previous qualitative descriptions of shoot growth (Bond, 1942; Barua, 1970).

Combining data from the morphological criteria and bud activity then enables a hypothesis to be developed to explain the control of shoot growth in tea. Shoot growth is rhythmic; yet by definition rhythmic growth implies that it be maintained under different environmental conditions. Consequently, shoot growth is modified by the environment, yet controlled by endogenous factors, as recognised by Barua (1970). Although widely accepted, the role of environment as a modifier of rhythmic growth of tea needs to be proven. It could be hypothesised that rapid expansion of leaves and internodes during bud burst alters the partitioning of assimilates from the bud in favour of the developing shoot. Similar observations have been made in *Quercus* (Hanson *et al.*, 1988; Alaoui-Sossé *et al.*, 1994; Barnola *et al.*, 1993). Subsequent shoot expansion maintains the dominance of developing shoots, such that further initiation of primordia is inhibited. Such inhibition could be a process of direct competition for assimilate between the bud and developing shoot, or alternatively may involve plant growth regulators as inhibitors and promoters of growth. Consistent

with this theory, bud activity is renewed following exposure of the dormant bud and shoot maturation; the bud is instead the dominant sink. Proceeding chapters consider the roles of environment, assimilate partitioning and plant hormones in the context of this hypothesis.

IV.2 Effect of environment on growth

2.1 Introduction

Shoot growth in tea was shown in Chapter IV.1 to occur in repeating cycles of growth and dormancy, where leaf primordia were initiated during dormancy. As an infradian rhythm, environment may modify the amplitude (yield) and frequency (duration) of the flush (Koukkari and Warde, 1985). Shoot extension and shoot length vary with air temperature (Tanton, 1982a; Squire, 1979) and day length (Tanton, 1982b), but the combined effect of these two factors on rhythmic growth in tea has not been reported. The aim of this chapter is to assess the effect of high and low night temperatures combined with short and long day lengths on flush duration, shoot extension, leaf initiation and production during rhythmic growth.

2.2 Materials and methods

2.2.1 Experimental design

Plants were pruned and maintained under long days (16 hours) and high night temperature (15 °C) conditions until bud burst. Twenty four plants were randomly allocated to four environment treatments (chapter III 4.3), of all possible combinations of long (16 hour) and short (9 hour) days with high (15 °C) and low (5 °C) night temperatures (Table 2.1). These conditions were maintained using the glasshouse and three growth cabinets in the glasshouse.

Table 2.1 Environment conditions

Day length	Temperature (°C)	
(hours)	15	5
16	LD HN	LD LN
9	SD HN	SD LN

Three shoots for each replicate plant per treatment were tagged. Extension growth and the number and length of internodes and leaves were measured every two to three days for two consecutive flushes, as described in Chapter III.5 of the general materials and methods.

The effect of environment on the number of primordia in the apical bud during rhythmic shoot growth was assessed in a separate experiment. Plants (32) were allowed to initiate buds under normal glasshouse conditions and then randomly allocated to two environments; LD HN and SD LN. The number of primordia and expanded leaves was measured for 10 shoots per treatment at regular intervals, as described in Chapter III.5 of the general materials and methods.

2.2.2 Statistical analysis

The maximum shoot length and number of leaves per shoot, the rate of shoot extension and rate of leaf unfolding for each shoot per replicate, treatment and flush was estimating by fitting data to the logistic model using Systat (version 5.2 for the Macintosh), as in Chapter III.7 of the general materials and methods.

Tests of significance for main effects and interactions between treatments of day length and temperature were then assessed using an ANOVA for two factors with replication (Excel 4.0) for each of the following:

For flushes one and two (1, 2):

- Shoot length
- Shoot extension
- Number of leaves per shoot
- Rate of leaf unfolding
- Length of leaves and internodes at positions 1 (start), position 2 (half way along flush) and position 3 (end) of flush
- Duration of first flush, dormancy and second flush.

The significance of treatment means was assessed at the 0.05 level using the least significant difference where interactions were significant.

2.3 Results

2.3.1 General features of the effect of environment on rhythmic growth

Two flushes of growth, separated by a period of dormancy of about 30 days (Table 2.2), occurred in each of the four environments (Figs. 2.1 and 2.2). This was less pronounced in LD LN, as shoot growth was slightly asynchronous and therefore the average response when presented graphically did not provide clear evidence for true dormancy (Figs. 2.1 and 2.2). The duration of the first flush at around 30 days was approximately half that of the second flush, except in low night temperatures where the duration of both flushes was similar at 60 days. The rate of shoot extension in the first flush was similar between all environments, where shoots attained a maximum length of 80 to 100 mm (Fig. 2.1). Shoot extension in the second flush was greatest in LD HN, at 290 mm, producing a combined shoot length (flush one and two) of 390 mm. This was approximately two and a half times the total shoot length of the other three environments, at 100 to 150 mm.

Leaf production (Fig. 2.2) during rhythmic shoot growth followed similar trends to shoot extension. In the first flush, leaf production was similar for all treatments, where shoots produced on average 4 to 5 leaves. In the second flush, leaf production was greater in LD HN, which produced on average 9 leaves, with a combined total (flush one and two) of 14 leaves. Plants in the remaining environments produced 2 to 5 leaves, reaching a combined total of 7 to 10 leaves for flushes one and two.

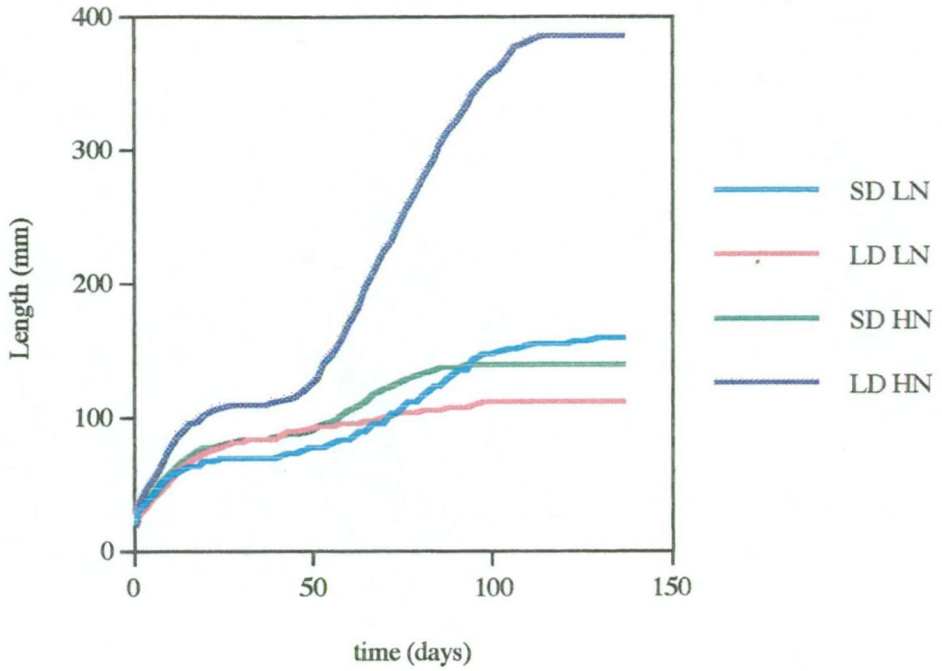

Figure 2.1 Effect of interaction between day length and night temperature on shoot length

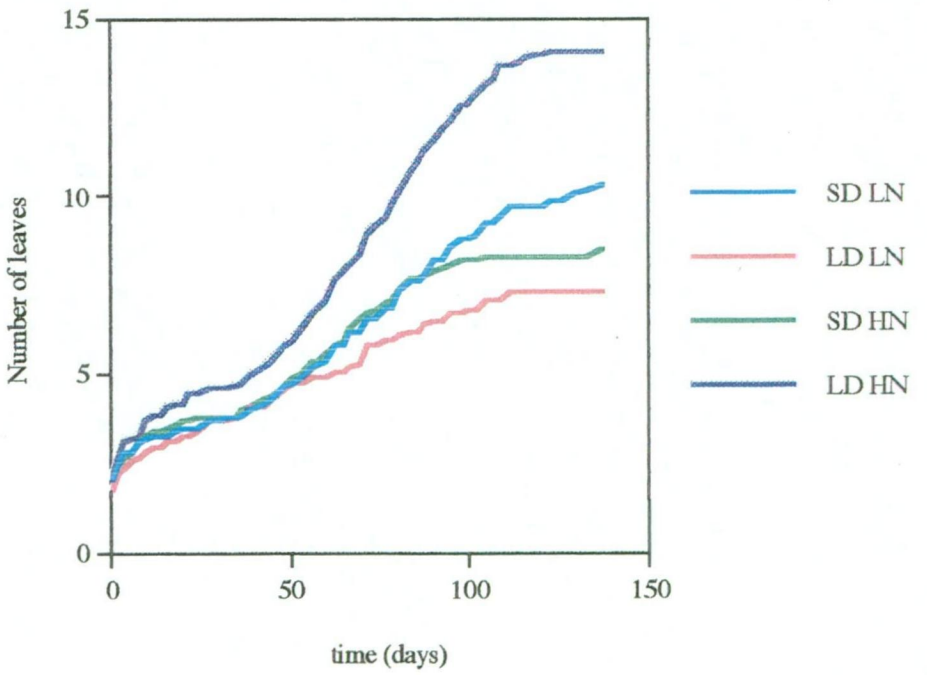

Figure 2.2 Effect of interaction between day length and night temperature on number of leaves per shoot

2.3.2 Flush duration

There was no observed effect of the imposed environment on the duration of the first flush, which was on average 30 days for all treatments (Table 2.2). Similarly, there was no effect of environment on the duration of dormancy which separated the first and second flushes of growth, which was from 30 to 37 days (Table 2.2). A significant interaction in the opposing environments of SD LN and LD HN increased the duration of the second flush to 70 and 56 days respectively (Table 2.3).

Table 2.2 Effect of day length and night temperature on duration of flushes one and two and dormancy ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		First flush (days)	Dormancy (days)	Second flush (days)
Day length	SD	28	34	60
(A)	LD	31	33	52
	F	NS	NS	NS
Temperature	LN	28	37	29
(B)	HN	31	30	49
	F	NS	NS	NS
AB	F	NS	NS	**

Table 2.3 Interaction between day length and night temperature for duration of second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	70ax	42bx
LD	49ax	56ax

2.3.3 Shoot extension and leaf production during rhythmic growth

In the first flush, shoot length was significantly increased by LD and HN (main effect, no interaction) to 94 mm compared to either SD or LN, at 73 mm (Table 2.4). There was no significant effect of environment on the rate of shoot extension (10 mm day^{-1}), the number of leaves per shoot (around 4), or the rate of leaf production (about 0.5 mm day^{-1}) in the first flush (Table 2.4). This may reflect the common environmental conditions (LD HN) which plants were subject to during leaf initiation.

In the second flush, there was a highly significant interaction between day length and night temperature (Table 2.5). Long days and a high night temperature increased shoot extension to almost 16 mm day^{-1} and hence shoot length to 315 mm (Table 2.6 and 2.7 respectively). Shoot extension in the other combinations of day length and night temperature was comparably slower, at only 2 to 6 mm day^{-1}, attaining a shoot length of 60 to 90 mm (Table 2.6 and 2.7). Of these, shoot extension in SD HN at almost 6 mm day^{-1} was greater than under LD LN at almost 2 mm day^{-1}, which may indicate that the effect of high night temperature predominated over long days (Table 2.6). The rate of leaf production was greater under HN (main effect, no interaction) at 0.34 leaves day^{-1}, compared to LN at 0.2 mm day^{-1} (Table 2.5). Day length was insignificant as the rate of leaf production was 0.26 mm day^{-1} for both SD and LD (Table 2.5). A highly significant interaction between LD and HN increased the number of leaves per shoot to 12 (Table 2.8). This was twice that produced in the other combinations of day length and night temperature (Table 2.8).

Table 2.4 Effect of day length and night temperature on first flush for rate of shoot extension, shoot length, leaf production and total number of leaves ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		Shoot extension (mm day^{-1})	Shoot length (mm)	Leaf production (leaves day^{-1})	Number leaves per shoot
Day length	SD	9.4	73.2	0.52	4.2
(A)	LD	10.1	93.8	0.46	4.3
	F	NS	**	NS	NS
Temperature	LN	9.0	74.6	0.48	4.2
(B)	HN	10.5	92.1	0.50	4.3
	F	NS	*	NS	NS
AB	F	NS	NS	NS	NS

Table 2.5 Effect of day length and night temperature on second flush for rate of shoot extension, shoot length, leaf production and total number of leaves ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		Shoot extension (mm day^{-1})	Shoot length (mm)	Leaf production (leaves day^{-1})	Number leaves per shoot
Day length	SD	4.3	85.7	0.26	6.3
(A)	LD	8.7	188.9	0.26	8.7
	F	***	***	NS	***
Temperature	LN	2.4	71.4	0.20	6.4
(B)	HN	10.7	203.2	0.34	8.6
	F	***	***	***	**
AB	F	***	***	NS	***

Table 2.6 Interaction between day length and night temperature for shoot extension of second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	3.0ax	5.7ax
LD	1.8ax	15.7by

Table 2.7 Interaction between day length and night temperature for shoot length of second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	80.5ax	90.9ax
LD	62.4ax	315.4by

Table 2.8 Interaction between day length and night temperature for number of leaves in the second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	7.2ax	5.4bx
LD	5.6ax	11.7by

2.3.4 Leaves and internodes

In both flushes, leaves in position one, as cataphylls, were shorter than leaves in either positions two or three. This indicated that the basic components of the flush were maintained across environments (Fig. 2.3). There were no consistent trends between environment and leaf length. In the first flush, an interaction between LD with LN increased the length of cataphylls in position one to 55 mm (Table 2.10). In the second flush, there was no significant interaction between day length and night temperature (Table 2.9). There was instead a main effect of LN that increased the length of cataphylls in position one to about 20 mm (Table 2.9).

In the first flush, a main effect of LD increased the length of leaves in position two to almost 100 mm (Table 2.11). Yet in the second flush, a significant interaction between LD HN increased leaf length in position two to about 90 mm (Table 2.12). In the first flush, an interaction between LD LN increased the length of leaves in position three to almost 100 mm (Table 2.15). In comparison, leaf length in the second flush in position three was significantly increased by a main effect of HN to almost 50 mm (Table 2.14).

In both flushes, internodes in position one, at the start, and position three, at the end of the flush were shorter than internodes at position two, half way along the stem (Fig. 2.3). In the first flush, there was no effect of environment on the length of internodes in position one, two or three (Tables 2.9, 2.11 and 2.14). In the second flush, the length of internodes in position one was not affected by environment, and at around 5 mm was similar in length to the first flush (Table 2.9). In the second flush, a significant interaction between LD HN increased the length of internodes in position two, to about 50 mm, and position three to 12 mm (Tables 2.13 and 2.16). This contributed to the increased final shoot length observed in Fig. 2.1.

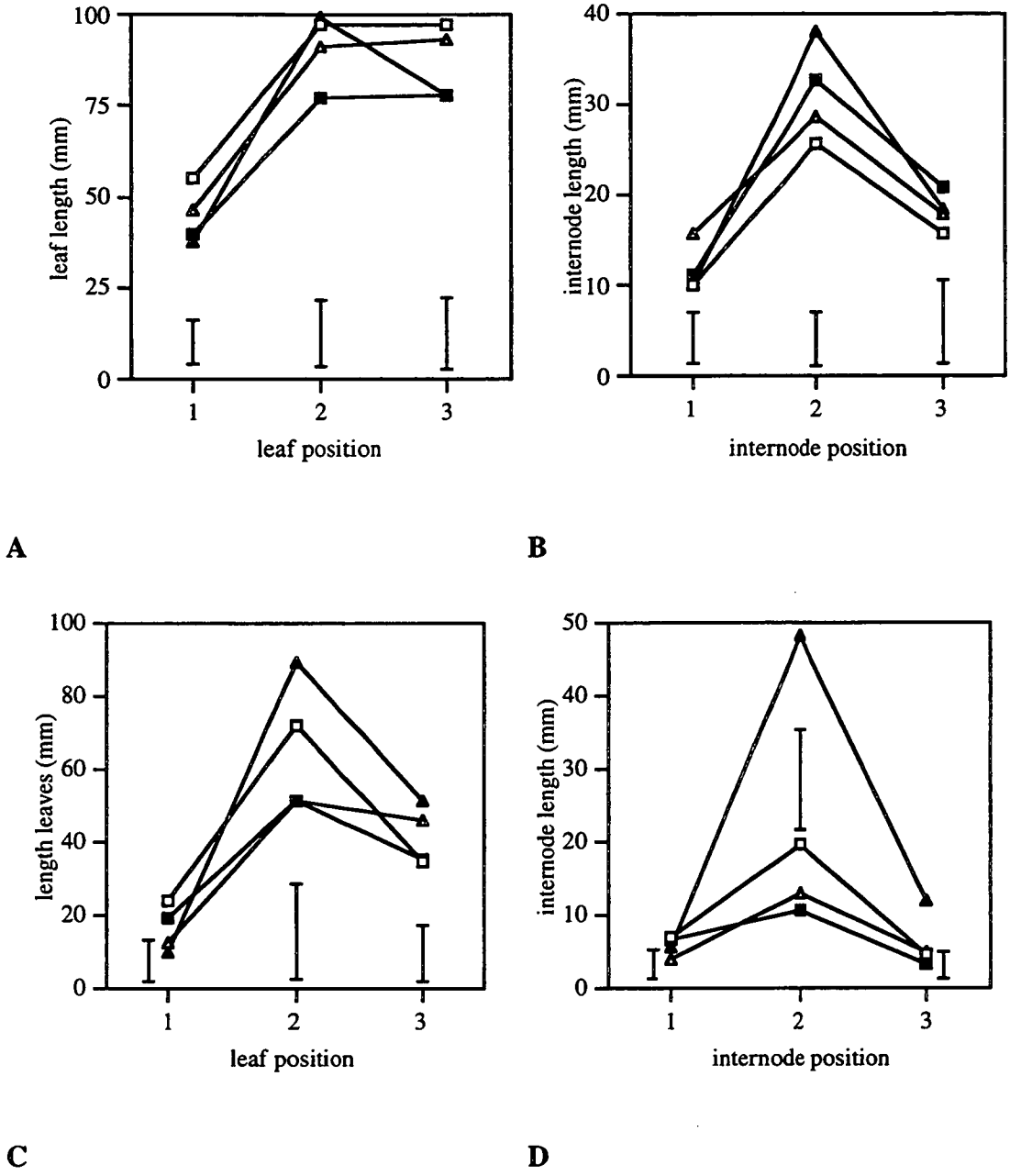

Figure 2.3 Effect of environment on length of leaves and internodes A) leaf length, flush 1; B) internode length, flush 1; C) leaf length, flush 2; D) internode length, flush 2; □) SD LN; ■) LD LN; Δ) SD HN; ▲) LD HN. Positions 1 to 3 refer to leaves or internodes at start, middle and end of shoot respectively; LSD's shown for each leaf or internode position

Table 2.9 Position one. Effect of day length and night temperature (main effects and interaction) on length of leaves and internodes for first and second flushes. Means in each column followed by the same letter are not significantly different ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		Flush 1		Flush 2	
		Leaves (mm)	Internode (mm)	Leaves (mm)	Internode (mm)
Day length	SD	42.8	12.9	17.8	5.4
(A)	LD	46.7	10.6	14.5	6.13
	F	NS	NS	NS	NS
Temperature	LN	47.5	10.6	21.3a	6.8
(B)	HN	42.0	12.9	11.1b	4.8
	F	NS	NS	**	NS
AB	F	**	NS	NS	NS

Table 2.10 Position one. Interaction between day length and night temperature for leaf length (mm) in first flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	39.6ax	46.1ax
LD	55.4ay	37.9bx

Table 2.11 Position two. Effect of day length and night temperature (main effects and interaction) on length of leaves and internodes for first and second flushes. Means followed by the same letter are not significantly different ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		Flush 1		Flush 2	
		Leaves (mm)	Internode (mm)	Leaves (mm)	Internode (mm)
Day length	SD	84.2a	27.3a	61.6	16.2a
(A)	LD	98.2b	35.2b	70.2	29.3b
	F	*	**	NS	**
Temperature	LN	87.1	29.1	61.6	15.0a
(B)	HN	95.2	33.3	70.2	30.5b
	F	NS	NS	NS	***
AB	F	NS	NS	**	***

Table 2.12 Position two. Interaction between day length and night temperature for leaf length (mm) in second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	71.9ax	51.3ax
LD	51.2ax	89.2by

Table 2.13 Position two. Interaction between day length and night temperature for internode length (mm) in second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	19.5ax	12.9ax
LD	10.4ax	48.1by

Table 2.14 Position three. Effect of day length and night temperature (main effects and interaction) on length of leaves and internodes for first and second flushes. Means followed by the same letter are not significantly different ($p<0.05$*; $p<0.01$**; $p<0.001$***)

		Flush 1		Flush 2	
		Leaves (mm)	Internode (mm)	Leaves (mm)	Internode (mm)
Day length	SD	85.4	16.9	40.1	4.9^a
(A)	LD	87.5	19.5	43.1	7.7^b
	F	NS	NS	NS	**
Temperature	LN	87.5	18.3	34.8^a	4.0^a
(B)	HN	85.5	18.1	48.5^b	8.6^b
	F	NS	NS	14.4*	***
AB	F	*	NS	NS	***

Table 2.15 Position three. Interaction between day length and night temperature for leaf length (mm) of the first flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	77.7ax	93.2ax
LD	97.3ay	77.8bx

Table 2.16 Position three. Interaction between day length and night temperature for internode length (mm) of the second flush. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Day length	Night temperature	
	LN	HN
SD	4.7ax	5.1ax
LD	3.3ax	12.0by

2.3.5 Leaf initiation

The number of primordia within the apical bud was assessed for two of the four environments, LD HN and SD LN. Plants initiated roughly five leaf primordia under the pretreatment conditions of LD HN (Fig. 2.4). These primordia expanded into leaves following the allocation of plants between the two environments. Leaf expansion in the first flush for both treatments resulted in a decline in the number of primordia within the bud to four. These four primordia, plus a further two primordia formed during the final stages of dormancy, expanded producing the six leaves of the second flush in LD HN. During shoot extension, a further four primordia were initiated in the bud. The number of primordia in the bud during shoot extension was therefore constant. These additional four primordia formed the cataphylls of the dormant bud after shoot extension was complete. Dormancy in SD LN was 25 % longer in duration than in LD HN, yet only one primordium was initiated (Fig. 2.5). Hence the second flush started later and produced fewer leaves.

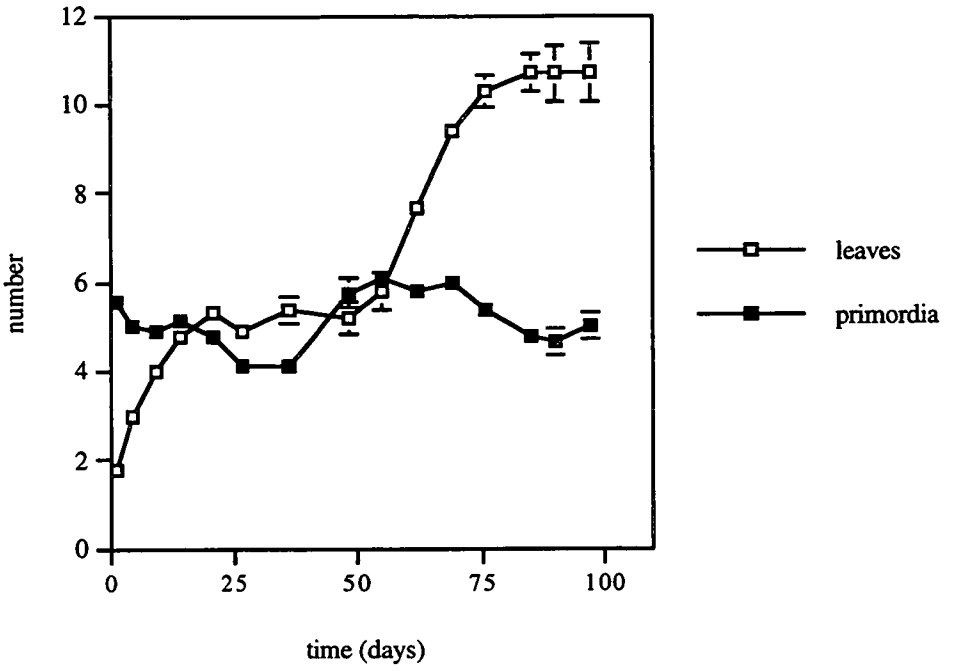

Figure 2.4 Number of leaves per shoot and primordia in apex over time under LD HN. Bars represent SE of 10 replicates

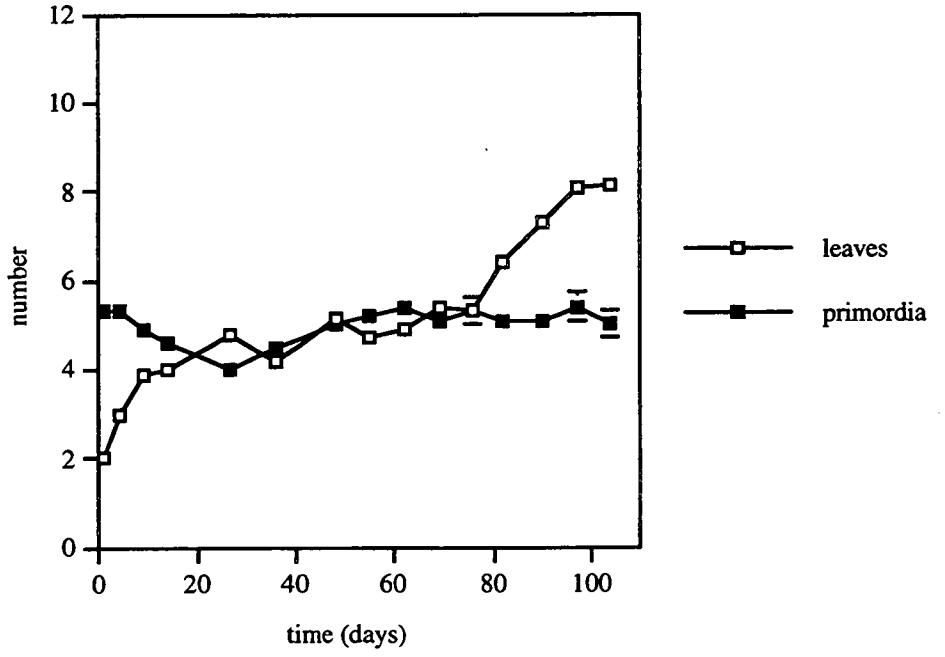

Figure 2.5 Number of leaves per shoot and primordia in apex over time under SD LN. Bars represent SE of 10 replicates

2.4 Discussion

Rhythmic growth was maintained under the four combinations of day length and night temperature. This was consistent with endogenous infradian rhythms, which are modified, but not controlled, by changes in environment (Koukkari and Warde, 1985). Although shoot morphology was similar in each of the four environments, the size of leaves and internodes varied. Increased size of leaves and internodes due to main effects and interactions between long days and a high night temperature would be likely to contribute to an increase in yield under these conditions. This is in agreement with the observations of Yanase (1980), where it was reported that leaf area was greater in high temperatures, although the effect of day length on leaf area was not considered.

The relationship between environment, leaf initiation and subsequent leaf production has not been previously assessed. From five to six leaf primordia were initiated in buds when plants were subject to the same environmental treatments following pruning and before allocation to treatments at bud burst. Four leaves were subsequently produced in the first flush, regardless of environment. As a result, environment only affected the rate of shoot extension and hence total shoot length. For example, the rate of shoot extension at 10 mm day^{-1} in both long days and high night temperatures (main effects, no interaction), resulted in significantly greater shoot lengths at around 90 mm in comparison to 75 mm for either short days or low night temperatures.

At unfolding of the final flush leaf, the apical bud contained four primordia. In LD HN, a further two primordia were initiated in the bud during dormancy, with additional leaf primordia initiated during growth of the second flush. These later formed the cataphylls and the first two foliage leaves of the second flush. This is in agreement with the observations of Bond (1942, 1945), where leaf primordia were reportedly initiated during dormancy and cataphyll primordia during shoot extension. In comparison, fewer leaf primordia were formed in the second flush in SD LN. As a result, a significantly greater number of leaves was produced under LD HN (12 in total), than under SD LN (7 in total). A shorter plastochron, at 0.3 leaves per day, also contributed to increased leaf production in LD HN. Shoot extension and hence shoot length was increased by a significant interaction between long day length and high night temperatures. Shoot extension in LD HN at 15 mm day^{-1} was much greater in comparison to 2 to 5 mm day^{-1} for other treatments; while similarly total shoot length under LD HN at 315 mm was significantly greater than the average of 75 mm obtained in the remaining treatments.

It is proposed that environment influences shoot growth in two distinct ways. Firstly, environment can modify the number of leaf primordia initiated both during dormancy and shoot extension; which subsequently affects the number of leaves produced during shoot growth. This new information then improves our understanding of the relationship between environment and leaf production in tea. Secondly, environment can modify the rate of shoot extension and hence total attainable shoot length. It was not possible from the experimental design used to assess whether the effect of temperature was a general one or related specifically to night temperature. The response to night temperature was however consistent with reports published by Tanton (1982a), Squire (1979) and Smith *et al.* (1993a), where shoot extension was reported to increase in response to an increase in temperature. The rate of shoot extension and total shoot length attained during rhythmic growth were similar to that reported in the literature, although the rate of shoot extension, total shoot length and number of leaves produced in the LD HN treatment appear to be exceptional. For example, typical shoot lengths of 30 to 50 mm, with a rate of growth of 0.9 to 1.5 mm day^{-1} were reported by Smith *et al.* (1990), while in the only other comparable treatment reported in the literature, shoots attained a maximum length of 160 mm under 13 hour day length and 20 °C night temperatures (Tanton, 1982a).

Similar leaf production for each environment in the first flush was reflected in the duration of the flush, at 30 days (4.3 weeks), for all environments. This was considerably shorter than the average flush duration of 12 weeks reported by Bond (1942, 1945), under constant conditions, and Tanton (1982b) in differing day lengths and night temperatures. The duration of the second flush was increased to 70 days (10 weeks) under LD HN compared to the other environments, due to the production of additional leaves and increased shoot length.

Results from the current study support the proposal of Das and Barua (1987) that shoot growth is affected by both day length and temperature, and not day length alone, as first reported by Barua (1969). The effect of day length and night temperature on shoot growth in the current study differed from both Das and Barua (1987) and Tanton (1982b). Das and Barua (1987), reported that plants became dormant under conditions of short days (11 hours) combined with high temperatures (27 °C); and long days (13 hours) combined with low temperatures (5 °C). In comparison, plants in the current study continued to grow in all combinations of short days (9 hour), long days (16 hours), high night temperatures (15 °C) and low night temperatures (7 °C), with dormancy only occurring as a result of the rhythmic growth cycle. Tanton (1982b) reported that a day length of 11 hours reduced shoot growth in black tea only when combined with high night temperatures, concluding that temperature had a more

significant effect than day length on shoot extension - even though not all possible combinations of day length and night temperature were assessed, as in the current study. These differences could, however, result from species differences. Black tea is primarily a subtropical crop, and may consequently lack the adaptation observed in green tea as a temperate crop to short day length and low night temperature.

In conclusion, rhythmic growth in green tea modified by environment, was observed through differences in leaf initiation, leaf production, shoot extension and shoot length. As tea is grown only for its vegetative shoots, a natural progression of the current study is to consider the effect of environment on harvested yield and quality of tea, as examined in Chapter IV.3. Changes in tea shoot physiology occurring during rhythmic growth will be examined in Chapters IV.5, IV.6 and IV.7.

IV.3 Effect of environment on harvest yield and quality

3.1 Introduction

Chapter IV.2 established that rhythmic growth in tea was modified by the environment. Growth of cultivated tea differs in that shoots are harvested at plucking standards of between two to four leaves and a bud before the rhythmic cycle is complete. New shoots are produced from axillary buds which have been released from apical dominance. It is reported that yield and quality of harvested tea varies with season, harvest and plucking standard under field conditions (Nakagawa *et al.*, 1977; Owuor and Othieno, 1991; Owuor and Odhiambo, 1993). Under these conditions it is difficult to separate the effect of each individual factor, yet there are few studies which report the effect of environment, plucking standard or harvest on yield and quality of green tea under controlled conditions. The aim of this chapter is to assess the effect of environment, plucking standard and harvest on harvest time, yield and quality of tea in controlled conditions.

3.2 Materials and methods

3.2.1 Experimental design

Plants (36) were pruned and nine plants randomly allocated to four environment treatments (chapter III 4.3). Four treatments (environments) of all possible combinations of long (16 hour) and short (9 hour) day lengths with high (15 °C) and low (5 °C) night temperatures were maintained using three controlled cabinets and glasshouse (Table 3.1).

Table 3.1 Environment conditions

Day length	Temperature (°C)	
(hours)	15	5
16	LD HN	LD LN
9	SD HN	SD LN

Each treatment of nine plants was further divided into sub plots of three plants (replicates). Sub plots represented three plucking standards, including harvests at two, three and four leaves and a bud per shoot respectively, for harvest one (H1) and harvest two (H2).

The start of growth and harvest date was recorded for each plant. At the time of harvest, the number of leaf primordia contained in three randomly chosen axillary buds was measured for each sub plot and treatment, to determine the potential of the new flush.

Following harvest, leaf samples were processed as in Chapter III.8 of the general materials and methods, and dry matter yield recorded. Samples were then ground using a hammer mill, and quality (total phenolics, nitrogen, NDF and aroma) determined as in Chapter III.9 of the general materials and methods.

3.2.2 Statistical analysis

Insufficient growth of plants in the SD LN treatment prevented any statistical analysis for interactions between day length and night temperature. Instead, the remaining three treatments of LD LN, SD HN and LD HN were grouped within the factor 'environment', and tested against the factors 'plucking standard' and 'harvest' for both main effects and interactions using an ANOVA for three factors with replication, using Genstat 5 (Second Edition). The significance of treatment means was assessed at the 0.05 level using Duncan's New Multiple Range Test where main effects or interactions were significant (Steel and Torrie, 1980).

3.3 Results

3.3.1 Time to harvest

Low night temperatures significantly increased the duration of dormancy preceding the start of growth of the first flush to 53 days in LD LN, when compared to only 2 days in LD HN (Table 3.2). Environment did not affect the duration of the first flush, which was on average 17 days. Duration of the first flush significantly increased from 13 days at plucking standards of two leaves, to 21 days for plucking standards of four leaves (Table 3.2). Dormancy (2) separating the first and second flushes was not affected by either environment or plucking standard and was on average 30 days (Table 3.2). Duration of the second flush (from bud burst to harvest) was greater in SD HN, at 21 days, than either LD LN or LD HN, which were on average 16 days in duration. A similar, although insignificant trend was observed in the first flush. These results suggest that leaf production under short day lengths was slower than in long day lengths. Duration of the second flush increased from 7 days at plucking standards of two leaves, to 28 days at plucking standards of four leaves. Harvests were staggered due to the increased duration of dormancy in LD LN treatment and differences in flush duration between plucking standards.

Table 3.2 Duration of dormancy and flushes (days) for first and second harvests ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means in each column followed by the same letter are not significantly different ($p<0.05$)

		dormancy 1	flush 1	dormancy 2	flush 2
Environment	LD LN	53.3a	16.3	29.9	15.7a
(A)	SD HN	9.9b	18.1	33.7	21.0b
	SD HN	2.5c	15.1	29.0	16.3a
	F	***	NS	NS	*
Plucking	two	22.2	12.6a	29.7	7.2a
standard (B)	three	20.5	15.6a	31.4	17.1b
	four	23.0	21.2b	31.1	28.7c
	F	NS	**	NS	***
AB	F	NS	NS	NS	NS

3.3.2 Number of primordia

Following harvest, new shoots were produced from the uppermost axillary bud on the remaining stub. Dissection of these buds revealed that the number of leaf primordia increased significantly from 3.8 primordia at a plucking standard of two leaves, to 4.4 primordia at a plucking standard of four leaves (Table 3.3). The number of primordia in buds at a plucking standard of three leaves was intermediate at 4.1 primordia (Table 3.3). The observed increase in number of primordia at a plucking standard of four leaves reflected an increase in bud age. This did not alter the length of dormancy, which was similar for all plucking standards (Table 3.2). Neither environment or harvest had any significant effect on the number of primordia. There were no interactions between factors environment, plucking standard or harvest.

Table 3.3 Number of primordia for environment, plucking standard and harvest and interactions ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		Primordia
Environment	LD HN	4.1
(A)	LD LN	4.1
	SD HN	4.2
	F	NS
Plucking (B)	two	3.8a
Standard	three	4.1b
	four	4.5c
	F	***
Harvest (C)	H1	4.1
	H2	4.1
	F	NS
AB	F	NS
AC	F	NS
BC	F	NS
ABC	F	NS

3.3.3 Yield

Yield varied with environment, significantly increasing from 3.2 g in LD LN to 4.9 g in LD HN (Table 3.4). Yield in SD HN at 4 g was then midway between LD LN and LD HN (Table 3.4). These observations and the lack of growth in SD LN may indicate that the effect of night temperature on yield may be greater than day length, although interactions between these two factors cannot be assessed from the current study. Yield significantly increased with plucking standard, from 1.7 g for two leaves, to 6.6 g for four leaves (Table 3.4). Yield at a plucking standard of three leaves at 3.8 g was then midway between that of two and four leaves (Table 3.4). There was no significant difference in yield between harvests one and two, which were on average 4 g. There were no interactions between factors environment, plucking standard or harvest.

Table 3.4 Yield (g) for environment, plucking standard and harvest and interactions ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		Yield
Environment	LD HN	4.86a
(A)	LD LN	3.28b
	SD HN	4.01ab
	F	*
Plucking (B)	two	1.74a
Standard	three	3.84b
	four	6.58c
	F	***
Harvest (C)	H1	4.09
	H2	4.00
	F	NS
AB	F	NS
AC	F	NS
BC	F	NS
ABC	F	NS

3.3.4 Aroma

A range of volatiles was identified using GC/MS from the headspace sample and solvent extract of dry and brewed tea respectively. Of the nine volatile identified in the headspace sample, only dimethyl sulphide and 3-methyl butanal were likely to be genuine volatiles. Of these, dimethyl sulphide was the most abundant. The remaining seven compounds were potential contaminants (Appendix III). A total of 43 peaks was identified in the hexane extract of brewed tea. The most abundant volatiles included linalool, geraniol, indole, ß-ionone-epoxide, dihydroactinidiolide, nerolidol and caffeine (Appendix IV). Menthone, isomethone, carvone and anethole were likely to have been contaminants. The large sample size of leaf required to analyse the aroma profile of tea restricted quality analysis to total phenolics, nitrogen and neutral detergent fibre.

3.3.5 Total phenolics

Total phenolics were estimated as catechin equivalents, catechin being one of the four phenolics present in tea. Total phenolics were present in higher amounts under long days, at on average 138 mg g^{-1}, compared to short days, at 119 mg g^{-1} (Table 3.5). Total phenolics significantly increased from 123 mg g^{-1} in harvest one to 141 mg g^{-1} in harvest two. There was a significant interaction between environment and harvest, where total phenolics significantly increased from the first harvest, at 114 mg g^{-1} to 157 mg g^{-1} in the second harvest in LD LN (Table 3.6). Total phenolics significantly decreased from a plucking standard of two leaves, at 150 mg g^{-1}, to 116 mg g^{-1} at a plucking standard of four leaves (Table 3.5). Total phenolics for the plucking standard of three leaves at 131 mg g^{-1} was then midway between the plucking standards of two and four leaves (Table 3.5). There were no interactions between plucking standard with either environment or harvest.

Table 3.5 Total phenolics (mg catechin equivalents g^{-1}) for environment, plucking standard and harvest and interactions ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		Phenolics
Environment	LD HN	141.7a
(A)	LD LN	135.2a
	SD HN	119.4b
	F	**
Plucking (B)	two	149.5a
Standard	three	130.9b
	four	115.9c
	F	***
Harvest (C)	H1	122.8a
	H2	141.4b
	F	***
AB	F	NS
BC	F	NS
AC	F	**
ABC	F	NS

Table 3.6 Interaction between harvest and environment for total phenolics (mg catechin equivalents g^{-1}). Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Environment	Harvest	
	H1	H2
LD HN	142.5ax	140.9axy
LD LN	113.5ay	156.8by
SD HN	112.3ay	126.4ax

3.3.6 Total nitrogen

Nitrogen contributes to flavour and high concentrations are desirable for tea quality (Goto, 1992). Nitrogen was significantly greater under low night temperatures, at 39 mg g^{-1}, compared to high night temperatures, which were on average 34 mg g^{-1} (Table 3.7). Nitrogen significantly declined with plucking standard, from 39 mg g^{-1} at two leaves, to 32 mg g^{-1} at four leaves (Table 3.7). Total nitrogen at a plucking standard of three leaves, at 36 mg g^{-1}, was midway between two and four leaves (Table 3.7). The observed decrease in nitrogen at plucking standards of three to four leaves was of greater magnitude for high temperatures, due to an interaction between environment and plucking standard (Table 3.8). In comparison, nitrogen showed little variation across environments for a plucking standard of two leaves, which was on average 40 mg g^{-1} (Table 3.8).

Table 3.7 Total nitrogen (mg g^{-1}) for environment, plucking standard and harvest and interactions ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p< 0.05$)

Factor		Nitrogen
Environment	LD HN	33.3a
(A)	LD LN	39.3b
	SD HN	34.7a
	F	***
Plucking (B)	two	39.4a
Standard	three	36.2b
	four	31.7c
	F	***
Harvest (C)	H1	36.6a
	H2	34.9b
	F	*
AB	F	***
BC	F	**
AC	F	*
ABC	F	NS

Total nitrogen declined from harvest one, at 37 mg g^{-1}, to harvest two, at 35 mg g^{-1} (Table 3.7). The observed decline in nitrogen in the second harvest was more pronounced in LD HN due to an interaction between environment and harvest (Table 3.9). Similarly, the observed decline in nitrogen in the second harvest was greater at a plucking standard of four leaves due to an interaction between harvest and plucking standard (Table 3.10).

Table 3.8 Interaction between environment and plucking standard for nitrogen (mg g^{-1}) for environment. Means in each row followed by the same letter (a-c) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Environment	Plucking standard		
	two	three	four
LD HN	38.7ax	33.0bx	38.2cx
LD LN	39.7ax	42.5cy	35.7by
SD HN	40.0ax	33.1bx	31.1bx

Table 3.9 Interaction between environment and harvest for nitrogen (mg g^{-1}) for environment. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x-z) are not significantly different ($p<0.05$)

Environment	Harvest	
	H1	H2
LD HN	35.5ax	31.1bx
LD LN	39.5ay	39.0ay
SD HN	34.8ax	34.6az

Table 3.10 Interaction between plucking standard and harvest for nitrogen (mg g^{-1}) for environment. Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x-z) are not significantly different ($p<0.05$)

Plucking standard	Harvest	
	H1	H2
two	40.1ax	38.8ax
three	35.9ay	36.4ay
four	38.8az	34.6by

3.3.7 Neutral detergent fibre

Neutral detergent fibre (NDF), which is a measure of tissue maturity, increased significantly with plucking standard, from 156 mg g^{-1} at two leaves, to 192 mg g^{-1} at four leaves (Table 3.11). NDF at three leaves, at 164 mg g^{-1}, was then midway between two and four leaves (Table 3.11). This reflected increased leaf maturity and proportion of stalk. The effect of environment on NDF, at 157 to 182 mg g^{-1} was insignificant (Table 3.11). There was no significant difference in NDF between harvests one and two, at on average 171 mg g^{-1} (Table 3.11). There were no interactions between environment, plucking standard or harvest (Table 3.11).

Table 3.11 Neutral detergent fibre (mg g^{-1}) for environment, plucking standard and harvest and interactions ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different at $p < 0.05$

Factor		NDF
Environment	LD HN	174
(A)	LD LN	157
	SD HN	182
	F	NS
Plucking (B)	two	156a
Standard	three	164a
	four	192b
	F	*
Harvest (C)	H1	168
	H2	174
	F	NS
AB	F	NS
AC	F	NS
BC	F	NS
ABC	F	NS

3.4 Discussion

Yield and quality of harvested tea was affected by environment, plucking standard and repeated harvests. Growth in SD LN was insufficient for harvesting, which therefore limited the analysis to the combined factors of day length and night temperature for the remaining treatments. Poor growth in SD LN combined with the significant delay in bud burst in LD LN relative to either SD HN or LD HN perhaps indicated that low night temperatures interacted with short day lengths to delay bud burst. This is in agreement with the observations of Kuranuki (1988), where it was reported that bud burst of the first crop following winter was delayed by low temperature, although the effect of day length on bud burst was not considered. These results also provide practical implications for site selection, where sites which experience relatively low temperatures during spring are likely to commence bud burst later than sites which experience higher temperatures. Delayed bud burst may then reduce the number of harvests per season.

The initial delay in bud burst in the LD LN treatment did not affect the duration of the subsequent flush. Instead, duration of the first and second harvests increased as additional leaves were harvested to obtain plucking standards of three and four leaves per shoot. This was consistent with reports on black tea, where increasing the round length increases the proportion of coarse shoots, which have more than two leaves (Cloughley *et al.*, 1983; Basu, 1989; Owuor and Odhiambo, 1990). Consequently, it is possible that more harvests may be achieved per season for a fine compared to a coarse plucking standard. Increased duration of the second flush in SD HN perhaps indicated that short day lengths increase the amount of time required for shoot growth. Similar observations were reported by Tanton (1982b), although it was concluded that low temperatures had a greater effect on shoot growth than day length. Yanase (1980) has similarly reported that flush duration is shorter for higher temperatures; although the effect of day length was not considered. Consequently, both the environment and plucking standard may alter the harvest schedule of green tea.

Increasing the plucking standard from two to four leaves increased the age of axillary buds on the remaining stub at harvest relative to harvests of two or three leaves. This was reflected in the increased number of leaf primordia. However, this did not affect the duration of dormancy which separated the first and second flushes. Variation in the number of leaf primordia in axillary buds has been reported by Goodchild (1968), where the number of leaf primordia in axillary buds increased as distance from the apical bud increased. A significant reduction in the length of dormancy may then only be expected should the plucking standard be greater than four leaves and a bud.

Yield was significantly affected by the plucking standard. As the plucking standard was increased from two to four leaves, the additional leaves and stalk increased yield, which is consistent with reports in the literature (Willson and Clifford, 1992). The effect of plucking standard on yield occurred independently of environment. Yield was greater in LD HN than in either LD LN or SD HN. Previously, only shoot extension (a component of yield) has been shown to increase in response to either increasing temperature (Yanase, 1980) or in combination with increasing day length (Tanton, 1982b) under controlled conditions.

In the literature review, the main quality parameters of green tea which may be affected by environment and plucking standard included aroma, phenolics, nitrogen and fibre. The major components of the aroma profile detected by headspace analysis and hexane extract of brewed tea included dimethyl sulphide, 3-methyl butanal, linalool, geraniol, indole, ß-ionone-epoxide, dihydroactinidiolide, nerolidol and caffeine; which is consistent with the literature (Kiribuchi and Yamanishi, 1963; Yamanishi *et al.*, 1970; Takei *et al.*, 1976; Kawabata *et al.*, 1977; Kawakami and Yamanishi, 1983; Yamanishi *et al.*, 1995). A variety of trace compounds were also detected, some of which were likely to be contaminants. However, the low yield of processed leaf per plant limited the assessment of quality to phenolics, nitrogen and neutral detergent fibre.

Although yield increased at a plucking standard of four leaves and a bud, leaf quality, as defined by phenolics, nitrogen and fibre, declined, while the effect of environment on leaf quality varied. Phenolics, or more specifically, the flavan-3-ols, contribute positively to tea quality. The total amount of phenolics increased under long days, which was consistent with the activity of the enzyme PAL, which is a key enzyme in the synthesis of phenolics and regulated by light (Sajio, 1980). Furthermore, there was a significant interaction between environment and harvest, where total phenolics significantly increased in the second harvest of the LD LN treatment to be comparable to that for harvests one and two in LD HN. It is however unclear as to why there was a higher relative proportion of phenolics in the second but not first harvest in LD LN. Phenolics were similarly greater when two (150 mg g^{-1}), rather than four leaves (120 mg g^{-1}) were harvested. This is in agreement with the observations of Owuor and Odhiambo (1990), for (oxidised) phenolics in black tea, and Forrest and Bendall (1969), who reported that phenolics were present in highest concentration in young tea tissue, especially the shoot tips. The maximum amount of phenolics, at 150 mg g^{-1}, was similar to that reported in 'very high quality' teas from China (Liang *et al.*, 1990).

The total amount of nitrogen was similarly greater in shoots harvested at two leaves and a bud, at 40 mg g^{-1} or 4 %, which reflects the tendency of nitrogen to accumulate in young tissue (Okano *et al.*, 1994). This is however low in comparison to the 6.5%

usually reported in high quality teas produced in Japan (Nakano *et al.*, 1996), although this reflects the higher level of fertiliser inputs currently used in Japan. When the plucking standard, and hence yield, was increased by harvesting three and four leaves and a bud, nitrogen declined. However, a significant interaction between environment and plucking standard revealed that the decline in nitrogen was greater for plucking standards of four leaves under a high night temperature. Total nitrogen also declined from the first to the second harvest. This decline was more pronounced for plucking standards of four leaves under LD HN, due to interactions between harvest and plucking standard, and harvest and environment respectively. Perhaps repeated harvests under these circumstances may result in a decline in the size of the nitrogen pool and thus nitrogen content of the harvested leaves (Okano and Matsuo, 1996). High temperatures are also reported to reduce the amount of amino acids in tea shoots, from which the ammonium side chain also contributes to nitrogen, although the effect of day length was not assessed (Yanase, 1980). This is in contrast to phenolics, which were instead present in higher amounts under long day lengths. The decline in green tea quality harvested in summer (Nakagawa *et al.*, 1977) may then be related to the negative effect of LD HN on nitrogen.

Neutral detergent fibre, in comparison to nitrogen and phenolics, is reported to have a negative correlation with tea quality (Goto, 1992). Increasing yield through harvesting more leaves tended to increase NDF because of the increased proportion of stem and older leaves. NDF of about 160 mg g^{-1} for two harvested leaves and 190 mg g^{-1} for four leaves was similar to quantities reported by Nakano *et al.* (1996). NDF also tended to be greater in high night temperatures (170 mg g^{-1}) than in low night temperatures (150 mg g^{-1}). Although this was insignificant, these results perhaps indicate that high night temperature increases plant fibre. This is consistent with Yanase (1980), where high temperatures were reported to increase thickening and lignification of sclerenchyma and development of the xylem.

Consequently, quality of tea is optimal at plucking standards of two leaves and conditions of long day lengths combined with low night temperatures. This maximises phenolics and nitrogen, which are desirable, while minimising fibre, which otherwise reduces tea quality. Increasingly long day lengths and low night temperatures are typical of spring conditions, which are associated with the production of high quality tea (Willson and Clifford, 1992). A compromise between the observed reduction in yield when two leaves are harvested and reduced quality when four leaves are harvested could be to harvest three leaves.

IV.4 Effect of pruning on harvest yield and quality

4.1 Introduction

Chapter IV.3 established that yield was maximised by harvesting at coarse plucking standards in long days and a high night temperature. In contrast, quality was maximised by harvesting at fine plucking standards in long days and a low night temperature. A compromise between harvested yield and quality can be achieved by harvesting three leaves and a bud under conditions of long days combined with a low night temperature. In field conditions yield is reported to decline with each additional harvest, due to increased bud dormancy (Wight and Barua, 1955). Pruning 5 to 20 cm of the maintenance foliage is reported to overcome the loss of plant vigour and promotes the regrowth of a new flush of leaves (Eden, 1965). Yet, in black tea it is reported that yield declines in the first year after pruning (Mwakha, 1989, 1991). There is a lack of readily available data in the literature that examines the implications of pruning on yield and quality of green tea. The aim of this chapter is to examine the effect of pruning during spring and summer on yield and quality of tea for coarse and fine plucking standards.

4.2 Materials and methods

4.2.1 Experimental design

Plants were maintained in a shade tunnel, as in Chapter III.4.2 of the general materials and methods. Thirty six plants were randomly allocated between three pruning treatments. Plants were pruned to a depth of approximately 5 cm below the maintenance foliage. These included (P-), pruning before the start of spring growth; (P+), pruning before the start of the second (summer) flush; and (C) a control, which was not pruned. Thus there were two harvests; (H1), harvest one, and (H2), harvest two. The pruning treatments were further divided into subplots according to plucking standard, which determined the number of leaves harvested per shoot. Plucking standards included two versus four leaves and a bud, leaving the first leaf (a cataphyll). The experimental design was thus a 3^2 x 2 factorial.

P-2 Pruning, before onset of spring growth, harvest of two leaves and bud

P-4 Pruning, before onset of spring growth, harvest of four leaves and bud

C2 Control, no pruning, harvest of two leaves and bud

C4 Control, no pruning, harvest of four leaves and bud

P+2 Pruning, after first harvest, harvest of two leaves and bud

P+4 Pruning, after first harvest, harvest of four leaves and bud

Dates of harvest were recorded. Following harvest, leaf samples were processed according to Chapter III.8, and yield and quality (NDF, total phenolics and nitrogen) determined as in Chapter III.9. Average maximum and minimum temperatures and day length for the duration of both harvests for each treatment are shown in Table 4.1.

Table 4.1 Average temperature and day length for first and second harvests, per pruning treatment

Harvest 1	time (dates)	max T (°C)	min T (°C)	day length (hr)
P-2	21/8 - 31/10	12.9	7.2	11.3
P-4	21/8 - 15/11	15.2	8.8	12.3
C2	21/8 - 17/10	13.7	7.8	12.1
C4	21/8 - 31/10	14.8	8.4	12.1
P+2	21/8 - 17/10	13.7	7.8	12.1
P+4	21/8 - 31/10	14.8	8.4	12.1
Harvest 2				
P-2	4/11 - 1/12	17.1	9.8	14.1
P-4	15/11 - 24/1	19.0	11.1	14.3
C2	17/10 - 1/12	17.3	10.0	14.1
C4	31/10 - 24/1	18.9	11.1	14.1
P+2	17/10 - 7/1	18.1	10.3	14.1
P+4	31/10 - 24/1	19.0	11.1	14.3

4.2.2 Statistical analysis

The significance of each variable (yield, nitrogen, phenolics and NDF) for factors 'pruning' (P-, C, P+), 'plucking standard' (two, four) and 'harvest' (H1, H2) were tested using an ANOVA for three factors with replication (StatView). Data for each variable was then tabulated as both main effects and interactions. The significance of treatment means was assessed at the 0.05 level using Duncan's New Multiple Range Test (Steel and Torrie, 1980) or the least significant difference where main effects or interactions were significant.

4.3 Results

4.3.1 Time to harvest

Pruning before the growth of the first flush in late winter increased the duration of the first harvest by 10 days, from 60 days in the C2 treatment, to 70 days in the P-2 treatment; and from about 75 days in the C4 treatment to about 85 days in the P-4 treatment (Fig. 4.1). Duration of the first harvest in the P+2 and P+4 treatments were identical to that of the controls, as pruning was not undertaken until after the first harvest. Pruning after the first harvest in the P+ treatments increased the duration of the second harvest from 60 to 90 days in the P+2 treatment, and from 75 to 95 days in the P+4 treatment. In the control, the duration of the second harvest for both the C2 and C4 treatments was increased by approximately 20 days compared to the that of the first harvest.

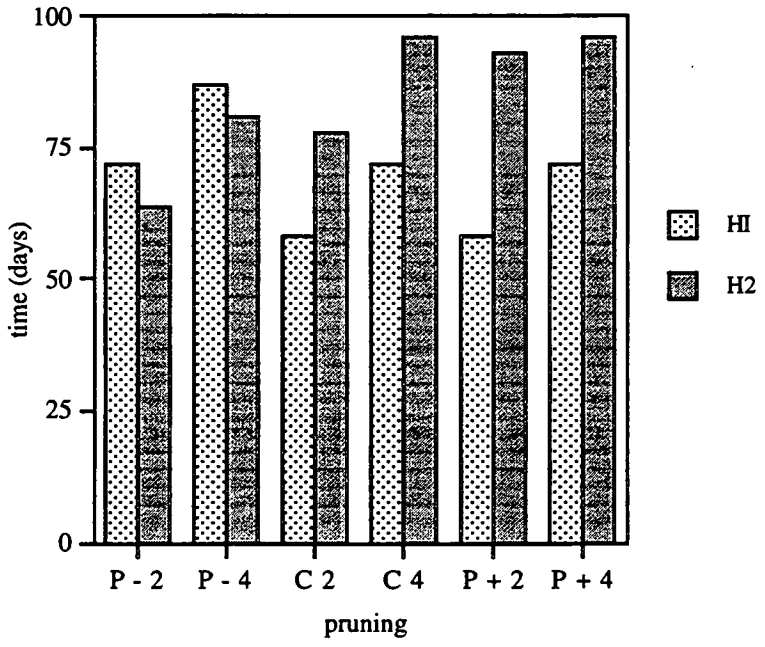

Figure 4.1 Duration of harvest for pruning treatment and plucking standard. LSD (H1)=1.0; LSD (H2)=14.7; LSD (H1-H2)=14.3

4.3.2 Yield

There was no effect of pruning on yield, which was on average 2.7 g (Table 4.2). Yield increased significantly when four leaves were harvested, at 3.8 g, compared to two leaves at 1.6 g (Table 4.2). There was a significant interaction between pruning and plucking standard, even though the main effect of pruning treatment on yield was insignificant (Table 4.3). This interaction resulted in yield linearly increasing from 3 g to 4.7 g for pruning treatments P-, C and P+, harvested at a plucking standard of four leaves. At the same time, yield for pruning treatments was not significantly different when two leaves were harvested (Table 4.3). There was no significant interaction between pruning and harvest, although yield significantly declined from 3.1 g to 2.3 g from harvest one to two (Table 4.2). A significant interaction between plucking standard and harvest indicated that the decline in yield from harvest one to two is of greater magnitude when four leaves are harvested than compared to two (Table 4.4). Consequently, pruning before the first spring flush appears to result in a decline in yield only when four leaves are harvested.

Table 4.2 Effect of pruning, plucking standard and harvest on yield (g) ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		Yield
Pruning (A)	P-	2.4
	C	2.7
	P+	3.0
	F	NS
Plucking (B)	two	1.6a
Standard	four	3.8b
	F	***
Harvest (C)	H1	3.1a
	H2	2.3b
	F	**
AB	F	**
AC	F	NS
BC	F	**
ABC	F	NS

Table 4.3 Interaction between pruning and plucking standard for yield (g). Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Pruning	Plucking standard two	four
P-	1.8ax	3.0bx
C	1.7ax	3.8bx
P+	1.3ax	4.7cy

Table 4.4 Interaction between plucking standard and harvest for yield (g). Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Plucking standard	Harvest one	two
two	1.7ax	1.5ax
four	4.6ay	3.1by

4.3.3 Total phenolics

There was no significant difference in total phenolics between either the pruning treatments (150 to 160 mg g^{-1}), plucking standard (155 mg g^{-1}) or harvest (155 mg g^{-1}) (Table 4.5). There were no significant interactions between the factors.

Table 4.5 Effect of pruning, plucking standard and harvest on total phenolics (mg g^{-1} catechin equivalents) ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		Phenolics
Pruning (A)	P-	151
	C	161
	P+	157
	F	NS
Plucking (B)	two	159
Standard	four	155
	F	NS
Harvest (C)	H1	154
	H2	159
	F	NS
AB	F	NS
AC	F	NS
BC	F	NS
ABC	F	NS

4.3.4 Total nitrogen

Total nitrogen tended to increase significantly from the P- treatment, at 30 mg g^{-1}, to the P+ treatment, at 33 mg g^{-1}; the control being between the two, at 32 mg g^{-1} (Table 4.6). There were no significant interactions between pruning treatment and either the plucking standard or harvest. Total nitrogen declined from 34 to 29 mg g^{-1} as the plucking standard increased from two to four leaves. Total nitrogen also declined from the first harvest, at 36 mg g^{-1}, to the second harvest, at 28 mg g^{-1}. However, there was a significant interaction between plucking standard and harvest, where the decline in nitrogen from a plucking standard of two to four leaves was greater in magnitude for harvest one than harvest two (Table 4.7).

Table 4.6 Effect of pruning, plucking standard and harvest on nitrogen. Means followed by the same letter are not significantly different ($p<0.05$*; $p<0.01$**; $p<0.001$***)

Factor		Nitrogen
Pruning (A)	P-	30a
	C	32ab
	P+	33b
	F	*
Plucking (B) Standard	two	34a
	four	29b
	F	***
Harvest (C)	H1	36a
	H2	28b
	F	***
AB	F	NS
AC	F	NS
BC	F	***
ABC	F	NS

Table 4.7 Interaction between plucking standard and harvest for nitrogen (mg g^{-1}). Means in each row followed by the same letter (a, b) are not significantly different ($p<0.05$). Means in each column followed by the same letter (x, y) are not significantly different ($p<0.05$)

Plucking standard	Harvest	
	one	two
two	40.1ax	28.6bx
four	31.7ay	27.0bx

4.3.5 Neutral detergent fibre

Neutral detergent fibre (NDF) was significantly increased in the P- treatment, at about 190 mg g^{-1}, compared to the control and P+ treatment, which were both about 165 mg g^{-1} (Table 4.8). However, it was perhaps unexpected that NDF was not similar for both the pruning treatments, instead of being higher only in the P- treatment. Increasing the plucking standard from two to four leaves significantly increased NDF from 148 to 196 mg g^{-1}, due to the additional mature leaves. NDF was also observed to significantly increase from the first harvest, at 154 mg g^{-1}, to 189 mg g^{-1} in the second harvest. There were no significant interactions between pruning, plucking standard or harvest.

Table 4.8 Effect of pruning, plucking standard and harvest on neutral detergent fibre (mg g^{-1}) ($p<0.05$*; $p<0.01$**; $p<0.001$***). Means followed by the same letter are not significantly different ($p<0.05$)

Factor		NDF
Pruning (A)	P-	189a
	C	163b
	P+	164b
	F	***
Plucking (B) Standard	two	148a
	four	196b
	F	***
Harvest (C)	one	154a
	two	189b
	F	***
AB	F	NS
AC	F	NS
BC	F	NS
ABC	F	NS

4.4 Discussion

Repeated harvesting is reported to reduce yield due to an increase in bud dormancy (Wight, 1955). Annual pruning is reported to renew plant vigour lost through repeated harvesting (Wight and Barua, 1955). Pruning, both before and after the first spring flush, delayed bud burst and hence the time of harvest. Contributing to this delay was presumably the removal of viable buds which would have otherwise produced the new flush. Time of harvest was also delayed by increasing the plucking standard from two to four leaves and a bud, which was consistent with observations reported by Grice (1982) and Owuor and Odhiambo (1990). Pruning, combined with a coarse plucking standard, may then reduce the number of harvests per season.

The increased duration of the second flush of the control plants could perhaps be attributed to the lack of canopy structure. In the first harvest, shoot growth was synchronised by spring bud burst, which was translated into high yields. However, in the second harvest, growth of new shoots from axillary buds was controlled by apical dominance, where shoots positioned furthest from the tip of the shoot were the first to develop. Both the duration of the flush and presumably yield then became a function of the number of shoots present in active growth at the time of harvest, similar to black tea (Willson and Clifford, 1992). Harvesting new shoots only from the tips of the maintenance foliage hence increased the flush duration and perhaps contributed to a decline in yield.

Harvesting additional leaves by increasing the plucking standard from two to four resulted in an increase in yield, which is consistent with the observations of Palmer-Jones (1977), Cloughley *et al.* (1983), Mwakha (1991) and Owuor and Othieno (1991). In comparison, pruning is reported by Mwakha (1989, 1991) to reduce yield in the following year. Results from this study are however contradictory. Pruning before the first harvest significantly reduced yield, but only at a plucking standard of four leaves. Pruning before the second harvest, according to the results, increased yield for plucking standards of four leaves. This observation is perhaps the result of high yield in the first harvest, which was not pruned, and a comparably poor yield in the control plants in the second harvest. Had shoot growth in the second harvest of control plants been synchronised, perhaps the yield of these plants may have been greater than that of the pruned plants.

Yield declined from the first (spring) harvest to the second (summer) harvest. This observation is consistent with that reported in the literature, both for green tea (Anon., 1990) and in black tea over repeated harvests (Wight and Barua, 1955). This

highlights the importance of regular (annual) pruning to renew plant vigour as used in green tea cultivation in Japan (Anon., 1990). These observations are however perhaps inconsistent with the slight increase in average temperature from the first to second harvest, which according to the literature should increase the rate of shoot extension and hence yield (Yanase, 1980; Tanton, 1982a).

Not only yield determines economic returns, but also quality. Phenolics are reported by Forrest and Bendall (1969) to be present in higher concentrations in young tissue. Therefore, it could be predicted that an increase in plucking standard will decrease the concentration of total phenolics. Contradictory to this, total phenolics did not vary with either the plucking standard or pruning. Instead, total phenolics at 150 to 170 mg g^{-1} were comparable to that reported by Liang *et al.* (1987) in 'very high quality' tea from China. The cultivation of plants under shade cloth may have contributed to the relatively high amounts of phenolics, the synthesis of which is reportedly enhanced under shade (Saijo, 1980). This perhaps then promoted the relatively high amounts of total phenolics detected in both fine and coarse plucking standards.

There was no significant effect of pruning on leaf nitrogen, which varied only with plucking standard and harvest. Although harvesting additional leaves at a coarse plucking standard increased yield, this was at the expense of nitrogen, which declined. This is consistent with the observations of Barua (1960), where it was reported that nitrogen declined from 7.3 % in the bud, compared to only 5.0 % in the third leaf. Stalk similarly has relatively low amounts of nitrogen, at around 4.4 % (44 mg g^{-1}). Total nitrogen declined from about 40 mg g^{-1} in the first (spring) harvest, to 30 mg g^{-1} in the second (summer) harvest, which was consistent with the observations of Nakagawa and Furuya (1975). The decline in nitrogen was more pronounced in the second harvest at a plucking standard of four leaves. The removal of young leaves in which nitrogen has accumulated could have potentially reduced the size of the nitrogen pool in subsequent flushes (Okano, 1994).

High amounts of fibre in tea is undesirable, as it reduces the ease of processing (Biswas and Biswas, 1971). Fibre varied with pruning treatment. The increase in fibre was only observed in the P- treatments, and not the P+ treatment, which is perhaps contradictory. Consequently, not only was there a decline in yield in the P- treatment compared to the control, but also an undesirable increase in fibre. NDF also increased from the first to the second harvest. This could potentially be attributed to differences in temperature between the first (spring) and second (summer) harvest, if this resulted in increased leaf size or stalk as reported by Yanase (1980). Harvesting additional leaves at a coarse plucking standard increased both yield and neutral detergent fibre (NDF), due to the inclusion of mature leaves stalk. NDF of about 150

mg g^{-1} for two harvested leaves and 200 mg g^{-1} for four leaves was similar to quantities reported by Nakano *et al.* (1996).

It can then be concluded that pruning delays bud burst and hence time to harvest, and may then potentially reduce the number of harvests per season. Pruning did not affect either total phenolics or nitrogen, but did increase the amount of fibre in harvested shoots. An undesirable decline in yield, nitrogen and increase in fibre only occurred at coarse, but not fine plucking standards. This could therefore be avoided by maintaining fine plucking standards after pruning. Repeated harvests reduced yield and quality (as decreased nitrogen and increased fibre). This highlights the importance of the first spring crop, and the necessity for pruning if this renews plant vigour.

IV.5 Photosynthesis

5.1 Introduction

Rhythmic growth in tea occurs in repeating cycles of growth and dormancy, which may be modified by environment (Chapters IV.1 and IV.2). Rhythmic growth can then be described according to changes in morphology, as examined in Chapter IV.1, and physiology. It was hypothesised in Chapter IV.1 that rapid expansion of leaves and internodes during bud burst may alter the partitioning of assimilates from the bud in favour of the developing shoot, as reported in *Quercus* (Hanson *et al.*, 1988; Alaoui-Sossé *et al.*, 1994; Barnola *et al.*, 1993). This may potentially be associated with change in net photosynthesis of both the newly expanding leaves and mature flush. In this chapter, the relationship between photosynthesis, light intensity, temperature and stage of development is examined using infra-red gas analysis.

5.2 Materials and methods

5.2.1 The IRGA

An infra red gas analyser (IRGA, Analytic Development Inc.) equipped with a portable Leaf Chamber Analyser (LCA) and Type 3 Parkinson Leaf Chamber (PLC), was used for measurement of gas exchange.

A diagram of an IRGA is shown in Fig. 5.1. This is described as an open system. The IRGA consists of three parts, the infra red source, gas cell and detector. Infra red radiation is emitted from the source into the gas cell. CO_2 in the gas cell decreases the amount of radiation reaching the detector, which reduces the detector output signal. The IRGA measures the difference in CO_2 mole fraction between the chamber inlet and outlet.

As shown in Fig. 5.1, air is supplied from a reference gas cylinder, passed through a volumetric flow meter and into the IRGA, where the mole fraction of the reference air is measured. Air then flows from the IRGA to the in situ plant leaf chamber (PLC); a small fan within the PLC ensures adequate air mixing in the chamber. Air then passes back to the IRGA, where the outlet mole fraction of CO_2 is measured. The CO_2 differential between the inlet and outlet represents the amount of gas exchange occurring by the leaf (Coombs *et al.*, 1985).

5.2.2 Measurement of photosynthetic rate

Plants were grown under normal glasshouse conditions as described in the general materials and methods in Chapter III.4.1.

Photosynthetic measurements were undertaken in an air conditioned room. Light was provided with a 400 W mercury vapour lamp, with various thicknesses of Sarlon shade cloth used to vary light intensity. Radiant heat from the mercury vapour lamp was minimised by a (glass) water bath positioned between the light source and IRGA, in which the water was continually flushed with tap water. Photon flux density (PFD) was measured using a Lambda L1-185 meter fitted with a quantum flux sensor. The quantum flux sensor measured photosynthetically active radiation (400 - 700 nm), and results were reported in μmol photons m^{-2} s^{-1}. Measurements of light intensity were taken at leaf height, and corrected for the perspex leaf chamber.

The most recently fully expanded leaf was chosen for light and temperature response curves. Net photosynthesis was measured on leaves attached to plants using reference air at 340 - 360 ppm CO_2, and CO_2 enhanced photosynthesis using air at 2 % O_2 and 360 ppm CO_2.

Light response curves were determined for four replicate median (third) leaves at each of the following stages of development, for flushes one (1) and two (2):

I. Bud burst, expansion of cataphylls
II. Shoot extension, involving leaf expansion and internode extension
III. Exposure of dormant bud, completion of shoot extension
IV. Completion of leaf expansion.

The labelling strategy is shown in Fig. 5.2.

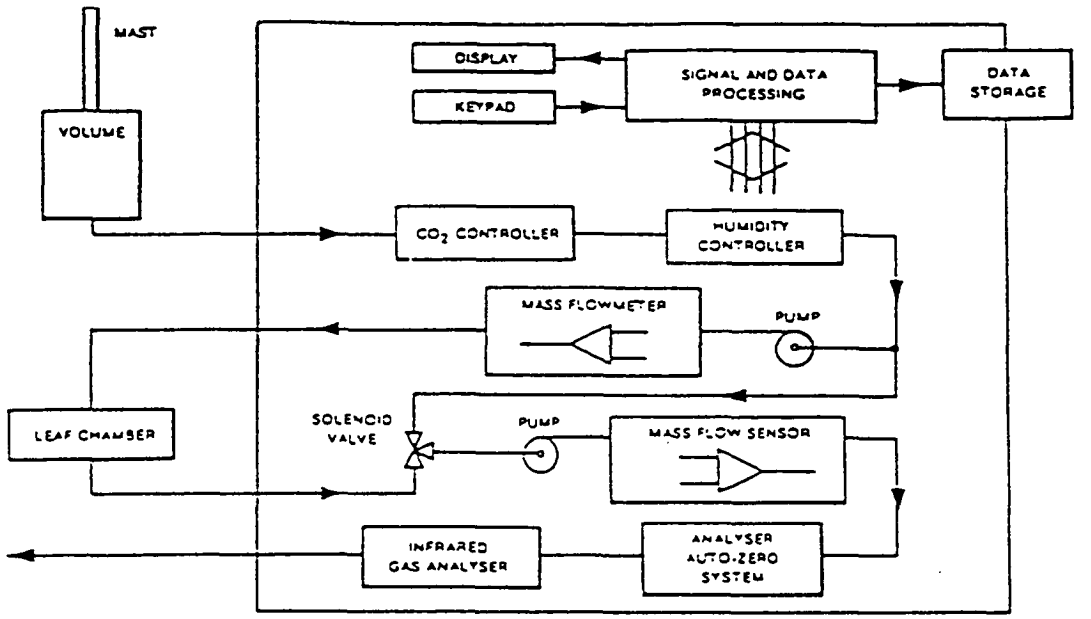

Figure 5.1 ADC IRGA - (mast replaced with gas cylinder)

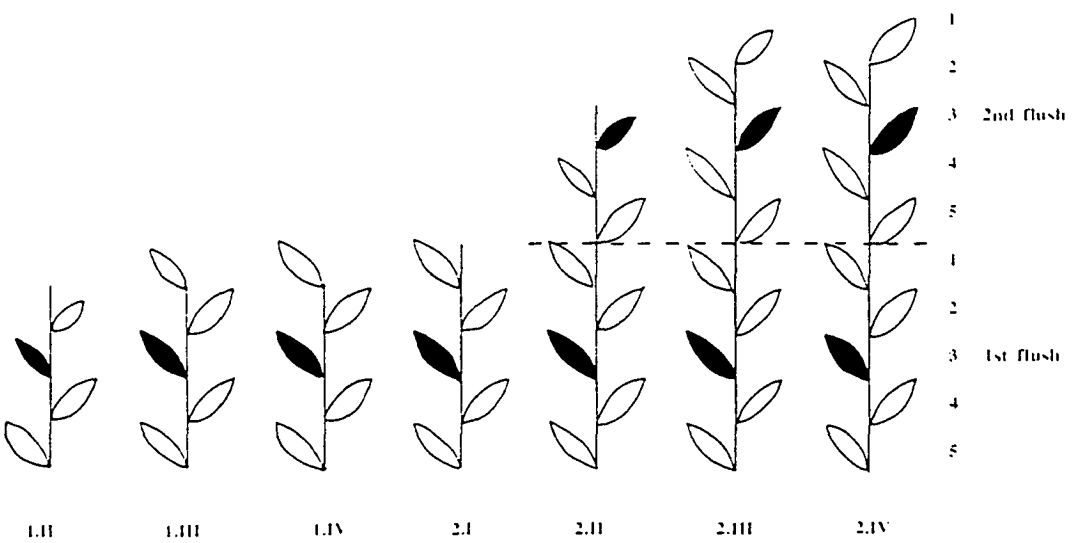

Figure 5.2 Sampling strategy for photosynthetic measurements for I) start of shoot growth; II) shoot extension; III) dormancy; and IV) completion of shoot growth; for 1) first and 2) second flushes. Numbers 1-5 denote leaf and internode position.

5.2.3 Calculation of photosynthetic rate

Photosynthetic rates were calculated from the following equation (Farquhar and Sharkey, 1982):

$f = (f_v/1000) \times (1/22.4) \times [273.15/(273.15 + T)] \times (p/101.3) \times (1/60)$

$X_o = H_o/100 \times V/1000 \times 22.4/18 \times [273.15/(273.15+T)] \times (p/101.3)$

$X_e = H_i/100 \times V/1000 \times 22.4/18 \times [273.15(273.15+T)] \times (p/101.3)$

$A = (f/s) \times \Delta c[(1-X_e)/(1- X_o)]$

where:

A	Assimilation rate (μmol CO_2 m^{-2} s^{-1})
Δc	CO_2 difference between inlet and outlet (μmol mol^{-1})
f	Mole fraction of air (mol s^{-1})
f_v	Volumetric flow of air (cm^3 min^{-1})
p	Atmospheric pressure during measurement (k Pa)
s	Leaf area (m^2)
X_o	Water vapour at outlet (mol mol^{-1})
X_e	Water vapour at inlet (mol mol^{-1})
H_o	Outlet relative humidity
H_i	Inlet relative humidity
V	Weight (g) of a m^3 of saturated aqueous vapour at T °C
T	Temperature

Leaf area was either 6.25 cm^2 (area of PLC), or measured from (traced) paper replicas using a planimeter. The flow of air was measured using a volumetric flow meter prior to entry into the IRGA system. The IRGA measured Δc, Ho, H_i and T. The atmospheric pressure, p, was taken as 101.3 k Pa. Correction for water vapour (X_o and X_e) were necessary, as otherwise sensitivity of the IRGA to water vapour transpired from the leaf overestimated the mole fraction of CO_2 of the outlet air.

Photorespiration was calculated by subtracting net photosynthesis in 2 % O_2 from net photosynthesis in 21 % O_2 at each temperature. True rates of photosynthesis were then calculated by subtracting dark respiration from net photosynthesis in 2 % O_2.

5.2.4 Statistical analysis

Parameters of maximum net photosynthesis, compensation point and dark respiration, were determined by fitting the data to the following equation using non linear least squares DUD with the statistical program SAS (Appendix V) (Hanson *et al.*, 1988):

$$Pn = Pmax \times (1-(1-Rd/Pmax)^{(1 - PFD/Pc)}$$

where:

Pn photosynthesis (μmol CO_2 m^{-2} s^{-1})
Pmax maximum net photosynthesis (μmol CO_2 m^{-2} s^{-1})
Pc compensation point (μmol photons m^{-2} s^{-1})
Rd dark respiration (μmol CO_2 m^{-2} s^{-1})
PFD photon flux density (μmol photons m^{-2} s^{-1})

Photosynthetic efficiency (Qi) was determined from the slope (dx/dy) of the linear portion of the light response curve. Significant differences between stages of development for each parameter was assessed using a repeated measures ANOVA in the statistical program StatView. The LSD was reported for each parameter (Appendix VI).

5.3 Results

5.3.1 Light response curve

The response of net photosynthesis to photon flux density consisted of two phases (Fig. 5.3). Firstly, there was a linear phase where net photosynthesis increased with increasing photon flux density, through the light compensation point of 19.8 μmol photons m^{-2} s^{-1}, where net exchange of CO_2 balanced O_2. Photosynthetic efficiency (the efficiency of light utilisation by photosynthesis), was given by the initial slope of the light response curve, at 0.082 μmol CO_2 μmol photons^{-1}. Secondly, as photon flux density further increased, light became limiting at the light saturated net photosynthetic rate of 14.2 μmol CO_2 m^{-2} s^{-1}, at a photon flux density of 400 μmol photons m^{-2} s^{-1}. Efflux of CO_2 in the dark, or dark respiration, was 2.4 μmol CO_2 m^{-2} s^{-1}.

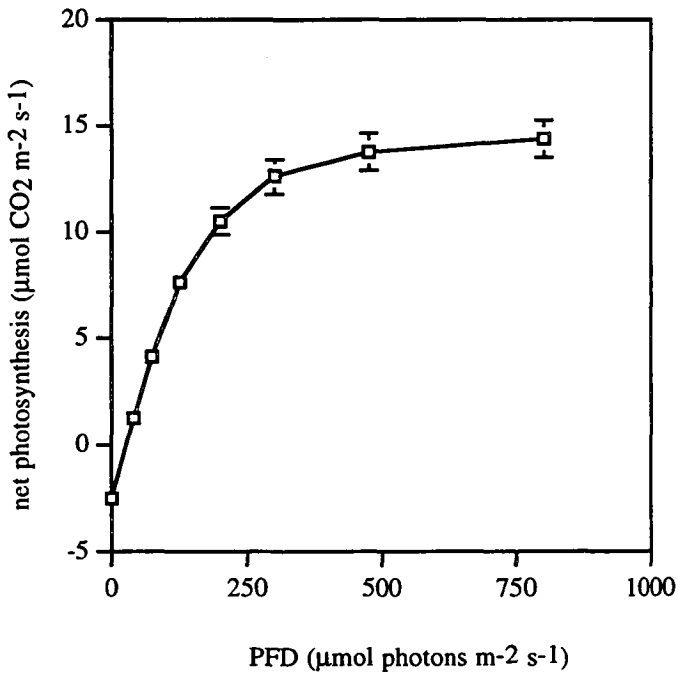

Figure 5.3 Light response curve. (Bars represent SE of four replicates)

5.3.2 Temperature response curve

Net photosynthesis in 21 % oxygen reached a maximum of 10 μmol CO_2 m^{-2} s^{-1} at 25 °C, and decreased with increasing temperature (Fig. 5.4). Dark respiration increased to a maximum of 2.5 μmol CO_2 m^{-2} s^{-1} with increasing temperature, due to increased rates of metabolism and enzymatic reactions at higher temperatures.

In conditions of low oxygen (2 % O_2), photosynthesis reached a maximum of 18 μmol CO_2 m^{-2} s^{-1} at 25 °C. This represented a 45 % increase in the observed net rate of photosynthesis compared to that in 21 % O_2.

Loss of CO_2 by photorespiration increased with increasing temperature, reaching a maximum of 8 μmol CO_2 m^{-2} s^{-1} at 20 °C. True photosynthesis, which eliminates oxygen inhibition, photorespiration and dark respiration from net photosynthesis, reached a maximum of 20 μmol CO_2 m^{-2} s^{-1} at 25 °C, and declined at higher temperatures.

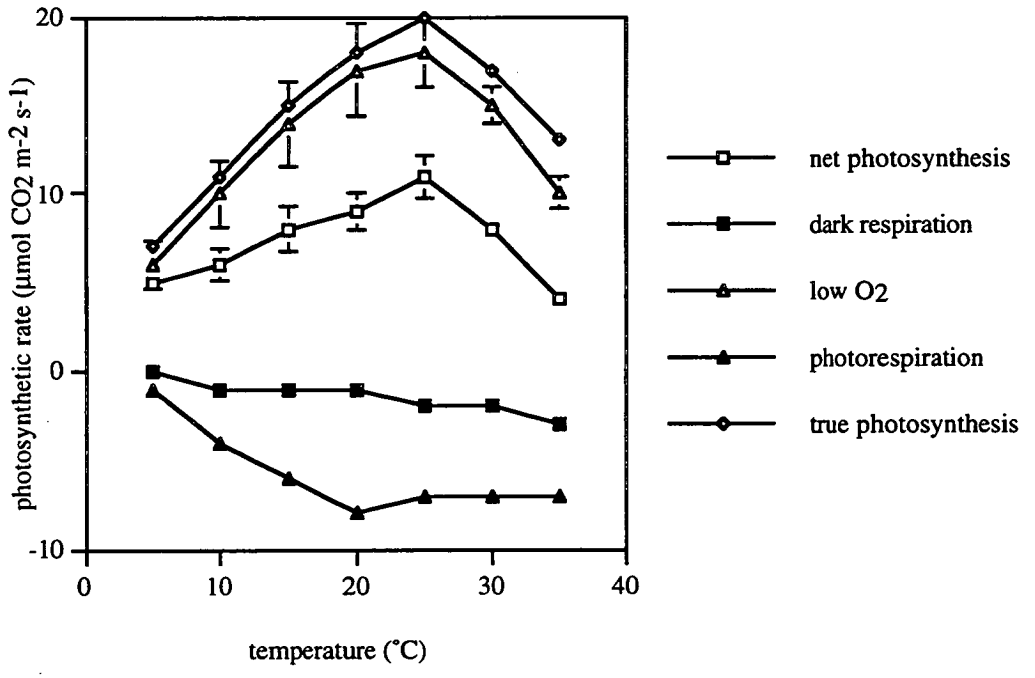

Figure 5.4 Temperature response curve and true rate of photosynthesis. (Bars represent SE of four replicates)

5.3.3 Net photosynthesis and stage of development

Net photosynthesis varied with photon flux density depending on the stage of growth (Fig 5.5). The four components of maximum net photosynthesis (Pn), dark respiration (Rd), compensation point (Pc) and photosynthetic efficiency (Qi) were calculated for each individual light response curve at each stage of development for the first and second flushes of growth (Figs. 5.6 and 5.7).

Maximum net photosynthesis of the first flush increased from 4.5 μmol CO_2 m^{-2} s^{-1} during stage 1.II, shoot extension, to 13.2 μmol CO_2 m^{-2} s^{-1} during stage 1.III, dormancy (Fig. 5.6 A). The comparatively low maximum net photosynthesis during stage 1.II was associated with a high compensation point, at 77 μmol photons m^{-2} s^{-1} compared to later stages of development (Fig. 5.7 A). Photosynthetic efficiency was 0.06 μmol CO_2 μmol photons^{-1} (Fig 5.7 B). During stage 1.III, both the compensation point and photosynthetic efficiency declined, reaching 25 μmol photons m^{-2} s^{-1} and 0.05 μmol CO_2 μmol photons^{-1} respectively by stage 1.IV (Figs. 5.7 A and 5.7 B). Maximum net photosynthesis declined slightly during stage 1.IV, the completion of shoot growth, to around 10 μmol CO_2 m^{-2} s^{-1} (Fig. 5.6 A). At the same time, dark respiration decreased from 5.5 μmol CO_2 m^{-2} s^{-1} during stage 1.I, shoot extension, to around 2 μmol CO_2 m^{-2} s^{-1} by stage 1.IV, the completion of shoot growth (Fig. 5.6 B). Low rates of photosynthesis, coupled with high respiration, are typical of immature leaves.

Light response curves during growth of the second flush were similar to those observed in the first flush (Fig. 5.5). Maximum net photosynthesis in the developing second flush increased from 4.5 μmol CO_2 m^{-2} s^{-1} during stage 2.II to 10 μmol CO_2 m^{-2} s^{-1} during stage 2.III, at dormancy, which was maintained into stage 2.IV, at the completion of shoot growth (Fig. 5.6 A). Changes in the compensation point, dark respiration and photosynthetic efficiency during growth of the (new) second flush were otherwise the same as observed during expansion of the first flush (Figs. 5.6 B, 5.7 A and 5.7 B).

Maximum net photosynthesis of first flush leaves significantly increased to 18 μmol CO_2 m^{-2} s^{-1} during stage 2.I, at the start of growth of the second flush (Fig 5.6 A). This was associated with an increase in photosynthetic efficiency, from 0.05 μmol CO_2 μmol photons^{-1} during stage 1.IV to 0.07 μmol CO_2 μmol photons^{-1} during stage 2.I (Fig. 5.7 B). Thereafter, maximum net photosynthesis and photosynthetic efficiency of the first flush declined from stages 2.II to 2.IV to maintenance levels of between 10 to 13 μmol CO_2 m^{-2} s^{-1} and around 0.05 μmol CO_2 μmol photons^{-1}

respectively (Figs. 5.6 A and 5.7 B). Both dark respiration and the compensation point of the first flush were stable at 2 μmol CO_2 m^{-2} s^{-1} and 23 μmol CO_2 m^{-2} s^{-1} respectively throughout growth of the second flush, from stages 2.I to 2.IV (Figs. 5.6 B and 5.7 A).

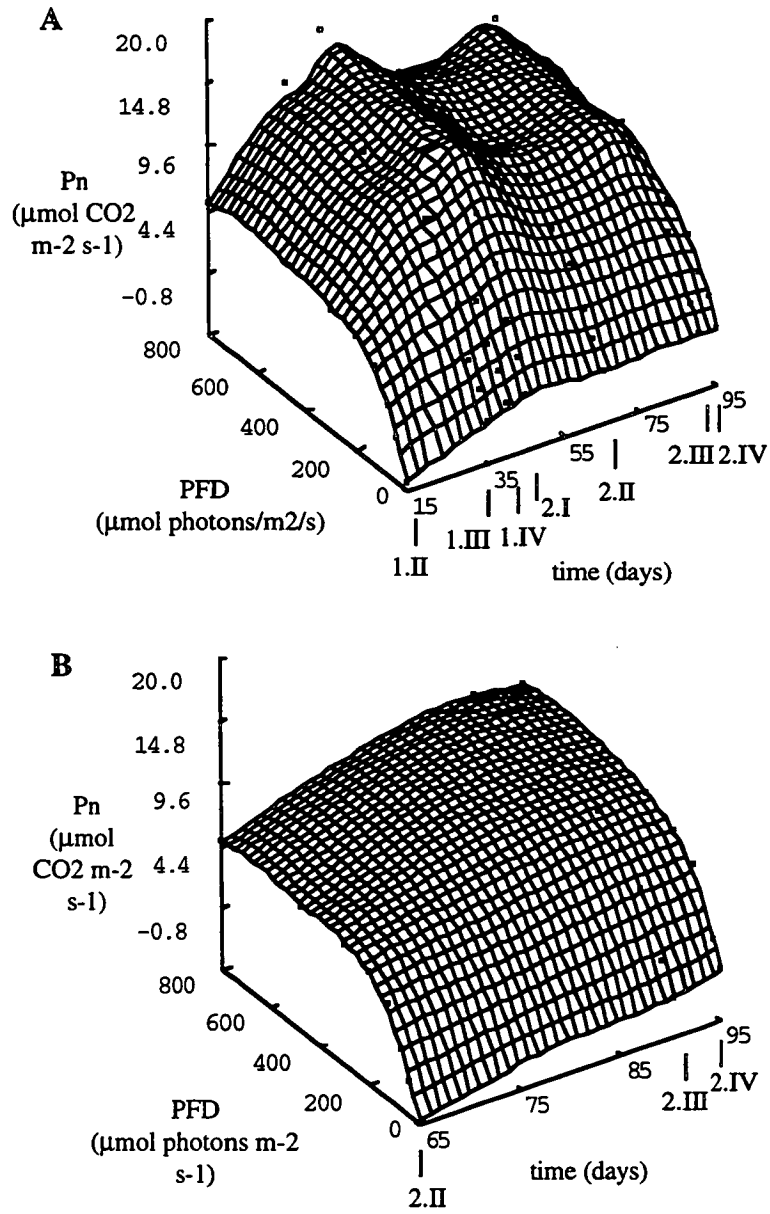

Figure 5.5 Light response curves relative to time and stage of development (1.II to 2.IV inclusive) for A) first flush; B) second flush.

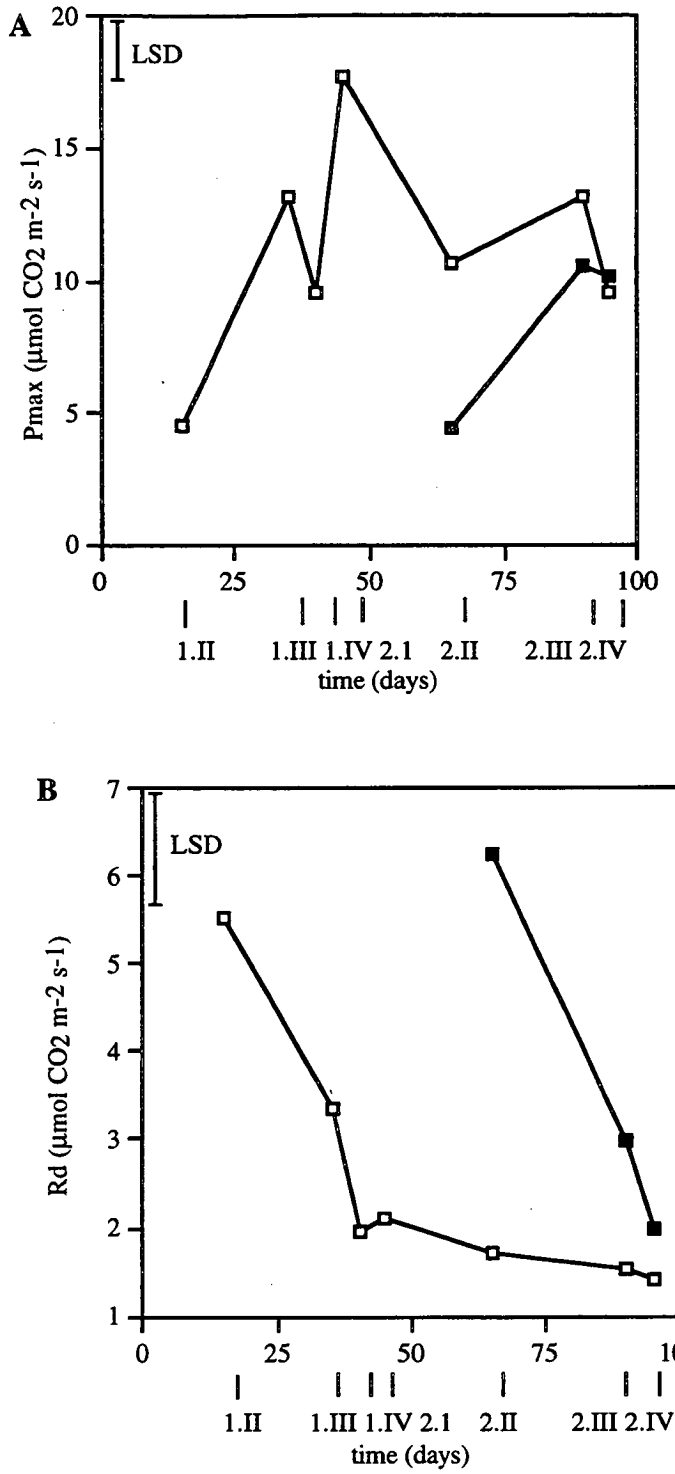

Figure 5.6 A) Maximum net photosynthesis; and B) dark respiration in relation to stage of development (1.II to 2.IV inclusive) and time after bud burst. □) first flush; ■) second flush.

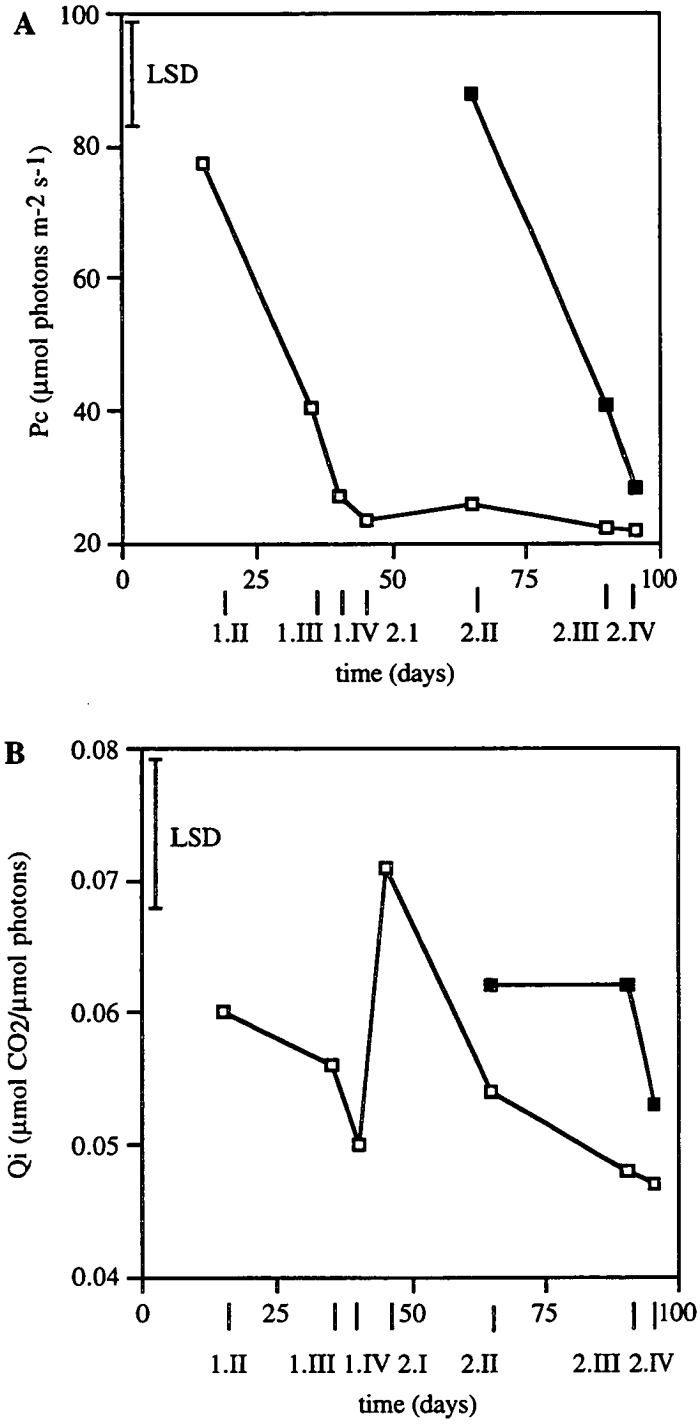

Figure 5.7 A) Compensation point; and B) photosynthetic efficiency in relation to stage of development (1.II to 2.IV inclusive) and time after bud burst. □) first flush; ■) second flush.

5.4 Discussion

The response of net photosynthesis to photon flux density was curvilinear, consistent with other plant species (Larcher, 1995). The light response curve consisted of a linear phase where net photosynthesis increased with increasing photon flux density, through the light compensation point of 19.8 μmol photons m^{-2} s^{-1}, where net exchange of CO_2 balanced O_2. This was in agreement with the observations of Okano *et al.* (1995), where the compensation point reportedly varied from 21 to 41 μmol photons m^{-2} s^{-1}; and other non-tropical evergreen species, in which the compensation point may vary from 10 to 30 μmol photons m^{-2} s^{-1} (Larcher, 1995). Photosynthetic efficiency (the efficiency of light utilisation by photosynthesis), was given by the initial slope of the light response curve, at 0.082 μmol CO_2 μmol photons^{-1}. As photon flux density increased further, light became limiting at the light saturated net photosynthetic rate of 14.2 μmol CO_2 m^{-2} s^{-1}, and photon flux density of 400 μmol photons m^{-2} s^{-1}.

Maximum net photosynthesis was similar to the majority of values reported in the literature (Barua, 1960, 1964; Squire, 1977; Sakai, 1975; Aoki, 1980, 1981), yet significantly differed from Smith *et al.* (1993b, 1994) who reported net photosynthesis of only of 3 μmol CO_2 m^{-2} s^{-1} in black tea. Closure of stomates due to low humidity, or lack of adjustment by leaves to the conditions in which measurements are being undertaken, has been observed to significantly reduce gas exchange, both in the current study and by Barua (1960). This could offer some explanation as to the low rates of photosynthesis reported by Smith *et al.* (1994). Further variation in net photosynthesis was also experienced when using the ADC mast to supply air to the IRGA, which failed to supply air at a constant flow rate. In the current study the mast was replaced with a gas cylinder, from which air flow could be regulated.

Maximum net photosynthesis at 14 μmol CO_2 m^{-2} s^{-1} and photon flux densities of 400 μmol photons m^{-2} s^{-1} were significantly lower than other C3 crop species, which vary from 20 to 40 μmol CO_2 m^{-2} s^{-1}, and 600 to 1000 μmol photons m^{-2} s^{-1} respectively (Larcher, 1995). The relatively low photon flux density at which net photosynthesis becomes saturated is reflective of the origins of tea, as an understorey plant adapted to conditions of low light and temperate climates (Eden, 1965). Furthermore, the relatively low net photosynthetic rate of tea is likely to contribute to the comparatively low annual biomass produced by tea, which is reportedly around 18 t ha^{-1} (fw) in comparison to 25 to 40 t ha^{-1} annual biomass produced by other temperate C3 crops (Larcher, 1995).

In some instances, low net photosynthesis can be a result of high rates of respiration. Under optimum temperatures of 25 °C, the efflux of CO_2 in the dark, or dark respiration, was 2.4 μmol CO_2 m^{-2} s^{-1}. This was slightly higher than the observations of Okano *et al.* (1995), where dark respiration was reported to be around 1.5 μmol O_2 m^{-2} s^{-1}, and similarly higher than other evergreen broadleaved temperate trees, at between 0.8 to 1.4 μmol CO_2 m^{-2} s^{-1} (Larcher, 1995). At temperatures greater than 25 °C, dark respiration continued to increase, for example, reaching 3 μmol CO_2 m^{-2} s^{-1} at 35 °C. At higher temperatures, dark respiration is likely to decline, as substrates for respiration become limiting. Photorespiration, resulting from competitive inhibition of ribulose biphosphate carboxylase by oxygen (Salisbury and Ross, 1985), was comparatively higher than dark respiration, and reached a maximum of 7 μmol CO_2 m^{-2} s^{-1} at 20 °C.

As photorespiration does not generate ATP, high rates of photorespiration represent a significant loss in photosynthetic efficiency. True photosynthesis, which accounted for losses from dark respiration and photorespiration, then reached a maximum of 20 μmol CO_2 m^{-2} s^{-1} at 25 °C. This was then only comparable to net rates of photosynthesis reported in other C3 crop species, mentioned previously (Larcher, 1995). At temperatures greater than 25 °C, true photosynthesis declined sharply, due to increased dark respiration and high photorespiration. Reported rates of true photosynthesis in tea at around 10 μmol CO_2 m^{-2} s^{-1} (converted from 1.5 g CO_2 m^{-2} hr^{-1}), have previously not considered the significant effect of photorespiration, only dark respiration (Sakai, 1975).

Net photosynthesis varied depending on the stage of growth and development. Net photosynthesis of first flush leaves increased to a maximum of 18 μmol CO_2 m^{-2} s^{-1} during stage 2.I, at the start of the second flush, and was associated with an increase in photosynthetic efficiency. The increase in net photosynthesis reflected increased demand for assimilate by the bud, in which immature leaves and internodes were rapidly expanding as they approached bud burst. Net photosynthesis has been similarly reported to increase in response to demand following defoliation, as reported by Aoki (1981) in tea after harvesting. Plant hormones may also play a role in directing assimilate partitioning, as in *Theobroma* (Orchard *et al.*, 1980), or by directly influencing photosynthesis (Caers and Vendrig, 1986; Tamas *et al.*, 1973; Arteca and Dong, 1981; Cummins *et al.*, 1971). Potential correlations between plant hormones and rhythmic growth will be assessed in Chapter IV.6.

During stage II, shoot extension, net photosynthesis declined in the previous flush leaves to maintenance levels of around 14 μmol CO_2 m^{-2} s^{-1}. It has also been reported that the photosynthetic capacity of maintenance leaves declines after bud burst as new

shoots develop because of mutual shading (Okano *et al.*, 1996). Although net photosynthesis of first flush leaves declined, the comparatively low net photosynthesis of newly expanded leaves during stage II, at 4.5 μmol CO_2 m^{-2} s^{-1}, was indicative that additional imported assimilate was probably required to maintain leaf growth. Such low rates of net photosynthesis may be attributed to high rates of dark respiration, which at 5.5 μmol CO_2 m^{-2} s^{-1} during stage II, was twice that observed during stage III, dormancy. Low net photosynthesis of recently unfolded leaves was in agreement with the observations of Barua (1960), where net photosynthesis was reported at around 3 to 4 μmol CO_2 m^{-2} s^{-1}.

Net photosynthesis has been similarly reported to increase at the start of growth of a new flush in *Quercus*, but unlike tea, high rates of net photosynthesis are maintained in the second flush of new seedlings until dormancy (Hanson *et al.*, 1988). In *Quercus*, as all leaves for the current flush expand concurrently, increased demand for assimilate by the new leaves is provided, in part, by increased rates of net photosynthesis throughout shoot extension. Additional assimilates are also provided during shoot extension in *Quercus* by carbohydrate reserves in the stem (Alaoui-Sossé *et al.*, 1994). In comparison, in tea, leaves and internodes unfold sequentially from the apex. Leaves first to unfold are then physiologically older than leaves which unfolded later in shoot extension, and may potentially contribute exported assimilate to the younger leaves during shoot growth. As a result, during shoot extension, demand for assimilate and hence net photosynthesis from previous flush leaves may decline (to be examined in Chapter IV.6). As demand for assimilates falls, the accumulation of sucrose within the chloroplast is likely to stimulate starch synthesis and reduce photosynthesis, providing feedback inhibition of photosynthesis (Stitt, 1990). Consequently, assimilates exported from nearby sources are presumably sufficient to support growth of the new flush.

In conclusion, net photosynthesis of previous flush leaves increased to a maximum during the start of growth of the new flush, and was likely to be in response to increased demand for assimilate by rapidly expanding leaves and internodes in the bud. Net photosynthesis then declined during shoot extension, perhaps reflecting decreased demand for assimilate. However, as leaves of the new flush have not reached full expansion, other potential sources of assimilate could include current photosynthate from mature leaves (at maintenance levels of photosynthesis), leaf and stem carbohydrate reserves. The role of exported photosynthate and carbohydrate reserves during rhythmic growth of tea will be examined in Chapter IV.6 using ^{14}C labelling and quantification of starch in leaves and internodes.

IV.6 Assimilate partitioning

6.1 Introduction

Rhythmic growth in tea occurs in repeating cycles of growth and dormancy, which as shown in Chapters IV.1 and IV.2 is not controlled by environment, and is instead endogenous. Rhythmic growth can then be described according to changes in morphology, as examined in Chapter IV.1, and physiology. It was hypothesised in Chapter IV.1 that rapid expansion of leaves and internodes during bud burst alters the partitioning of assimilates from the bud in favour of the developing shoot. This was shown in Chapter IV.5 to result in increased rates of net photosynthesis in second flush leaves. This is however transient, where net photosynthesis declined to maintenance levels during shoot extension, even though additional imported assimilates (either current photosynthate or carbohydrate reserves) are likely to be required to maintain shoot growth of immature leaves during shoot extension. It is proposed that shoot expansion maintains the dominance of developing shoots, such that further initiation of primordia is inhibited. Such inhibition could be a process of direct competition for assimilate between the bud and developing shoot, or alternatively may involve plant growth regulators as inhibitors and promoters of growth (Crabbé and Barnola, 1996). The bud may become the dominant sink following exposure of the dormant bud and shoot maturation. In this chapter, the relationship between assimilate partitioning, shoot growth and development in green tea is examined in the context of this hypothesis, by assessing both the partitioning of radioactive ^{14}C labelling from mature leaves to the developing flush and starch accumulation.

6.2 Materials and methods

6.2.1 Labelling with $^{14}CO_2$

Sodium bicarbonate ($NH^{14}CO_3$) (Amersham International) with a specific activity of 58 mCi $mmol^{-1}$ and concentration of 1.00 mCi was diluted to 10.0 mL with distilled water and adjusted to pH 9.4 with 0.01 M NaOH to make a diluted solution of 0.5 mCi mL^{-1}.

Plants were grown as in Chapter III.4.1 of the general materials and methods. The first subtending leaf of a new shoot was labelled with $^{14}CO_2$, at each stage of development. Three plants were labelled for each developmental stage. Refer to Fig. 6.1.

I. Bud burst, extension of cataphylls
II. Shoot extension, involving leaf expansion and internode extension
III. Dormancy, completion of shoot extension
IV. Completion of leaf expansion.

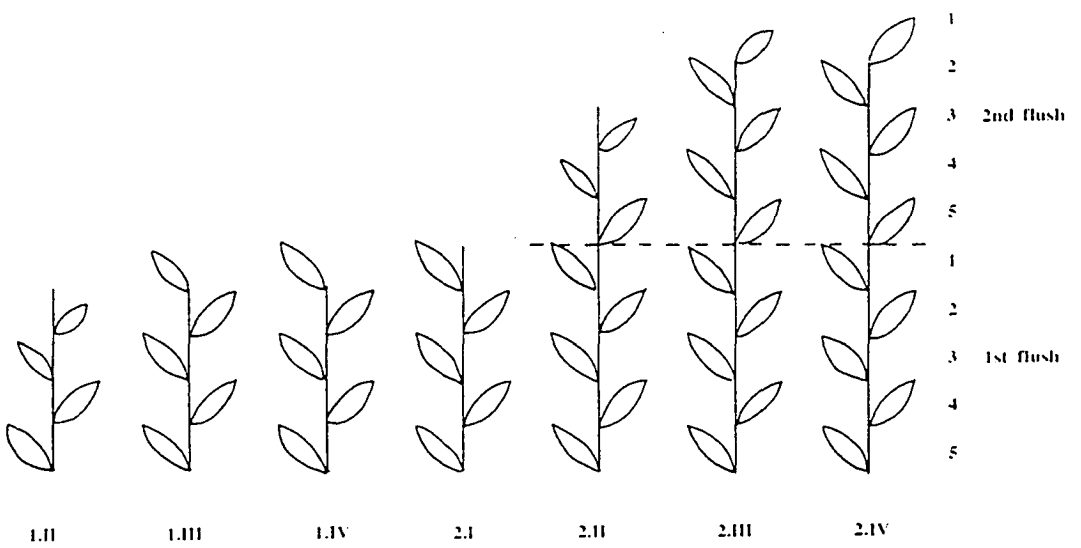

Figure 6.1 Plant sampling strategy for I) start of shoot growth; II) shoot extension; III) dormancy; and IV) completion of shoot growth; for 1) first and 2) second flushes. Numbers 1-5 denote leaf and internode position.

Leaves were labelled with 5 μCi of radioactive CO_2, based on the methods of Beveridge *et al.* (1992). Leaves were enclosed in an air tight chamber, constructed of perspex, which measured 90 cm long, 27 cm wide and 9 cm deep. The chamber was in two halves, which were clamped together during $^{14}CO_2$ labelling. Indentations in the top half of the chamber accommodated the petioles of the leaves, and plasticine was used to plug any gaps. Approximately 20 μCi (5 μCi per plant) of $^{14}CO_2$ was liberated within the chamber by adding 2.0 mL of 1N HCL to the $Na_2{}^{14}CO_3$ in a small vial. Fed leaves were left in the chamber for 1 hour, following which the chamber was evacuated of residual $^{14}CO_2$ by flushing with air supplied by a gas cylinder. The supply of $^{14}CO_2$ was largely exhausted at the end of the 1 hour uptake period and any residual CO_2 was collected in soda lime. The chamber was then dismantled, and plants were maintained in the glasshouse for a further 24 hours. The period of time from uptake to harvesting was based on published data (Sanderson and Sivapalan, 1966b) and confirmed experimentally.

After harvesting from the plants, shoots were further dissected into two internodes below the source leaf, the source leaf, and leaves and internodes above the source leaf. Leaves and internodes were numbered accordingly.

6.2.2 Extraction and purification

Following freeze drying, leaves and internodes were ground in liquid nitrogen, using a stainless steel mortar and teflon pestle.

Ground samples were extracted in 10 mL of 60% aqueous ethanol at 60 °C overnight.

The aqueous (soluble fraction) portion was purified using PVPP, to remove polyphenolics and chlorophyll which otherwise quenched scintillation counting. The remaining solid was washed with a further 5 mL of 60% ethanol, centrifuged, and purified through the PVPP column. The PVPP column was washed with a further 3 mL of 60% ethanol, to collect any residual extract.

Two mL of Ready Safe scintillation mixture (Beckman) was added to 1 mL of purified extract in 3 mL polypropylene scintillation vials.

The solid residue (insoluble fraction) was dried at 60 °C overnight. 10 to 100 mg of dry sample was suspended in a 1:1 mixture of Insta-gel scintillant (Canberra-Packard) to distilled water in 4 mL scintillation vials.

Both soluble and insoluble fractions were counted using a Beckman Scintillation counter. Results were reported as %DPM 100 mg^{-1} dry tissue.

6.2.3 Starch determination

Starch concentrations were determined for three replicate samples of each developmental stage. The Megazyme total starch (amylglucosidase/α amylase) assay procedure was used.

The sample was freeze dried and ground using liquid nitrogen. 50 mg of sample were placed into a 6 mL glass vial. Soluble sugars were extracted with 4 mL of 80% ethanol at 60 °C for 1 hour. The sample was then centrifuged at 3000 rpm for 10 minutes, and the supernatant discarded. The extraction was repeated 3 times.

Resistant starch was denatured using 1 mL of DMSO at 100 °C for 5 minutes.

Starch was then hydrolysed to glucose with 1.5 mL of α-amylase (300 U in MOPS buffer: 50 mM, pH 7.0) at 100 °C for 6 minutes; followed by 0.05 mL amylglucosidase (20 U in sodium acetate buffer; 200 mM, pH 4.5) at 50 °C for 30 minutes.

Samples were then centrifuged at 3000 rpm for 10 minutes. An aliquot of 1 mL was diluted to 10 mL with distilled water. A further aliquot of 0.1 mL was added to 3 mL of GOPOD reagent, and colour was allowed to develop over 20 minutes at 50 °C.

Absorbance was determined at 510 nm against a reagent blank of 0.1 mL of distilled water with 3 mL of GOPOD.

6.2.4 Statistical analysis

Tests of significance were conducted using a repeated measures ANOVA for stage of development in the statistical program StatView, and LSD's reported for each of the following factors (Appendix VII):

- Dry weight
- Buds
- Leaves
- Internodes
- Source leaf
- Internodes below source leaf
- Leaves and internodes positioned one to five

6.3 Results

6.3.1 Dry matter partitioning at different stages of growth

During stage 2.II, leaf and internode expansion, dry weight increased from around 3 mg for leaf one, to 10 to 20 mg for leaves two and three (Fig. 6.2). Leaves four and five, as cataphylls, were considerably smaller in dry weight, at around 3 mg, than either of leaves two or three (Fig. 6.2). At the same stage in development, the dry weight of internode five was slightly greater, at around 10 mg, than internodes one to four, in which dry weight increased slightly from around 3 mg in the first internode to 7 mg in the fourth internode (Fig. 6.3).

During stage 2.III, dormancy, leaf dry weight increased from about 25 mg for the first leaf, to around 60 mg in leaves two and three, which reflected the gradient in leaf age (Fig. 6.2). Leaves four and five had completed expansion and attained a maximum dry weight of around 3 and 60 mg by stage 2.II and 2.III respectively. In comparison, dry weight of leaves one to three did not attain full expansion until stage 2.IV; reaching a maximum of around 100 mg for leaf one, and 175 mg for leaf three (Fig. 6.2).

During stage 2.III, dormancy, dry weight increased from about 3 mg in internode one, to a maximum of almost 30 mg in internode three, reflecting incomplete internode extension (Fig. 6.3). Dry weight then declined to around 20 and 15 mg in internodes four and five respectively (Fig. 6.3). The lack of any further significant increase in dry weight in internode five perhaps indicated that internode extension was complete (Fig. 6.3). The comparatively low dry weight of internode five may then be reflective of the shorter length of internodes at the start of a flush.

During stage IV, completion of flush growth, dry weight of internodes four and five significantly increased. These internodes were then either greater in size than internodes sampled during stage III, or secondary thickening had commenced, which may contribute relatively more weight, as wood, than unlignified stem tissue. Internodes one to three increased by approximately 10 mg during stage IV, although, as observed during stage III, dry weight increased from internode one, then around 15 mg, to almost 40 mg in internode three. The decline in dry weight from internode one to three reflected the tendency for internodes at the end of a flush to be shorter in length.

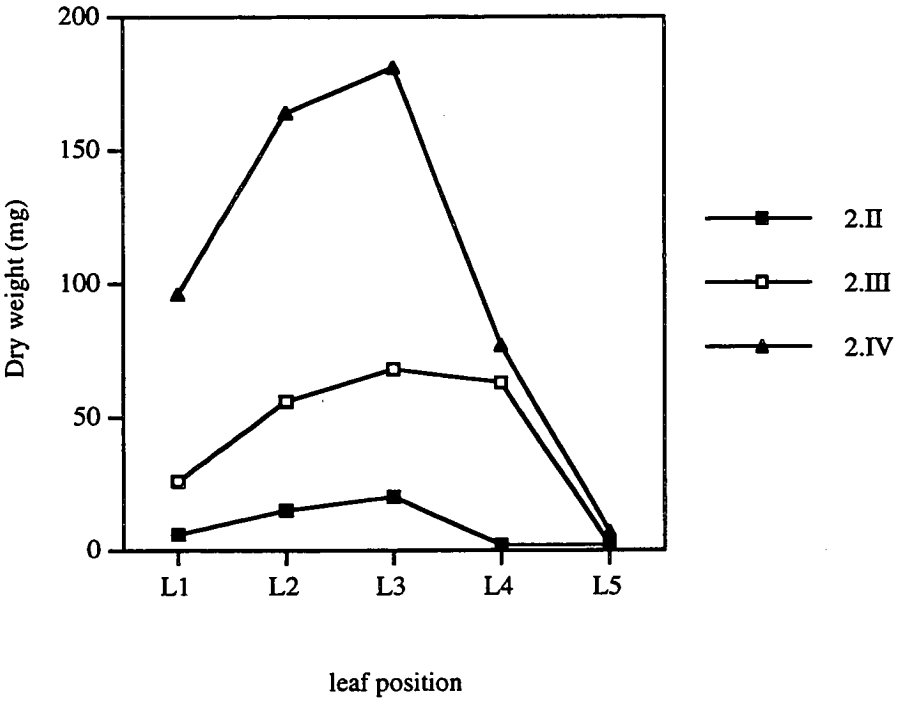

Figure 6.2 Dry weight of leaves for stages of development
LSD (II.2)=5.4; LSD (III.2)=62.0; LSD (IV.2)=51.4.

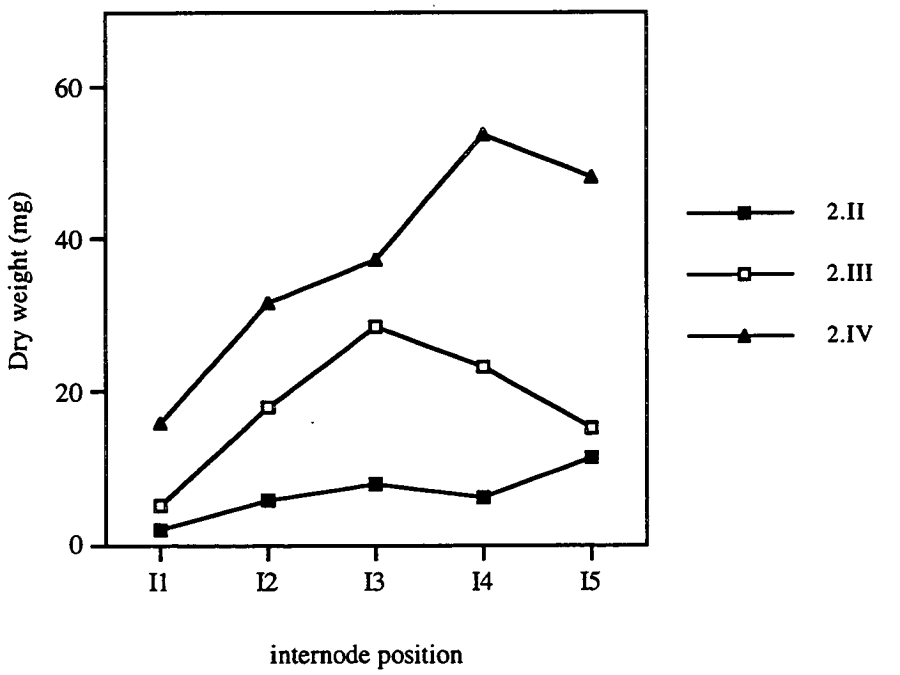

Figure 6.3 Dry weight of internodes at different stages of development
LSD (II.2)=4.7; LSD (III.2)=10.5; LSD (IV.2)= 27.0.

6.3.2 Partitioning ^{14}C label at different stages of growth

During stage 2.I, at the start of shoot growth, the bud was the dominant sink, attracting 50% of the ^{14}C label from the source leaf that subtended the new flush, which retained 20 % of ^{14}C label (Fig. 6.4). The majority of ^{14}C label retained by the source leaf was partitioned to the soluble fraction (18 %), which was likely to be comprised of soluble carbohydrates (sucrose), and other compounds which may be extracted by aqueous ethanol (Table 6.1). The remaining ^{14}C label in the source leaf (3.7 %) was partitioned to the insoluble component, which was likely to consist of starch and structural compounds, including cellulose and lignin (Table 6.1). Increased partitioning of label to the bud was also reflected in the comparatively greater proportion of ^{14}C label present in the soluble component, at around 38 %, which was three times that of the insoluble component, at 12.3 %. The remaining 25 % of ^{14}C label was partitioned to internodes below the source leaf. As for buds, a similar proportion of ^{14}C label detected in internodes below the source leaf was partitioned to the soluble and insoluble components.

During stage 2.II, expanding leaves and internodes replaced the bud as the dominant sink, where 15 % of label was partitioned to the bud, while 30 and 40 % of ^{14}C label was partitioned to leaves and internodes respectively (Fig. 6.4). Only around 6 % of ^{14}C label was retained in the source leaf, which was three times less than that retained in the source leaf during stage 2.I, which perhaps reflected an increase in demand for assimilate by the expanding leaves and internodes (Fig. 6.4). Approximately one-third of the ^{14}C label detected in internodes was partitioned to the insoluble fraction, at 14 %, potentially as either structural components or carbohydrate reserves (Table 6.1). In comparison, in both leaves and buds, one-quarter of ^{14}C label was partitioned to the insoluble fraction (Table 6.1). Relatively little ^{14}C label was partitioned to the internodes below the source leaf, at around 6 % (Fig. 6.4). Of this, the ^{14}C label was partitioned roughly in equal amounts between the insoluble and soluble fractions (Table 6.1).

During stage III, dormancy, partitioning of ^{14}C label to leaves declined to 20 % from around 30 % in stage II, but this was overall insignificant (Fig. 6.4). In comparison, partitioning of ^{14}C label was maintained at 40% in internodes, although shoot extension was complete. This label could either be utilised in secondary growth of the stem, or perhaps represents transported assimilate in the phloem. Partitioning of ^{14}C label to internodes below the source leaf and buds remained the same from stage II to stage III, at around 5 and 15 % respectively. As partitioning of ^{14}C label to leaves had declined from stage II to stage III, while partitioning to buds and internodes, both above and below the source leaf, remained the same, an increased proportion of ^{14}C

label was instead retained in the source leaf (Fig. 6.4). This increase was however statistically insignificant. Approximately one-quarter of ^{14}C label was partitioned to the insoluble fraction for all leaves and internodes, including the source leaf (Table 6.1). In comparison, ^{14}C label partitioned to the soluble fraction in buds, at around 12 %, was significantly greater than the insoluble fraction, at around 1 %.

During stage 2.IV, shoot growth was complete, which was reflected in the decline in ^{14}C label partitioned to leaves (20 to 5 %) and internodes (50 to 25 %) when compared to stages 2.II and 2.III (Fig. 6.4). Partitioning of ^{14}C label to the bud also declined from stage II to III, from 15 to 5 %, although this was insignificant (Fig. 6.4). Reduced demand for assimilate by fully expanded leaves and internodes instead resulted in increased retention of ^{14}C label in the source leaf, from 10 to 45 % from stages 2.III to 2.IV (Fig. 6.4). Similarly, partitioning of ^{14}C label to internodes below the source leaf increased significantly to 15 % (Fig. 6.4). Partitioning of ^{14}C label was three times greater in the soluble fraction of internodes both above and below the source leaf (Table 6.1). A similar trend was observed for soluble and insoluble fractions retained in the source leaf. In leaves, however, only 0.5 % of ^{14}C label was partitioned to the insoluble fraction, which was twelve times less than that in the soluble fraction, at around 6 %. A negligible amount of ^{14}C label was detected in the insoluble fraction in buds.

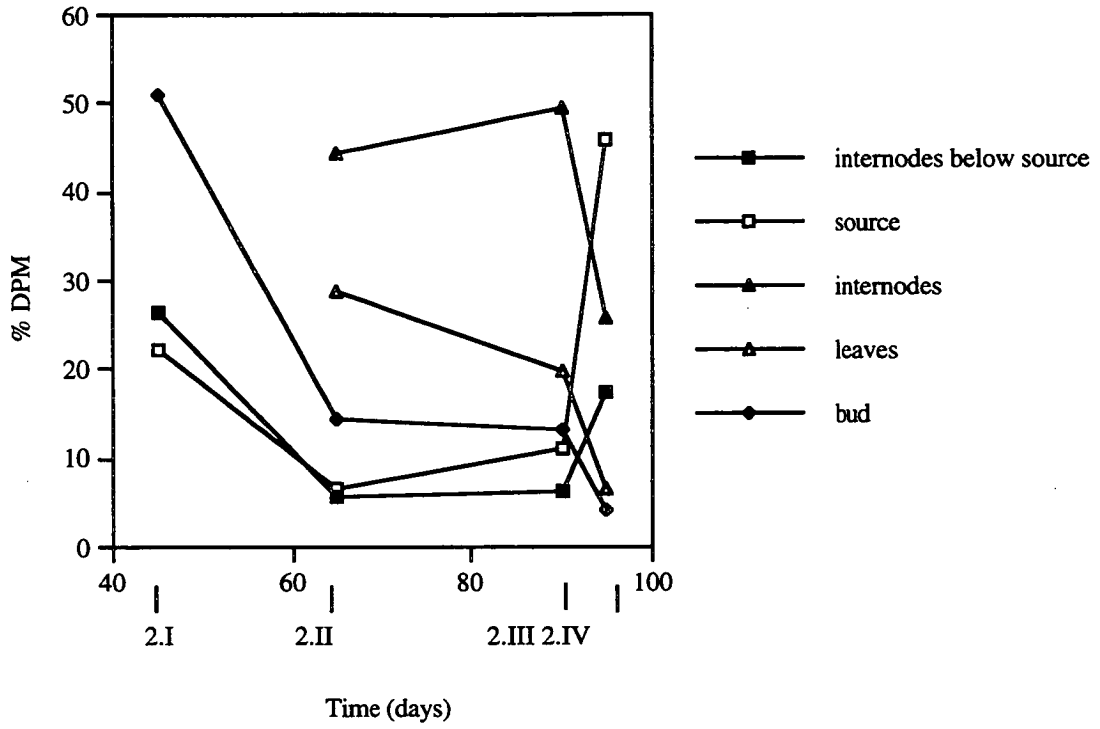

Figure 6.4 Partitioning of assimilate from subtending leaf into new shoot at different stages of development. LSD (internodes below)=7.8; LSD (source)=15.9; LSD (internodes above)=8.1; LSD (leaves)=17.9; LSD (bud)=12.4

Table 6.1 Soluble and insoluble fractions (% DPM) at each stage of development

	2.I		2.II		2.III		2.IV	
	sol.	insol.	sol.	insol.	sol.	insol.	sol.	insol.
bud	38.7 ±4.2	12.3 ±2.1	11.0 ±3.9	3.5 ±1.5	11.6 ±3.8	0.8 ±0.1	4.0 ±2.7	0.1 ±0.1
leaves	-	-	21.2 ±1.8	7.5 ±0.7	15.2 ±3.9	5.8 ±2.3	6.1 ±2.6	0.5 ±0.2
internodes	-	-	30.2 ±0.7	14.2 ±1.8	32.9 ±1.4	13.3 ±1.1	19.9 ±2.6	6.0 ±1.0
source	18.7 ±2.0	3.7 ±0.6	5.1 ±1.1	1.5 ±0.2	10.6 ±2.8	3.1 ±0.9	34.5 ±7.9	11.6 ±2.0
internodes below	18.9 ±3.1	7.6 ±2.0	3.1 ±0.2	2.6 ±0.7	5.5 ±1.5	1.9 ±0.5	12.4 ±0.2	5.1 ±1.1

6.3.3 Export of ^{14}C label to leaves during shoot development

As leaves unfold in succession from the apex, there is a gradient in leaf maturation from leaf five, first to unfold from the apex, to leaf one, last to unfold from the apex. This gradient in leaf age was reflected in the partitioning of ^{14}C label.

During stage 2.II, shoot extension, significantly greater quantities of ^{14}C label were partitioned to the first leaf, at 7.5 %, and second leaf, at 15 %, than to leaves three to five (Fig. 6.5). Of this, approximately one-quarter of ^{14}C label was partitioned to the insoluble fraction (Table 6.2). The small quantities of ^{14}C label partitioned to leaves three to five indicated that these leaves were net exporters of assimilate. The partitioning of ^{14}C label to leaves one to two indicated that imported assimilate was still required to sustain leaf development and growth.

During stage 2.III, dormancy, negligible quantities of ^{14}C label was partitioned to the third to fifth leaves, which was consistent with that observed during stage 2.II (Fig. 6.5). Partitioning of ^{14}C label to leaf two declined to 7.5 % and was half that imported during stage II, reflecting leaf maturation. Partitioning of ^{14}C label within the second leaf to the soluble fraction was four times greater than the insoluble fraction (Table 6.2). Around twice the amount of ^{14}C label was partitioned to the first leaf, at around 12 %, when compared to the second leaf, reflecting the younger age of leaf one. Of this, around one-third of the ^{14}C label was partitioned to the insoluble fraction.

Partitioning of ^{14}C label to the second leaf declined from 7.5 % during stage 2.III to 0 % by stage 2.IV, at the completion of shoot growth (Fig. 6.5). In comparison, 5 % of ^{14}C label was still partitioned to leaf one, which was half of that partitioned to the equivalent leaf during stage III. The majority of the ^{14}C label partitioned to the first leaf was detected in the soluble component (Table 6.2). Consequently, although the decline in import of ^{14}C label in the first leaf reflected leaf maturation, continued import of ^{14}C label (although comparatively small) indicated that the first leaf had not yet approached net export status at the time of sampling.

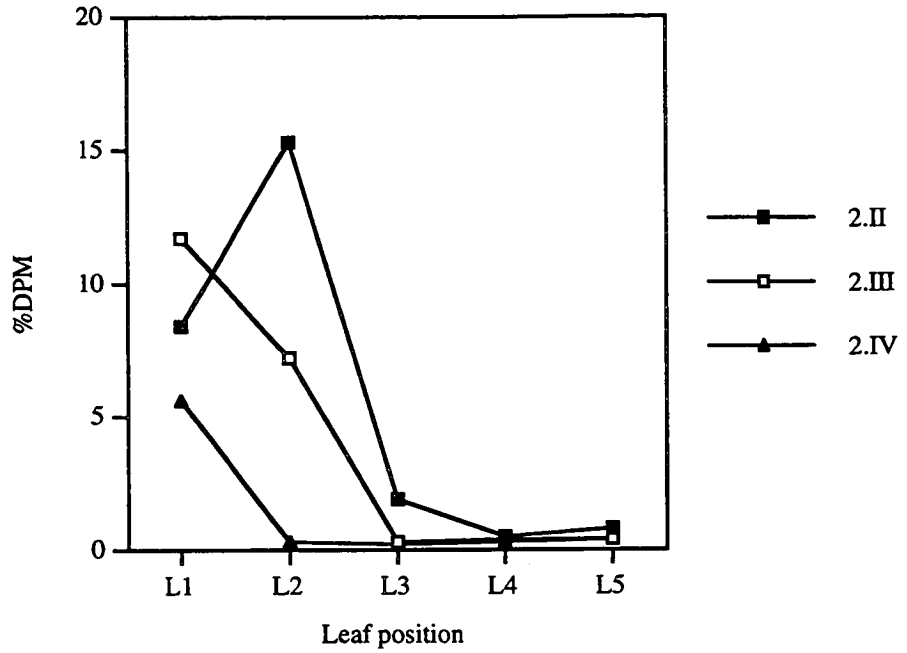

Figure 6.5 Export of ^{14}C label to individual leaves.
LSD (II.2)=4.9; LSD (III.2)=8.0; LSD (IV.2)=3.8.

Table 6.2 Soluble and insoluble fraction of ^{14}C label (%DPM) in leaves

	2.II		2.III		2.IV	
	sol.	insol.	sol.	insol.	sol.	insol.
L1	5.85 ±1.90	2.52 ±0.68	7.47 ±2.70	4.23 ±1.89	5.17 ±2.46	0.43 ±0.22
L2	11.40 ±0.12	3.88 ±0.98	6.90 ±0.44	1.53 ±0.44	0.21 ±0.04	0
L3	1.50 ±0.12	0.31 ±0.07	0.20 ±0.06	0	0.15 ±0.04	0
L4	0.46 ±0.46	0.04 ±0.04	0.33 ±0.15	0	0.25 ±0.06	0
L5	0.69 ±0.06	0.02 ±0.02	0.27 ±0.13	0	0.27 ±0.27	0.03 ±0.03

6.3.4 Export of ^{14}C label to internodes during shoot development

As discussed previously, leaves unfold in succession from the apex, such that there is a gradient in leaf maturation from leaf five, the first to unfold from the apex, to leaf one, the last to unfold from the apex. A similar gradient in internode maturation is observed, as the expansion of internodes and unfolding of leaves occur concurrently. This gradient in internode age was reflected in the partitioning of ^{14}C label.

During stage 2.II, shoot extension, around 10 to 12 % of ^{14}C label was partitioned to internodes one to three, decreasing significantly to 6 to 7 % in internodes four and five (Fig. 6.6). This was likely to reflect an increased requirement for imported assimilate to sustain internode expansion. In all internodes, around one-third of the partitioned ^{14}C label was detected in the insoluble fraction, potentially as either structural components or stem carbohydrate reserves (Table 6.3).

During stage 2.III, dormancy, partitioning of ^{14}C label significantly decreased from the first to third internodes, from 20 to 7 % respectively. The amount of ^{14}C label partitioned to the first internode was roughly twice that partitioned to the equivalent internode during stage II. Of this, the majority of ^{14}C label was partitioned to the soluble fraction, at 16 %, which was five times greater than that partitioned to the insoluble fraction (Table 6.3). In comparison, only about 5 % of ^{14}C label was partitioned to internodes four and five, which was similar to that observed during stage 2.II (Fig. 6.6).

During stage IV, completion of shoot growth, around 6 % of ^{14}C label was partitioned to the first internode (Fig. 6.6). Of this, the majority of ^{14}C label was partitioned to the soluble fraction, at about 5 %, which was almost three times that of the insoluble fraction, at about 2 %. In comparison, only around 2 % of ^{14}C label was partitioned to internodes two to four, most of which was partitioned to the soluble fraction. Partitioning of ^{14}C label to the fifth internode increased significantly to 14 % (Fig. 6.6). The majority of ^{14}C label partitioned to the fifth internode was present in the soluble fraction, at around 11 %, which was almost four times greater than that partitioned to the insoluble fraction, at around 3 % (Table 6.3). Increased partitioning of ^{14}C label to the fifth internode relative to internodes one to four could reflect secondary growth, or partitioning away from the shoot, in which shoot expansion is complete and hence demand for assimilate less than in stages 2.II or 2.III.

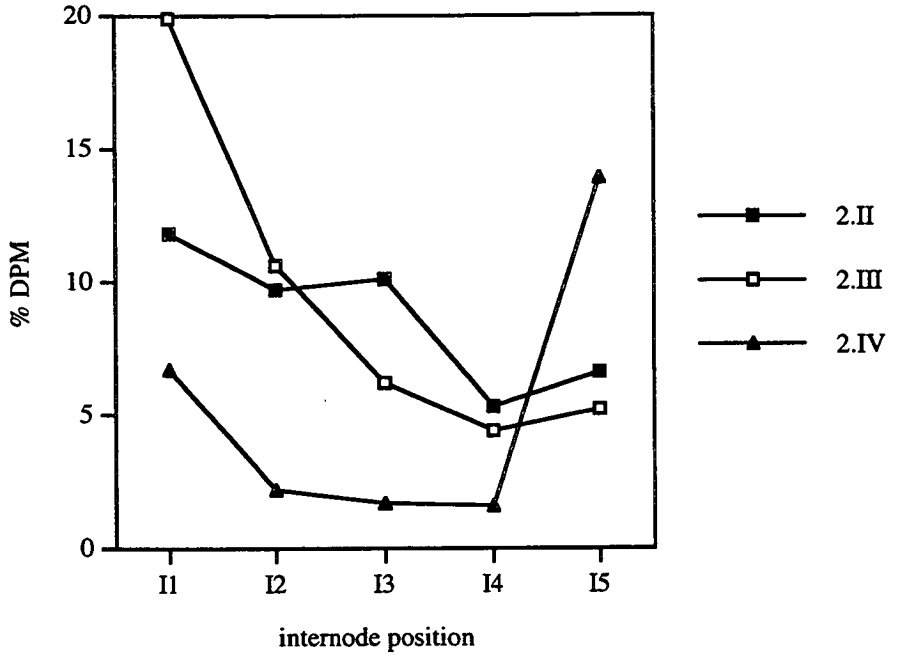

Figure 6.6 Export of ^{14}C label to individual internodes.
LSD (II.2)=2.7; LSD (III.2)=9.6; LSD (IV.2)= 6.1.

Table 6.3 Soluble and insoluble fraction of ^{14}C label (%DPM) in internodes

	2.II		2.III		2.IV	
	sol.	insol.	sol.	insol.	sol.	insol.
I1	8.24 ±1.6	3.54 ±1.2	16.43 ±3.9	3.40 ±1.2	4.85 ±2.6	1.84 ±0.8
I2	7.52 ±1.4	2.10 ±0.5	6.83 ±1.4	3.73 ±0.8	1.75 ±0.7	0.41 ±0.1
I3	6.89 ±0.1	3.20 ±0.9	3.27 ±0.9	2.93 ±0.8	1.31 ±0.4	0.36 ±0.1
I4	3.28 ±0.6	2.02 ±0.8	2.27 ±0.6	2.13 ±0.8	1.18 ±0.3	0.36 ±0.1
I5	4.34 ±0.3	2.22 ±0.0	4.07 ±0.9	1.10 ±0.0	10.82 ±1.5	3.06 ±0.3

6.3.5 Starch in leaves and internodes

Quantity of starch was in general greater in leaves than internodes, and reflected shoot growth and development. Shoot maturation during growth of the new flush was accompanied by the accumulation of starch in internodes. Starch in internodes increased significantly from negligible amounts during stage 1.II, shoot extension, to about 5 % during stage 1.IV, at the completion of growth of the new flush (Fig. 6.7). A similar trend was observed in starch accumulation in leaves, where starch increased from about 5 % during stage 1.II, during shoot extension, to 15 % during stage 1.IV, at the completion of shoot growth (Fig. 6.7).

During stage 2.I, at the start of growth of the second flush, starch in leaves previously accumulated by stage 1.IV significantly declined from 15 % to 10 % (Fig. 6.7). The decline in internode starch was more pronounced than in leaves, where starch previously accumulated during stage 1.IV was completely hydrolysed during stage 2.I at the start of shoot growth (Fig. 6.7).

During growth of the second flush, starch again accumulated in first flush leaves, doubling from 10 % during stage 2.I at the start of shoot growth to 20 % during stage III, at dormancy (Fig. 6.7). This was 5 % greater than the maximum quantity of starch previously accumulated when leaves had completed their first flush of growth. In comparison, during stage 2.IV, at completion of growth of the second flush, starch again declined to about 7.5 % (Fig. 6.7). This was slightly less than the previous decline in starch to 10 % which occurred during stage 2.I at the start of growth (Fig. 6.7).

Similarly, starch accumulated in internodes during growth of the second flush, increasing to 5 % during stage 2.II, during shoot extension, which was comparable to the amount of starch previously detected in internodes during stage 1.IV of the first flush (Fig. 6.7). The amount of starch in internodes of the second flush then tended to slowly decline, reaching about 2.5 % by stage 2.IV, at completion of growth of the second flush (Fig. 6.7).

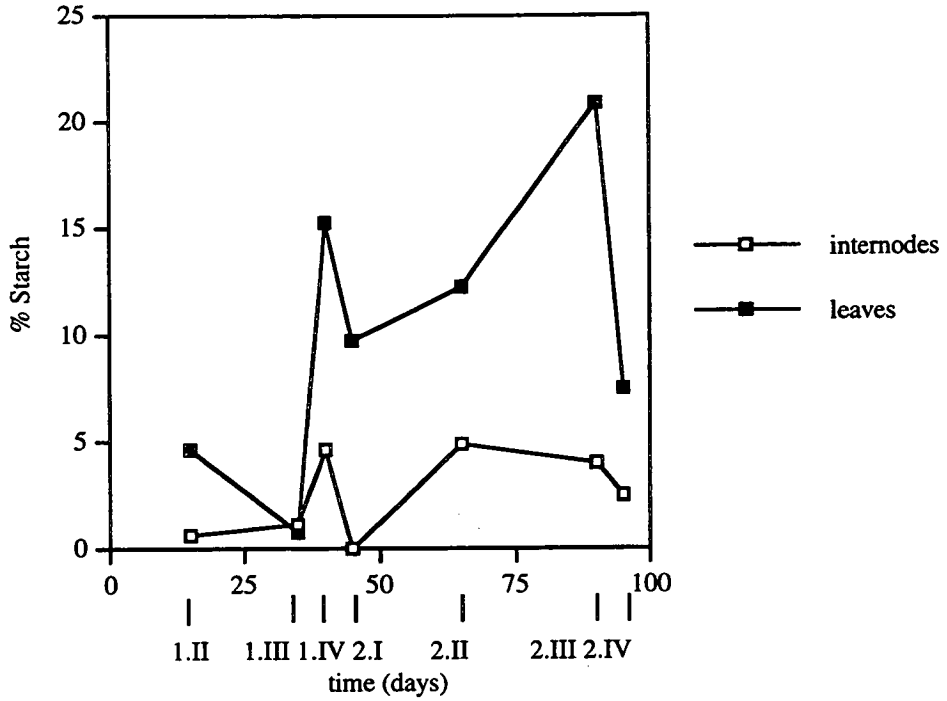

Figure 6.7 Starch in previous flush leaf at different stages of development. LSD (leaves) = 3.73, LSD (internodes) = 1.78

6.4 Discussion

The partitioning of assimilates in vegetative shoots of tea is influenced by shoot growth and development. The bud is the dominant sink at the start of growth, where rapidly expanding leaves and internodes of the bud attract the majority (50 %) of ^{14}C label from the leaf subtending the new flush. This is in agreement with Sleigh *et al.* (1984), where it was shown that the majority of ^{14}C label was partitioned to buds of *Theobroma* at the start of shoot growth. Barnola *et al.* (1993) likewise have shown that the accumulation of ^{14}C DMO increased in buds at the start of shoot growth in *Quercus*. Additional reserves for bud burst are supplied by hydrolysis of starch in internodes, and to some degree from leaves in spring growth of tea, as observed by Hakmata (1983), and in the third flush of *Quercus robur* seedlings (Alaoui-Sossé *et al.*, 1994). It is possible that the hydrolysis of starch may occur in a gradient down the stem, as shown in *Theobroma* (Sleigh *et al.*, 1984) and *Quercus* (Alaoui-Sossé *et al.*, 1994).

During stage 2.II, shoot extension, expanding leaves and internodes replaced the bud as the dominant sink, attracting 30 and 40 % of ^{14}C label respectively. In comparison,

partitioning of ^{14}C label to buds declined from 50 % during stage I to only 6 %. This is in agreement with the observations of Barnola *et al.* (1993), where partitioning of ^{14}C labelled DMO decreased in the bud during shoot extension in *Quercus*, yet differs from Sleigh *et al.* (1984), where partitioning of ^{14}C labelled assimilate was maintained to buds of *Theobroma* until the completion of growth, when it declined.

Increased demand for assimilate by expanding leaves and internodes during stage 2.II resulted in only 6 % of ^{14}C label being retained in the source leaf, which was significantly less than during stage I, at 25 %. This is similar to the observations of Sleigh *et al.* (1984), where export of ^{14}C label increased to 35 % during shoot extension in *Theobroma*. Dickson *et al.* (1990) has also reported increased export of ^{14}C label during flush growth in *Quercus*. However, at the same time, the quantity of starch in first flush leaves increased slightly from 10 % during stage 2.I, to 12.5 % during stage 2.II. This was 7.5 % less than the maximum amount of starch accumulated in first flush leaves during stage 1.III, and indicates that both starch reserves and current (^{14}C labelled) assimilate are utilised to sustain leaf and internode expansion during stage 2.II. This differs from *Theobroma* (Sleigh *et al.*, 1984) and second flush *Quercus* seedlings (Barnola *et al.*, 1993), where starch is instead mobilised in leaves and internodes only during shoot extension.

Leaves unfold from the apex sequentially, and then expand concurrently with their associated internodes. As a result, there is a gradient in leaf age, where leaves and internodes which are the first to unfold from the apex will be chronologically older than those which unfold later from the apex. By stage 2.III, the last three leaves of the new flush attained full expansion, as reflected by constant dry weight. These leaves did not import ^{14}C label from the source leaf, and were presumably net exporters of assimilate. Similar observations are reported by Sanderson and Sivipalan (1966a), although using qualitative autoradiography, unlike in the current study, in which ^{14}C was quantitatively measured using scintillation counting. At the same time, partitioning of ^{14}C label to the second leaf had declined by half, indicating the progression in leaf maturation from stage 2.II to 2.III. Immature leaves are reported to both import and export assimilates due to basipetal leaf maturation (Larson *et al.*, 1972), not becoming net exporters of assimilate until they are 30 to 60 % fully expanded (Turgeon, 1989). Partitioning of ^{14}C label to leaves during stage III, dormancy, then declined from 30 % during stage II to 20 %, and although this was insignificant, it perhaps reflected reduced demand for assimilate. This is in agreement with observations of Manivel and Hussain (1982a), where partitioning of ^{14}C label declined with shoot development, but unlike in the current study, this was not related to growth and export status of developing leaves.

Although partitioning of ^{14}C label to leaves declined during stage 2.III, partitioning of ^{14}C label to buds and internodes, both of the new and previous flush, remained the same. As a result, retention of ^{14}C label doubled from 6 to 12 % in the source leaf, reflecting decreased demand for exported assimilate by the new flush. At the same time, the amount of starch increased to a maximum of 20 %, which was roughly twice that present in first flush leaves during stage 1.I and 1.II. Consequently, it was likely that only current assimilate, rather than reserves of carbohydrate, were used in leaf expansion during stage 2.III.

The distribution of ^{14}C label between internodes of the new flush varied depending on internode position and hence age during stages 2.II and 2.III, although the total amount of ^{14}C label was similar. For example, during stage 2.II, increasing quantities of ^{14}C label were partitioned to internodes one to three, which had not attained full expansion, as reflected by their respective dry weights. In comparison, during stage 2.III, less ^{14}C label was partitioned to internode three, which had attained maximum weight, and instead partitioned in increased amounts to internodes one and two. The overall total ^{14}C label in internodes was then similar between stages 2.II and 2.III.

The ^{14}C label partitioned to the different components of the flush was further divided into the soluble and insoluble fractions, using aqueous ethanol extractions. The soluble fraction was likely to include soluble carbohydrates, including glucose, fructose and sucrose (Sanderson and Perera, 1965), the ethanol-insoluble fraction starch, and structural compounds including pectins, hemicelluloses and cellulose (Selvendran and Selvendran, 1972). Internodes and to a lesser extent leaves of the new flush contained little starch during stages 1.II and 1.III; consequently the ethanol-insoluble fraction was then likely be comprised of structural compounds, used in the formation of cell walls and xylem during primary growth. In comparison, during stage 1.IV, the ethanol-insoluble fraction of leaves and internodes was likely to contain both structural compounds and starch, the latter of which had increased in leaves and internodes to 15 and 5 % respectively.

During stage 2.IV, completion of shoot growth, partitioning of ^{14}C label to leaves continued to decline, from 20 % during stage 2.III, to 5 %, due to leaf maturation, as observed in the attainment of maximum dry weight and net export status. Partitioning of ^{14}C label to internodes similarly declined, from 50 % during stage 2.III, to 25 %. This resulted from a reduction in ^{14}C partitioning to internodes two to four, in which expansion had ceased, as reflected in the dry weight, which had stabilised. Although some ^{14}C label was partitioned to the first internode, the majority of ^{14}C label instead accumulated in the fifth internode, mainly in the soluble component. This could indicate either secondary growth, or transport of ^{14}C label away from the shoot, as

demand for assimilate had declined as shoot growth was complete. Partitioning of ^{14}C label to the bud also declined, from 15 to 5 %, even though at this stage of development leaf initiation has recommenced, and was consistent with the observations of Sleigh *et al.* (1984). The decline in partitioning to the bud is not necessarily inconsistent with increased bud activity, as the energy requirements for leaf initiation are likely to be comparatively smaller than for other stages of development.

Reduced demand for assimilate was reflected by increased retention of ^{14}C label within the source leaf, from 10 % during stage 2.III, to 45 % during stage 2.IV. Partitioning to internodes below the source leaf similarly increased, and presumably the ^{14}C label had been partitioned to other parts of the plant, including the roots (Fordham, 1972; Manivel and Hussain, 1982b; Hakamata, 1983; Kandiah and Wimaladharma, 1978), and is consistent with the observation that root growth alternates with shoot growth in tea (Fordham, 1972; Kandiah *et al.*, 1984). Increased partitioning of ^{14}C label also occurs during dormancy in *Theobroma* (Sleigh *et al.*, 1984). Although retention of ^{14}C label increased in the source leaf, three-quarters of which was partitioned to the soluble fraction, at the same time starch declined by about half of that during stage 2.III, to only 7.5 %. It then appears that leaves preferentially hydrolyse starch reserves at the completion of shoot growth for export, rather than exporting recent photosynthate. The destination for the exported reserves must be away from the now developed shoot, in which the demand for assimilate has declined.

In conclusion, both current photosynthate and reserves from the previous flush support growth of new shoots. Expanding leaves and internodes during bud burst and shoot extension are the dominant sink for assimilate. Competition for assimilates between expanding leaves and the bud apex may contribute to the formation of cataphylls in the apex, although this has not been confirmed.

IV.7 Plant Hormones

7.1 Introduction

Plant hormones, as inhibitors and promoters of growth, are potentially implicated in the regulation of rhythmic growth. Those hormones likely to be involved include abscisic acid (ABA), cytokinin and gibberellin (GA), the concentrations of which were quantified in apical buds and leaves of tea at the four stages of development using a radioimmunoassay procedure. Auxin has not previously been associated with rhythmic growth in either tea or other species (Kulasegaram and Kathiravetpillai 1972, Manivel *et al.* 1981, Barman and Manivel 1989), and as such was not quantified.

7.2 Materials and methods

7.2.1 Tissue sampling

Plants were grown as in Chapter III.4.1 of the general materials and methods. Terminal buds and subtending leaves were sampled for each stage of development (Fig. 7.1). Tissue samples from three plants were bulked in three replicates, to ensure sufficient weight of tissue for analysis.

I. Bud burst, expansion of cataphylls
II. Shoot extension, involving leaf expansion and internode extension
III. Dormancy, completion of shoot extension
IV. Completion of leaf expansion.

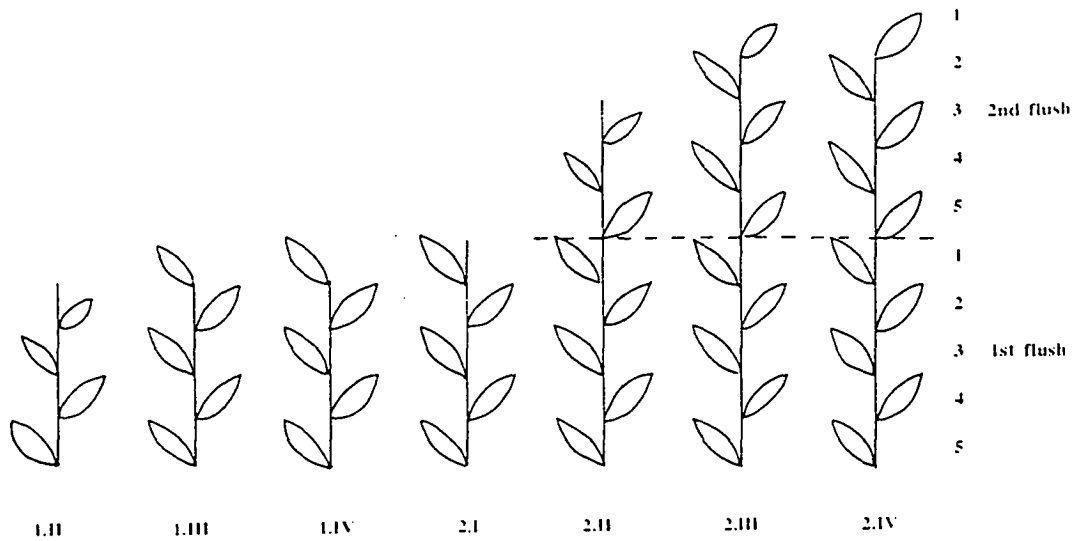

Figure 7.1 Plant sampling strategy for I) start of shoot growth; II) shoot extension; III) dormancy; and IV) completion of shoot growth; for 1) first and 2) second flushes. Numbers 1-5 denote leaf and internode position.

7.2.2 Extraction and purification

Following harvest, samples were freeze dried and then stored at -18 °C. Samples were ground with liquid nitrogen using a stainless steel mortar and teflon pestle. 0.5 g of dry ground samples were weighed into teflon vials, and extracted with 10 mL of 80 % methanol (containing 20 mg L^{-1} butylated hydroxytoluene) by shaking overnight at 4 °C.

Samples were then centrifuged at 20 000 rpm for 12 minutes, the supernatant decanted into a 20 mL vial, and the remaining solids washed with a further 10 mL of 80 % MeOH and recentrifuged. Methanol was removed from the supernatant using a Speed Vac concentrator (Savant, USA), and the extract resuspended in 10 mL of 0.01 M ammonium acetate.

The extract was purified through connected columns of polyvinylpolypyrrolidone (PVPP, 5 mL bed volume), DEAE Sephadex (3 mL bed volume) and SepPak C18 cartridges (Millipore Waters Associates). The PVPP and Sephadex were preconditioned with 20 mL of 1.0 M ammonium acetate, followed by 25 mL of 0.01 M ammonium acetate. SepPak cartridges were preconditioned separately, with 5 mL

of 100% MeOH for basic hormones and 5 mL 0.01 M ammonium acetate for acidic hormones. The extract was passed through the columns, followed by an additional 30 mL of 0.001 M ammonium acetate.

The SepPak cartridge containing the cytokinins was removed from the combined column, and rinsed with 5.0 mL of distilled water. The cytokinins were then eluted with 5 mL of 60 % MeOH. The PVPP column was then detached from the Sephadex column and a fresh SepPak C18 cartridge (preconditioned for acidic hormones with 5 mL of 100% MeOH and 5 mL of 1% acetic acid) was attached to the Sephadex column. The acidic hormones were eluted from the Sephadex with 15 mL of 1.0 M acetic acid. The SepPak cartridge was removed and rinsed with 5 mL of distilled water, and the acidic hormones eluted with 80% MeOH.

The cytokinin and acidic fractions were then evaporated to dryness, redissolved in MeOH and a 0.1 mg aliquot (dry sample) dispensed in triplicate into 3 mL polypropylene test tubes. The acidic hormones were methylated using diazomethane, and evaporated to dryness. Samples were stored at -18 °C until analysis.

Percentage recoveries were assessed by adding known quantities of tritiated hormone to the extraction columns, and quantifying the amount of recovered hormone using RIA. Percentage recoveries were generally greater than 90% for all hormones.

7.2.3 Radioimmunoassays

Quantification of hormones used the RIA procedures of Cutting *et al.* (1983, 1986), Hofman *et al.* (1985, 1986) and Hofman (1990). Standards were made in MeOH, and the required amounts dispensed into polypropylene test tubes. The MeOH was removed and the standards and samples dissolved in 0.1 mL of radioactive tracer (containing approximately 10 000 cpm) in phosphate buffered saline (pH 7.4) containing 0.1 % gelatine. [1,2(n)-^{3}H] gibberellin A_1 and DL-cis,trans-[G-^{3}H] abscisic acid were purchased from Amersham International while ^{3}H zeatin was previously produced in the laboratory. Antibody (0.1 mL, diluted to provide 30 to 50% binding) and 0.25 mL of 0.1% bovine serum (or phosphate buffered saline for the ABA assay) were then added, the mixture vortexed, and incubated at 37 °C for 30 minutes. Ammonium sulphate (0.75 mL of 90 % saturated) was added to precipitate the bound fraction and centrifuged at 7 000 rpm for 16 minutes. The supernatant was decanted, and the solid residue washed with 1 mL of 50 % ammonium sulphate, recentrifuged and decanted. The remaining residue was redissolved in 0.25 mL of distilled water, 2.5 mL of Ready-Mix Scintillation fluid added, the tubes capped and shaken, and radioactivity determined using a Beckman LS 5801 scintillation counter.

As polyclonal antibodies were used in this experiment, there was significant cross reactivity between the assayed and related hormones. The zeatin antibody had significant cross reaction with zeatin riboside, while the GA antibody had significant cross reaction with GA_1 and GA_3, with a lesser cross reaction to GA_{20}. The ABA antibody had no significant cross reaction with any other plant hormones.

7.2.4 Validation of radioimmunoassay

Validation for all plant hormone analysis using RIA has been described by Pengelly (1985). Two procedures were used to detect for the presence of competitive and non competitive inhibitors. Leaf samples were analysed for each hormone to be assayed, using internal standards and extract dilution methods to validate the immunoassay. Standard addition involves adding constant amount of plant extract to each point on the standard curve, and counting the standard curve as an unknown as previously described. The amount of plant hormone detected is then plotted against the amount of plant hormone added, and if non-linear indicates the presence of inhibitors of plant hormone binding. The extraction dilution technique involves assaying a range of extract weights, and plotting extract weight against the amount of plant hormone found. If the plot of detected plant hormones to sample weight should be linear and show additivity, the extract is free of inhibitors. The results from the internal standards and extract dilution methods indicated the lack of inhibitors in the extract.

7.2.5 Statistical analysis

Tests of significance were conducted using a repeated measures ANOVA for stage of development for concentration of hormones GA, ABA and ZR in leaves and buds using the statistical program StatView (Appendix VIII).

7.3 Results

7.3.1 Abscisic Acid

Abscisic acid was detected in buds and subtending leaves in all stages of development (Fig. 7.2). ABA levels in leaves did not vary significantly during the different stages of development, ranging between 18 to 23 ng g^{-1}. ABA was significantly greater at 35 ng g^{-1} in dormant buds during stage 2.III in comparison to around 24 ng g^{-1} at the other stages of development.

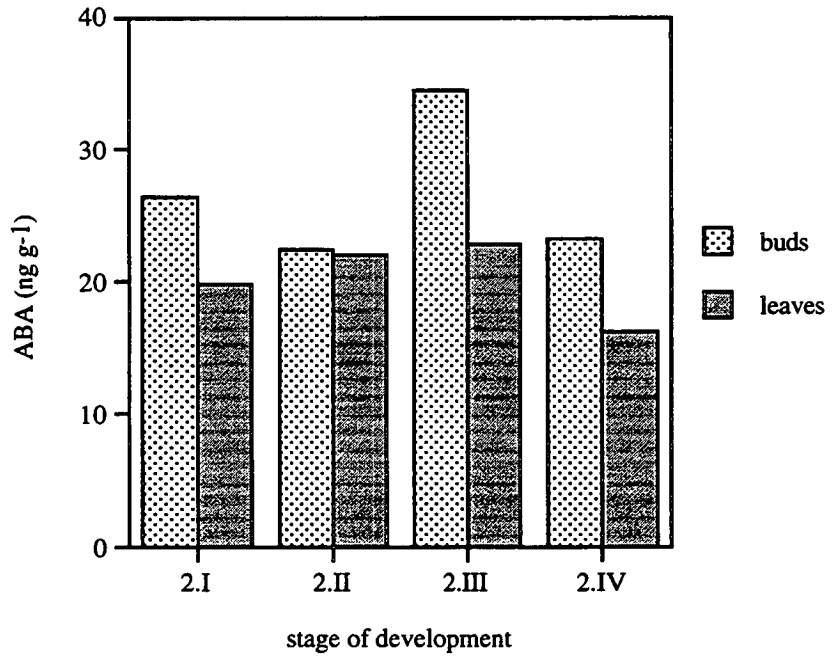

Figure 7.2 Abscisic acid in buds and subtending leaf according to stage of development. (LSD (buds)=5.1 ng g^{-1}; LSD (leaves)=9.3 ng g^{-1})

7.3.2 Gibberellin

Significant amount of GA (23 ng g^{-1}) were detected in buds at bud burst during stage 2.I, during which internodes and leaves were undergoing rapid extension growth (Fig. 7.2). This is consistent with the role of GA in cell extension in immature tissue, especially internodes (Davies, 1995). No significant quantities of GA were present in buds at the remaining stages of development, concentration ranging from nil to 1 ng g^{-1}. Lack of GA in subtending leaves during stage 2.I and stage 2.IV reflects leaf maturity. In comparison, subtending leaves at stage 2.II and stage 2.III are immature; yet GA was only detected in significant concentrations of 33 ng g^{-1} at stage 2.III. GA was otherwise negligible in subtending leaves during stage 2.II.

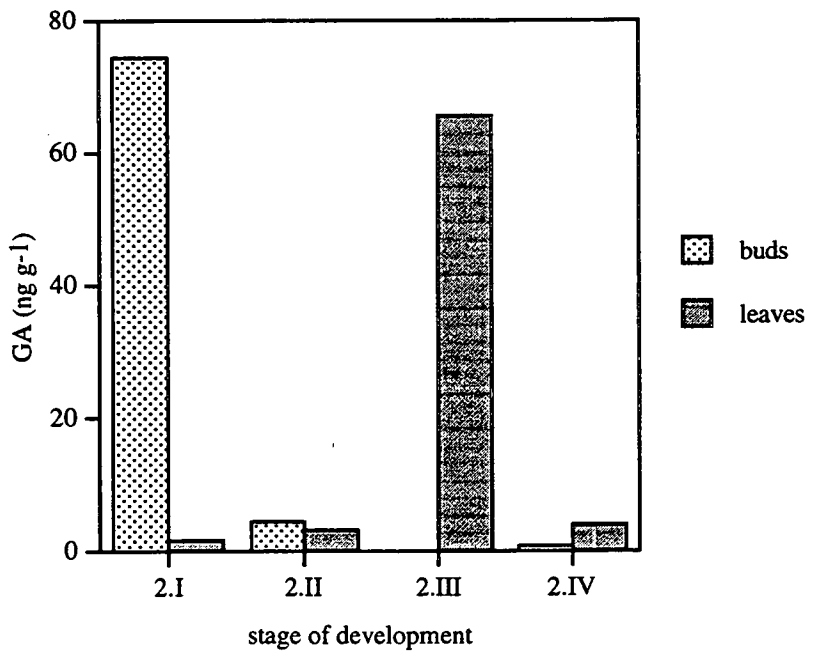

Figure 7.3 Gibberellic acid in buds and subtending leaf according to stage of development. (LSD (buds)=12.2 ng g^{-1}; LSD (leaves)=6.2 ng g^{-1})

7.3.3 Zeatin riboside

Zeatin riboside was detected in buds and leaves at all stages of development (Fig. 7.3). In buds, concentration of ZR declined by approximately 50 % during shoot extension in stage 2.II, to 30 ng g^{-1}. Concentrations of ZR otherwise ranged from 75 to 90 ng g^{-1} at the other stages of development. As cytokinins are associated with cell division (Davies, 1995), the decline in ZR concentration during shoot extension could be due to reduced activity in the bud (Chapter IV.1). In leaves, ZR was present in significantly higher concentration at 70 ng g^{-1} during bud burst, at stage 2.I; the concentration of ZR then declined as leaves reached full expansion, reaching a minimum of around 25 ng g^{-1} during stage 2.IV.

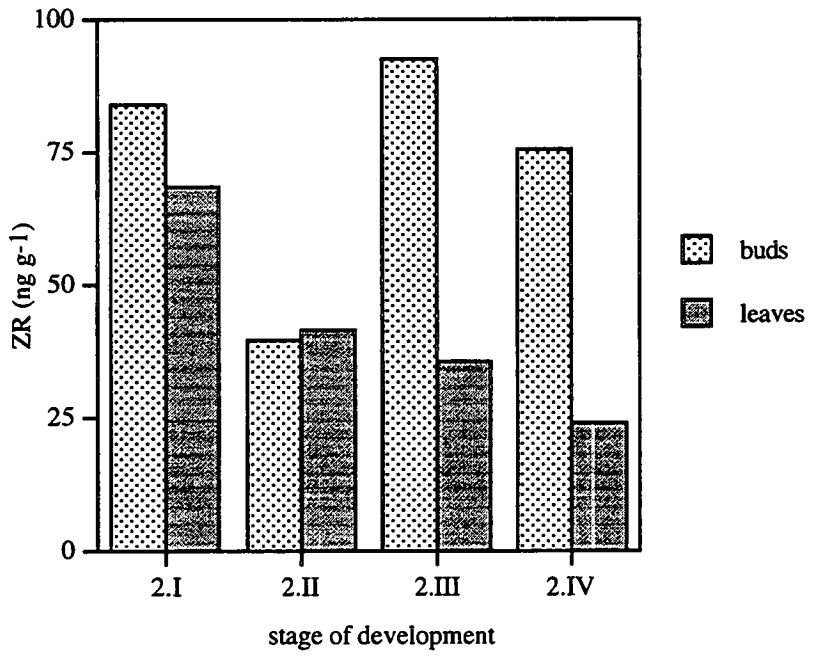

Figure 7.3 Zeatin riboside in buds and subtending leaf according to stage of development. (LSD (buds)=21 ng g^{-1}; LSD (leaves)=22 ng g^{-1})

7.4 Discussion

The discovery that rhythmic shoot growth in perennial species was endogenous led many to believe that hormones, as growth inhibitors and promoters, controlled rhythmic growth in tea. Early experiments showed that grafts between actively growing buds and scions of tea increased plant height and length of leaves and internodes (Kulasegaram, 1969b). In contrast, less growth occurred in grafts between dormant buds and scions - leading to the proposal that plant hormones, or some transportable substance, controlled dormancy in tea. The removal of leaves or cataphylls, such as in *Gnetum* (Mialoundama *et al.*, 1984) has been shown to promote bud activity and hence maintain shoot growth, leading to the hypothesis that the substance(s) responsible for bud dormancy originated in the leaves.

Currently, most reports linking bud dormancy and the promotion of shoot growth in tea to plant hormones are based on observations from applied hormone studies. For example, applied gibberellin (GA) and cytokinin (such as benzyl adenine) are reported to promote the release of bud dormancy in tea (Kulasegaram and Kathiravetpillai, 1972; Manivel *et al.*, 1981; Barman and Manivel, 1989); while applied ABA can in contrast promote bud dormancy, as reported in *Gnetum* (Mialoundama *et al.*, 1984). However, it has not been proven that there is a causal relationship between exogenous application of hormones and the observed response (Lavender and Salim, 1987), which therefore limits interpretation. These limitations can be overcome through quantifying endogenous hormone concentration; as in the present study where shoot growth and development in tea was correlated with changes in concentration of the plant hormones abscisic acid (ABA), gibberellin (GA) and the cytokinin, zeatin riboside (ZR).

Abscisic acid is traditionally regarded as a growth inhibitor (Davies, 1995) and is implicated in the regulation of the induction, maintenance and release of bud dormancy in perennial species (Dennis, 1994). For example, the concentration of ABA is reported to increase over winter in species including *Salix* (Junttila and Jensen, 1988), *Corylus* (Rodríguez *et al.*, 1991) and *Betula* (Rinne *et al.*, 1994). In tea, Nagar (1996) proposed that a decline in free to bound ABA in shoots over winter could both induce and maintain bud dormancy. Similarly, during rhythmic growth, although ABA in leaves was relatively constant at 18 to 23 ng g^{-1}, ABA in buds increased significantly during dormancy from 24 ng g^{-1} to 34 ng g^{-1}. This is consistent with the observations of Kulasegaram and Kathiravetpillai (1979), where growth inhibitors (perhaps ABA) were associated with lateral shoot dormancy in tea. This differs from the observations of Orchard *et al.* (1980) and Abo-Hamed *et al.* (1981) in *Theobroma*,

where high concentrations of ABA in leaves in the last phase of dormancy were reported to maintain, rather than induce bud dormancy occurring during rhythmic growth. It may also be possible that the increase in ABA during rhythmic growth is limited to the cataphylls, as the meristem is entering the phase of greatest apical activity as new primordia are initiated.

The quantity of ABA detected in leaves and buds in tea was comparable to that reported in *Pseudotsuga* leaves (Graham *et al.*, 1994), and in leaves of *Glycine max* (Hein *et al.*, 1984). In contrast, the ABA concentration associated with dormancy in other species is considerably higher, varying between 500 to 3500 ng g^{-1} in *Citrus* (Plummer *et al.*, 1991), *Betula* (Rinne *et al.*, 1994), *Corylus* (Rodríguez *et al.*, 1991). In tea, Nagar (1996) reported that ABA reached a maximum of 3200 ng g^{-1} dw in dormant buds of tea during winter, declining to 350 ng g^{-1} at bud burst. This was then ten times greater than the concentration of ABA in buds and leaves in the current study.

Physiological changes associated with ABA in these examples experience relatively high changes in concentration. For example, in *Citrus* leaves (Plummer *et al.*, 1991), ABA increases nine fold from shoot emergence to shoot extension; while ABA content increases 10 fold in *Silex* buds during winter dormancy (Barros and Neill, 1986), and nine fold in tea shoots during winter dormancy (Nagar, 1996). In comparison, while the increase in ABA at dormancy during rhythmic growth was significant compared to the other stages of growth, the increase was not as pronounced as that which is reported to occur during winter dormancy in the previous examples.

The endogenous concentration of GA has not been previously reported for tea, nor whether or not GA concentration varies during shoot growth. To date, the only reports linking shoot growth to GA are observations that exogenously applied GA can reduce the length of bud dormancy in tea; which may bear little relation to endogenous changes in GA activity (Kulasegaram and Kathiravetpillai, 1972; Manivel *et al.*, 1981; Barman and Manivel, 1989). In buds, GA was present in significant concentrations only during the start of growth, during the period of rapid shoot elongation. It has similarly been reported that levels of GA during early expansion of buds in *Alnus*, *Betula* and *Populus* are relatively high (Zanewich and Rood, 1994), while shoot tips of *Brassica* have similarly high concentrations of GA (20 ng g^{-1} dw) in buds (Zanewich and Rood, 1993). Observations regarding GA in buds in the current study are consistent with the primary role of GA, in stimulating cell elongation and therefore stem extension (Davies, 1995). It is however inconsistent that GA was present in fully expanded leaves during dormancy but not during shoot elongation, when the leaves have not yet reached full expansion and are therefore immature.

Cytokinin is associated with cell division, and, like GA, tends to be present in highest concentrations in young tissues (Davies, 1995). In *Theobroma*, increased cytokinin concentration in leaves is reported to promote bud burst during rhythmic growth (Orchard *et al.*, 1981). It was proposed that increased activity of roots during dormancy promoted cytokinin synthesis (Orchard *et al.*, 1981). Consistent with these observations, the concentration of ZR in tea leaves was significantly greater during bud burst, declining from stages 2.II and 2.IV. The concentration of ZR in buds was around 75 ng g^{-1}, but significantly declined during stage 2.II, shoot extension. This could be associated with reduced activity in the bud, as growth of primordia has ceased (Chapter IV.1). Although the polyclonal antibody to ZR may have had some cross reactivity, there is a possibility that other cytokinins, such as zeatin and isopentyl adenine could also be involved in the regulation of bud burst in tea, which should be examined in further studies.

In conclusion, changes observed in concentration of GA, ABA and ZR during shoot growth could indicate a potential role of these hormones in the control of shoot development in tea; ABA in the regulation of the initial stages of dormancy, and GA in the release of buds from dormancy. The exact nature of the role of these hormones in the regulation of rhythmic growth of green tea however requires the development of more precise techniques than those which are currently available, to study hormone activity at the cellular and molecular level, and hormone receptor sites.

V. General Discussion

The yield and quality of tea is determined by the growth pattern of the shoot system, and in particular the development of young leaves. This discussion begins by considering the significance of changes in shoot morphology and physiology during rhythmic growth of green tea; and secondly, the effect of environment on rhythmic growth, harvested yield and quality is considered in light of commercial outcomes relevant to industry.

Shoot growth of the three green tea cultivars, Okuhikari, Sayamakaori and Yabukita was assessed at two sites, Grove and Westerway. The cultivar Okuhikari had the fastest rate of growth at both sites, but an early frost at Grove limited total shoot length in the 1994-95 season to around 200 mm. Variability in the average total shoot length both between varieties and sites can perhaps be attributed to differences in the microclimate, and as such, highlights the importance of site selection in plant productivity in terms of maximising yield and quality.

Shoot extension in field conditions at Grove did not reflect the cyclic pattern of shoot growth reported by Bond (1945), as the pattern of shoot extension was approximately linear with respect to time, the rate of shoot extension declining only at the onset of winter dormancy. At Westerway, a slight discontinuity in the rate of shoot extension at the start of January could perhaps be consistent with a flush. The presence of fish leaves along the new shoots at both sites however indicated that growth did occur in flushes, as these leaves delineate successive flushes (Bond, 1942); but the scattered distribution of these leaves along shoots perhaps indicated that flush growth became asynchronous as the season progressed. According to the literature, this is not unusual, as at any one time shoots on the one plant are reported to be in either the active or dormant phase of the growth cycle (Wight, 1955; Wight and Barua, 1955; Pethiyogoda, 1964). Asynchronous growth in the free-growing plant is unlikely to affect flush growth during harvesting, which in itself is reported to synchronise flush growth (Fordham and Palmer-Jones, 1975).

Growth of tea shoots in constant day length and temperature was shown to occur in repeating cycles of growth and dormancy. This is common with other species which exhibit rhythmic growth, such as *Quercus* (Hanson *et al.*, 1986), *Hevea* (Hallé and Martin, 1968) and *Theobroma* (Greathouse *et al.*, 1971) and indicated that the rhythm is regulated by endogenous mechanisms. These observations are the first to date that actually provide evidence that rhythmic shoot growth in tea is endogenous; as the only previous study of rhythmic shoot growth in tea by Bond (1942, 1945) was undertaken

in field conditions, where the cycle may have been influenced by changes in environment.

Cataphylls, which as the name suggests are of distinct morphology when compared to foliage leaves, enclose the bud during dormancy, and delineate successive cycles in growth along a shoot. Understanding the processes which culminate in cataphyll formation is central to understanding the control of rhythmic growth. The lack of marginal serrations and small size of cataphylls originally led to the proposal that cataphylls were reduced foliage leaves, that is, a result of arrested development (Goebel, 1880; cited Foster, 1931a). Subsequent evidence in *Carya* (Foster, 1931b), and *Malus* (Abbott, 1970) has however dispelled this theory, as cataphyll morphology is reported to be determined as young primordia. The production of cataphylls during rhythmic growth is instead thought to reflect changes in shoot ontogeny and physiology occurring during shoot growth and development, as examined in this study.

A common trend amongst plants exhibiting rhythmic growth is that leaf initiation is not continuous, and instead alternates with shoot development, as observed in *Fraxinus* (Nougarède *et al.*, 1996) and *Hevea* (Hallé and Martin, 1968). As a result, leaf primordia are initiated in the bud during dormancy, and then expand during shoot extension forming the new flush. During shoot extension in these species, initiation of primordia is reported to cease at the meristem, an observation which is supported by the observed decline in mitotic activity in the meristem in these two examples. However, in tea, Bond (1945) reported that leaf initiation was continuous, with some variation in rate, while shoot extension occurred in flushes. Not only is this pattern of growth inconsistent with rhythmic growth exhibited in other plant species; but is also inconsistent with the observations on leaf initiation during rhythmic growth in tea in this study. Although there was variation in rate of initiation of leaf primordia, which is consistent with the observations of Bond (1945), there was a distinct cessation in leaf initiation during shoot extension. During this period, the rate of extension of the last (three) primordia to initiate during dormancy becomes negligible; these primordia instead form the cataphylls and the first foliage leaf of the next flush. Hence, in tea there is an alternating pattern in bud activity and shoot extension, which is held in common with other plant species which exhibit a similar pattern in shoot growth.

Rhythmic growth can be described not only by changes in morphology and ontogeny, but also physiology, including assimilate partitioning and changes in plant hormone concentration. The theory that assimilate partitioning is a function of sources and sinks, and of competition between sinks, as reviewed by Wardlaw (1990), is applicable to rhythmic growth in tea. During rhythmic growth in tea shoots, there are

effectively two sinks, the bud, and the flush leaves and internodes. As activity of the bud and the shoot (leaves and internodes) alternates, it would be expected that the competitive ability of these two sinks will vary with shoot ontogeny. Current theory (Crabbé and Barnola, 1996) is that the immature leaves and internodes positioned just below the apex at bud burst replace the latter as the dominant sink, in their need for imported assimilate to promote leaf expansion and internode extension. In tea, not only does the bud attract the majority (50 %) of ^{14}C label as current photosynthate from the leaf subtending the bud; there is also strong evidence supporting an overall increase in demand for assimilate during bud burst.

In the event that current photosynthate is inadequate to meet demand, additional carbohydrate is mobilised from storage reserves, such as starch, or by a change in the partitioning of carbon between storage and translocation in leaves, or in the rate of photosynthesis (Wardlaw, 1990). In tea, during bud burst, additional carbohydrates are mobilised from starch reserves in the stem, which is completely hydrolysed. At the same time, starch declines in leaves, while export of ^{14}C label from equivalent leaves increases; together indicating increased partitioning in leaves to soluble rather than storage carbohydrates. In addition, at bud burst, leaf net photosynthesis increases from 14 to 18 μmol CO_2 m^{-2} s^{-1}. Increased net photosynthesis and partitioning to soluble carbohydrates is consistent with 'feed forward' theories on the control of assimilate partitioning in leaves. An increase in photosynthesis is proposed by Stitt (1990) to increase the concentration of fructose 1,6 phosphate, which overcomes inhibition of fructose 1,6 phosphate by fructose 2,6 phosphate, leading to, by the accumulation of substrate and increased sensitivity of sucrose phosphate synthetase, increased sucrose synthesis in the cytosol over starch synthesis in the stroma. The mobilisation of storage carbohydrate, and increase in net photosynthesis then indicates an increase in demand for assimilate during bud burst in tea during rhythmic growth.

An increase in demand for assimilate at bud burst during rhythmic growth is reported in other plant species. In *Quercus*, increased net photosynthesis during both bud burst and shoot extension was interpreted by Hanson *et al.* (1988) to indicate an increase in demand for assimilate by the developing flush. This was later also shown to involve mobilisation of starch reserves from internodes, and to some extent leaves of the previous flush (Alaoui-Sossé *et al.*, 1994). However, partitioning of assimilates in *Theobroma*, which also exhibits rhythmic growth, differs in that there is no reported increase in net photosynthesis at any stage during shoot growth (Baker and Hardwick, 1973). It then appears that there are variations in the partitioning of assimilates during rhythmic growth exhibited in different plant species, and it is perhaps dependant on the

availability of carbohydrate reserves and current photosynthate, which was shown to occur in tea.

During shoot extension, the development of photosynthetic capacity of leaves modifies the partitioning of assimilates from the previous flush, as the source, to the developing flush, which has replaced the bud as the dominant sink. Leaves are reported to commence photosynthesis quite early during their development (Dickmann, 1971), although the generated assimilate is inadequate to sustain growth (Larson *et al.*, 1972). Rates of photosynthesis in immature leaves are typically reported to be less than mature leaves, reflecting to some extent chloroplasts that are not fully active and the rate of carboxylation which has not attained full capacity, and is therefore coupled with high rates of respiration (Larcher, 1995). Consistent with this, net photosynthesis of median tea leaves during shoot expansion, at 5 μmol $CO_2 m^{-2} s^{-1}$, is around one third of mature leaves, while high dark respiration rate, at 7 μmol $CO_2 m^{-2} s^{-1}$, reflects the energy requirement of the newly expanding leaves. Barua (1960), has similarly reported low net photosynthesis in newly expanded leaves in tea; although photosynthetic measurements were on detached leaves, which may underestimate the rate of photosynthesis. As leaf photosynthesis has not attained full capacity, leaves will then require imported assimilate to maintain leaf development (Larson and Dickson, 1973), as indicated by the partitioning of ^{14}C label into recently unfolded leaves from the apex; similar, although qualitative, evidence was reported by Sanderson and Sivipalan (1966b) using autoradiography. This technique, however, cannot reveal the gradual decline in imported label which occurs as tea leaves age; as shown along the shoot, where there is a gradient in the accumulation of ^{14}C label from the first to final leaf unfolded from the apex.

There is evidence, however, to suggest that shoot extension is still associated with a high demand for assimilate in tea, due to a decline in the retention of ^{14}C label in the source leaf from 25 % during bud burst, to 5 % during shoot extension. The exported ^{14}C label instead accumulates in the internodes and leaves, although this is a function of leaf position. Crabbé and Barnola (1996) proposed that the expanding leaves and internodes as the dominant sink maintain the inactivity of the meristem during shoot extension; this theory is supported by the lack of further initiation of primordia at the meristem in tea. Increased export of ^{14}C label, coupled with the lack of further accumulation of starch in mature leaves indicates the predominance of partitioning to soluble over storage carbohydrates. However, a different situation prevails during bud burst, where photosynthesis declines from the previous maximum of 18 μmol $CO_2 m^{-2} s^{-1}$, to maintenance levels of around 14 μmol $CO_2 m^{-2} s^{-1}$, while the quantity of starch reserves in internodes increases. Therefore it would appear that maintenance

rates of photosynthesis and increased turnover of soluble carbohydrates in previous flush leaves are adequate to sustain the requirement for imported assimilate by the new flush.

Hence, as the demand for assimilate falls, partitioning to carbohydrate reserves is favoured over soluble carbohydrates. The accumulation of photosynthate and hence sucrose in the cytosol of leaves inactivates sucrose phosphate synthetase, resulting in increased fructose 2,6 phosphate, which inhibits fructose 1,6 phosphate (Stitt, 1990). As a result, more carbon in retained in the chloroplast where it is instead partitioned to starch. This is the likely mechanism resulting in the increase in starch reserves in leaves and internodes during dormancy, in both the previous flush, reflecting the decline in demand, and in the new flush, but in this case indicating tissue maturation. It is proposed by Crabbé and Barnola (1996) that the attainment of full expansion of leaves and internodes during dormancy in *Quercus* reduced the demand for assimilate, therefore favouring the bud as dominant sink, in which mitotic activity and leaf initiation recommences. The fact that leaf initiation in tea recommences during dormancy supports this theory, but additional sinks for assimilate are indicated by the partitioning of ^{14}C label to the internodes below the source leaf. Likely sinks for assimilate could include the roots (Kandiah, 1971), growth of which is reported to occur during dormancy in tea (Fordham, 1972).

The partitioning of assimilates between the bud and leaves during rhythmic growth, can further clarify the relationship between sources and sinks in commercially produced tea, which is regarded to influence yield and quality harvested shoots (Aoki, 1981; Okano *et al.*, 1995). Increased net photosynthesis after harvesting (Aoki, 1981) and a decline in carbohydrate reserves after pruning (Kandiah, 1971; Selvendran and Selvendran, 1972) are reported to occur in response to change in the sink-source balance; yet similar changes in assimilate partitioning are characteristic of tea as a result of balanced activity between the bud and shoot. The observation that carbohydrate reserves are mobilised during shoot extension in tea from not only the stem, but also leaves has not been reported previously, although it was reported by Selvendran *et al.* (1972) that leaves accumulated starch. As mature leaves (and stem) contribute carbohydrates for shoot growth, there may then be an advantage in leaving more than one leaf, as is the current practice, after harvesting.

It is proposed that plant hormones, as potential inhibitors and promoters of growth, together with changes in assimilate partitioning, regulate rhythmic growth. ABA, acting as an inhibitor, may be involved in the regulation of dormancy during rhythmic growth. In *Gnetum*, prolonged application of exogenous ABA was reported by Mialoundama *et al.* (1984) to reestablish bud dormancy during rhythmic growth,

which it was proposed inhibited the initiation of primordia at the apex. However, applied hormones do not necessarily reflect the associated change in physiological state in question; for example, dormancy induced in buds by the application of ABA is frequently transient (Trewavas and Jones, 1991). Also, little is known about the absorption, metabolism and compartmentalisation of applied hormones (Lavender and Salim, 1987). Such problems can be overcome by quantifying endogenous hormone concentration in buds or leaves; results from which have indicated a potential role of the plant hormones ABA, GA and cytokinins in the regulation of rhythmic growth. There is also the possibility that tissue sensitivity, rather than hormone concentration, may regulate growth (Trewavas, 1982).

It has been proposed that the control of dormancy during rhythmic growth resides in the cataphylls, which enclose the bud during dormancy, as growth is reestablished upon their removal, as observed in *Gnetum* (Mialoundama *et al.*, 1984), and *Quercus* (Barnola *et al.*, 1993). This could however involve either plant hormones, or changes in assimilate partitioning, where removal of cataphylls favours the apex as the dominant sink. In *Theobroma*, high concentrations of ABA in leaves in the last phase of dormancy were reported by Orchard *et al.* (1980) and Abo-Hamed *et al.* (1981) to maintain, rather than induce bud dormancy occurring during rhythmic growth. ABA has also been implicated in the regulation of the first two of three physiological stages of dormancy, which include the induction, maintenance and release from dormancy (Dennis, 1994); although evidence supporting an increase in ABA during winter is conflicting. For example, there is a general lack of support in the literature that the increase in ABA is due to change in photoperiod, but the potential effect of temperature on ABA has not really been discounted. Over winter, in tea, a decline in free to bound ABA in shoots was proposed by Nagar (1996) to both induce and maintain bud dormancy. In tea, during rhythmic growth, there is a significant increase in ABA in buds during dormancy. The increase in ABA could be potentially limited to the cataphylls, as otherwise the meristem is entering the phase of greatest activity as new primordia are initiated, the rate of which approaches a maximum during the final stages of dormancy, when ABA concentration in the bud declines to previous levels. Limitations in sample weight prevented analysis of hormone concentration in the meristem versus the cataphylls. ABA in leaves does not significantly vary in concentration during shoot growth; this observation differs from that reported in *Theobroma*, where ABA increased during the later stages of dormancy. Therefore, there is evidence supporting ABA in the regulation of the initial stages of dormancy during rhythmic growth in green tea. The possibility of tissue sensitivity can however not be discounted. The exact nature of the role of ABA in the regulation of dormancy

requires the development of more precise techniques than those currently available, to study activity at the cellular and molecular level, and hormone receptor sites.

Although ABA is thought to be in some way involved in the induction and maintenance of dormancy, there is less evidence supporting a role for ABA in the release from dormancy; which is instead thought to involve other factors which override ABA (Powell, 1987). Two possibilities include GA and cytokinins, which may promote internode extension and leaf expansion respectively (Crabbé and Barnola, 1996). Evidence supporting this proposal in relation to rhythmic growth usually cites reports that applied GA and cytokinin promote bud burst both in tea (Kulasegaram, 1969 a, 1969b; Kulasegaram and Kathiravetpillai, 1972; Manivel *et al.*, 1981) and in other species exhibiting rhythmic growth (Champagnat *et al.*, 1986; Parmentier *et al.*, 1991; cited Crabbé and Barnola, 1996). As discussed previously, bud burst promoted by applied hormones may not necessarily reflect endogenous activity. In *Theobroma*, the increased activity of roots during dormancy, as a potential site of cytokinin synthesis, is thought to stimulate the reported increase in cytokinin concentration in leaves, promoting bud burst during rhythmic growth (Orchard *et al.*, 1981). Consistent with this, the concentration of the cytokinin zeatin riboside (ZR) in leaves significantly increased to about 70 ng g^{-1} at bud burst in tea during rhythmic growth. In contrast, the concentration of ZR in buds was typically around 75 ng g^{-1}, but significantly declined during shoot extension to a minimum of around 30 ng g^{-1}, which corresponds with the cessation of leaf initiation at the apex. Furthermore, although the polyclonal antibody used to quantify the concentration of zeatin riboside may have some cross reactivity with other cytokinins, there is the possibility that cytokinins such as zeatin or perhaps isopentyl adenine could also be involved in the regulation of bud burst in tea.

High concentrations of GA have been correlated with internode extension (Davies, 1995); and some reports propose environmental cues promoting dormancy block the synthesis of GA (Junttila and Jensen, 1988), which may even be mediated by ABA. Increased GA in buds at bud burst observed in these studies is then consistent with the role of GA in regulating internode extension, which is occurring in buds along with leaf expansion; while the increased concentration of ABA during dormancy could be involved in the inhibition of GA, the concentration of which is insignificant in buds at dormancy. The significance of this is uncertain given that GA in buds was also negligible during shoot extension and late dormancy, neither of which were associated with increased ABA.

Plant hormones have also been implicated in the regulation of assimilate partitioning, which in this discussion is presented as an important factor in the regulation of shoot

growth in tea. For example, in some instances there is an increase in net photosynthesis following the application of GA and auxin, and a decline in net photosynthesis for ABA. There is the possibility that the increase in demand for assimilate, reflected in increased mobilisation of stem and leaf reserves, and increased net photosynthesis, could then involve plant hormones. There is however, no evidence, supporting that these responses to an increase in demand for assimilate were the result of a change in hormone concentration, which were of no significance in the leaves at bud burst.

Consequently, changes in ontogeny and sink activity culminate in rhythmic growth of tea. These changes affect photosynthesis, the partitioning of assimilates and plant hormone concentration during shoot growth. Future work should focus on factors that control the physiological changes occurring during rhythmic growth. This could include more specific studies on the role of plant hormones, especially in the measurement of hormone concentration, transport and compartmentation within the shoot system. This, however, requires the development of more precise analytical methods than those used here. Assimilate partitioning could be considered in terms of transport, phloem loading and unloading, and enzymic reactions involved in photosynthesis and partitioning. Microdensiometry could be used to quantitatively assess cellular division at the apex, to determine whether mitosis is suspended as in other species exhibiting rhythmic growth. The role of roots in rhythmic growth, not only as a sink for photosynthate, but also as a source of plant growth regulators, could also be studied in the context of the current findings.

Quantitative criteria defining shoot growth and development in green tea can be collated from the combined data, not only from quantitative measurements of shoot morphology, but also physiological characteristics including photosynthesis, assimilate partitioning and hormone analysis. The advantage of quantitative criteria is that they minimise sample variation otherwise associated with qualitative assessments. Previously, only qualitative criteria have been used by Bond (1942, 1945) to relate subjectively identified changes in shoot morphology during rhythmic growth of tea, to changes in shoot extension, leaf initiation and production.

Rhythmic growth in tea is controlled by endogenous factors, and is then an infradian (greater than 48 hours in duration) rhythm. Then, by definition, rhythmic growth should be maintained in different environmental conditions; although potentially the amplitude and frequency of the rhythm may be modified (Koukkari and Warde, 1985). The obvious exception is winter dormancy, which will be induced should conditions exceed the environmental limitations for growth. Typically in other species, such as *Quercus* (Barnola *et al.*, 1993), growth was reported as rhythmic when the cycle

continued under controlled conditions, but has not been shown to be maintained in different environments. That rhythmic growth in tea is maintained in different combinations of high (15 °C) and low (7 °C) night temperatures and long (16 hour) and short (9 hour) day lengths, which modify shoot extension and leaf production (amplitude) and duration (frequency) of the rhythm, is then consistent with the definition of an infradian rhythm. Shoot growth in these conditions can then be used to clarify the effect of environment on tea production.

The aim of current research in environment and tea production is to maximise yield and quality. Harvesting young shoots results in successive flushes, of up to four per season, the yield and quality of which depend on several components, such as plucking standard, skiffing and pruning. As tea production is seasonal, due to winter dormancy experienced by tea plants in temperate regions (Barua, 1969), there are also reported peaks in yield of green tea associated with favourable environmental conditions (Fordham and Palmer-Jones, 1975). The effect of each component on yield and quality is however complex, and typically addressed under field conditions which introduce other variables which can hamper the interpretation of results.

A variety of models describe the relationship between shoot extension and temperature (Tanton, 1982a; Stephens and Carr, 1990; Smith *et al.*, 1990) . Only Tanton (1982b) has reported on the effect of both day length and temperature on shoot extension, even though day length is also likely to affect shoot growth, given that winter dormancy occurs in response to change in both day length and temperature. Tanton (1982b) concluded that temperature was the main factor influencing yield, not day length, although the interpretation of the data was limited by not including all possible combinations of day length and night temperature, which prevented the assessment of interactions between the two factors on yield.

In contrast, results from this study provide evidence for a strong interaction between day length and night temperature and shoot growth. A long day length (16 hours) and a high night temperature (15 °C) interact to significantly increase yield of tea during rhythmic growth by increasing the rate of shoot extension, final shoot length, the plastochron and leaf production. Less favourable conditions then limit shoot extension and hence total shoot length. However, shoots which are harvested to produce tea do not attain their potential yield. In comparison to other C3 crop species, yield is limited not only by harvesting only young shoots, at around 30 % (Tanton, 1979), but also by relatively low rates of net photosynthesis, which at 14 μmol CO_2 m^{-2} s^{-1} are significantly lower than other C3 crop species, which vary from 20 to 40 μmol CO_2 m^{-2} s^{-1} (Larcher, 1995). The relatively low rate of net photosynthesis in tea is accompanied by high rates of photorespiration, which when eliminated using air with

2 % oxygen, increases the photosynthetic rate to 20 μmol CO_2 m^{-2} s^{-1}. Reported rates of true photosynthesis in tea have previously only included dark respiration, not photorespiration (Sakai, 1975). Yield also declines by about one-third after pruning, but this was only significant when plants were harvested using coarse plucking.

Yield can be increased by increasing the plucking standard from two to four leaves; but just as shoot extension is favoured by the combination of long days and a high night temperature, so too is yield, as a result of increased leaf and internode length. Unfortunately, it cannot be determined whether the increase in yield is a result of the interaction between long days and a low night temperature, due to the lack of sufficient growth to warrant harvesting in the short day length and low night temperature treatment, which limited the statistical analysis of results.

Differences in yield are not just attributable to the effect of environment on shoot extension, but also on leaf initiation, which has been ignored in the literature; although the effect differs between rhythmic growth compared to harvesting of shoots to produce tea. During dormancy in the rhythmic growth cycle, leaf primordia are preformed in the bud before shoot extension; this determines the number of leaves produced per shoot. An environmental treatment which increases the number of primordia initiated during dormancy will then result in increased leaf production, as observed in long days and a high night temperature. Conditions less favourable (both short and long days combined with a low night temperature) to leaf initiation will not only limit the number of leaves produced per shoot, but apparently also the rate of initiation, therefore significantly delaying bud burst. As a result, increased yield in plants in long days and high night temperatures is due to the combined effect of these factors on increased leaf initiation during dormancy, and an increased rate of shoot extension.

When young shoots are harvested to produce tea, lateral buds on the remaining stub are released from apical dominance, and their subsequent growth produces the next flush. Normally, growth of lateral buds does not occur during rhythmic growth until a new flush commences (Barua, 1970), indicating that the apical dominance of the terminal bud is only effective in a current flush. Consistent with this, Goodchild (1968) reported that there were more primordia in lateral buds as distance from the terminal bud increased. However, there are no reports as to whether harvesting or environment affects the number of primordia in lateral buds. Increasing the plucking standard from two to four leaves and a bud significantly increases time to harvest, due to the production of additional leaves. Similar trends are observed in black tea, although this is a function of the plucking round, rather than plucking standard, which is usually maintained at two leaves and a bud (Cloughley, 1983; Basu, 1989; Owuor

and Odhiambo, 1990). At harvest, although lateral buds are of greater physiological age for a plucking standard of four leaves, there is a small but insignificant increase in the number of primordia, which is around four for plucking standards from two to four leaves. Environment does not affect the number of primordia in lateral buds at harvest. That four primordia are initiated is significant in that this is the minimum number of primordia remaining at the commencement of dormancy during rhythmic growth. As a result, there is no difference in the duration of dormancy after harvesting for either plucking standard or environment.

Pruning or skiffing of tea, in comparison to harvesting, removes the outer maintenance layer of foliage, and growth of new shoots proceeds from remaining (previously) dormant buds (Fordham and Palmer-Jones, 1975). Pruning is reported to reduce yield in the following year (Magambo and Waithaka, 1985; Mwhaka, 1989). In this study it was shown that pruning prior to the spring flush or later not only reduces yield, but also delays the harvest as viable buds are removed and the remaining dormant buds must presumably initiate further primordia prior to bud burst.

The effect of day length and night temperature on shoot extension and yield may be explained by differences in photoperiod and temperature on assimilate partitioning. Long photoperiods increase the time period for fixation of carbon, which increases the availability of photosynthate for growth. Starch reserves accumulated in the chloroplast during the day can then be mobilised at night to supply soluble carbohydrate (Servaites *et al.*, 1989). High night temperatures increase the rate of enzymic reactions when compared to low night temperatures, therefore maintaining plant metabolism at night. This is reflected in tea, in which dark respiration is close to zero at 5 °C, but increases to around 4 μmol CO_2 m^{-2} s^{-1} above 25 °C. Then, in conditions of long day lengths combined with high night temperatures, presumably more carbon is fixed during the long photoperiod, and used at night during respiration, therefore maintaining shoot extension. In comparison, metabolism at night is likely to be minimal in long day lengths with low night temperatures, therefore growth is limited to the day and is overall reduced in comparison to long days with high night temperatures. In short days, plant growth would be limited by the amount of carbon fixed during the day, and unless some of this is partitioned to starch there will otherwise be a shortage of available carbohydrate to maintain growth, even if this is combined with a high night temperature.

Tea production is not only concerned with maximising yield, but also quality. This relationship is complicated as it is reported that factors which increase yield, do so at the expense of quality. Quality of green tea can be defined according to both organoleptic, physical and chemical properties, the former of which have been

criticised as subjective, but are still in use (Biswas and Biswas, 1971). Physical characteristics of importance, such as fibre, and chemical components, such as phenolics and nitrogen may be quantified using analytical methods. In green tea, the first spring harvest is reported to have a higher yield and quality than the later summer harvests (Nakagawa *et al.*, 1977), suggesting either a seasonal or harvest effect; not easily separated as such experiments are done under field conditions, which introduces several variables. Yanase (1980) proposed that the seasonal distribution of yield and quality could be attributed to temperature, reporting that higher summer temperatures reduced plucking round, and reduced quality by increasing fibre and bitter tannins, while decreasing the amount of theanine and amino acids. Yanase however ignored day length, which, as shown, has a strong interaction with night temperature on yield. Another important factor is the plucking standard, which when increased in black tea increases yield, due to extra stem and mature leaves, but at the expense of tea quality. For example, some of the important quality components, such as leaf nitrogen and phenolics, are reported to decline with leaf age, therefore contributing to the decline in quality of the product, when the plucking standard is increased. Black tea, from the perspective of tea quality, is however a totally different product from green tea.

Long days, which increase yield, tend to increase the concentration of (desirable) phenolics, where increases in photoperiod may extend the activity of the light-regulated enzyme, phenyl alanine ammonia lyase, which is involved in the synthesis of the flavan-3-ols (Saijo, 1981). The effect of plucking standard on both phenolics and fibre occurred independently of environment, where although yield increased with coarser plucking standards, quality declined. Not only does harvesting additional leaves increase the proportion of stem and mature leaves, which reportedly reduces the ease of processing and final quality of tea (Selvendran *et al.*, 1972); but phenolics declined. Consistent with this are reports that phenolics accumulate in young tissue, therefore declining with age (Forrest and Bendall, 1969).

In comparison, high nitrogen, which is desirable in tea, declined in high night temperatures. The decline in nitrogen was more pronounced in high night temperatures for coarse plucking, which reduced nitrogen by around half. The decline in nitrogen was greater in the second harvest when combined with a long day length and high night temperature or a plucking standard of four leaves. Total nitrogen also declines in the second harvest following pruning when two leaves and a bud are harvested, which may be related to amount of available nitrogen. For example, nitrogen tends to accumulate in young leaves (Salisbury and Ross, 1985); but may become a deficit when these leaves are harvested to produce tea. This, and reports that nitrogen uptake peaks during spring, and declines until autumn (Okano *et al.*, 1996),

may indicate that the amount of available nitrogen declines in the second summer harvest of tea.

Quality is then maximised under conditions of long day lengths with a low night temperature and fine plucking standards of two leaves and a bud, which maintain high amounts of phenolics and nitrogen. However, a suitable compromise between tea yield and quality could instead be to harvest three leaves and a bud. Although pruning reduced yield, there was overall no effect on quality.

Results from this study provide preliminary information concerning factors which control yield and quality of tea. Future work should focus on the physiological factors that control the effect of environment on shoot growth, yield and quality of tea. Genetic variation in yield and quality also requries further examination to maximise plant productivity. Further work is also required to define the physical, chemical and organoleptic quality of green tea produced in Tasmania. The effect of processing on final quality also needs consideration. This information can then be used to make informed decisions regarding the production and potential manipulation of growth to improve yield and quality of green tea.

In summary, shoot growth in tea is rhythmic, and is the culmination of coordinated activity between the apex and shoot, and controlled by endogenous factors. Leaf primordia initiate during dormancy. Rapidly expanding leaves and internodes at bud burst replace the meristem as the dominant sink for assimilate, in which leaf initiation has ceased. The rapid expansion of internodes is perhaps associated with an increase in endogenous GA. Increased demand for assimilate requires the import of carbohydrate from starch reserves and current photosynthate from the previous flush, in which net photosynthesis has increased. During shoot extension, the supply of assimilate from expanded leaves of the new flush reduces the demand for assimilate, as reflected in the decline in net photosynthesis of previous flush leaves. Imported assimilate is still required by recently unfolded leaves, and is supplied from the previous flush leaves. Initiation of primordia at the apex does not recommence until dormancy, when the bud and lower internodes replace the fully expanded flush as the dominant sink. At dormancy, there is an increase in the concentration of ABA in buds, which may maintain dormancy in cataphylls. The observed changes in morphology, assimilate partitioning and endogenous hormone concentration may then be collated to define quantitative criteria which describe rhythmic growth in tea.

The newly expanded leaves of the flush are harvested, forming the green tea crop. The rhythmic cycle is maintained under different environmental conditions, although the amount of growth significantly increases under conditions of long days (16 hour)

and a high night temperature (15 °C). Yield and quality of harvested tea appear to be optimised under conditions of long day lengths and low night temperatures (7 °C) at a plucking standard of three leaves and a bud.

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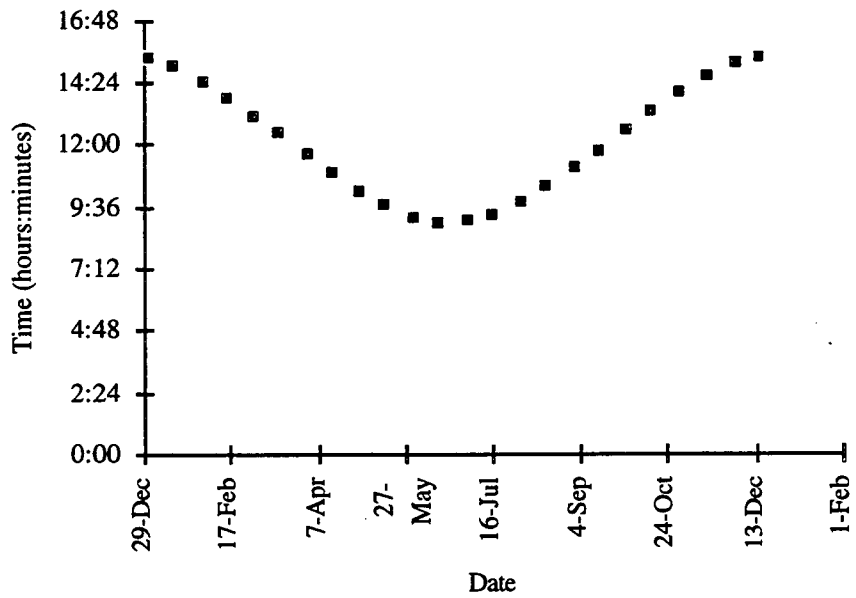
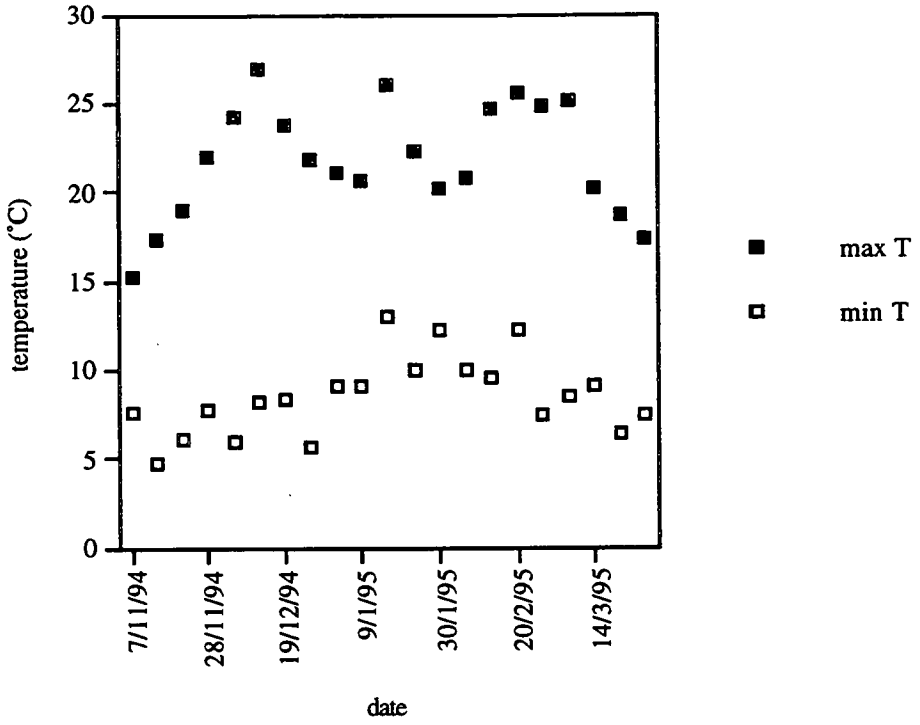
VII. Appendices

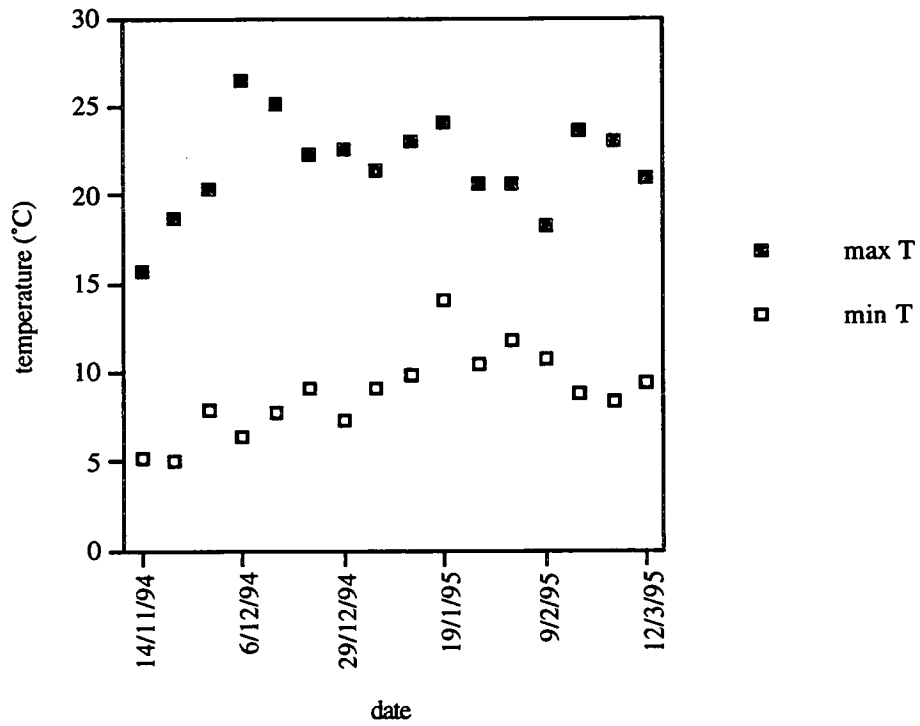
Appendix I. Environment (Chapter IV.1)



Average day length for Tasmania



Maximum and minimum air temperatures at Westerway for 1994-95 season



Maximum and minimum air temperatures at Grove for 1994-95 season

Appendix II. Logistic curve (Chapter IV.1, IV.2)

Data for independent variable 'time' versus dependant variable 'length' was fitted to the logistic curve, using the non-linear model in Systat version 5.2 for the Macintosh:

length = a/(1+ exp (-b*(time-m)))

where a, b and m are parameters of the curve. The parameter c gives the upper asymptote of the curve (the maximum attainable shoot length), m gives the time at the point of inflection (ie. the time of maximum absolute growth rate) which occurs when the shoot has length ce^{-1}, and b, often referred to as the slope parameter, gives the relative growth rate at the point of inflection and hence the maximum absolute growth rate bce^{-1}.

Iteration	Loss	Parameter	Values	
	0.1162240d+06	0.0000d+000	0.0000d+000	0.0000d+00
	0.1829440d+05	0.1616d+030	0.0000d+000	0.0000d+00
	0.1635152d+05	0.1697d+030	0.2187d-010	0.0000d+00
	0.5192584d+04	0.1742d+030	0.3837d-010	0.2058d+02
	0.4645971d+04	0.1753d+030	0.4149d-010	0.2417d+02
	0.3438240d+04	0.1576d+030	0.5227d-010	0.1661d+02
	0.2525866d+04	0.1299d+030	0.7697d-010	0.9845d+01
	0.1986670d+04	0.1326d+030	0.8027d-010	0.1349d+02
	0.1778396d+04	0.1287d+030	0.8528d-010	0.1202d+02
	0.1194465d+04	0.1242d+030	0.1012d+000	0.1061d+02
	0.2936656d+03	0.1279d+030	0.1340d+000	0.1238d+02
	0.1647294d+03	0.1221d+030	0.1466d+000	0.1144d+02
	0.1123739d+03	0.1159d+030	0.1693d+000	0.1045d+02
	0.7876273d+02	0.1180d+030	0.1677d+000	0.1081d+02
	0.6472552d+02	0.1182d+030	0.1795d+000	0.1085d+02
	0.6167371d+02	0.1175d+030	0.1796d+000	0.1075d+02
	0.6162429d+02	0.1174d+030	0.1800d+000	0.1073d+02
	0.6162360d+02	0.1174d+030	0.1801d+000	0.1073d+02
	0.6162358d+02	0.1174d+030	0.1801d+000	0.1073d+02
	0.6162358d+02	0.1174d+030	0.1801d+000	0.1073d+02

Dependent variable is length

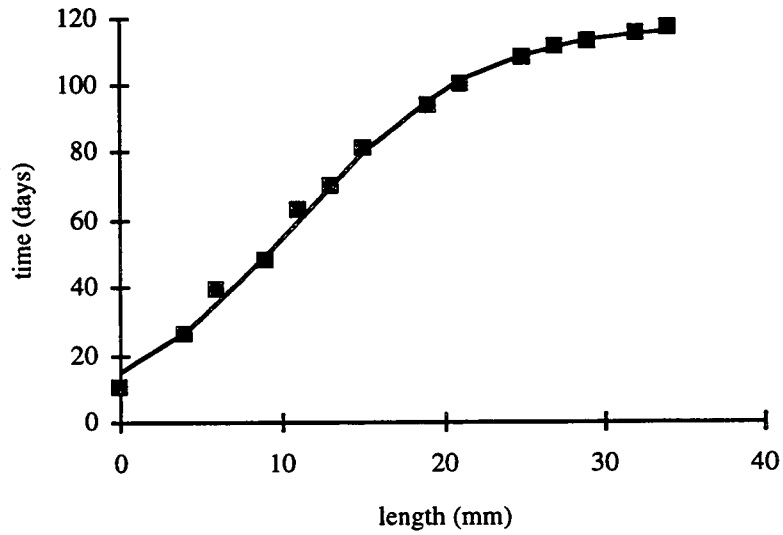
Source	Sum-of-squares	df	Mean-square
Regression	116162.377	3	38720.792
Residual	61.624	12	5.135
Total	116224.000	15	
Corrected	18294.400	14	

Raw r-squared (1-residual/total) = 0.999

Corrected r-squared (1-residual/corrected) = 0.997

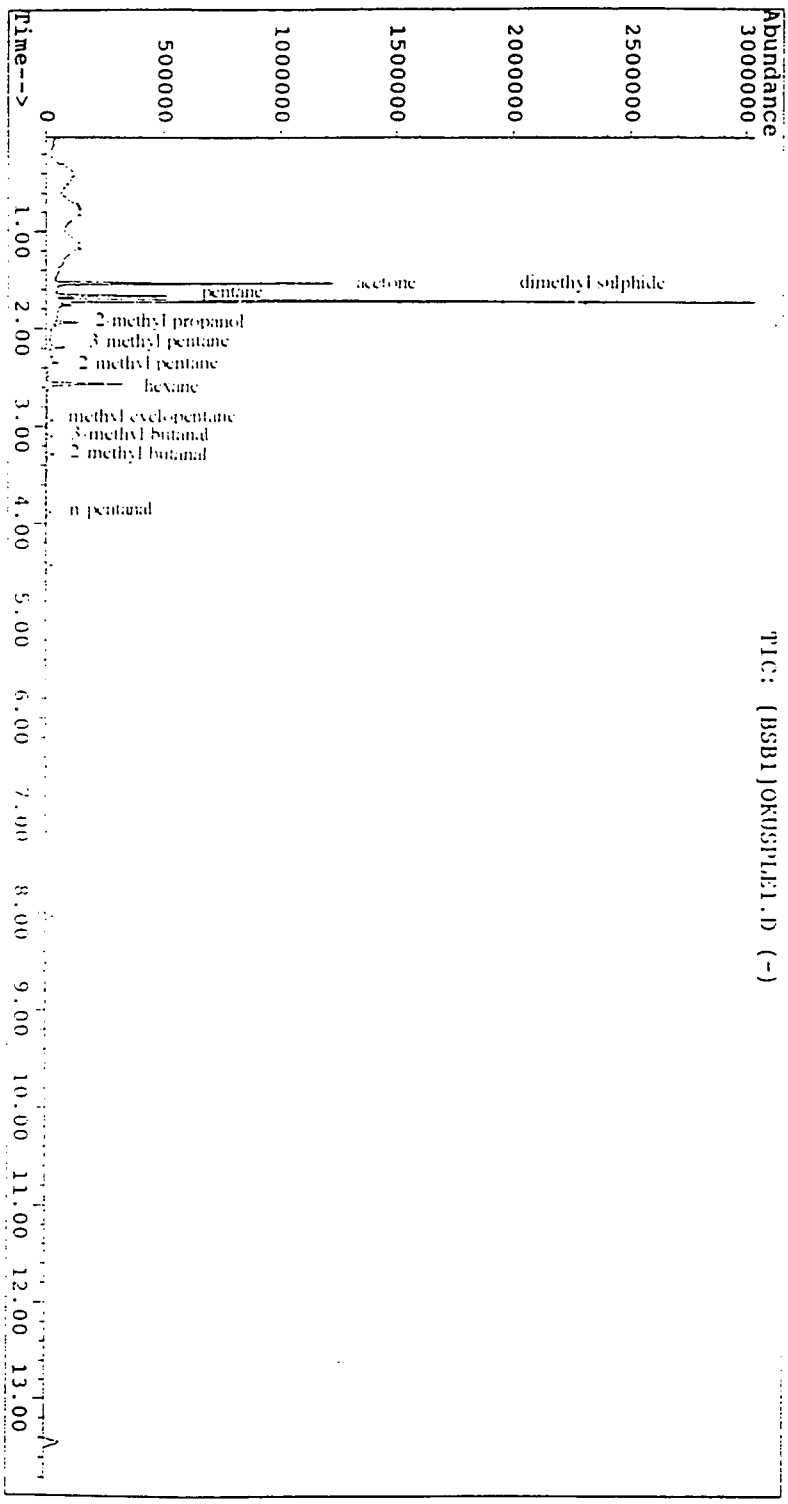
Parameter	Estimate
A	117.371
B	0.180
M	10.728

therefore, the maximum rate of growth was 5.90 mm day^{-1}

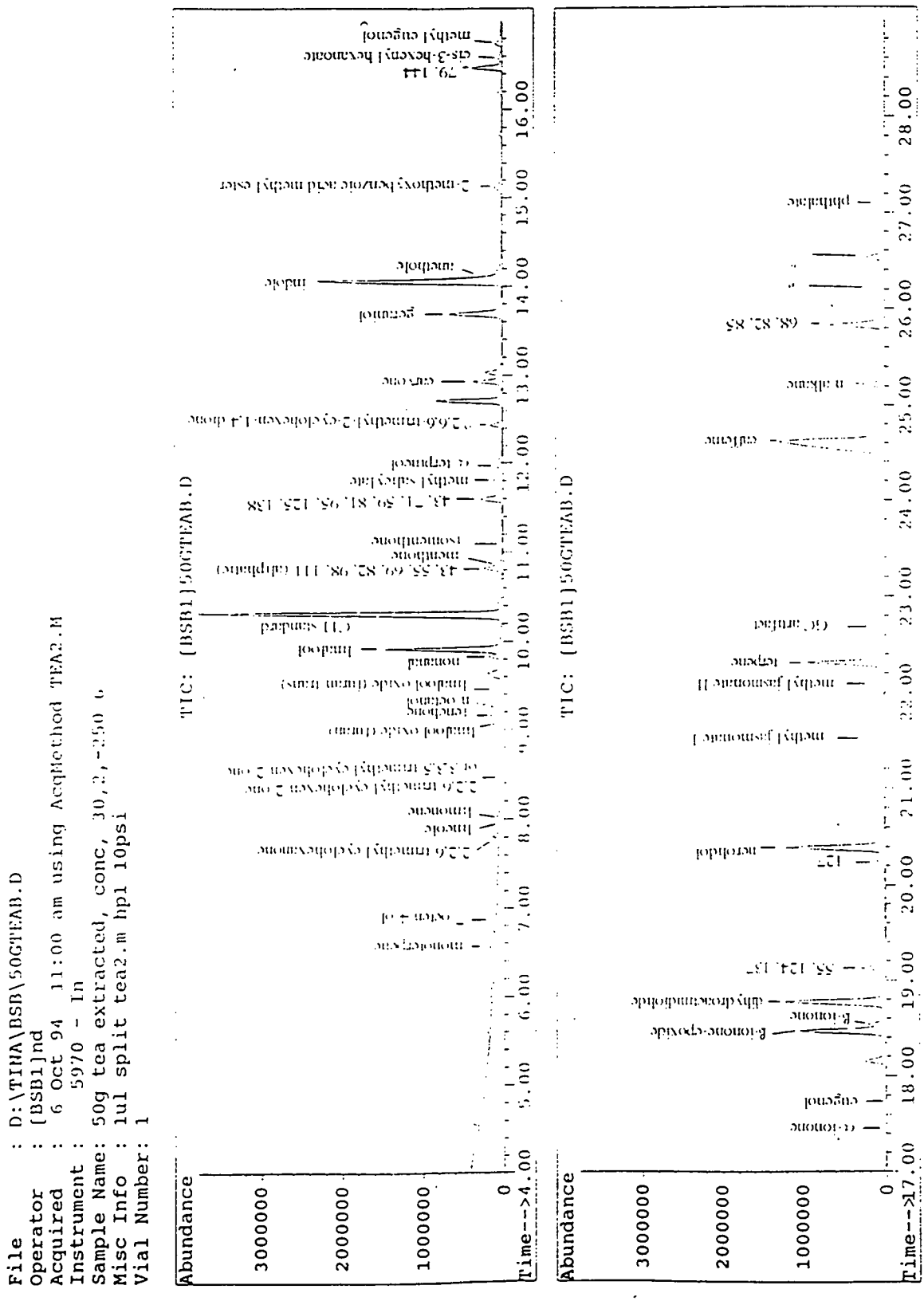


Data for length, first flush, fitted to logistic curve (from IV.1)

Appendix III. Aroma profile, headspace analysis using GC/MS (Chapter IV.3)



Appendix IV. Aroma profile of extract using GC/MS (Chapter IV.3)



Appendix V. Fitting of photosynthetic light curves to non-linear model, to estimate Pmax, Pc, Rd and Qi (Chapter IV.5)

The SAS System

12:31 Wednesday, May 22, 1996

Program:

```
data a;
input light assim;
cards;
0 -2.5
40 1.19
80 4.02
160 7.24
250 10.46
400 12.52
620 13.69
1000 14.28
;
run;
proc nlin data=a method=dud;
parms b1=15 b2=20 b3=2.5;
model assim=b1*(1-(1-b3/b1)**(1-light/b2));
output out=b p=rpred r=resid l95=lpred u95=upred;
run;
proc print data=b;
var light assim rpred resid;
run;
```

NOTE: b1=Pmax; b2=Pc; b3=Rd; and Qi calculated from the slope of the curve

Non-Linear Least Squares DUD Initialisation

Dependent Variable ASSIM

DUD	B1	B2	B3	Sum of Squares
-4	15.000000	0.000000	-2.500000	14.907840
-3	16.500000	30.000000	-2.500000	14.901848
-2	15.000000	33.000000	-2.500000	24.348634
-1	15.000000	30.000000	-2.750000	10.238282
0	15.000000	30.000000	-2.750000	10.238282
1	13.560097	15.736423	-2.193848	2.724869
2	14.382323	20.613346	-2.411235	0.220350
3	14.346324	19.941485	-2.330429	0.170875
4	14.206406	20.172125	-2.400339	0.134128
5	14.213919	19.856287	-2.382768	0.122186
6	14.199778	19.760705	2.366778	0.121050
7	14.199712	19.758677	-2.366232	0.121050
8	14.197445	19.766178	-2.367872	0.121041
9	14.197595	19.764298	-2.367612	0.121041
10	14.197587	19.764160	-2.367597	0.121041

Non-Linear Least Squares Iterative Phase

Dependent Variable ASSIM

Method: DUD

Iter	B1	B2	B3	Sum of Squares
11	14.197592	19.764229	-2.367611	0.121041

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Dependent Variable ASSIM

Source	DF	Sum of Squares	Mean Square
Regression	3	738.05	246.01
Residual	5	0.12	0.024
Uncorrected Total	8	738.17	
(Corrected Total)	7	269.99	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
B1	14.19	0.11	13.89	14.50
B2	19.76	0.83	17.60	21.92
B3	-2.36	0.13	-2.70	-2.02

Asymptotic Correlation Matrix

Corr	B1	B2	B3
B1	1	-0.01	0.20
B2	-0.01	1	-0.922
B3	0.20	-0.92	1

OBS	LIGHT	ASSIM	RPRED	RESID
1	0	-2.50	-2.3676	-0.13239
2	30	1.19	1.0899	0.10007
3	60	4.02	3.8258	0.19419
4	120	7.54	7.7036	-0.16363
5	190	10.46	10.4368	0.02317
6	300	12.52	12.6036	-0.08362
7	465	13.69	13.7578	-0.06776
8	750	14.28	14.1500	0.12999

Appendix VI. Statistical analysis for parameters of light curve at different stages of development (Chapter IV.5)

ANOVA: Pmax at different stages of development (repeated measures). LSD = 1.90

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	3	6.2693	2.098	.1254	.9444
Within subjects	36	599.7264	16.6591		
treatments	9	553.3625	61.4847	35.8056	.0001
residual	27	46.364	1.7172		
Total	39	605.9957			

Reliability Estimates for- All treatments: -6.972 Single treatment: -.0958

ANOVA: Pc at different stages of development (repeated measures). LSD = 15.78

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	3	877.8163	292.6054	.4042	.751
Within subjects	32	23167.0833	723.9714		
treatments	8	19976.3945	2497.0493	18.7825	.0001
residual	24	3190.6889	132.9454		
Total	35	24044.8996			

Reliability Estimates for- All treatments: -1.474 Single treatment: -.0709

ANOVA: Qi at different stages of development (repeated measures). LSD = 0.021

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	3	.0004	.0001	.9011	.4514
Within subjects	32	.0042	.0001		
treatments	8	.0021	.0003	2.8991	.0206
residual	24	.0021	.0001		
Total	35	.0046			

Reliability Estimates for- All treatments: -.1097 Single treatment: -.0111

ANOVA: Rd at different stages of development (repeated measures). LSD = 1.31

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	3	5.4106	1.8035	.4776	.7001
Within subjects	32	120.8447	3.7764		
treatments	8	101.2242	12.653	15.4773	.0001
residual	24	19.6205	.8175		
Total	35	126.2552			

Reliability Estimates for- All treatments: -1.094 Single treatment: -.0616

Appendix VII. Statistical analysis for partitioning of ^{14}C label to leaves 1 to 5 at different stages of development (Chapter IV.6)

ANOVA: 14C label partitioned to leaves (1 to 5) during stage II.2 (repeated measures). LSD = 4.92

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	1	1.4138	1.4138	.0187	.8946
Within subjects	8	604.642	75.5803		
treatments	4	592.0396	148.0099	46.9782	.0013
residual	4	12.6024	3.1506		
Total	9	606.0558			

Reliability Estimates for- All treatments: -52.46 Single treatment: -.02442

ANOVA: 14C label partitioned to leaves (1 to 5) during stage III.2 (repeated measures). LSD = 8.02

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	32.7355	16.3677	.4327	.6585
Within subjects	12	453.8932	37.8244		
treatments	4	339.2264	84.8066	5.9167	.0162
residual	8	114.6668	14.3334		
Total	14	486.6287			

Reliability Estimates for- All treatments: -1.311 Single treatment: -.128

ANOVA: 14C label partitioned to leaves (1 to 5) during stage IV.2 (repeated measures). LSD = 3.69

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	9.1112	4.5556	.5271	.6034
Within subjects	12	103.7133	8.6428		
treatments	4	72.9476	18.2369	4.7421	.0295
residual	8	30.7657	3.8457		
Total	14	112.8245			

Reliability Estimates for- All treatments: -.8972 Single treatment: -.1045

Appendix VIII. Statistical analysis for plant hormones in buds and leaves at different stages of development (Chapter IV.7)

ANOVA: ABA in buds at different stages of development (repeated measures).

LSD = 5.06

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	12.4517	6.2258	.1832	.8356
Within subjects	9	305.7775	33.9753		
treatments	3	267.1692	89.0564	13.84	.0042
residual	6	38.6083	6.4347		
Total	11	318.2292			

Reliability Estimates for- All treatments: -4.457 Single treatment: -.2566

ANOVA: ABA in leaves at different stages of development (repeated measures).

LSD = 9.32

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	14.7117	7.3558	.3216	.733
Within subjects	9	205.8775	22.8753		
treatments	3	75.2358	25.0786	1.1518	.402
residual	6	130.6417	21.7736		
Total	11	220.5892			

Reliability Estimates for- All treatments: -2.11 Single treatment: -.2043

ANOVA: GA in buds at different stages of development (repeated measures).

LSD = 12.2

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	2.9443	1.4722	.0122	.9879
Within subjects	9	1086.0671	120.6741		
treatments	3	1071.8924	357.2975	151.2397	.0001
residual	6	14.1747	2.3625		
Total	11	1089.0115			

Reliability Estimates for- All treatments: -80.97 Single treatment: -.3279

ANOVA: GA in leaves at different stages of development (repeated measures).

LSD = 6.2

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	8.9559	4.478	.0177	.9825
Within subjects	9	2282.3571	253.5952		
treatments	3	2270.2434	756.7478	374.8219	.0001
residual	6	12.1137	2.019		
Total	11	2291.313			

Reliability Estimates for- All treatments: -55.63 Single treatment: -.3255

ANOVA: ZR in buds at different stages of development (repeated measures).

LSD = 21.12

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	90.8073	45.4037	.0742	.929
Within subjects	9	5505.9695	611.7744		
treatments	3	4835.0275	1611.6758	14.4127	.0038
residual	6	670.942	111.8237		
Total	11	5596.7768			

Reliability Estimates for- All treatments: -12.47 Single treatment: -.3011

ANOVA: ZR in leaves at different stages of development (repeated measures).

LSD = 22.10

Source:	df:	SS:	MS:	F-test:	P value:
Between subjects	2	72.524	36.262	.0822	.9217
Within subjects	9	3969.1479	441.0164		
treatments	3	3234.5819	1078.194	8.8068	.0129
residual	6	734.566	122.4277		
Total	11	4041.6718			

Reliability Estimates for- All treatments: -11.16 Single treatment: -.2978