Carbohydrate as a Factor Controlling Leaf Development in Cocoa

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"And God said, "Let the earth put forth vegetation, plants yielding seed, and fruit trees bearing fruit in which is their seed, each according to its kind, upon the earth". And it was so."

Genesis 1.11

To my mother, brothers and sisters,

To my friends.

Contents

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Abstract

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Cocoa shows growth periodicity of the shoot apex where periods of active new leaf development (flushing) alternate with periods of dormancy (Interflush). This thesis presents the results of an investigation into the characteristics of leaf growth, and the production and translocation of photosynthate/carbohydrate between source and sink leaves aimed to investigate the possible role of plant carbohydrate status in the control of the intermittent leaf production.

Patterns of growth of individual leaves showed that the commencement of leaf unfolding within one flush is not simultaneous, the last leaf unfolds several days after the first. Later produced leaves in one flush are also progressively smaller and have lower expansion rates. Removal of the two earlier developing leaves in a flush significantly increased areas of the remaining leaves in the flush compared with control plants, showing that later leaves in the flush have higher size potential than they show in a normally developed flush.

New leaves were continuously produced during an extended period of young leaf removal, and usually a greater number of larger leaves were produced in the flush following the cessation of leaf removal. New primordia were found to be produced during the whole flush cycle in intact seedlings, but since only a proportion of them develop to form one flush of leaves, this shows that the number of leaves in one flush is not limited by availability of primordia.

The photosynthetic capacity of mature leaves did not increase during the phase of major increase in carbohydrate consumption by developing leaves but rather decreased slightly. Translocation of assimilated ^{14}carbon from mature leaves was however significantly increased during phase of rapid expansion of the new leaves. Compensatory changes in the ^{14}carbon-export from a single remaining source leaf after defoliation showed that mature leaves normally operate much below both their maximum photosynthate loading capacity and export potential.

Partial removal of developing leaves within one flush resulted in increased ^{14}C-photosynthate import into the remaining (sink) leaf showing that a developing leaf has a greater import and unloading potential than that utilized during its development in one normal flush.

Quantitative estimations of the sizes of carbon sources and sinks, and transport data showed that the carbohydrate supply from current photosynthate was likely to be insufficient to meet the demands of several simultaneously expanding leaves.

Collectively the results are considered to show that a carbohydrate stress develops at the apex during the flushing phase which limits the size and number of leaves within one flush. Carbohydrate reserves, which are depleted to complete development of the current flush, are replenished during interflush to a level which stimulates renewed leaf production. Carbohydrate availability is thus considered a major factor controlling the flush cycle in cocoa. The possible interactions with plant growth regulators as co-operative control components is also discussed.

Chapter 1

1.1 General Introduction

In some perennial plants, shoot extension growth is characterised by continuous leaf production through the year, whilst in others it is periodically terminated by the formation of resting buds, and it is from these buds that growth is later resumed (Wareing, 1970). Usually these latter plants are therefore characterised by a discontinuous mode of leaf production. In this category of plants are included: i) deciduous species, which produce one "crop" of leaves per year and ii) plants such as cocoa and many other tropical trees which produce several "crops" of leaves per year. Species from the second group are known as plants which show "leaf flushing" since they exhibit alternating periods of active growth and rest. Shoot growth in cocoa, for example, is not continuous throughout the year. Periods of active growth, with production and rapid expansion of a set of new leaves and stem elongation are separated by periods with no leaf production, known as the dormant phase. The number of new leaves produced per flush (flush intensity) depends on seedling age, growth vigour and environmental conditions. This type of growth is usually referred to as "flush growth", and the period from when the apex becomes active, producing the first leaf of one flush up to the corresponding stage for the next flush is called a "flush cycle" (Orchard, 1977). In this thesis, "flush" and "flush growth" are used to conceptualise the period of active leaf production and expansion and "flush cycle" to denote the whole series of events

from production of the first leaf up to the corresponding stage in the next flush.

Mechanisms controlling periodicity in vegetative growth of deciduous plants are known to be related to well defined seasonal variations in, for example, daylength, temperature and rainfall. But where a non-seasonal climate prevails (as in the tropics) the mechanisms controlling flushing behaviour are not understood. From a basic science viewpoint, it is important to understand how flushing is controlled because it is the mode of leaf growth of very many tropical species of economic importance. Fruit is harvested from many species which flush and it is well known that fruit production is related to leaf production and canopy size. Thus from the viewpoint of understanding the control of productivity; information on the control of leaf production and leaf formation is also very important.

The flush cycle in cocoa has been previously described and for ease of reference divided into different stages by Greathouse et al. (1971); Orchard (1977) and Vogel (1975). The nomenclature used here to characterise the stages is originated from a combination of the above mentioned terminologies. The flush cycle is thus divided into a period of leaf production and expansion and an interflush period during which the newly produced leaves mature and the apical bud remains inactive. The first stage of the flush period is denoted Flush-one (F-1) during which the bud swells and bursts and, the outer stipules of the bud spread apart revealing the outer leaves which will form the first leaves of the new flush. This stage ends when the first leaf reaches about 1% of its final area (Table 1; Figure 1.1 Plate A). The subsequent stage is Flush-two (F-2).

Table 1. Stages in the flush cycle of *Theobroma cacao*

Stage of flush cycle	Characteristic events of each stage of the flush cycle
F-1	Apical bud swells and bursts; stipules spread apart and some leaves emerge from the bud. This stage ends when the first leaf produced in the flush reaches about 1% of its final area.
Early F-2	Other leaves start to expand and the first leaf expands from 1 to 5% of its final area.
Mid F-2	The first leaf expands from 5 to 80% of its final area. During this substage the leaves are "flaccid" and hang vertically. The leaves are coloured red to pale green. This substage terminates when the last leaf reaches about 20% of its final size.
Late F-2	The first leaf completes expansion; rapid internode growth occurs and the last leaf of the flush expands from 20 to 80% of its final size. The apical bud is apparently "dormant".
I-1	The last leaf terminates expansion and all the flush leaves are greening up and they take up horizontal orientation. Apical bud remains "inactive".
I-2	All the leaves are fully expanded and they attain the dark-green colour typical of mature cocoa leaves. This stage ends when the apical bud swells and bursts to start a new flush cycle (F-1 stage).

Figure 1.1 - Plates showing different stages of the flush cycle.


A


B


C

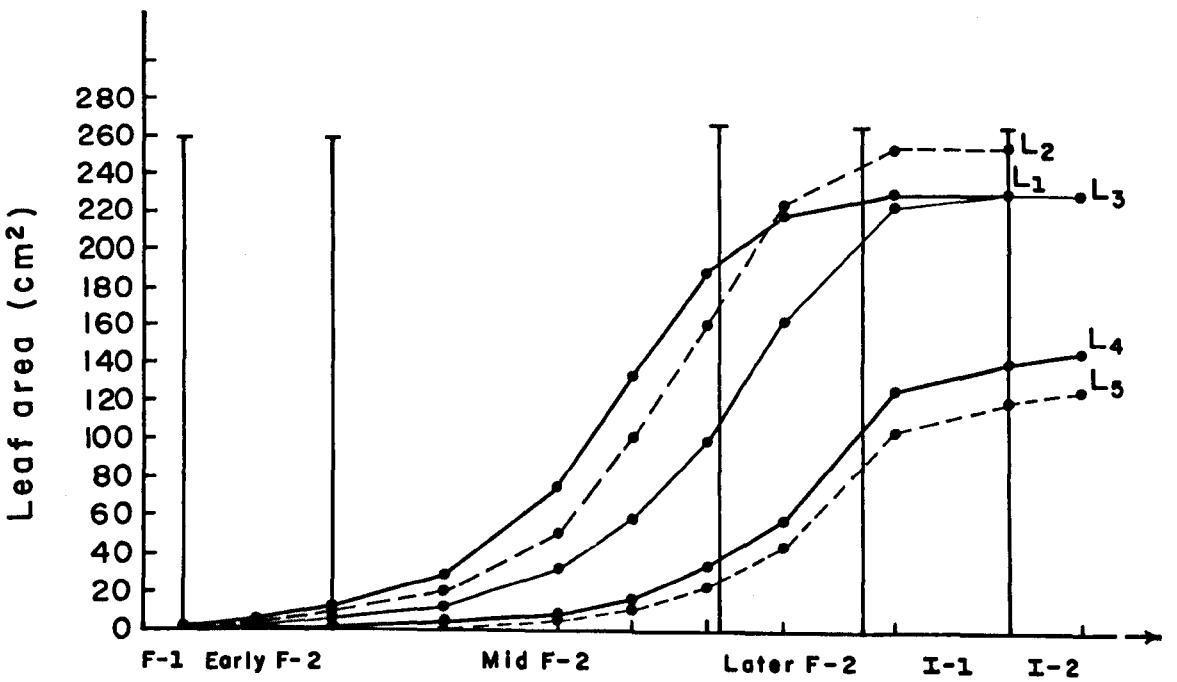

D


E

During this stage other leaves emerge from the bud and all except the last leaf expand to their full size: the last leaf reaches about 80% of its final size at the end of this phase (Figure 1.2). The leaves at this stage are "flaccid" (very thin, soft and flexible) and their colour varies from a bright red to pale green, due to varying amounts of anthocyanin. The F-2 stage is mainly characterised by rapid expansion of the leaf blades and rapid internode elongation (Plates B, C). It is divided into substages (Plates B, C) which are detailed in Table 1. During this stage, the apical bud is said to be "reversibly" dormant, because, if developing leaves of the flush are removed, there is a rapid resumption of leaf emergence from the apical bud (Orchard, 1977). During the subsequent stage, Interflush-one (I-1) the last leaf terminates expansion (Figure 1.2), rapid greening of the new leaves occurs and they also become more "rigid" and the blade takes up a horizontal orientation (Plate D). Previously the orientation is approximately horizontal. During Interflush-two (I-2), the final stage of the flush cycle, the leaves complete their development and become dark-green, the colour typical of mature leaves in cocoa (Plate E). During I-1 and I-2 the new part of the stem is still green and the apical bud remains "quiescent" or "dormant". There is no immediate resumption of the apical bud activity (new leaf expansion) if newly expanded leaves are removed during I-2 stage and the bud is thus termed "irreversibly dormant". This stage (I-2) ends when the apical bud swells and bursts again and then a new flush cycle starts. The flush cycle is summarised in Table 1.

In mature cocoa trees branching and flowering are also

Figure 1.2 - Changes in leaf area of flush leaves during a flush cycle.
(X - axis = Flush cycle - Time in days,
Scale 1 cm = 2 days).


Leaf area (cm²)
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
L2
L1
L3
L4
L5
F-1
Early F-2
Mid F-2
Later F-2
I-1
I-2

correlated temporally with the leaf flush cycle (Alvim, 1966; Greathouse and Laetsch, 1969). This indicates that the leaf growth rhythm of the shoot apex may involve other events of the whole shoot.

In mature cocoa trees the leaf growth periodicity is very clear showing in general five or six flush cycles per year (Greenwood and Posnette, 1950; Williams and Okoloko, 1972). There usually are two or three intensive flushes per year when most branches produce leaves and an equal number of other minor ones with only a few branches producing leaves (Alvim, 1977). It has been found that the time taken for a typical flush cycle to be complete is about seven to eight weeks for mature plants (Greathouse et al., 1971; Williams and Okoloko, 1972) and about four to six weeks for young plants (Orchard, 1977).

In the field, some flushes in cocoa appear to be the result of an endogenous rhythm and others the result of environmental changes. For example, water stress and low temperature or radiation tend to inhibit bud development and some flushes are clearly related to release from these conditons (Hutcheon, 1976). A great deal of information is available for cocoa plants on the periodicity of leaf production in relation to change in environmental factors, especially the effects of temperature (Charrier, 1969; Couprie, 1972; Hurd and Cunningham, 1961; Murray and Sale, 1967; Sale, 1968); solar radiation (Boyer, 1970; Snoeck, 1979) and rainfall (Alvim and Alvim, 1978; Boyer, 1970; Williams and Okoloko, 1972).

Young cocoa plants show a slightly different behaviour. They flush more frequently and the climatic elements are reported to have less effect upon their growth rhythms (Greenwood and Posnette,

1950). Results from young plants grown under "constant" conditions showed clearly a persistent growth rhythm and no clear synchronisation of leaf flushing between plants (Greathouse et al., 1971). An asynchronous growth rhythm between plants, and even between branches from the same plant, has been observed in mature plants under "constant" conditions. They are known as "polyrhythmic" plants. On these plants the foliage is distributed in distinct and dephased layers in relation to developmental cycles (Vogel, 1975). Polyrhythmy has also been observed in jorquetted plants grown in pots. In these plants the only two existing branches showed different periods of flushing. It is clear from the foregoing information that a basic internal mechanism controls the growth rhythm in cocoa, but that it may also be modified by environmental factors. Growth control is thus basically through an endogenous mechanism in cocoa and it is different from the exogenous control of the discontinous leaf production in deciduous species.

Before discussing the various hypotheses proposed to explain the endogenous control of the growth rhythm in cocoa, it is valuable to consider hypotheses for other tropical species which show flushing. Although the pattern of periodic leaf production is similar in many species showing flush growth, the control of the flush growth may differ among species. Therefore, some of the mechanisms which have been suggested for the control of flushing will be briefly discussed here. In mango for example, an inhibitor from mature leaves appears to inhibit bud growth and new leaves are initiated with removal of this inhibitor, although the further development of these new leaves is dependent upon an adequate water supply in the apex (Holdsworth, 1963; Scarrone, 1964). For tea,

Bond (1945) suggested that dormant periods were the result of water deficits in the apex. It has also been suggested that a competition for water between the apical bud and the young developing leaves is the controlling factor of the flushing in the rubber (Hallé and Martin, 1968).

To explain the endogenous control of the growth rhythm in cocoa, Vogel (1975) suggested several forms of control, including competition between leaves and apical bud; inhibition of leaf primordia and apical bud growth by other developing leaves in the flush; "membrane messages" and "biological oscillations". According to the competition theory, a stronger growing organ (active organ) prevails in the competition for water or carbohydrate over the less competitive organ (sensitive organ). This phenomenon could produce a periodic inbalance between active and sensitive organs and consequently establish the growth rhythm of the shoot apex. The theory of inhibition proposes that a young leaf primordium in the apical bud could have its growth prevented by a possible inhibitor translocated from other semi-developed leaves within the developing flush. Two less plausible theories "membrane messages" (a possible action through cell to cell contact, or Na^+ and K^+ gradients) and "biological oscillations" (resonance of cellular oscillations) are also suggested to explain the control mechanism of the rhythm of shoot growth. There is no experimental evidence yet to support either of these last two theories.

Orchard (1977) suggested that "dormancy" of the apical bud is maintained during the leaf expansion phase, as the result of competition between expanding leaves and apical bud for nutrients

and growth promotors. This author suggested that during the period of leaf expansion, nutrients and growth substances are preferentially translocated to expanding leaves, resulting in a deficit of these compounds in the apical bud, which therefore prevents any further leaf expansion by the bud. It has also been postulated by Alvim et al. (1974) that changes in the abscisic acid (ABA):cytokinin balance could control the activity of the apical bud; shoot growth being stopped by an increased ABA:cytokinin ratio. These workers suggest that in the field at times of drought a water stress will lead to an increase in ABA within mature leaves and apical bud which will stop further leaf production. The ABA content of the plant will fall if some leaves abscise (a phenomenon frequently seen in the field at times of drought) or the water stress is removed. In both of these situations, the ABA levels at the apex will fall and the consequent decrease in the inhibitor (ABA): promotor (cytokinin) level in the apex will stimulate resumption of leaf growth.

There is evidence that the intensity of flushing in cocoa is partly related to existing leaf area index, since trees with a low leaf area index tended to flush more vigorously than those with larger canopies (Hutcheon, 1976). In fact, flushing and leaf fall (which reduces leaf area index) have been considered as related phenomena (Alvim, 1977; Boyer, 1974) and intensive flushes have been observed either simultaneously with, or just after (or even just before) pronounced leaf fall (Alvim, 1977). Two hypotheses were proposed by Alvim et al. (1969) to explain the relation between flushing and leaf fall: i) as mentioned above with leaf fall the concentration of the inhibitor, suggested to be ABA (Alvim et al.,

1974), originating from mature leaves and responsible for bud dormancy, would be reduced and thus the bud would become active again; ii) senescence and abscision of mature leaves could be accelerated by a "strain" for nutrients and/or hormones imposed by the flushing. The first hypotheses could explain the flushes occurring simultaneously with or just after leaf fall and the second one could be applied to those flushes which occur before the leaf fall.

Young leaf removal, (at the early F-2 stage), has been shown to modify the growth rhythm. More new leaves were continuously produced by the terminal bud if removal of young leaves was continued (Orchard, 1977; Vogel, 1975; Vogel et al., 1982). Other results obtained with leaf removal at different flush stages suggested an inhibitory effect of other developing leaves within the new flush on the apical bud growth (Iserentant, 1976). Usually, one or two leaves fail to expand and abscise between consecutive flushes (Vogel, 1975). This worker suggested that these abscised leaves may have had their growth prevented at a very sensitive phase of their life, which is clearly supportive of the principle of the theory of competition for water or carbohydrate (or other metabolites) mentioned above. Vogel's work does not clarify whether the abscised leaves would be later leaves in the currently developing flush or the first leaves of the next flush, it only refers to leaves abscising between consecutive flushes.

In respect of the hypotheses that water status of the plant may control the apical bud activity, Borchert (1973), in his mathematical model simulated for cocoa suggested that a disturbance

of the water status of the plant during the leaf production was the possible factor mediating an interaction between shoot growth and root growth. According to this model, when leaf area was reduced (reducing the transpiration and consequently the water deficit), continuous leaf production and shoot growth occurred. According to this simulation, leaf production was halted by an internal water deficit in the plant as the result of an increased shoot:root ratio following new leaf production and restarted when the shoot:root ratio was restored by root growth and consequent increase in water uptake removed the water deficit. Orchard (1977) further suggested that with the complete development of the stomata of the expanding leaves, which only occurs at the end of the F-2 stage, and the consequent increase in transpiration coinciding with a period of decline in root growth (Sleigh, 1981; Vogel, 1975) a periodic water stress could develop in the leaves and thus be the factor responsible for the bud dormancy. The water stress throughout the plant could decrease cytokinin production by roots and increase inhibitor levels in the leaves, which could then be responsible for the bud dormancy. With accelerated root growth during the I-2 stage (Sleigh, 1981), the water stress could be reduced as the result of an increased water uptake by the new roots, and this could also increase the levels of root-produced cytokinins and gibberellins in the apex inducing the bud to burst (Orchard, 1977). Recently however, Hardwick et al. (1985) found no indication of water stress developing in cocoa leaves of seedlings at any stage of the flush cycle, and thus these authors have tended to refute Borchert's hypotheses that an internal water deficit would be the factor responsible for interrupted leaf production at the cocoa shoot apex.

Furthermore Hardwick et al. (1982) observed that leaf emergence in a new flush occurred only a few days after the increase in root growth rate and certainly before any significant change in shoot:root ratio. Actual bud burst must therefore have occurred several days before leaf emergence, i.e. almost coincident with the accelerated growth of the roots. These workers suggested that both new leaf production and increased root growth are initiated more or less simultaneously through a greater availability of carbohydrate following the maturation of the preceding flush of leaves. However, no detailed study was made at that time about the possibility that carbohydrate availability might be the factor controlling the cycle of production and development of cocoa leaves, i.e. the flush cycle.

It is clear from the earlier discussion that the former hypotheses for the mechanism of control of the flush cycle based on plant growth regulators or water stress cannot provide the full explanation of the mechanism controlling the flush cycle in cocoa seedlings and that an involvement of carbohydrate balance within the seedlings in the control is likely. It is also appropriate at this stage to emphasise that a developing flush represents a very strong and large sink for carbohydrate since the several simultaneously developing leaves will be consuming large amounts of carbohydrate to support their development particularly since they are pale green with little chlorophyll and very low photosynthetic ability until after their expansion is complete (Baker, 1974).

Although a cocoa seedling of the age used in this study has three or four complete/mature flushes of leaves which could supply photosynthate to support the developing leaves the canopy represents

a collection of leaves of several ages. Sleigh (1981) observed that younger mature leaves were more active both in assimilating ^{14}carbon-dioxide and in exporting the assimilate. It is therefore possible that the older leaves can make only a relatively limited contribution to the overall carbohydrate source available. There is evidence for other species that the photosynthetic capacity of mature leaves increase in response to an increased demand for photoassimilates (Gifford and Evans, 1981; Neales and Incoll, 1968) but from preliminary experiments (Bird and Hardwick, 1982) it was suggested that mature cocoa leaves appeared to have a different behaviour since they did not seem to show changes in net photosynthesis during the development of a new flush of leaves.

There is clearly a need for a major systematic examination of the photosynthetic and translocation capacities of leaves of cocoa seedlings which will have both fundamental scientific interest and allow examination of the hypotheses that a stress for carbohydrate may develop in the shoot apex during development of a new flush of leaves and that it may be the main factor limiting flush size and the flush cycle in cocoa seedlings.

This work is thus mainly concerned with an evaluation of the hypothesis that change in plant carbohydrate status is a major factor controlling leaf production in cocoa seedlings. The study was also designed to provide essential contributions to our basic knowledge of leaf production and its control in cocoa seedlings, which in total must provide explanations for why: i) leaf development from the apex starts at the F-1 stage, ii) leaf development stops at F-2 and iii) why the shoot apex remains "inactive" through later F-2, I-1 and I-2 stages. In order to place

the evaluation of carbohydrate control in context and to provide a more complete understanding of leaf growth, investigations were directed to provide information about:-

1 - patterns of individual leaf development within one flush including parameters such as expansion rates, cell area and cell division;

2 - photosynthetic performance of mature leaves of different ages, throughout the flush cycle;

3 - relative importance of leaves of different ages as suppliers of photosynthates to one developing. This study involved ^{14}carbon-dioxide feeding and determinations of patterns of the ^{14}carbon translocation from individual leaves of three previous flushes;

4 - detailed microscopic investigations of the shoot apex, at each stage of the flush cycle, were also planned so as to define the basis on which the apex can be classified as active or dormant during the flush cycle.

More detailed discussions on the reasons for each component of the investigation are presented in each experimental chapter.

Chapter 2

Leaf Development

2.1 Introduction

It is appropriate to emphasise that although there have been many hundreds of investigations into leaf development and its control, the overwhelming majority is have been related either to species in which leaf production is continuous for an extended period or to those producing a single "crop" of leaves each year, the temperate deciduous species. In the case of cocoa, even when it is grown under conditions where light, temperature, water and nutrients are not limiting, leaf production remains intermittent. The mechanism proposed for the control of the periodicity and "pattern" of development of individual leaves in the species referred to above, are not likely therefore to be directly applicable to cocoa.

All studies related to leaf production and development in cocoa have concentrated attention on the periodicity of flushing. It was decided that a much more detailed description of the timing of leaf production and mode of development of the individual leaves was required to complete the description of the flush cycle, and to form a much sounder base from which hypotheses concerned with the mechanism of control of flush growth could be developed and experimentally evaluated. This chapter then is concerned with reporting the collection and evaluation of growth characteristics of flushes occurring in intact and partially defoliated seedlings, designed to provide the basic information and to support the main

concern of this thesis, which is the evaluation of the possible role of carbohydrate as a factor controlling leaf production and development in cocoa.

Cursory investigation of seedlings reveals that at maturity the leaves within one flush differ in size, the later formed leaves tending to be smaller. If this can be confirmed and there are systematic differences in size between the successive leaves then this suggests a precise control of the development of each leaf within the flush, perhaps with changing levels of a promotor, or supporter of leaf development, or even increasing levels of an inhibitor acting throughout a flush. Because of the low inherent photosynthetic ability of the developing leaves, it has been suggested earlier that carboyhydrate (as a supporter of growth) may be a factor limiting the number of simultaneously developing leaves (flush size) which a seedling can support. If this is so, then as carbohydrate becomes increasingly limited towards the end of one flush, one might predict that later produced leaves would grow less. It was, thus, considered very important to determine the size distribution of leaves within individual flushes.

The characteristics of leaf development have been determined in a number of species and increasing interest has been shown in attempts to determine the growth characteristics of single leaves through studies of "growth curves" of the leaves (Amer and Williams, 1957; Dennett et al., 1978; Hackett and Rawson, 1974; Maksymowych, 1973). One of the advantages of studying leaf growth curves is that they provide both a convenient summary of the complete growth course

and an appreciation of the growth characteristics at any particular time (Causton and Venus, 1981). For example, Constable and Rawson (1980b) analysing leaf development in cotton, found that duration of leaf expansion varied with size and position of the leaf on the main stem. The smallest leaves (upper part of stem) showed a shorter duration of expansion than the larger leaves on the lower stem. Duration of leaf growth and expansion rate have been considered as the most important characteristics determining the final size of the leaf (Rawson et al., 1980). Following from detailed study of growth curves it is thus possible to interpret better how development of a leaf is controlled and it is thus appropriate to determine growth curves for individual cocoa leaves in a flush.

Interpretations of differences in leaf size of a species have also been made on the basis of cellular studies. Reference has been made to differences in leaf size being a consequence of changes in either the number or size of epidermal cells (Ashby, 1948; Ashby and Wangermann, 1950; Newton, 1963) and in the total number of cells (Milthorpe and Newton, 1963; Steer, 1971; Sunderland, 1960; Wilson, 1966). Some of these authors, e.g. Ashby and Wangermann (1950), Milthorpe and Newton (1963), have sugested that the number rather than size is the chief determinant of final leaf size, i.e. larger leaves are larger because they have more cells (of similar size) rather than a similar number of larger cells. The physiological conditions controlling cell division and expansion are considered to be different. Cell division is promoted by high levels of auxins and cytokinins (Moore, 1979) and also carbohydrate (Milthorpe and Newton, 1963), whereas cell expansion, although stimulated by auxins

and gibberellins, is frequently determined in intact plants by plant water status (Hsaio, 1973). Since these various plant growth regulators (Alvim et al., 1974; Hardwick et al., 1982; Orchard, 1977) and plant water status (Borchert, 1973; Hardwick et al., 1985) have been implicated in the control of cocoa leaf development and since evaluation of any control by carbohydrate is part of this thesis; it was important to investigate whether leaf size differences in cocoa were due to differences in cell number or size or a combination of both. Since there is generally good correlation between the pattern of cell enlargement in the epidermal layers and leaf area (Dengler, 1980), and it was not the purpose of this thesis to make an extensive cellular study of cocoa leaves, it was decided to concentrate attention on the upper epidermal cells.

In their studies of the relationships between leaf size, cell size and cell number, Milthorpe and Newton (1963) also found that the mean rate of cell division in leaves was greatly influenced by the level of incident radiation, whereas the cell size of a particular leaf was more influenced by the number and growth rates of other leaves developing at the same time. From this it was suggested that cell division in the young leaves was closely related to carbohydrate supply. There is evidence from observations on mature field-grown cocoa that vigorous flushing (higher number of leaves per flush) and highest rates of growth of leaves, occurred with increased light intensity following shade removal and it was presumed that this resulted from increased net photosynthesis (Hurd and Cunningham, 1961). Owusu et al. (1978) also observed that

increased sugar level in the plant was accompanied by a more intensive flushing and flowering, emphasising that carbohydrate availability most likely played an important role in the control of these processes.

It is known that for many species, young developing leaves are totally dependent upon supply of metabolites from other parts of the plant (Dale, 1976; Dale and Milthorpe, 1983). The relative level of import into a young developing leaf depends on its stage of development; its own photosynthetic capacity, and vascular connections with exporting leaves (Larson and Dickson, 1973; Shiroya et al., 1961; Wardlaw, 1968). It has been suggested that, in general, the strength of a leaf as an importer organ is small during the early developmental stage, rises rapidly to a peak value at approximately 10% of final size and declines as leaf expansion is completed (Swanson and Hoddinott, 1978). The absolute level of import of major metabolites such as carbohydrate into a leaf to support its development will vary with the level of photosynthesis shown by the developing leaf, e.g. if, as in the case of cocoa, rapidly expanding leaves have very low photosynthetic ability, then the dependence on supply from the rest of the plant (mature leaves) will be greater and last for longer. Indeed net import of carbohydrate into cocoa leaves was calculated not to cease until several days after attainment of full size (Baker, 1974). Thus at the cocoa shoot apex there are several simultaneously developing leaves, but with each at a different stage of development. This will represent a very major demand for supportive carbohydrate from the rest of the plant and may also mean that, individual leaves may be competing for

resources, particularly if the supply is limited. Those with higher relative growth rates will likely be dominating as importers of metabolites (Starck and Ubysz, 1974; Wardlaw, 1980). In such a situation the development of the individual leaves would be linked to development of the other flush leaves.

In order to see whether the development of different sizes of leaves in one flush was determined by competition between leaves, or was predetermined and thus size differences maintained where competition between leaves was reduced, experiments involving partial defoliation treatments were set up. The subsequent leaf development characteristics of remaining leaves was followed by determining growth curves, cell number and cell sizes. If any compensatory/enhanced growth of the remaining leaves occurred, it was considered that interpretation and an understanding of the reasons for the particular response would be more rational if cell size and number were available.

There are results with cocoa showing that, if very young leaves are excised before they attain 2.0 cm in length, new leaves emerge continuously during the excision period (Vogel, 1975; Vogel et al., 1982). Sleigh (1981) reports that the average frequency of leaf emergence was much greater during the excision period than in normal flush growth. This would also suggest the rate of leaf emergence, and the number of leaves produced in a flush, depends on interaction between the simultaneously developing leaves of one flush. It could also indicate that when carbohydrate demand is reduced, by removal of leaf sinks, that continuous leaf production is possible, thus

adding evidence to carbohydrate demand and limitation being causal in normal flushing events. When, as in these continued excision periods, normal leaf development is prevented and yet continued photosynthesis of the mature leaves is possible, it might be expected that the carbohydrate balance of such treated plants would increase. If the hypothesis of carbohydrate balance limiting normal flush development is correct, then when leaves were allowed to develop normally after an extended period of excision, one might expect the flush behaviour, i.e. number and sizes of leaves produced, would be different. Experiments were therefore also set up involving excision of different numbers of successively developing young leaves (excised at approximately 2.0 cm in length) and the development of leaves after excision ceased, was followed. Leaf number in a flush and leaf size and cell size were determined.

A slightly different approach was focused on the following problems. Firstly, the extent to which development of leaves within one flush is linked and secondly their dependence on current photoassimilate. The former was tackled by shading treatments, to reduce current photoassimilate production, and the latter by various defoliation treatments to alter the sink:source relationships. These experiments are very appropriate because Sleigh (1981) suggested that a considerable proportion of the $^{14}CO_2$ assimilated by a mature cocoa leaf was exported to expanding leaves (This aspect is also studied in detail and reported in chapter 4 of this thesis.). The proportion of recent products of photosynthesis exported is thought, from work on a range of species, to be affected by a number of factors including sink:source ratios (Fondy and

Geiger, 1980; Ho et al., 1983; Wyse and Saftner, 1982). In many other instances sink:source ratio parameters have been examined by partial leaf removal or by shading of source leaves (Hanson and West, 1982; Hussey, 1963; Maggs, 1964; Singh and Pandey, 1980). To date experiments on shading with cocoa have been restricted to observations on general growth of the plant in the field and its pod production capacity. None have been directed to an understanding of the utilisation of current photosynthate by developing leaves and so, such experiments were considered important for an understanding of the general control of leaf flushing pattern and the development of individual leaves.

In summary, the experiments in this chapter were designed to provide i) more basic information on the mode of expansion of flush leaves; ii) information on the extent to which development of leaves within one flush was linked, iii) information on the dependence of leaf development on current photoassimilate and iv) the sink:source relationships between the developing flush and the rest of the plant.

2.2 Leaf Growth

2.2.1 Materials and Methods

2.2.1.1 Plant culture.

The plants used for all experimental work were grown in a glasshouse at Liverpool University. Seedlings (Amelonado) were grown at a temperature of 20-25°C, and a relative humidity of at least 75% was maintained by a humidifier. Supplementary lighting was supplied by

white fluorescent tubes to maintain a minimum photoperiod of 12 hours. The plants were grown in John Innes No. 1 compost and fed every three weeks with a solution of N.P.K. and micronutrients. Additional plants were maintained in a "tropical glasshouse" at Ness Garden, Neston, Wirral, until required. The plants used in all experiments had three or four mature leaf flushes and were between eight to ten months old.

2.2.1.2 Leaf expansion

For studies of the expansion of individual leaves, a set of ten uniform plants, at F1 stage (see Table 1) were selected. Measurements of length and width of the lamina of each expanding leaf were made every two or three days from early F-1 to I-2 stages (Table 1). Leaf area was calculated by the following equation (Almeida and Machado, 1984).

Log_{10} (leaf area) = -0.138293 + (1.015078 x $\log_{10}$ leaf blade length + 0.964304 x$\log_{10}$ leaf blade width)

Units used are cm and cm^2

Data to originate this equation were obtained from sampling 400 leaves of several sizes from mature plants of different cultivars grown under field conditions and from seedlings grown in a glass house. This thus allows the equation to be used for wide ranging conditions.

To produce this equation, length and width of the blade of each

leaf were recorded before measuring the blade area with an Automatic Area Meter, model AAM-S. With values for length, width and leaf areas (obtained with the Area Meter) the equation which best describes the relationship between leaf area and leaf length and width was determined with an IBM 370 Computer. The equation yields a correlation coefficient between leaf area calculated with the equation and that obtained from the Area Meter of 0.99 and a variation coefficient of 1.01%. This equation allows estimation of areas for a larger range of wide and narrow leaves with an error below 10% for mature leaves and 15% of very young leaves.

The description of leaf development in this work is based mainly on leaf area changes with time; average rate of leaf lamina expansion; maximum rate of lamina expansion (expansion peak); final leaf area (Amax) and duration of leaf growth and leaf expansion. Curves for each leaf were obtained by plotting individual leaf areas against time and calculating leaf expansion characteristics between two limit points on the growth curves in the way described previously for other species (Constable and Rawson, 1980b; Dennett et al., 1978; Elston et al., 1976; Rawson et al., 1980). The fixed areas to represent these limit points on the curve, representing the lower and upper limits respectively, are arbitarily chosen for each study, but were chosen here to be 0.05 and 0.95 Amax to correspond with values commonly chosen by other workers. The time when 0.05 and 0.95 Amax reached by a leaf was found to the nearest day. The maximum rate of blade expansion was obtained, graphically, from the plot of leaf area against time and duration of expansion was considered as the number of days between 0.05 and 0.95 Amax on the

growth curves. The duration of leaf growth was also considered as the period of time (days) between the time of leaf unfolding (as defined in Table 2.1) and maximum leaf area. All the parameters used for description of leaf development are shown in Table 2.1.

Table 2.1 Parameters for the description of leaf development in cocoa

Parameters	Description	
	leaf size	time
Unfolding	2.0 cm^2 in area	T_1
Amax	Final leaf area	T_f
Start of rapid leaf blade expansion period	0.05 Amax	T0.05
End of rapid leaf blade expansion period	0.95 Amax	T0.95
Mean absolute rate of leaf blade growth	$Amax/(T_f-T_1)$	-
Mean rate of rapid blade expansion	0.90 Amax/(T0.95-T0.05)	-
Duration of leaf blade growth	-	(T_f-T_1)
Duration of rapid leaf blade expansion	-	(T0.95-T0.05)
Maximum/peak of expansion	Determined graphically from each leaf growth curve.	

2.2.1.3 Total upper epidermal cell number per leaf

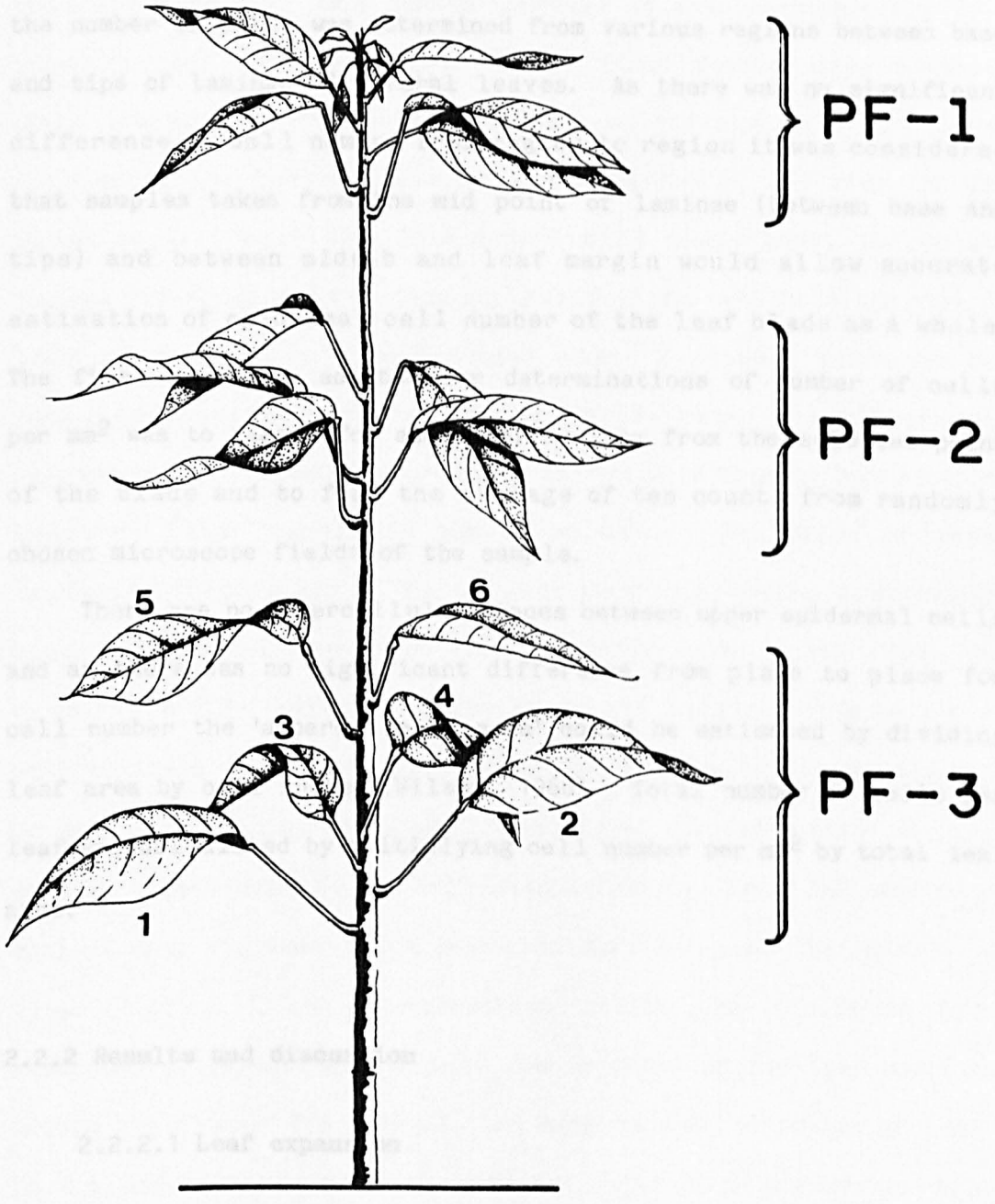
Cell number was determined for mature and expanding leaves. The cell number of mature leaves was determined in leaves from

different positions within each flush and from four flushes in the seedlings. Numbered positions of the leaves within one flush and of the flushes in the seedling were defined as shown in Figure 2.1.

The number of cells was counted from impressions of the upper epidermis. For mature leaves impressions were made with 'nail-varnish', but for expanding leaves 'nail-varnish' could not be used because it caused some leaf damage to young leaves and could therefore not be applied several times to the same position to determine the number of cells during the lamina expansion. An alternative impression compound "Silflo" was then used. This did not damage the leaf surface and could therefore be used several times at the same region of the leaf laminae. The impression compound was obtained by making a mixture of "Silflo" (a silicone rubber impression material) with "Silflex" catalyst in a proportion of 0.2 cm^3 of catalyst to 2.0 cm^3 of Silflo. The mixture was made quickly, but carefully, avoiding air bubble formation. From the impression made with "Silflo" a second impression was obtained with 'nail-varnish' which was used for cell determinations. Impressions from mature and expanding leaves were placed on microscope slides and their cells were microscopically counted. Cell number per unit area was obtained by counting the number of whole cells in a field of a known area and adding a correction for the number of cells partly included in the field. The correction was made by counting two half cells as one whole cells

Before embarking on estimation of total leaf blade upper epidermal cell number from determinations on a small selected part

Figure 2.1 - Diagrammatic representation of a typical cocoa seedlings with three previous flushes. Numbers for leaves in PF-3 indicate numbering system for leaves within any one flush. Leaf one is always the oldest/first formed within one flush.


PF-1
PF-2
PF-3
1
2
3
4
5
6

of the leaf blade and then multiplying by a factor for the area sampled to total area, sampling efficiency was checked. For this the number of cells was determined from various regions between base and tips of laminae of several leaves. As there was no significant difference in cell number from region to region it was considered that samples taken from the mid point of laminae (between base and tips) and between midrib and leaf margin would allow accurate estimation of epidermal cell number of the leaf blade as a whole. The final procedure adopted for determinations of number of cells per mm^2 was to sample for each leaf an area from the selected point of the blade and to find the average of ten counts from randomly chosen microscope fields of the sample.

There are no intercellular spaces between upper epidermal cells and as there was no significant difference from place to place for cell number the 'apparent cell area' could be estimated by dividing leaf area by cell number (Wilson, 1966). Total number of cells per leaf was calculated by multiplying cell number per mm^2 by total leaf area.

2.2.2 Results and discussion

2.2.2.1 Leaf expansion

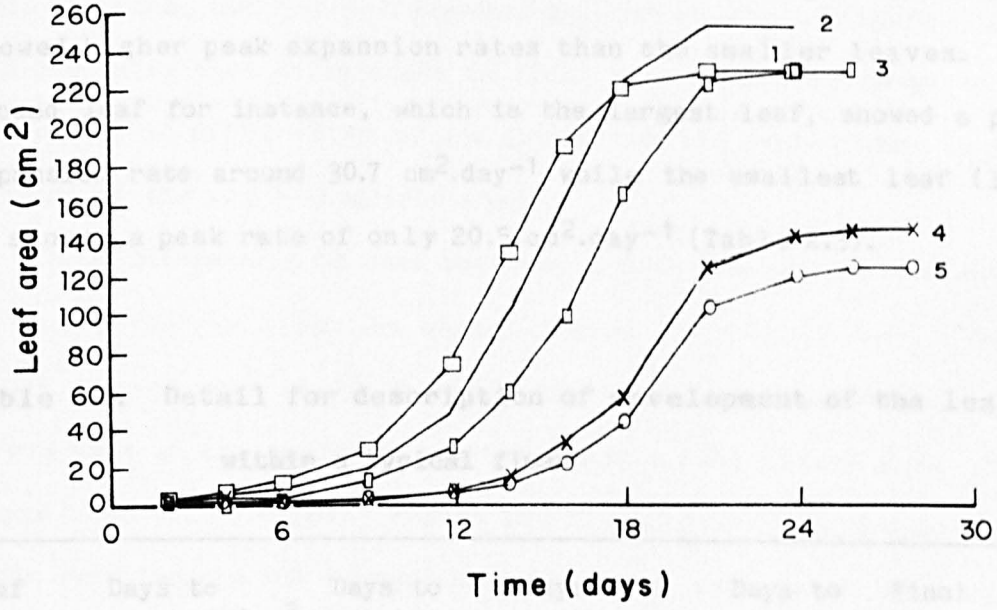
Growth curves for the five leaves within one typical flush are shown in Figure 2.2. The curves show the usual pattern of leaf growth curves with a lag phase followed by a phase of rapid growth and finally a phase in which the growth approaches zero. It is

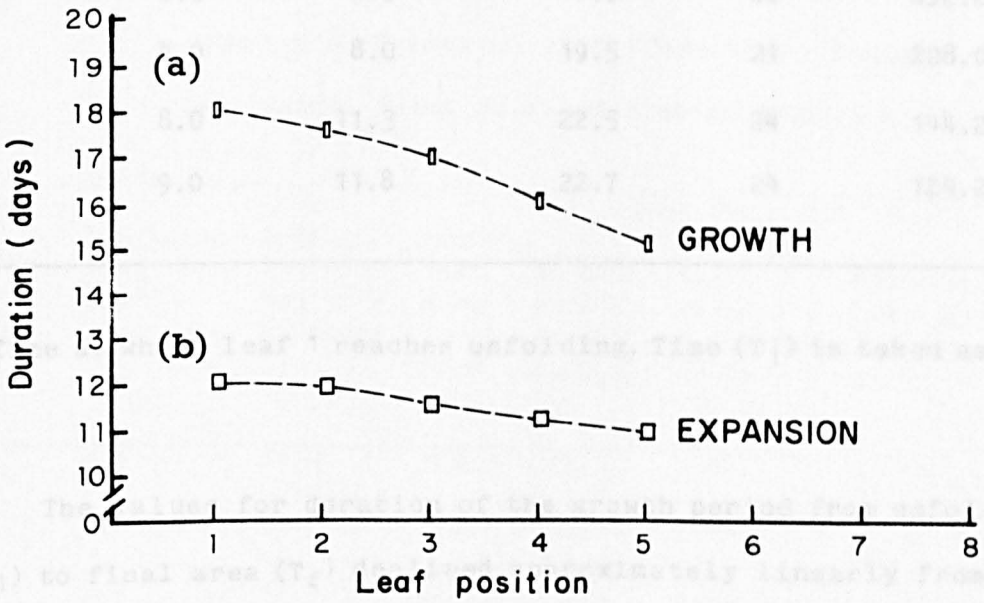
noted from Figure 2.2 that during the early phase of growth the leaves show a similar pattern of development. This early phase is followed by one of very marked increase in area corresponding to a phase of very rapid expansion. In the final further developmental phase the area of the leaves continues to increase at a much lower rate until the leaves become fully expanded at I-1 stage of the flush cycle (Table 1). In a superficial comparison, the growth curves for cocoa leaves show a sigmoid shape which is typical of leaf growth curves in many other plant species. Although the individual curves show similar patterns, there are differences between them. The earlier produced leaves show steeper curve slopes than the later leaves in the flush during the phase of rapid expansion (Figure 2.2). The earlier produced leaves also always have larger final areas than those produced later in the flush (see also Table 2.3). These changes in growth curves of cocoa leaves with position in a flush give a general picture of the development of each leaf within the flush. Analysis of the rapid phase of blade expansion (between 0.05 and 0.95 Amax.Table 2.2) provided convenient estimates of the mean rapid expansion rate for each leaf within one flush (Table 2.3), and an interesting finding from this is the clear reduction of expansion rate with the sequence of the leaf positions within the flush. For example, the mean rate of expansion of leaf 5 is 8.6 $cm^2.day^{-1}$ lower than that for leaf 2, which represents a decrease of around 45.5% of the mean rate of leaf expansion of leaf 5 compared with leaf 2.

Similarly the earlier produced leaves within the flush showed higher values for maximum peak expansion rates in relation to leaves

Figure 2.2 - Changes with time in area of the leaves within one flush. 1 = first produced leaf in the flush, other numbers in sequence to 5 - last produced leaf.

Figure 2.3 - Variation, with leaf position, of the duration of the growth period and phase of rapid expansion of the leaves within one flush.


Leaf area (cm 2)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
6
12
18
24
30
Time (days)
1
2
3
4
5


Duration (days)
20
19
18
17
16
15
14
13
12
11
10
0
(a)
(b)
GROWTH
EXPANSION
1
2
3
4
5
6
7
8
Leaf position

produced later in the flush (Table 2.3), i.e. the larger leaves also showed higher peak expansion rates than the smaller leaves. The second leaf for instance, which is the largest leaf, showed a peak expansion rate around 30.7 $cm^2.day^{-1}$ while the smallest leaf (leaf 5) showed a peak rate of only 20.5 $cm^2.day^{-1}$ (Table 2.3).

Table 2.2. Detail for description of development of the leaves within a typical flush

Leaf positon within one flush	Days to reach 2.0cm^2 (unfolding) T_1	Days to reach 0.05Amax $T_{0.05}$	Days to reach 0.95Amax $T_{0.95}$	Days to reach Amax T_f	Final leaf area cm^2 Amax
L_1	1.0*	5.0	17.0	19	228.0
L_2	2.5	6.5	18.5	20	252.2
L_3	4.0	8.0	19.5	21	228.0
L_4	8.0	11.3	22.5	24	144.2
L_5	9.0	11.8	22.7	24	124.2

* Time at which leaf 1 reaches unfolding. Time (T_1) is taken as day 1.

The values for duration of the growth period from unfolding (T_1) to final area (T_f) declined approximately linearly from the first to the last leaf position (Figure 2.3a). For the duration of rapid expansion (between T0.05 and T0.95) the values follow a

similar trend, but the differences are not so great (Figure 2.3b). This indicates that differences in final area of leaves seems to be the result of differences of the growth period rather than in the duration of the rapid expansion phase.

It is interesting to note that the latest leaves unfolded about 6-8 days after the first and second leaves (T_1 - Table 2.2). These latest leaves were still in early growth phase when the earliest leaves were in the rapid phase of expansion (see Figure 2.2). The second leaf (the largest leaf in the flush) also showed a greater growth rate (around 43% higher) than the fifth leaf, over the period between unfolding (T_1) and the onset of rapid expansion - T0.05 (Table 2.3).

Table 2.3 **Leaf area at different developmental stages defined by details from Table 2.2 and rates of growth and expansion of leaves at different positions within one typical flush detailed also in Table 2.2.**

Leaf position in flush	Unfolding area (cm^2)	Area at T0.05 (cm^2) 0.05Amax	Final leaf area (cm^2) Amax	Mean rates ($cm^2.day^{-1}$)		
				Absolute growth (Amax/ T_f-T_1)	Rapid expansion 0.90 Amax T0.95-T0.05	Maximum/Peak Determined from graph curve
L_1	2.0	11.4	228.0	12.6	17.0	29.2
L_2	2.0	12.6	252.20	14.4	18.9	30.7
L_3	2.0	11.4	228.0	13.4	17.8	31.0
L_4	2.0	7.2	144.2	9.0	11.6	22.0
L_5	2.0	6.2	124.2	8.3	10.3	20.5

The difference in the peak expansion rate between these two leaves was about 33%, being less for the leaf 5. Leaf 1 and leaf 3, however, showed the same values for rates of early growth (between T1 and T0.05) and also the same final area. However leaf 3 showed a higher rate of maximum expansion. This indicates that growth differences between ultimately larger and smaller leaves originate at a very early phase of leaf growth in the flush. Nevertheless, the case of leaf 3 with the same rate of early growth and final area, but with slightly greater rate of expansion than leaf 1, shows that the final leaf area, in cocoa, may also be the result of an increased rate of expansion, since leaf 3 had a slightly shorter duration of rapid expansion period than leaf 1. Similarly, it has been observed in other species that the final leaf size varies with leaf position and that this may be related to an increased rate of expansion over much the same or even a shorter expansion phase (Dale and Milthorpe, 1983; Steer, 1971).

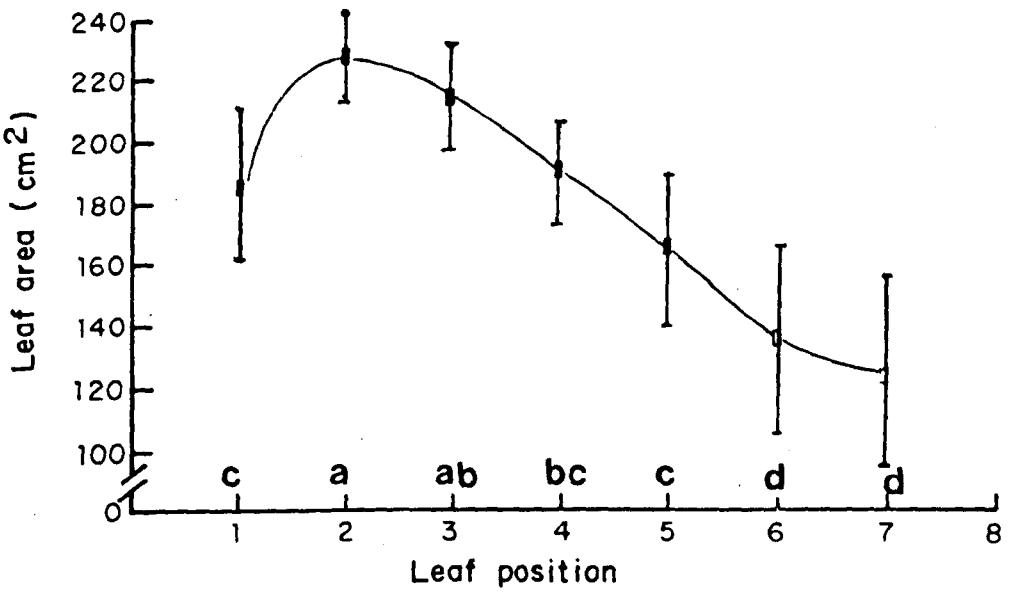
In Figure 2.4 are shown average values of final leaf areas for corresponding leaf positions from three previous (i.e. mature) flushes of 10 plants. The data from this figure, clearly confirm that the largest final leaf areas are produced at the second position and then decline, progressively, until the last positions. This pattern may, however, occasionally be different, if the first leaf, and sometimes also the second leaf, initiated in a flush, spontaneously abscise after they have unfolded or if their development is disturbed. In these cases leaf 3 then shows a larger

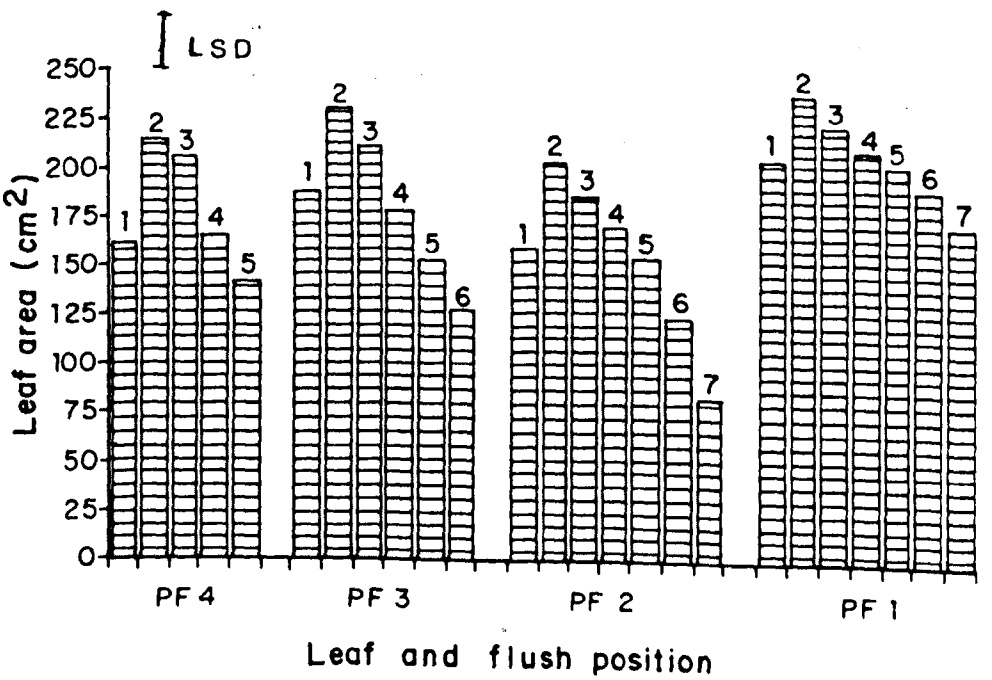
area in relation to other leaves in the flush. The second leaf was statistically significantly larger than the rest of the leaves within the flush ($p = 0.05$), with the exception of leaf 3, which although smaller than leaf 2, was not significantly different in final size (Fig. 2.4). The last leaf was always the smallest leaf in the flush. The negative gradient relating leaf area with position, was approximately linear from the second to sixth position, but with a less marked decline between positions six and seven.

Average values for individual leaf areas of leaves at different levels of insertion within a flush and for each flush of five plants with four mature flushes are shown separately in Figure 2.5. Changes in leaf areas with level of insertion of the leaves show the same pattern for all the four flushes. As observed in the previous paragraph (Figure 2.4), the largest leaves are again at the second position and the smallest leaf at the last position in all the flushes. These results (Figures 2.4 and 2.5) confirm that the differences in final leaf size, both within a particular flush and among different flushes on one plant are not random. Therefore an "internal factor" must be responsible for the variation in leaf size within the flush. It is also noted that the leaves in the later produced flushes (Figure 2.5 PF1 youngest latest produced to PF4 oldest flush) have larger areas than those from earlier flushes of the plants. Usually, larger flushes, both in respect to total number of leaves and average areas of leaves within a flush are produced by seedlings as they become older. This is probably associated with the normal increasing development of the plant as a whole. Even in the successively larger flushes there is still a

Figure 2.4 - Variation of the final area with position of the leaves within one flush. Value for each leaf shows the mean and standard deviation for ten replicates.

Figure 2.5 - Final area of the leaves within each flush of five seedlings. 1 = first formed leaf in each flush.
LSD = Least significant difference (Tukey = 0.05) between leaf positions from the four flushes.


Leaf area (cm 2)
240
220
200
180
160
140
120
100
0
c
a
ab
bc
c
d
d
1
2
3
4
5
6
7
8
Leaf position


LSD
Leaf area (cm 2)
250
225
200
175
150
125
100
75
50
25
0
PF 4
PF 3
PF 2
PF 1
Leaf and flush position

regular decrease of leaf area with position and the area of the last leaf is smallest.

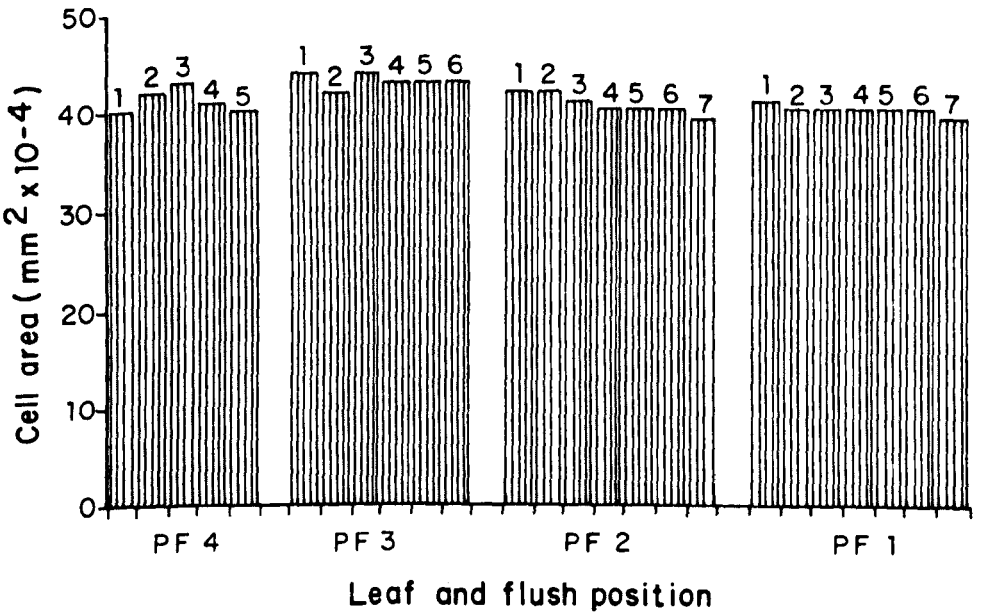
2.2.2.2. Cell area and cell number per leaf

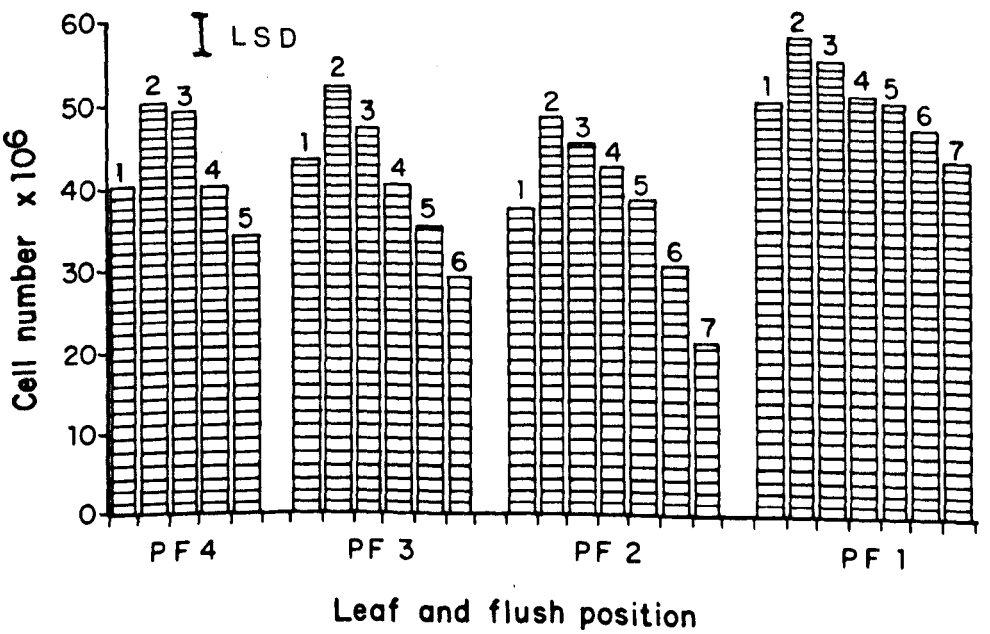
Average areas of cells in the upper epidermal layer of mature leaves from each position within each flush and for all the flushes on the seedlings are shown in Figure 2.6. The values for cell area were obtained from the leaves used for final leaf area comparisons in Figure 2.5. It is clear that cell areas did not show significant differences (unlike leaf area) within a flush. There was no significant correlation between leaf area and cell area, although there was a very slight decrease in cell area both with level of insertion of leaves within one flush and between flushes of one plant: the first formed leaves and earlier flushes (with the exception of PF4) having slightly larger cell areas. This shows, emphatically that differences in leaf area are not determined by different cell areas in the leaves and therefore that the factor(s) responsible for decrease in leaf area does (do) not affect cell enlargement.

Figure 2.7 shows average numbers of epidermal cells for the same leaves in which leaf area and cell area were determined above. The relationship between leaf position and number of cells per leaf shows a very similar pattern to that for leaf area, i.e. the greater cell number was observed in large leaves in the early positions in the flush. It is also evident from Figure 2.7 that there is a gradient of decreasing cell number per leaf from leaf 2 to the last

Figure 2.6 - Areas of cells of the upper epidermal layer of mature leaves of each of the four flushes shown in Figure 2.5. Each value represents the mean of five seedlings.

Figure 2.7 - Number of cells in the upper epidermal layer of mature leaves of each of the four flushes shown in Figures 2.4 and 2.5. Each value represents the mean of five seedlings.
LSD = Least significant difference (Tukey = 0.05) between leaf positions from the four flushes.


Cell area (mm 2 x 10-4)
50
40
30
20
10
0
1 2 3 4 5
1 2 3 4 5 6
1 2 3 4 5 6 7
1 2 3 4 5 6 7
PF 4
PF 3
PF 2
PF 1
Leaf and flush position


Cell number x 10⁶
60
50
40
30
20
10
0
LSD
1 2 3 4 5
1 2 3 4 5 6
1 2 3 4 5 6 7
1 2 3 4 5 6 7
PF 4
PF 3
PF 2
PF 1
Leaf and flush position

leaf in each flush. As for leaf area, cell number of the second leaf was significantly different (at level of 0.05 by Tukey's test) from that of all other leaves except to the third leaf. The number of cells per leaf was also positively and significantly correlated with final leaf area (n=25) showing a coefficient of correlation around 0.966. The petiole length of mature leaves (data not shown here) was also positively correlated with leaf area and cell number per leaf, showing respective coefficients of correlation of 0.897 and 0.788. Since the latest leaves in the flush showed smaller areas and leaf areas and petiole size are positively correlated this means that the later leaves also have shorter petioles. The petiole grows during leaf lamina expansion, increasing in length and in circumference (Dale, 1982). It therefore seems that factor(s) controlling lamina expansion will also control petiole growth.

Since cell number was so positively correlated with leaf size and there was no significant difference between cell area of any leaves within a flush, then clearly the final leaf area is effectively determined by cell number. The differences between final areas of large and small leaves thus reside in differences in number of cells which, in turn, must reflect differences in the cell division characteristics between leaves. These differences in cell division between large and small leaves may result from differences either in the rate of cell division or the duration of the cell division phase or even from a combination of differences in both features. Reductions in cell division rates of successively produced leaves were found in cucumber by Milthorpe and Newton (1963) who suggested that considerable differences in carbohydrate

supply available to the leaf primordia could occur from node to node, which could contribute to reduction in rate of cell division in the primordia. It is possible that similar differences may also occur in carbohydrate supply to individual leaves at the cocoa apex, during development of one flush of leaves, as a result of different sink strengths of the leaves at different developmental stages. As mentioned in the introduction to this chapter, organs with higher growth rates are dominant acceptors of metabolites, even under conditions of limiting supply (Starck and Ubysz, 1974; Wardlaw, 1980). It is possible that in cocoa, the earlier developing leaves of one flush may monopolise most of the available carbohydrate for their development and thus limit the supply for later leaves, which in turn, could reduce the rate or duration of the cell division phase in later leaves.

Figure 2.8 refers to cell number in the upper epidermal layer of expanding leaves. When the first determinations were made at day 0, leaf 1 was at 3.9% of its final area and had around 26.4% of its final number of cells. Leaves 2 and 3 were, respectively, at 2.5 and 1.6% of final areas and had around 16 and 10% of their final cell number. Leaf 1 reached maximum cell number at about 20.7% of its final area, while leaf 2 and leaf 3 reached their maximum number of cells at between 25 to 30% of their respective final areas (compare figures 2.8 and 2.10). This shows that about 75-80% of cells in the upper epidermal layer are formed at a very early stage of lamina expansion, in fact, prior to the leaves reaching 30% of their final areas. This also indicates that cell division in the

upper epidermal layer of cocoa leaves ceases before the leaf lamina reaches 35% of its final area.

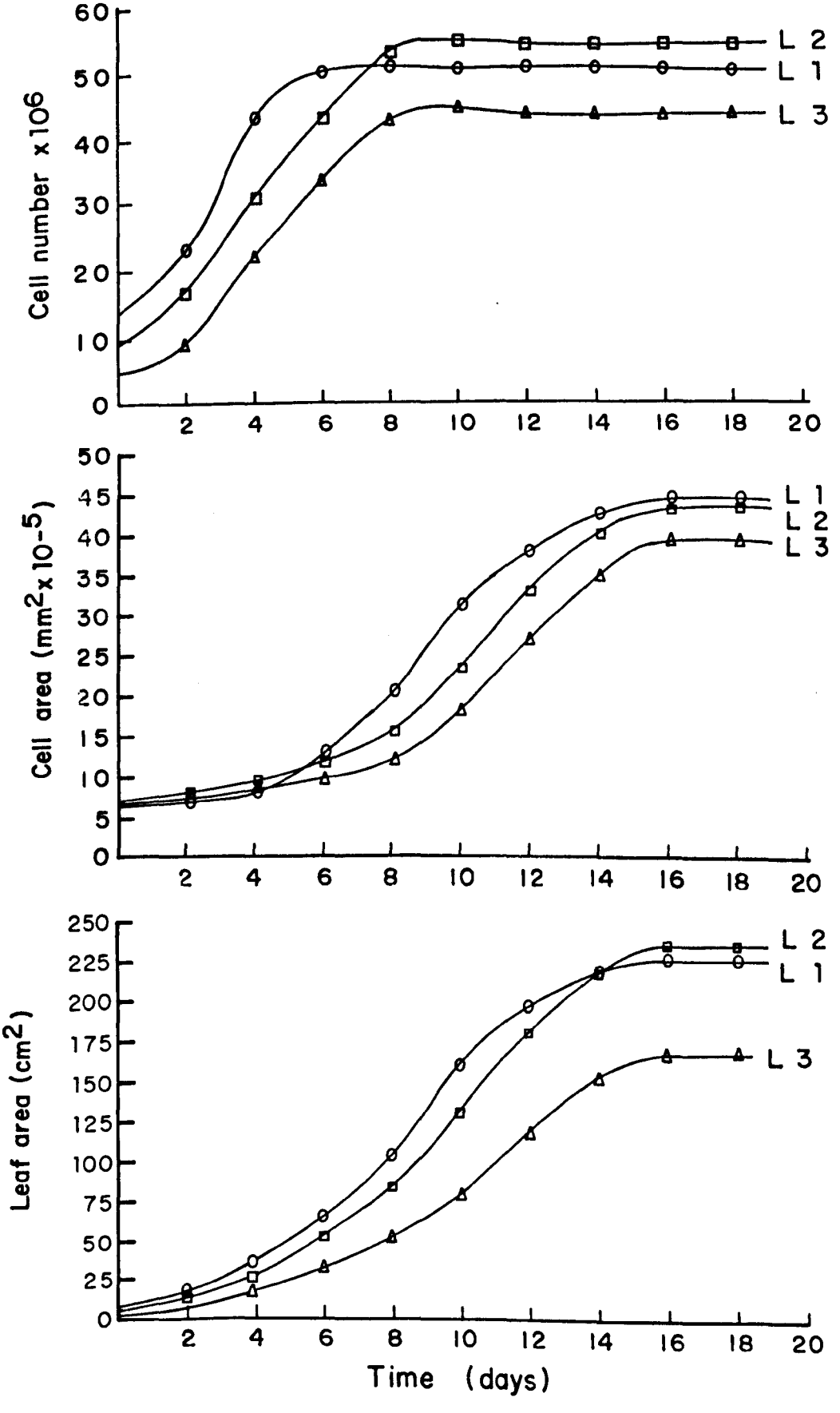
Leaf 2 showed a greater number of cells and a larger final area compared with leaf 1 and leaf 3. Presumably, this is due to the fact that leaf 2 has shown a higher increase in mean cell number per day, around 5.70×10^6 versus 5.48×10^6 for leaf 1 and 4.77×10^6 for leaf 3. It is interesting to note that leaf 1 showed a higher rate of cell division (as expressed in terms of increased cell number per day) between the second and third determinations (Day 2 and Day 4 in Figure 2.8), but leaf 2 maintained a high rate of daily increase in cell number for a relatively longer time than leaf 1. On the other hand leaf 3 showed a lower rate of increase in cell number per day, but over a similar total period of division than leaf 2. So that, the greater final cell number of leaf 2, in relation to leaf 1 is due to a longer duration of cell division, while relative to leaf 3, the greater size of leaf 2 may be due to a higher rate of cell division over a similar total period of division. The differences in final number of cells among leaves in one flush therefore appear to be determined by differences in duration and/or by differences in rate of cell division in the early stages of lamina development. In both cases the differences in final area between large and small leaves will result in differences in the final number of cells per leaf. Results found here correspond well with findings for a number of other species, i.e. that cell division in a leaf ceases at around one third of final size (Clough and Milthrope, 1975; Rawson et al., 1980; Williams and Bouma, 1970; Yegapan et al., 1980) and that cell number is the

Figure 2.8 - Changes with time in cell number of the upper epidermis of expanding leaves in one typical flush. L_1, L_2 and L_3 are respectively first, second and third formed leaves.

Figure 2.9 - Increase in area of epidermal cells of expanding leaves in one typical flush. L_1, L_2 and L_3 as above are the first, second and third formed leaves.

Figure 2.10 - Changes in leaf area of expanding flush leaves in one typical flush. L_1, L_2, L_3 as for Figures 2.8 and 2.9.

N.B. Data for Figures 2.8, 2.9, 2.10 are from the same leaves of one flush.


Cell number x10⁶
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
L 2
L 1
L 3
Cell area (mm²x10⁻⁵)
50
45
40
35
30
25
20
15
10
5
0
L 1
L 2
L 3
Leaf area (cm²)
250
225
200
175
150
125
100
75
50
25
0
L 2
L 1
L 3
Time (days)

chief determinant of leaf size (Milthorpe and Newton, 1963).

In Figure 2.9 are shown data for cell area for the same leaves in which cell number was determined. The cell area was obtained by dividing a known leaf area by the cell number contained in this area. By comparison of this figure with figure 2.8, it is observed that the greater proportion of cell enlargement has occurred from after the phase when the leaves reached the maximum cell number. Thus it seems that more enlargement of cells in the upper epidermal layer of cocoa leaves occurs after cessation of cell division. As mentioned above, the maximum cell number in the epidermal layer is reached at a point between 20 to 30% of final leaf area, the remaining 70-80% of increase in area is therefore due to enlargement of the existing cells in the epidermal layer. From a comparison of figures 2.9 and 2.10 it can be seen that the phase over which most cell enlargement occurs (Figure 2.9) coincides with that of most rapid leaf blade expansion (Figure 2.10). Thus, it is apparent that the final size of a leaf is mainly determined by the cell number reached in the early developmental phase of the leaf (until 20 to 30% of final area), but the cell enlargement has an important role in the completion of the lamina expansion. Similarly, it has been observed in a number of other species, that maximum rates of cell enlargement occur after the cessation of cell division (Denne, 1966; Koehler, 1973). So it is for cocoa, as has been previously suggested, for other species that the 'potential size' of a leaf is determined by the cell number present in the early stage of development (whose size varies with the species) and the 'actual

(final) size' by expansion of those cells (Barlow, 1970; Hancock and Barlow, 1960).

The work so far in this chapter shows conclusively that size differences between leaves within flushes are systematic (not random) and that later formed leaves are always smaller leaves. Differences in leaf size are due to differences in cell number since cell area does not vary significantly. The factor controlling cell number per leaf is thus one which determines cell division characteristics and not expansion. Since later leaves are smaller, this maintains the hypothesis that some restriction or stress limits leaf production in one flush. It is not clear though why there is a lower limit to leaf size. e.g. Why are leaves of less than around 60 cm^2 not formed? and why is leaf 2 and not leaf 1 the largest leaf?

The hypothesis being developed, and supported by findings up to date, is that there is a stress which intensifies as a flush develops which limits both leaf number per flush and causes the reduction in size of leaves later in the flush. This hypothesis must be tested and questions such as: how fixed are the characteristics of leaf size and is there interaction between leaves during expansion? must be answered. These questions are explored in the next section of this chapter through experiments involving defoliation treatments.

2.3 Excision of young leaves

2.3.1 Materials and Methods

Cocoa plants were grown in a glasshouse under the conditons

described in section 2.2.1. Plants with three previous flushes were selected at the early F-2 stage of flush four. From this set, seven plants constituted a control treatment, in which there was no leaf excision, and in the other seven plants the first and second developing leaves of flush four were excised to constitute the partial leaf removal treatment. Excision was made by cutting through the petiole when the leaves reached an area of approximately 2.0 cm^2 (unfolding stage, Table 2.1). The remaining leaves in the flush were allowed to grow normally and length and width were recorded every two days until full expansion was reached. Leaf area was calculated from the equation shown in section 2.2.1.2. Final leaf area; duration and rate of laminae expansion were considered as characteristics of leaf expansion and calculated for each leaf. A separate analysis of variance was processed for final leaf area and rate of expansion for each of leaves 3, 4 and 5, through a randomized design with seven and six replications respectively.

For experiments with continued excision of developing leaves, plants with four previous flushes were submitted to the following treatments, each replicated three times:

(1) No leaf removal (control).

(2) Eight leaves were removed (corresponding to between one and two flushes).

(3) Sixteen leaves were removed (corresponding to two to four flushes).

(4) Twenty-four leaves were removed (corresponding to three to six flushes).

Removal commenced with the excision of flush leaves at the

early F-2 stage, when the leaves were about 2.0 cm in length. Further newly emerged leaves were excised when they reached, approximately, 2.0 cm in length. This was done at intervals of three days by cutting through the petiole with a sharp scalpel. After the predetermined number of leaves had been excised, further formed leaves were again allowed to develop normally to their full size. Final leaf area of all leaves; number of new leaves produced, and the upper epidermal cell numbers were then determined. These measurements were taken from the smallest and largest leaves only, from the complete flush produced after excision ceased.

2.3.2 Results and discussion

Excision of the first and second developing leaves resulted in increased (compared with control plants) final size of the remaining leaves in a flush (Figure 2.11). Although the average size of leaf 3 from the partial leaf removal treatment was larger than leaf 3 of the control plants, there was no statistically significant difference between them at 0.05% of probability. Leaves 4 and 5 were significantly larger than those in corresponding positions of the control plants. It was not possible to compare leaves from the sixth position onwards, because control plants did not produce leaves at these positions.

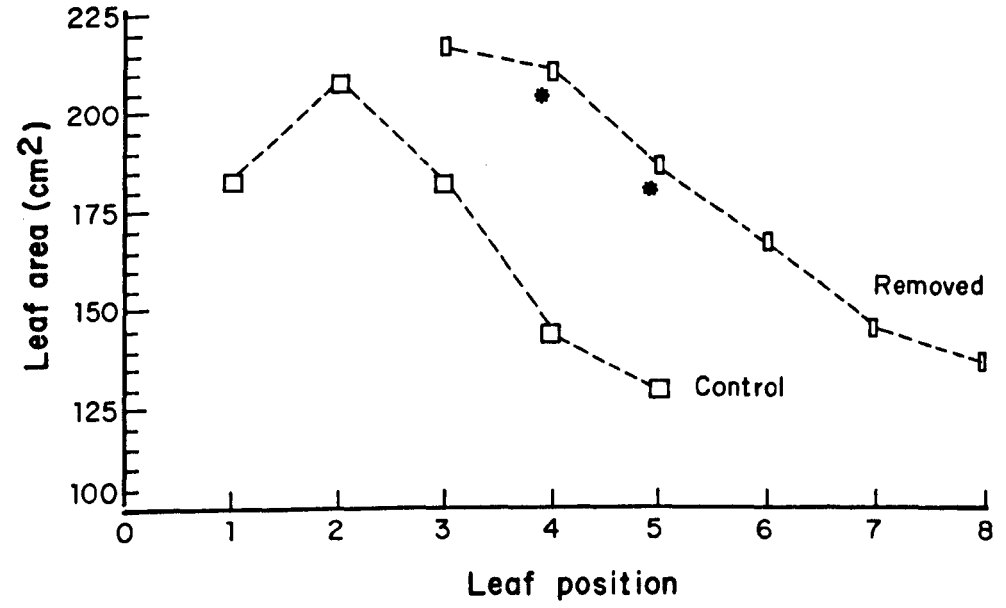
Data for rate and duration of leaf blade expansion (between 0.05 and 0.95 Amax) are shown in Figure 2.12 and 2.13. It is interesting to observe that the rate of leaf blade expansion was significantly increased for all the remaining leaves of the flush in

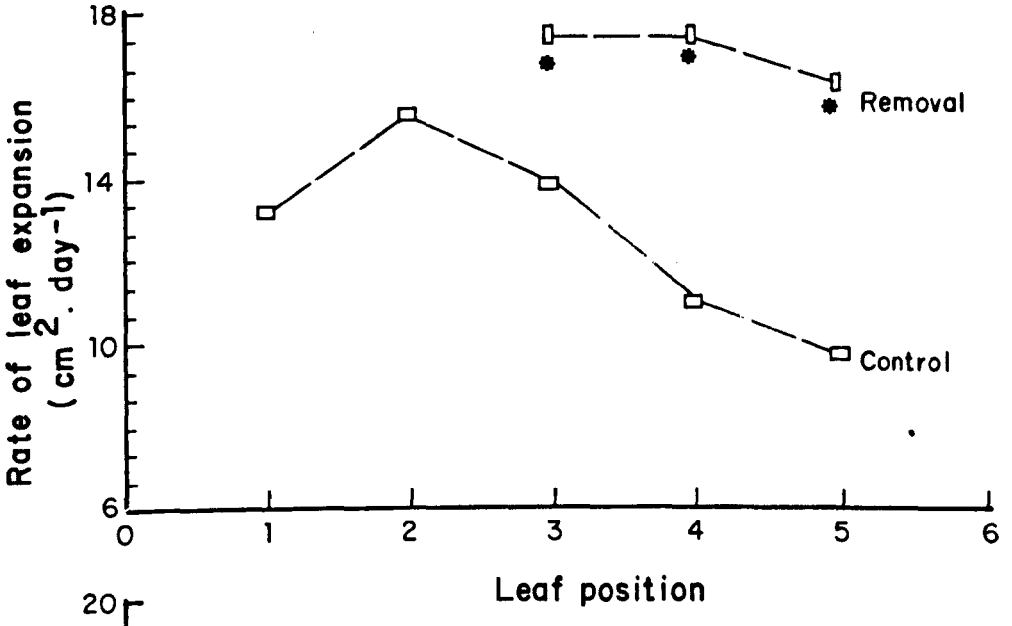
Figure 2.11 - Final areas of remaining leaves in a flush following partial young leaf removal. (Removed), compared with a control seedling. Leaves 1 and 2 excised from the 'removal' treatment.

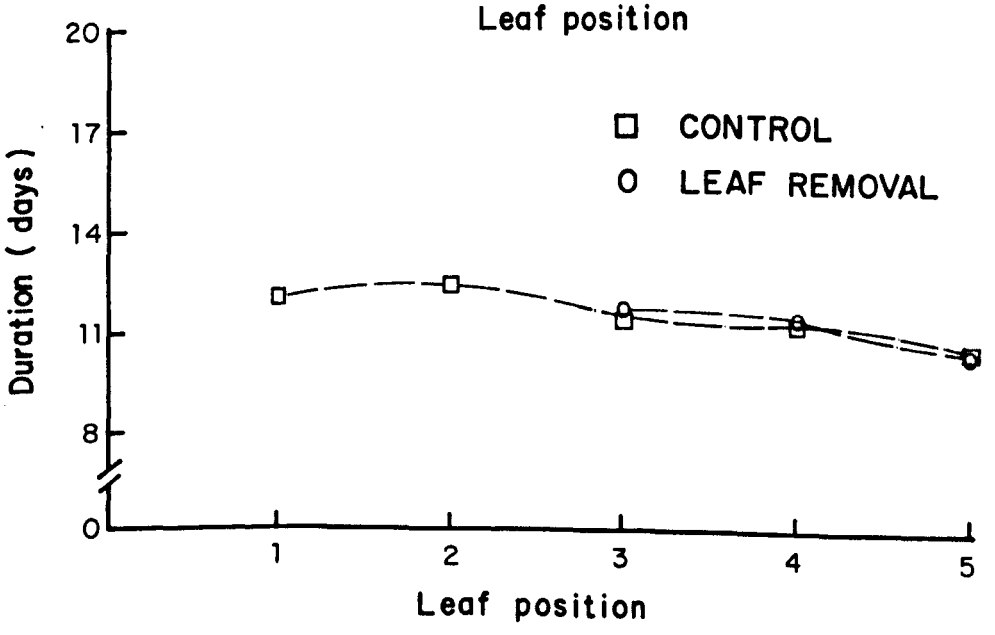
Figure 2.12 - Rate of leaf blade expansion of the remaining leaves in a flush following partial leaf removal, compared with a control seedling. Leaves 1 and 2 excised from the 'removal' treatment.

Figure 2.13 - Duration of leaf blade expansion of the remaining leaves in a flush with partial leaf removal, compared with a control seedling. Leaves 1 and 2 excised from the leaf removal treatment.

N.B. Data presented in Figures 2.11, 2.12, 2.13 are from the same seedlings. Results were statistically analysed and ● indicates means from treatments which are significantly different at P = 0.05 (Tukey) from the leaves at the same position in the flush of control plants.


Leaf area (cm2)
225
200
175
150
125
100
0
1
2
3
4
5
6
7
8
Removed
Control
Leaf position


Rate of leaf expansion
(cm 2. day-1)
18
14
10
6
0
1
2
3
4
5
6
Removal
Control
Leaf position


Duration (days)
20
17
14
11
8
0
1
2
3
4
5
CONTROL
LEAF REMOVAL
Leaf position

plants with partial leaf excision when compared with control plants (Figure 2.12). However, there were no significant differences between treatments, for duration of blade expansion (Figure 2.13). This indicates that the increased individual leaf areas obtained with partial leaf removal are the result of increased rates of expansion. It was also observed that, although a considerable increase in area had occurred in the remaining leaves of the partial defoliated treatment, the later leaves in the flush were still progressively smaller leaves. Partial defoliation had a clear effect on the leaf expansion of the remaining leaves and on the average number of leaves produced in one flush, which resulted in a larger total leaf area of the flush of defoliated plants compared with control plants. Since leaf size of identically positioned leaves was increased with partial leaf removal, it is clear that these leaves have a higher "size-potential" than they show in a normally developed flush. This clearly indicates that the potential expansion of the leaves, at least of those later leaves, is being controlled by the development of the earlier leaves in the flush.

It was shown in the previous section, that the smallest leaves have the lowest number of cells. It has been suggested that, generally, the number of cells in a leaf is governed by the amount of essential metabolites imported by the leaf (Dale, 1976). This indicates that, the final area of the smallest leaves of a flush and even development of larger leaves in a flush, may be being limited by a reduced supply of a supportive factor (possibly carbohydrate) for cell division. As mentioned in the introduction of this chapter (section 2.1) the expanding leaves in one flush are at different

stages of development and also that the sink strength of a developing leaf depends on its developmental stage. It has also been suggested (Sleigh, 1981) that developing cocoa leaves may constitute a major drain for carbohydrate, on the seedlings. So, it is possible that carbohydrate supply for the development of the later leaves may be limited, in particular, as a consequence of monopolization of available carbohydrate by the earliest expanding leaves in the flush. Orchard (1977) suggested a competition may exist between the apical meristem and young flush leaves for carbohydrate and/or cytokinins. Similarly, a competition among the expanding leaves within one flush may occur. It is noted from Figure 2.11 that the difference, in final area, between leaf 3 and 4, in the flush with partial leaf removal, was considerably less than that of the leaves on corresponding positions of control plants, i.e. 2.5% for treated plants versus 20.7 on control plants. This confirms the suggestion that leaf 4 of treated plants had a more favourable supply of some factor, maybe carbohydrate, for its requirements than leaf 4 on control plants, and it is strongly suggestive of relief from a competitive effect of the two first developing leaves (removed in the defoliation treatment) in a normal flush.

Usually, many more new leaves were produced in the first flush allowed to develop after a period of continuous leaf removal than were produced by control plants at the same flush stage, i.e. flush number five. With eight leaves removed, sixteen new leaves were produced in the flush following the leaf removal. On the plants

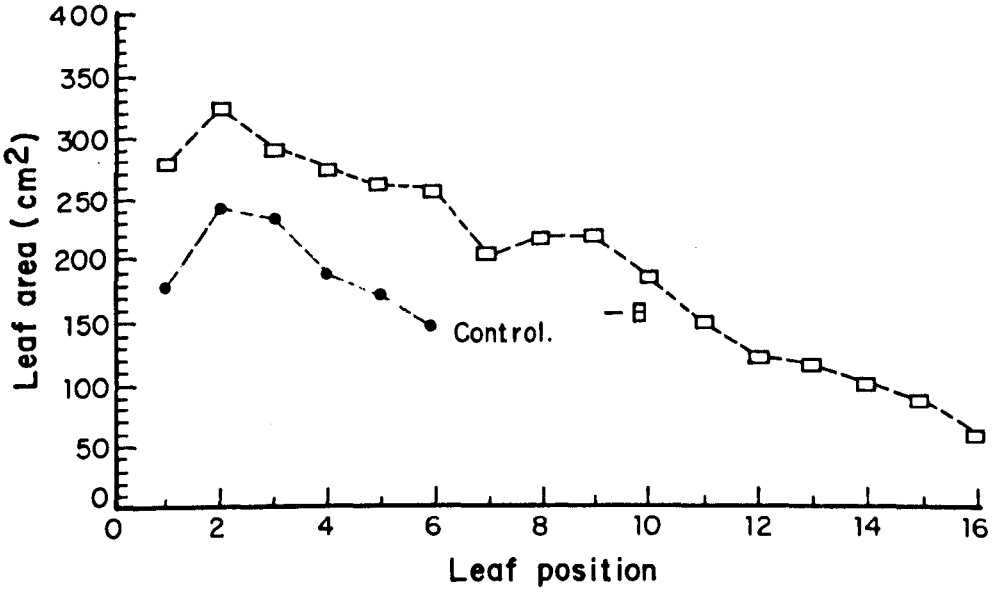
where sixteen and twenty-four leaves had been removed, fourteen and eight new leaves were produced respectively in the first flush completed after excision ceased (Figure 2.14 and 2.15).

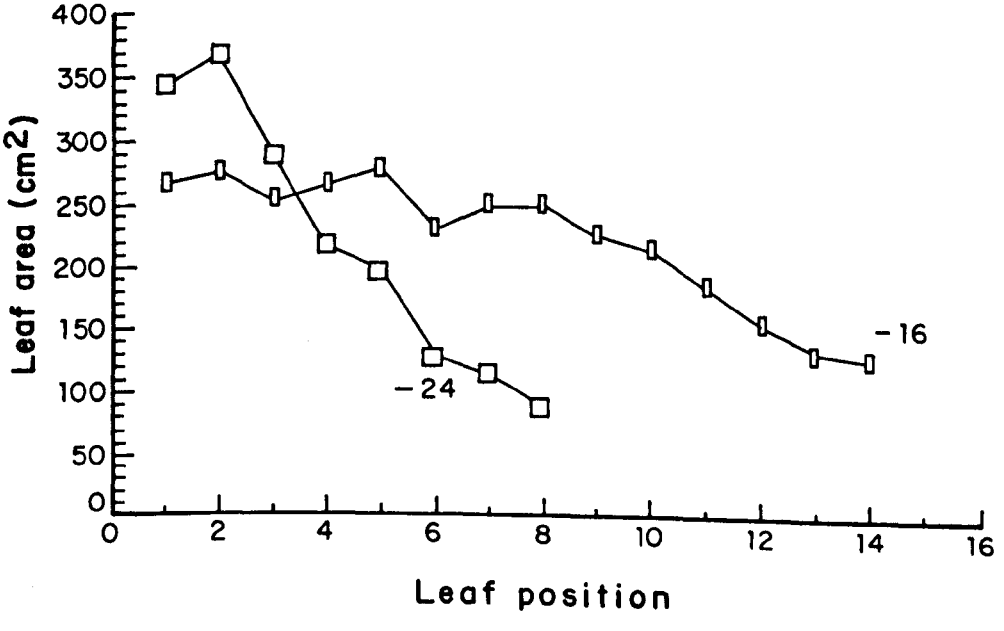
These results show clearly that the shoot apex has the capacity to produce, and the seedling to support the expansion of, many more leaves in one flush than are normally produced at a particular stage in seedling ontogeny. Average flush size in control plants with four previous flushes completed is six leaves, yet the flush sizes in plants with eight, sixteen and twenty-four leaves removed were 16, 14 and 8 leaves respectively.

Average values of final leaf area from the flush following the cessation of pruning when eight leaves had been removed are presented in Figure 2.14. A considerable increase in the leaf area of the early formed leaves was observed in pruned plants in relation to control plants. A similar response was shown by plants with sixteen and twenty-four leaves removed (Figure 2.15). Although a marked increase in maximum individual leaf area had occurred with leaf excision, the progressive decrease of leaf area from largest leaf throughout the rest of the flush, was still observed for all the treatments. The progressive reduction in leaf area of successive leaves in the flush was "faster" when twenty-four leaves were removed (Figure 2.15). In this treatment the sizes of leaves one and two were also larger than those in the other treatments. The largest leaf being on average 50% bigger than the largest leaf in control plants. The total leaf area per flush also rises considerably after defoliation, being 2.7, 2.7 and 1.5 times the control with respectively 8, 16 and 24 leaves removed (Figure 2.14

Figure 2.14 - Final areas of leaves in the first flush following the pruning of eight young leaves (-8), compared with a control seedling. Values represent means of three replicate observations.

Figure 2.15 - Final area of leaves in the first flush following the pruning of sixteen (-16) and twenty four (-24) young leaves. Values represent means of three replicate observations.


Leaf area (cm2)
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
Leaf position
Control.
-8


Leaf area (cm2)
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
Leaf position
-16
-24

and 2.15).

Table 2.4 contains values for cell number, cell area and final leaf area of the largest and smallest leaves in the flushes formed after defoliation from individual representative plants with eight, twenty-four and no leaf removal (control).

Table 2.4 Cell number, cell area and final leaf area of the smallest (S) and largest (L) leaves from individual representative pruned and unpruned plants.

Treatments (leaves removed)	cell number x 10^6		cell area ($mm^2.10^{-4}$)		leaf area (cm^2)	
	S	L	S	L	S	L
None	37.1	59.0	3.91	4.00	145.0	235.9
Eight	38.4	102.2	3.79	3.74	146.0	382.2
Twenty-four	28.1	105.6	3.80	3.73	107.0	393.9

It is noted that there are considerably greater numbers of cells in leaves from pruned plants, while for cell area there are no significant differences between pruned and unpruned plants. The very clear effect of leaf removal is thus on the cell number per leaf. These results confirm the findings in the previous section (2.2.2) that differences in final leaf area result from differences in cell number per leaf. Similar results were also found in Ipomoea caerula, in which young leaf removal produced compensatory growth in

the next formed leaf. This had an increased final area and total cell number and the increased area was due to increased cell number since there was no increase in cell size (Njoku, 1956).

There are a number of common features of the results from the continuous defoliation treatments. Irrespective of how many leaves are removed, the flush which follows always has: (i) more leaves and (ii) larger individual leaves at corresponding positions in the flush, than control plants. The number of leaves produced per flush is very different, being inversely related to the number of leaves removed. Cumulative leaf area per flush also decreases at the highest defoliation level. How can these results be explained? The greater number of leaves per flush following defoliation (and larger leaves) may reflect an attempt by the plants to restore shoot:root ratios. Such reactions are very common for partially defoliated plants (Brouwer, 1963; Humphries, 1958; 1960; Maggs, 1964). In the case of cocoa, Sleigh (1981) showed that root growth continued during continuous defoliation and thus shoot:root ratios declined. Compensatory rapid production and growth of leaves will quickly restore the ratios to normal. This greater rate of leaf production than normal will require a sustained period of high export of carbohydrate to support the growth. It is clear however that during defoliation carbohydrate consumption will be much reduced and continued photosynthesis of mature leaves will have led to a build up of storage carbohydrate above the level produced in a normal rate flush period. It is likely therefore that there will be sufficient carbohydrate available to support a larger than normal flush. When storage levels are reduced and current carbohydrate provides

support, leaf production will stop. Leaf number decreases more than leaf area in flushes produced with increased defoliation. The former might indicate the apex "running short" of primordia after the more extended defoliation, since leaf removal rate may have exceeded new primordia formation. In support of this suggestion it will be seen later (Chapter 5) that most new primordia are formed during F_2 and this stage is increasingly "delayed" with greater defoliation. It might be expected that leaf number and cummulative flush leaf area would be greater with extended defoliation because there would be a longer time for carbohydrate build up. During extended defoliation however, stem elongation continues and it may be that during a longer period with no new mature leaves produced, stem growth has reduced stored carbohydrate levels below those of plants with less extended defoliation. Additionally mature leaves would also be older when new growth was allowed in the extended defoliation treatments and so would have reduced photosynthetic capacities (see also Chapter 3).

To summarise then, the continuous defoliation experiments confirm that: i) final leaf size is not predetermined at the primordial stage before the leaf emerges from the bud; ii) there is interaction and competition between leaves in one flush even when unusually large, and iii) because early formed leaves are large and later ones progressively smaller a factor such as carbohydrate remains a strong candidate for major controlling influence on leaf number per flush and individual leaf size. Other findings with tomato (Hussey, 1963) and Dactylis glomerata (Davidson and

Milthorpe, 1966) also led these workers to suggest that carbohydrate availability was the important factor determining leaf growth after defoliation.

2.4 Shading of mature leaves

2.4.1. Materials and Methods

For the shading experiments, seedlings with three completed flushes, and at the early F-2 stage of a new flush, were shaded immediately above the level of the most recently expanded flush (PF_1), leaving out of shade the new developing flush. Another equivalent set of plants with no shade constituted the control. Shade was provided by covering plants with green nylon mesh screen. Light intensity was measured using a PAR Quantum Sensor below and above the screens. Light intensity above the screens averaged about 120 $\mu mols.m^{-2}.sec^{-1}$. Shading was arranged to give around 60 and 35% of 120, which corresponds approximately to 70 and 40 $\mu mols.m^{-2}.sec^{-1}$ respectively. These light intensities were found to depress photosynthesis significantly (R. Raja Harum, personal communication). The screens were supported on metal supports and secured with thin wire. All mature leaves (source leaves for photosynthates) from PF_1 down were maintained under shade during the development of one flush.

Final leaf area, cell number per leaf and upper epidermal cell area were determined when the new flush of leaves were fully expanded.

2.5.2 Results and discussion

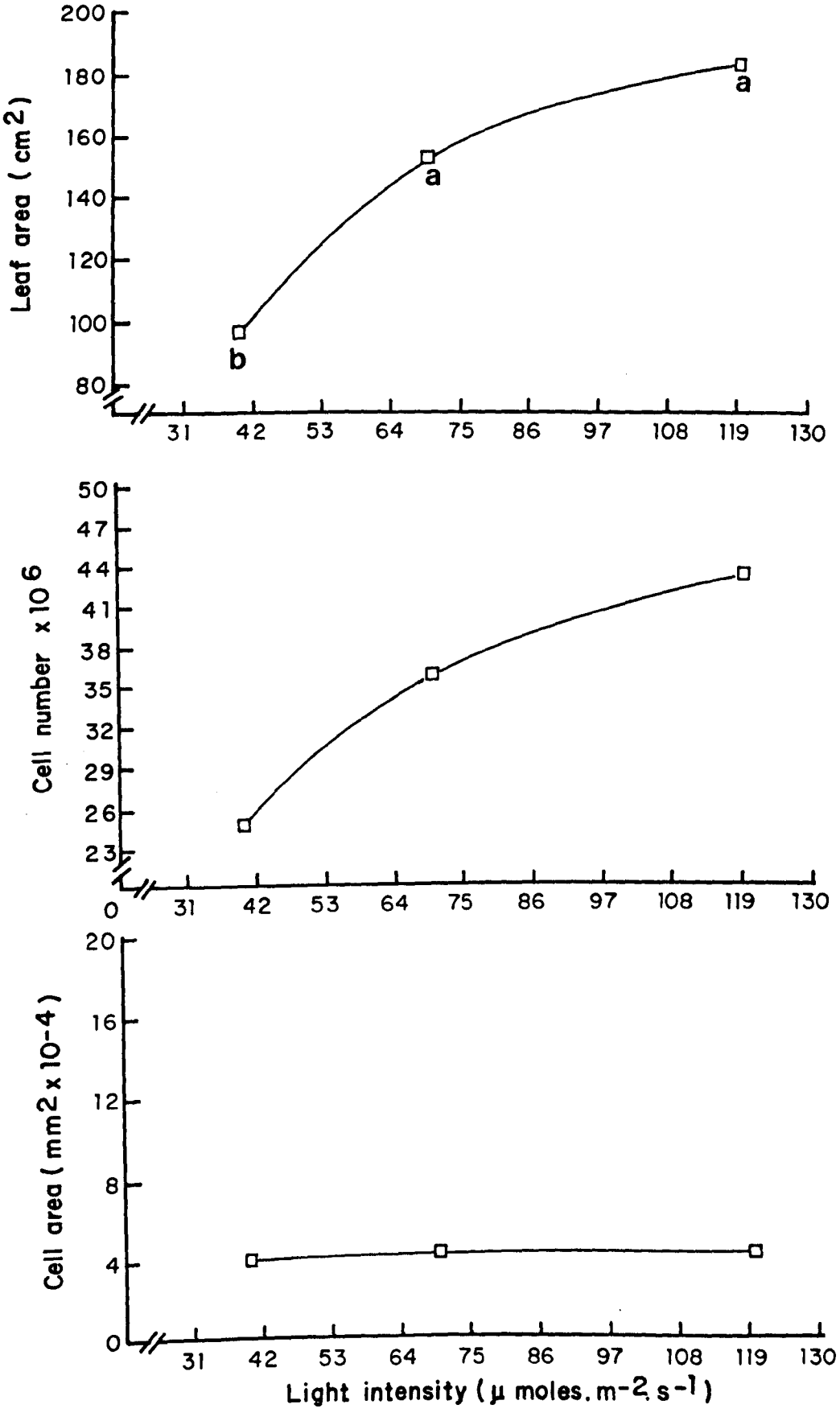
Figures 2.16 and 2.17 show results of leaf area and cell number per leaf for the new flush leaves expanded after shading of mature leaves. The reduction of 40% in light intensity to 70 μmols. $m^{-2}.sec^{-1}$ decreased the final average leaf area per flush and cell number per leaf by about 17% when compared with control plants. Cell number and leaf area were more considerably depressed with light intensity reduction from 120 to 40 μmols.m^{-2}. sec^{-1}. The reduction in light intensity from 70 to 40 μmols.$m^{-2}.sec^{-1}$, also significantly decreased leaf area and cell number per leaf. The cell number was decreased by 17 and 42% when the light intensity was reduced to 70 and 40 μmols.$m^{-2}.sec^{-1}$ respectively. It is interesting to note that the cell area (upper epidermis) was only very slightly decreased (about 9%) even when light intensity was reduced to 40 μmols.$m^{-2}.sec^{-1}$. In addition to the decrease which occurred in individual leaf areas, there was also a considerable decrease on the number of leaves expanded in the new flush when plants were shaded. On average, 35% of the newly produced leaves (10 in 28) abscised soon after they emerged from the bud and before they reached 2.0 cm long, especially the later leaves produced in the flush. Clearly this is most likely to be the result of a reduced supply of photosynthate for the shoot apex, as a consequence of decreased photosynthesis by the shaded leaves, but changes in any growth regulator(s) supplied from mature leaves to apex may also have occurred.

These results support those discussed in previous sections of this chapter i.e. that a limited current carbohydrate supply affects

Figure 2.16 - Mean leaf area of the flush produced following the shading of all the mature leaves. Values represent means from five plants. Means with different letters are statistically different at P = 0.05 (Tukey).

Figure 2.17 - Cell number of the upper epidermis of one representative leaf of the flush developed following the shading of the mature leaves.

Figure 2.18 - Area of cells of the upper epidermis of the representative leaf of the flush developed following the shading of the mature leaves.


Leaf area (cm2)
200
180
160
140
120
100
80
a
a
b
31
42
53
64
75
86
97
108
119
130
Cell number x 10 6
50
47
44
41
38
35
32
29
26
23
0
Cell area (mm2 x 10-4)
20
16
12
8
4
0
Light intensity (µ moles. m-2 s-1)

the cell division and normal development of further leaves in the flush. It is well known that the number of cells in a young leaf is governed, at least in part, by photosynthetic activity in older leaves which supply it (Dale, 1976; Wilson and Cooper, 1970). This same principle could be applied to explain the spontaneous abscission of later unfolded leaves in a flush, but in the sense that the later leaves are receiving insufficient carbohydrate to maintain cell division started in the primordial stage for a subsequent lamina expansion. If cell division rate is not maintained (and increased) then normal auxin production rates of the young leaf will not be maintained. A well known consequence of lowered auxin flow from leaf blade, through the petiole base, is the development of the abscission layer and leaf shedding will thus occur (Dale, 1982; Sheldrake, 1973).

The cell number decrease in leaves expanded after the shading of mature leaves supports the hypothesis that cell division in expanding cocoa leaves is limited by the reduced supply of current photosynthates. From the results obtained here, it is suggested that the size of the flush leaves in cocoa is largely determined by the carbohydrate availability from mature leaves.

To investigate this hypothesis further succeeding chapters in this thesis are concerned with the production and translocation of photosynthates from mature leaves to developing flush leaves. Final discussion on the carbohydrate control of leaf development is thus left for later.

CHAPTER 3

Carbohydrate production in cocoa seedlings

3.1 Introduction

In Chapter 2, it was suggested that the progressive reduction of leaf size and even the number of leaves in one flush may be the result of a limited carbohydrate availability at the shoot apex. This was, in part, evidenced from the larger areas and greater number of leaves produced in one flush following the extended removal of leaves at the stage of leaf unfolding and compensatory growth of the remaining leaves of one flush after partial defoliation of developing leaves. As a process which consumes carbohydrate, leaf development will represent a carbohydrate sink, and the mature leaves of the plant a carbohydrate source. In order to examine the hypothesis that carbohydrate controls flush growth, it is thus necessary to investigate sink:source relationships of cocoa seedlings throughout complete flush cycles. It is necessary to determine: i) the photosynthetic performance and ii) carbohydrate export characteristics of mature leaves of all ages on seedlings. The work on photosynthetic performance is described in this chapter and that on carbohydrate export in Chapter 4.

It has been observed that, in cocoa leaves, major synthesis of chlorophyll and 'self-sufficiency' for photosynthate only occur after the termination of leaf expansion (Baker, 1974). Thus it might be expected that leaf maturity is also reached several days after the complete expansion of the blade leaf, which coincides with a later phase in the flush cycle, the I-2 stage. Once maturity is

reached, cocoa leaves remain on the plant for a relatively long time. Their life-expectancy is between 10-12 months. This "normal" life-expectancy may be shortened by adverse environmental conditions, such as water stress and mineral deficiency, which are considered to accelerate leaf senescence. Although the leaf remains on the plant for a long time, very little is known on the duration of its function life as a provider of photosynthate for the plant as a whole. It has been considered that leaves of other tropical perennial crops may have a long period of photosynthetic activity, but it has rarely been studied (Corley, 1983). Most of the studies on tropical perennial plants have only examined photosynthetic capacity over the first weeks of leaf life, although Yamaguchi and Friend (1979) had observed little decrease in the photosynthetic activity of Coffea arabic leaves up to 150 days after emergence. For cocoa, some information is available concerning the photosynthetic activity during leaf development (Baker, 1974) and the lowering of photosynthesis when the leaf senesces (Hutcheon, 1976). However, there has been no work to estimate the photosynthate contribution which individual mature flush of different ages make to support new leaf development.

The total contribution of a leaf to the carbohydrate budget of the plant depends upon the period during which it is photosynthetically active and in a whole plant the photosynthetically active duration of the leaves is more important than their maximum photosynthetic rate at any particular time (Samsuddin and Impens, 1979). The contribution of a single leaf to the carbon economy of the whole plant might be expected to change

with age and relative position on the plant. As the plant grows, a particular leaf will be increasingly shaded by new leaves "above". Thus, when an expanded leaf is "relegated" from an upper to intermediate, and then lower position, its photosynthetic rate is believed to diminish (Ismail and Sagar, 1981). Leaf photosynthesis can be influenced by many other plant factors such as leaf age, leaf position, number and sizes of carbohydrate sinks and mutual shading. All the factors need to be considered in determining the carbon production of an individual leaf and of a whole plant (Constable and Rawson, 1980a). Among these factors, leaf age has been considered as a very important factor influencing photosynthetic activity in a number of plant species (Benjamin et al, 1981; Joggi et al, 1983; Osman and Milthorpe, 1971). In general, metabolic activity increases from some value in the developing leaf, reaches a maximum when leaf expansion is completed and then decreases as the leaf ages (Lin and Ehleringer, 1982). Constable and Rawson (1980a) observed a linear fall in maximum photosynthesis of cotton leaves from age 25 days, and at age 68 days the maximum photosynthesis was only 20% of the value on day 25. These workers observed that transpiration also changed with leaf age from day 25 and followed a similar pattern of decline to photosynthesis. Concurrent with changes in photosynthesis, decreases in stomatal conductance, transpiration and chlorophyll content with age have also been observed in many other species (Davis et al., 1977; Lin and Ehleringer, 1982; Raschke and Zeevaart, 1976).

Photosynthesis has also been observed to decrease, in many species, when the sink:source size is reduced by sink removal (Lawn and Brun, 1974; Loveys and Kriedmann, 1974; Mondal et al., 1978).

It was observed that utilization of assimilates by sink organs (sink-demand) and the translocation of assimilates to these organs may be an important factor in the control of photosynthesis in intact plants (Ho, 1979; Harold, 1980). Thus the photosynthetic potential of a leaf may be limited by the degree to which photosynthates are being removed from the photosynthesising leaf to the sinks (Thorne and Koller, 1974). Therefore, it might be expected that in a whole plant net assimilation would always be in balance with the net consumption of assimilates (Wareing and Patrick, 1975). There is some evidence for soybean that photosynthesis of one leaf increased when sink demand was increased by shading of all but that single leaf (Thorne and Koller, 1974). It has also been observed in maize, bean, willow and pine that increased photosynthetic rate of a "remaining" leaf occurred when other source leaves were removed (Wareing et al., 1968; Wareing, 1970). In these cases, it was suggested that the enhanced photosynthetic rate following defoliation resulted from increased levels of the carboxylating enzymes, RUBISCO (ribulose bisphosphate carboxylase) or phosphoenol pyruvate carboxylase, in remaining leaves. It was also found in these plants that an increase in the protein and chlorophyll content of the remaining leaves occurred following the defoliation (Wareing, 1970). Increase in protein content following the defoliation was considered to be a result of a reduced competiton between remaining leaves for nutrient and metabolites from other parts of the plant (Wareing, 1970).

Protein is an important component of cells and has been used as parameter to assess the physiological age of a tissue. The

senescence of leaves is, usually, associated with loss of soluble protein, predominantly RUBISCO, which catalizes the process of carbon dioxide fixation (Wareing and Phillips, 1978). Degradation and remobilization of protein during the course of leaf senescence is considered to provide an important source of nitrogen and sulphur for developing parts of the plant (Friedrich and Huffaker, 1980). Of the many degenerative changes occurring during the course of ageing of a leaf, declines in protein and chlorophyll content have been found to be most closely related to decreases in photosynthetic rates of many species (Hardwick and Woolhouse, 1967; Hardwick et al., 1968; Maunder and Brown, 1983; Peoples et al., 1983).

During leaf ontogenesis, chlorophyll is known to accumulate up to some maximum level after which the rate of degradation processes overtake the synthetic processes (Šesták, 1977). In the majority of species, most chlorophyll synthesis occurs during leaf expansion, but in cocoa leaves, as mentioned above, it is known that maximum synthesis of chlorophyll only occurs after the termination of leaf expansion (Baker and Hardwick, 1973). By the I-2 stage, a newly expanded leaf has attained most of its final complement of chlorophyll, and reached a high photosynthetic capacity. There is, however, no information on either the level of chlorophyll or photosynthetic capacity during the succeeding and major part of the leaf's mature phase. It is important to determine these parameters of cocoa leaf physiology both to improve the basic knowledge of cocoa leaf functioning and most importantly for the present work to allow an understanding of the capacity of mature leaves to produce and export carbohydrate to support and possibly control, flush leaf development. The latter is particularly important since developing

cocoa leaves have very low photosynthetic capacity and cannot support their own development.

For reasons mentioned above it is clear that the development of a new flush creates a major carbohydrate sink and it is suggested that a carbohydrate stress may develop within the plant during flushing. It is thus possible that the increased demand may result in increases of photosynthetic rate of the young mature cocoa leaves. It was important, therefore, to measure the photosynthetic performance of mature cocoa leaves throughout a complete flush cycle. In order to understand fully the functional role of mature cocoa leaves of all ages on seedlings as providers of photosynthates and the role of developing leaves as sinks for carbohydrates, several lines of investigation are presented in this chapter. These are:-

i) Determination of the photosynthetic capacity of leaves from each mature flush.

ii) Affects of manipulating sink:source relationships by leaf removal to investigate more fully the flexibility of photosynthetic performance of cocoa leaves (This was done particularly since experiments in i) showed no stimulation of photosynthesis of mature leaves during development of a new flush of leaves). Such studies are also of relevance as a basis for understanding the possible responses of leaves to major reproductive sinks i.e. developing cocoa pods. This in turn has implications for the theoretical determinations of maximum cocoa pod production capacity of trees.

iii) To complement photosynthetic determinations, and to allow interpretations of the findings in relation to the research

discussed above on the causes of changes in photosynthetic capacity with leaf age, protein and chlorophyll determinations were also made on mature leaves through several successive flush cycles.

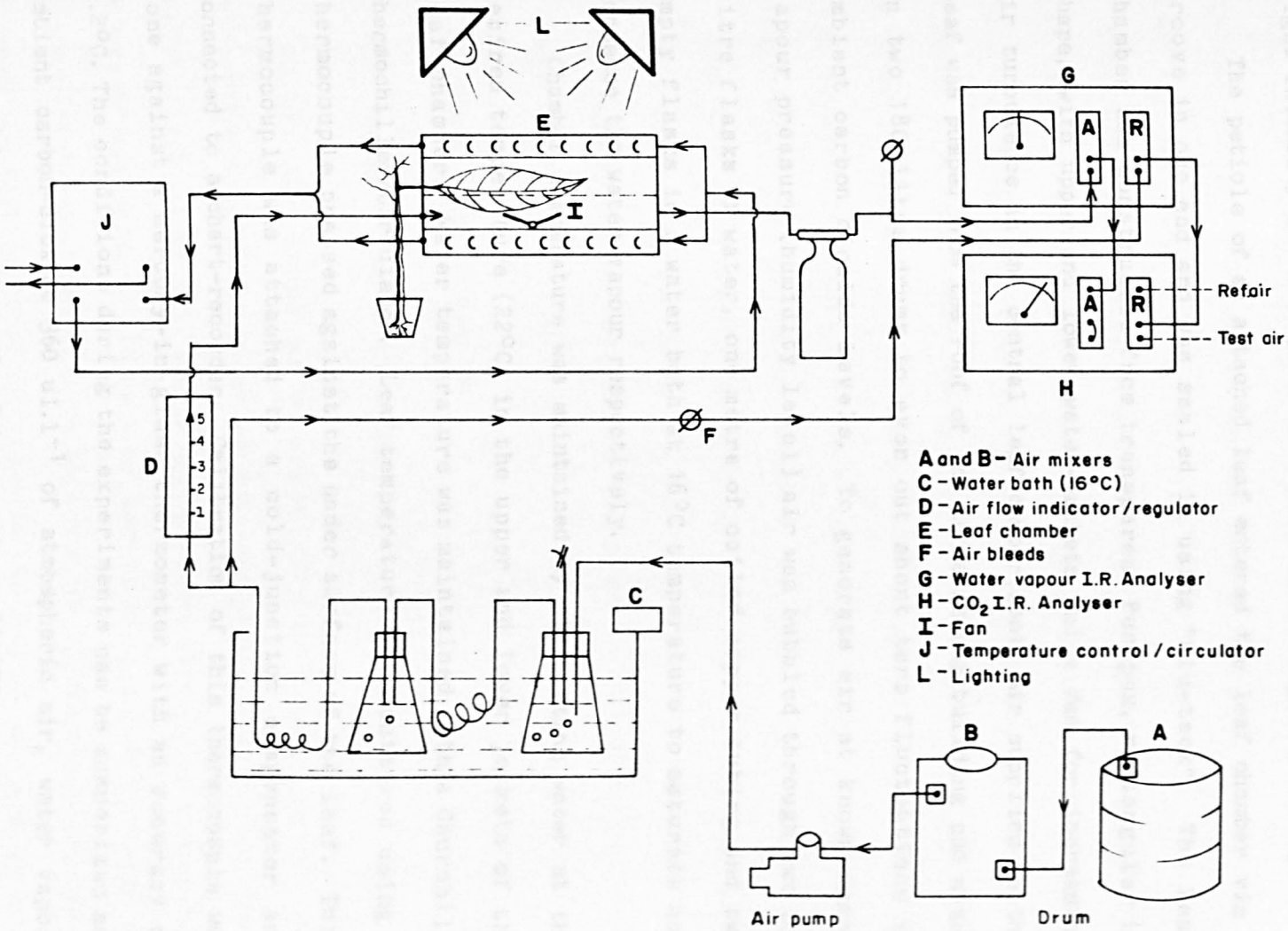
3.2. Photosynthetic performance

3.2.1 Materials and methods

All the plants used in these experiments were grown in the glasshouse under conditions as described in Chapter 2. In this study changes in rates of photosynthesis and transpiration were investigated in attached mature leaves of different ages and at several levels of light intensity. Measurements were always made on the third formed leaf (L3) in the flush. Changes in photosynthetic rates were also measured in leaves of the younger previous flush (PF_1) during the development of a new flush of leaves. These last determinations were made on intact plants during five consecutive weeks from the F-1 to early I-1 stages of the flush cycle. Changes in photosynthetic rates of PF_1 leaves were also determined during mid F-2 stage, in plants with major removal of mature leaves to manipulate source:sink relationships. All the determinations were repeated three times on cocoa seedlings with three or four previous flushes completed.

The carbon-dioxide and water vapour exchanges of leaves were measured using separate infra-red gas analysers (Analytical Development Company, Hoddesdon, Hertfordshire, U.K. Model 225 Mk II for CO_2 and 225 Mk III for H_2O vapour) as shown in diagram in Figure 3.1. The gas circuit used was an open system. Analysers were calibrated and used in differential mode.

Figure 3.1 - Flow diagram of gas circuit for intra-red gas analysis studies.
(On both gas analysers, A = Analysis cell, R = Reference cell, C = Water bath and humidity control).


L
E
I
J
G
A
R
Refair
Test air
H
D
5
4
3
2
1
F
C
A and B - Air mixers
C - Water bath (16°C)
D - Air flow indicator/regulator
E - Leaf chamber
F - Air bleeds
G - Water vapour I.R. Analyser
H - CO_2 I.R. Analyser
I - Fan
J - Temperature control/circulator
L - Lighting
B
A
Air pump
Drum

An adaption period of 24 hours was allowed for each leaf to equilibrate within the chamber before any measurements were taken. A minimum time of 20 minutes was allowed for stabilization at each light intensity in the experiments with varied light intensity.

The petiole of an attached leaf entered the leaf chamber via a groove in one end and was sealed in using "Blu-tack". The leaf chamber was constructed from transparent Perspex, rectangular in shape, with upper and lower water-jackets and a fan for increasing air turbulence in the central leaf compartment. Air supplied to the leaf was pumped from the roof of the three storey building and mixed in two 180 litre drums to even out short term fluctuations in ambient carbon dioxide levels. To generate air at known water vapour pressure (humidity level) air was bubbled through two 2.5 litre flasks of water, one metre of coiled copper tubing and two empty flasks in a water bath at 16°C temperature to saturate and condense the water vapour respectively.

Chamber temperature was maintained by circulating water at the desired temperature (22°C) in the upper and lower jackets of the leaf-chamber. Water temperature was maintained with a Churchill Thermochiller/Circulator. Leaf temperature was monitored using a thermocouple pressed against the under surface of the leaf. This thermocouple was attached to a cold-junction compensator and connected to a chart-recorder. Calibration of this thermocouple was done against a mercury-in-glass thermometer with an accuracy of 0.2°C. The conditions during the experiments can be summarized as: ambient carbon-dioxide 360 $ul.l^{-1}$ of atmospheric air, water vapour pressure 16 mb; average leaf temperature 22.5°C and water vapour pressure deficit 11.51 mb, equivalent to 60% relative humidity at

22.5°C.

Light used in the experiments was from two high-pressure mercury lamps with a 10 cm water screen to reduce their heating effects. Light intensities were varied by using muslin screens and monitored using a PAR Quantum Sensor (Crump Instruments, Essex, England - Model 502).

Respiration rates were determined under the above conditions, but in darkness for 12 hours during the night. No account was taken of possible difference between light and dark respiration rates. Throughout this work, calculations for leaf photosynthetic capacity were based on average photosynthetic rates between 10.00 and 13.00h. As can be seen in figure 3.2 there were significant changes in photosynthetic rates of leaves throughout one day.

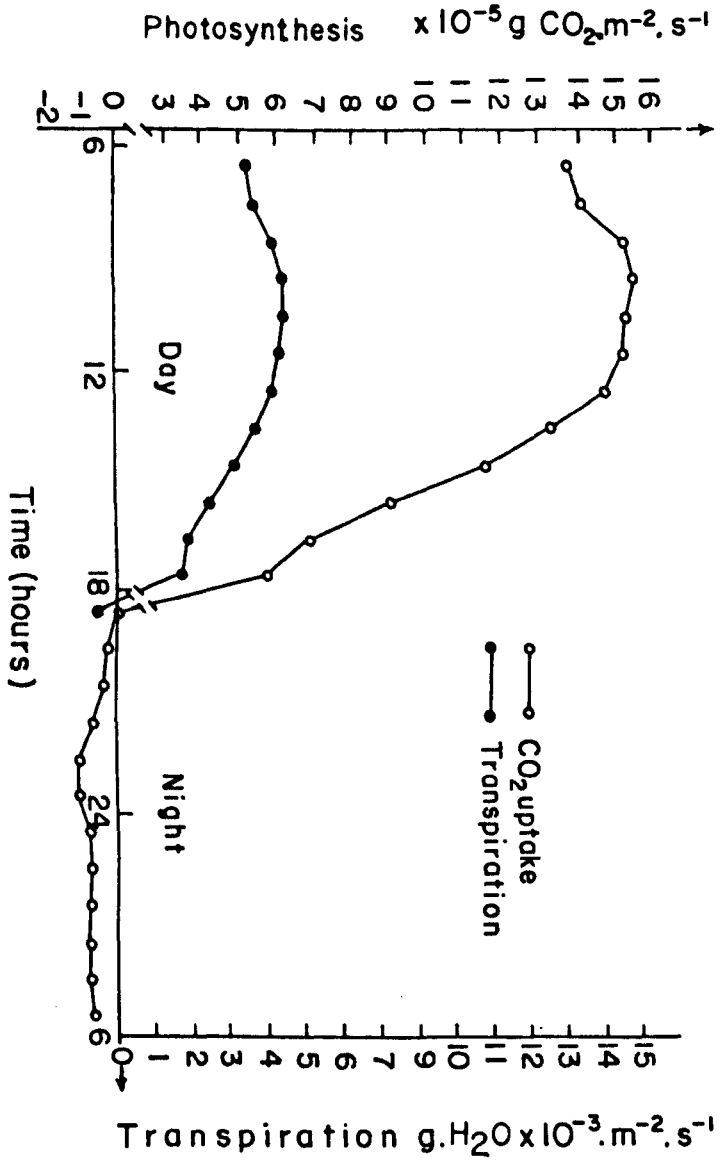
Leaf area was recorded for each leaf immediately prior to gas analysis and was estimated by the equation given in section 2.2.1. All the photosynthesis results are expressed as net photosynthesis in grams $CO_2.m^{-2}$ leaf area.s^{-1}.

3.2.2. Results and discussion

3.2.2.1 Daily course of photosynthesis in mature leaves

Figure 3.2 shows an example of the daily course of photosynthesis, night respiration and transpiration of a leaf from the PF_1 flush. From this figure two features which were common for leaves of all ages. As can be seen, one is the gradual increase in photosynthesis and transpiration to a maximum during the first three hours of the light period, the other is the gradual decrease in both parameters towards the end of the light period. The increasing

Figure 3.2 - Daily course of photosynthesis and respiration in a mature leaf. Change over from Day to Night at 18 hours.


Photosynthesis x 10⁻⁵ g CO₂.m⁻².s⁻¹
Time (hours)
Day
Night
CO₂uptake
Transpiration
Transpiration g.H₂O x 10⁻³.m⁻².s⁻¹

rates of photosynthesis and transpiration would be expected as a consequence of stomatal opening. The gradual decline of photosynthesis in parallel to that of transpiration, may be the result of partial stomatal closure induced by changes in water status of the leaf in the later part of the day. A similar decrease in photosynthesis, in the later part of the day, has been previously observed for some other species e.g. Cucumis sativus (Hopkinson, 1964).

The rate of dark respiration did not show any major changes through the dark period, only a slight increase during the first hours and from then it decreased and thereafter remained very constant throughout the rest of the night.

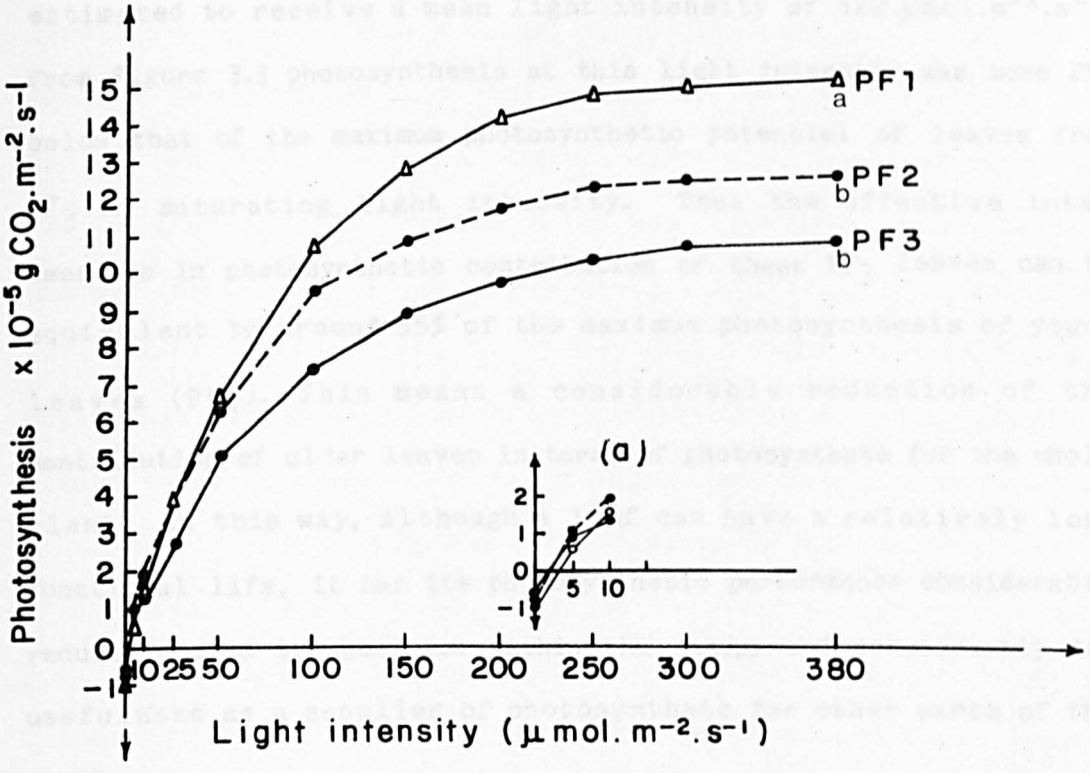
3.2.2.2. Photosynthetic light response curves for leaves of different ages - These are given in Figure 3.3. The relationships between photosynthesis and irradiance were all of the normal typical patterns; i.e. showing linearly rising photosynthetic rates at low and increasing irradiance followed by approach to a plateau at saturating irradiance.

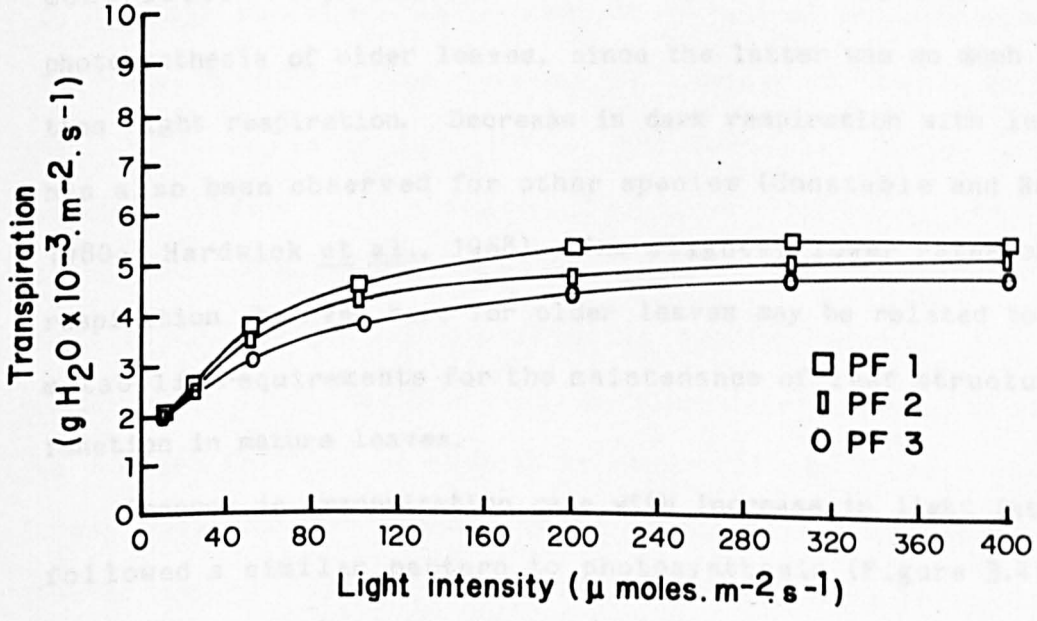
The relationships between photosynthetic rate and irradiance at lowest light intensities, where the relationship is strictly linear, gives a measure of the (quantum) efficiency of photosynthesis, often interpreted as the maximum capacity of the photochemical process. It appears that leaves of different ages have the same efficiency at low light levels, up to around 10 $\mu mol.m^{-2}.s^{-1}$, since curves for each leaf are on the same straight line (Figure 3.3a). Departure from linearity of the curves for different age leaves however, occurs at different irradiance, showing that the ability of the

leaves to use higher light levels is significantly different. In general the older the leaf the earlier, in increasing irradiance, that the photosynthesis light curve departs from linearity: PF_3 at aproximately 10 $\mu mol.m^{-2}.s^{-1}$; PF_2 and PF_1 above this value. It is very difficult to be absolute about the values. The oldest flush (PF_3) showed a considerably lower slope from a light intensity of 25 $\mu mol.m^{-2}.s^{-1}$ compared with PF_2 and PF_1 flushes. At saturating light intensities, the youngest leaves (PF_1) showed statistically significant higher photosynthetic rates than both PF_2 and PF_3 older leaves. The photosynthetic difference between PF_1 and PF_2 became more marked from an irradiance of 100 $\mu mol.m^{-2}.s^{-1}$, showing a higher efficiency of the younger leaves to respond to higher light levels. It is interesting to note that a greater difference in maximum photosynthesis was observed between PF_1 and PF_2 than between PF_2 and PF_3 flushes. It appears that a greater decline in maximum (light saturated) photosynthesis occurs during ontogenetic changes of leaves from PF_1 to PF_2 ages (from younger to middle age) and thereafter the decline becomes less marked with more advanced age of the leaves. This suggests that a mature cocoa leaf is able to maintain a good photosynthetic activity, although at a lower rate, for a relatively long part of its life. In fact, a decrease of around 29% from PF_1 to PF_3 is quite low, considering that PF_3 leaves can be averaging about 170-180 days old. Nevertheless, in a seedling in the field, the potential total photosynthetic contribution of these older leaves would be markedly reduced as a consequence of mutual shading within the canopy, when increasing numbers of leaves are formed above then. From other experiments,

Figure 3.3 - Light response curves of net photosynthesis for mature leaves of different ages. Sampled leaves were leaf 3 from each flush. Inset (a) shows detail around origin of large graph. Axes unit as for main graph.
Means with different letters are significantly different at P = 0.05 (Tukey).

Figure 3.4 - Light response curves of transpiration for mature leaves of different ages. Sampled leaves as in Figure 3.3.


Photosynthesis x10⁻⁵g CO₂.m⁻².s⁻¹
Light intensity (μmol. m⁻².s⁻¹)
PF1
PF2
PF3
a
b
b
(a)


Transpiration
(g H₂O x10⁻³.m⁻².s⁻¹)
Light intensity (μ moles. m⁻² s⁻¹)
PF 1
PF 2
PF 3

(data shown in Chapter 4 on carbohydrate balance), PF_3 leaves were estimated to receive a mean light intensity of 122 $\mu mol.m^{-2}.s^{-1}$. From Figure 3.3 photosynthesis at this light intensity was some 26% below that of the maximum photosynthetic potential of leaves from PF_3 at saturating light intensity. Thus the effective total decrease in photosynthetic contribution of these PF_3 leaves can be equivalent to around 55% of the maximum photosynthesis of young leaves (PF_1). This means a considerable reduction of the contribution of older leaves in terms of photosynthate for the whole plant. In this way, although a leaf can have a relatively long functional life, it has its photosynthetic performance considerably reduced due to its position within the canopy and consequently its usefulness as a supplier of photosynthate for other parts of the plant.

From line-intercepts (Figure 3.3a) it is possible to see a very slight decreases in dark respiration (y-axis) and light compensation points (x-axis) as leaves age. These changes, however, will contribute very little to the observed decrease in net photosynthesis of older leaves, since the latter was so much higher than night respiration. Decrease in dark respiration with leaf age has also been observed for other species (Constable and Rawson, 1980a; Hardwick et al., 1968). The slightly lower rates of dark respiration observed here for older leaves may be related to lower metabolic requirements for the maintenance of leaf structure and function in mature leaves.

Changes in transpiration rate with increase in light intensity followed a similar pattern to photosynthesis (Figure 3.4), i.e. there was a consistent increase in transpiration with increasing

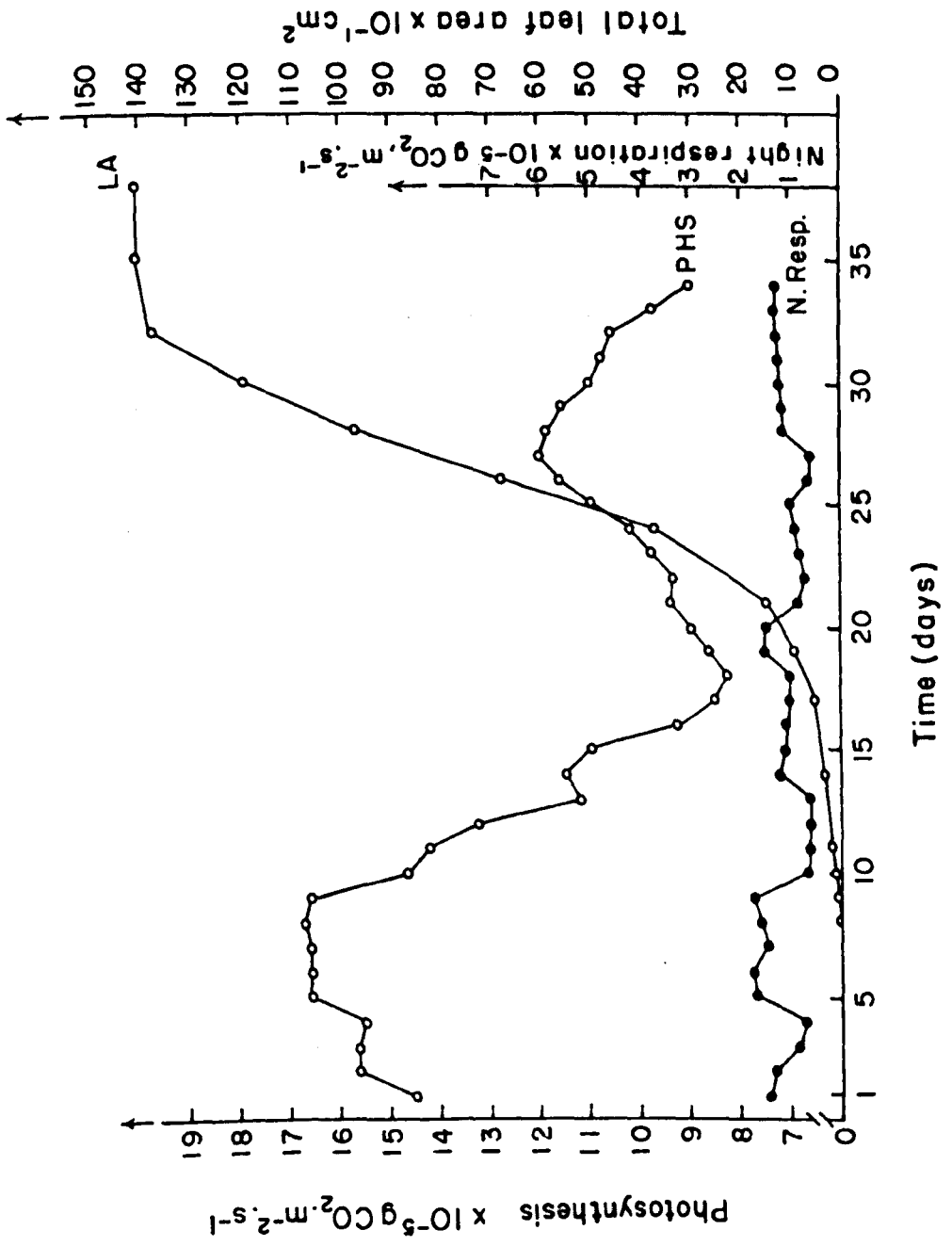
irradiance up to a maximum value. It is observed that about 90% of maximum transpiration rate was reached at 200 $\mu mol.m^{-2}.s^{-1}$ for all the leaves. Maximum rates of transpiration also decreased with leaf age, but decline in transpiration was less marked than that in photosynthesis. Decline in photosynthesis from PF_1 to PF_3 was about 29%, while in transpiration it was only around 13%. It is also noted that leaves with higher photosynthetic rates also maintained higher rates of transpiration. A significant positive correlation ($r = 0.98$) between photosynthesis and transpiration was obtained for all mature leaves. Since photosynthesis and transpiration were positively correlated, and older leaves showed lower rates of transpiration, clearly, the photosynthetic rates of these leaves were reduced, at least in part, by reduction in stomatal aperture. The observation here is very similar to the finding of Hutcheon (1976) that reduction of photosynthetic rate of cocoa with leaf age, was largely parallel to the rise in stomatal resistance. Increases in stomatal resistance and reductions in rate of transpiration with leaf age, have also been reported for a number of species (Aslam *et al.*, 1977; Davis *et al.*, 1977). The fact that lower rates of transpiration were obtained for older leaves at saturating irradiance indicates that stomatal opening in old leaves was never equivalent to the maximum of young leaves even at very high irradiance. This indicates that the capacity of stomata to respond to changes in light is reduced in older leaves. These results in total show that the decline in photosynthetic efficiency as the leaves age, is closely correlated with stomatal resistance, although other "internal factors" are also involved in the reduction of leaf

photosynthetic capacity.

3.2.2.3 - Photosynthetic performance of mature leaves during the development of a new flush - Changes in rates of photosynthesis and night respiration of a leaf from the PF_1 flush during development of a new flush are shown in figure 3.5. No increase in photosynthesis or in night respiration was observed during the development of the new flush. In contrast, a considerable drop in photosynthesis of mature leaves was noted during the initial phase of rapid leaf expansion (F2 stage). This is quite different from the situation reported for many other species in which photosynthesis is enhanced by increased carbohydrate demand and rates of carbohydrate removal from source leaves to developing organs (Ho, 1979; Harold, 1980; Mondal et al., 1978). As observed by Sleigh (1981) and subsequently shown in this study in Chapter 4, a considerable percentage of the ^{14}carbon assimilated by mature leaves was imported by expanding leaves at the F.2 stage. This means that a high proportion of photosynthate is removed from mature cocoa leaves during the F.2 stage and thus a corresponding increase in photosynthesis in mature cocoa leaves could also be expected on this basis and on the basis of findings for other species (Mondal et al., 1978; Mayoral et al., 1985; Neales and Incoll, 1968; Thorne and Koller, 1974). Clearly, this demonstrates that carbon requirements for growth, at least of leaves, do not appear to control directly the photosynthesis of mature cocoa leaves.

In regard to the drop in photosynthesis of mature leaves during new leaf development (Figure 3.5) it is possible that this represents ontogenetic changes within the leaves, since at the end

Figure 3.5 - Net photosynthetic (PHS) and night (dark) respiration (N. Resp.) rates of leaf 3 from PF_1 flush during the development of a new flush of leaves.
Total area of leaves within new flush = LA line.


Photosynthesis x 10⁻⁵g CO₂.m⁻².s⁻¹
Night respiration x 10-5 g CO₂, m⁻²s⁻¹
Total leaf area x 10⁻¹cm²
Time (days)
LA
PHS
N.Resp.

of the experimental period these leaves were five weeks older. Nevertheless the most marked decrease in photosynthesis by mature leaves was observed during the initial phase of new leaf blade expansion (F.2 stage) when the mature leaves were still relatively young. This marked drop is more difficult to explain. It is possible that part of this drop had been caused by an adverse effect on the leaf of inclusion in the assimilation chamber for a relatively long time. However, from day 18 onwards, photosynthetic capacity of the leaf recovered very significantly, so that by 27 days the overall drop was only around 28%. The phase of recovery occurred throughout the phase of major leaf expansion of the new flush. The conclusion must be that, although the chamber may have had some effect, there was also a significant effect caused by the events of the flush cycle. It is possible that the most marked fall in photosynthesis may be, in part, caused by withdrawal of metabolites other than carbohydrates from mature leaves to the developing flush of leaves. Results on chemical composition of cocoa leaves (Santana and Igue, 1979) showed that total nitrogen levels decreased significantly in mature leaves during the development of a new flush. This decline was attributed to an increased mobilization of this element to expanding leaves. It is possible that, in this study also, nitrogen mobilization may have occurred and caused a decrease in leaf nitrogen levels, which may involve protein/enzyme reductions, and which in turn may have depressed the photosynthetic capacity in mature leaves. The recovery of the photosynthetic rates may represent a relief of the nitrogen stress as a result of cessation of the nitrogen mobilization by expanding leaves when they have synthesised most of their cell protein. The late part of the

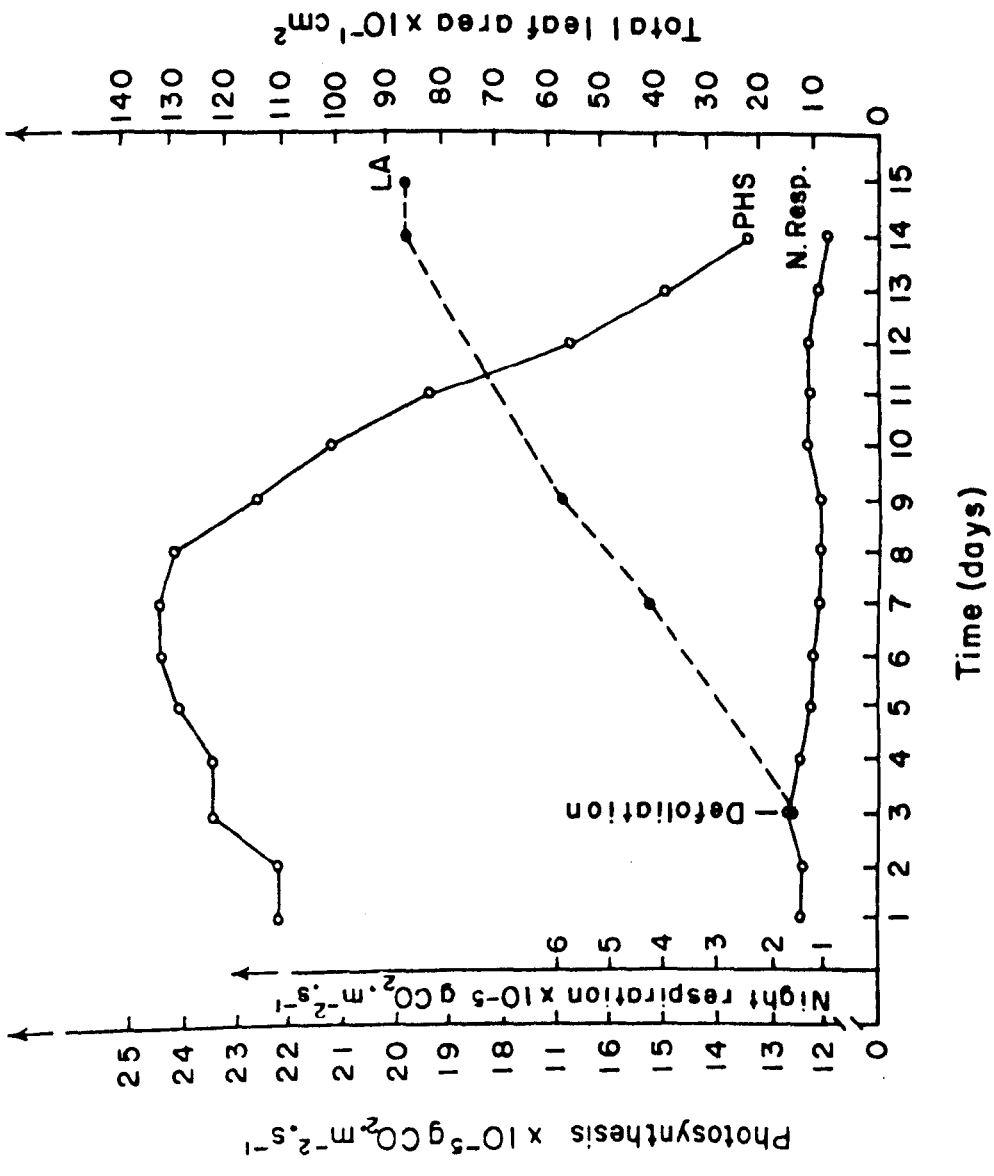
phase of leaf expansion, which coincides with the recovery of photosynthetic capacity, involves mostly cell vacuolation and cell wall extension involving mainly water uptake and cellulose synthesis not protein synthesis. Similar decreases in photosynthesis of adjacent and nearest leaves to a fruiting site, during boll growth, have also been observed in cotton (Constable and Rawson, 1980a). These authors suggested that, in all probability, the bolls constituted a sink for nitrogen and withdrawal of this from the leaves could have depressed photosynthesis. Further discussion of the reasons for the change in photosynthetic capacity of cocoa leaves is reserved until the results of protein and chlorophyll determinations are presented in the next section, for here too it will be seen that there are significant changes in these components during the flush cycle.

For dark respiration, no major changes occurred in mature leaves during the development of the new flush of leaves. There was only a tendency of dark respiration to decline during the period over which photosynthesis dropped, with no recovery to initial rates. Presumably, this reduction in respiration with leaf age indicates some changes in metabolic activity as the result of ontogenetic changes of the mature leaves, but no specific effect of the flush cycle.

Rates of photosynthesis and night respiration of a PF_1 leaf following mature leaf defoliation at F.2 stage, are shown in Figure 3.6. No increase in photosynthesis was observed in the one remaining source leaf after defoliation, i.e. even with a much increased carbohydrate sink demand in relation to source. A marked

Figure 3.6 - Rates of net photosynthesis (PHS) and night respiration (N. Resp.) of the remaining PF_1 source leaf following mature leaf defoliation day 3.

LA shows the total area of developing leaves within the new flush.


Photosynthesis x 10⁻⁵g CO₂.m⁻².s⁻¹
Night respiration x 10⁻⁵ g CO₂.m⁻².s⁻¹
Total leaf area x 10⁻¹ cm²
Time (days)
LA
PHS
N. Resp.
Defoliation

decline in photosynthesis of the mature leaves was again observed during the expansion of new leaves. These results differ from those found for some other species (Hodgkinson, 1974; Wareing et al., 1968) in which enhanced photosynthesis occurred in remaining leaves following a partial defoliation treatment. As mentioned in the introduction to this chapter, it has been suggested that increased photosynthetic rates (in leaves remaining after partial defoliation) of bean, maize, willow and pine resulted from increases in the level of the carboxylating enzymes RUBISCO and phosphoenol pyruvate carboxylase (Wareing et al., 1968). There was no direct evidence from results here to relate the reduction in photosynthesis of mature cocoa leaves to any changes in level of the RUBISCO enzyme. It has been observed in other species that this enzyme level is related to nitrogen levels in the plant, i.e. low enzyme levels being associated with low nitrogen levels and thus with much reduced photosynthetic rates (Osmond et al., 1980). As mentioned above, nitrogen is reported to be mobilized from mature cocoa leaves to expanding leaves, and this could lower the nitrogen level in mature leaves. Therefore a lower nitrogen level in the mature leaves could affect the RUBISCO levels and consequently the photosynthesis. Clearly, there was a pronounced decrease in photosyntehtic rates during part of the new flush leaf expansion. Further studies are urgently required in cocoa on levels of RUBISCO in mature leaves during the flush cycle.

3.3 Protein and Chlorophyll contents in mature leaves

3.3.1. Materials and methods

Plants for these determinations were grown in the glasshouse under conditions described in Chapter 2. All the plants had three or four previous flushes and were about eight months old. The experimental period was 16 weeks with weekly determinations of protein and chlorophyll contents of leaves from three previous flushes (PF_1, PF_2 and PF_3 as diagram in Figure 2.1). For each determination of protein or chlorophyll one disc of 1.8 cm diameter was taken from two leaves of each previous flush from each of the four plants. Each leaf constituted a replicate sample per plant and each plant an experimental unit.

For protein determinations, leaf discs were homogenised in 5% (W/V) trichloracetic acid (TCA) with a glass mortar and pestle. The homogenate was centrifuged at 1000 g for five minutes. The supernatant was decanted and the pellet containing the precipitated protein washed four times with 5% (W/V) TCA, to guarantee complete removal of any TCA soluble compounds which could interfere with the colorimetric determination of the protein. The pellet was treated with 5 cm^3 of 1 M sodium hydroxide solution (NaOH), at room temperature, for three hours during which time it was shaken continuously in order to fully solubilise the protein. The mixture was centrifuged at 1000 g for five minutes, the supernatant collected, and the pellet washed twice with 2cm^3 of 1 M sodium hydroxide solution. The supernatant and washings were pooled and then made up to 10 cm^3 standard volume and the protein content

determined by the method of Lowry et al., (1951). Bovine serum albumin (fraction V) was used to produce the standard protein calibration curve.

Chlorophyll determination. - Chlorophyll was extracted in 80% acetone in water using the method of Arnon (1949).

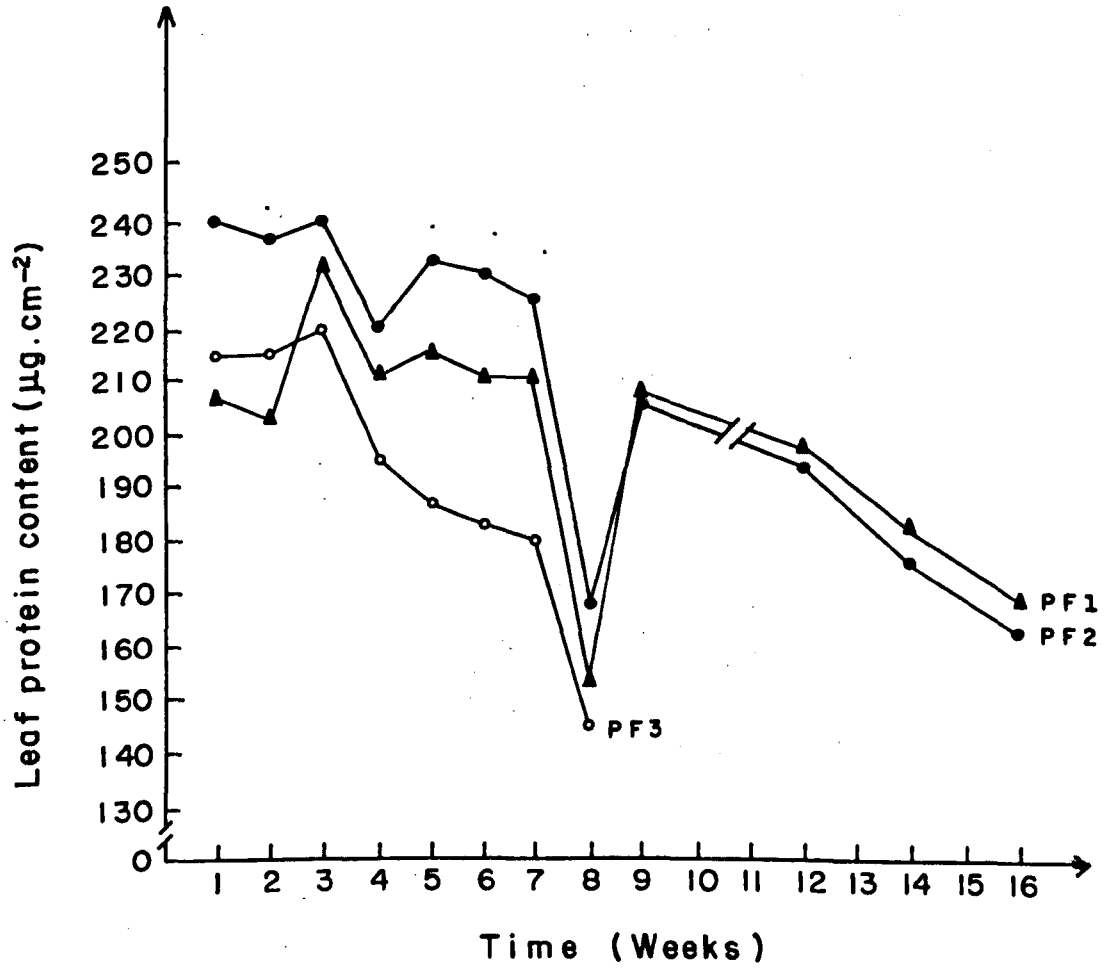
Both protein and chlorophyll contents are expressed in μg per unit leaf area (cm^2).

3.3.2 Results and discussion

Changes in the protein contents of mature leaves of several ages are shown in Figure 3.7. Each point on the graph represents the average of eight determinations. Over the whole period of the experiment there is a trend of reduction in protein content, amounting to approximately 19% in PF_1, but over 30% in PF_2 and PF_3 flushes. There is a particularly large decrease in leaf protein between the third and fourth weeks in each of the older flushes (PF2 and PF_3). Between weeks 4 and 5 there was a recovery in protein content for PF_1 and PF_2 flushes, but not the oldest flush (PF_3). Levels then stayed fairly constant in the youngest flush until week seven. The decline in protein content between weeks 3 and 4 occurred when young leaves of new flushes were in initial phases of expansion in each plant (average three leaves per flush). Between weeks 7 and 8 there was another sharp fall in protein with a significant recovery between weeks 8 and 9 for PF_1 and PF_2, after which time, levels continued the gradual decline. After week 8 the leaves of PF_3 were becoming unevenly yellow and senescing rapidly, thus it was not possible to take further reliable samples.

The second period of fall in leaf protein coincided again with

Figure 3.7 - Changes in protein content per unit leaf area of leaves from three mature flushes (PF_3 had reached advanced senescence by week 8).


Leaf protein content (µg.cm⁻²)
250
240
230
220
210
200
190
180
170
160
150
140
130
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (Weeks)
PF1
PF2
PF3

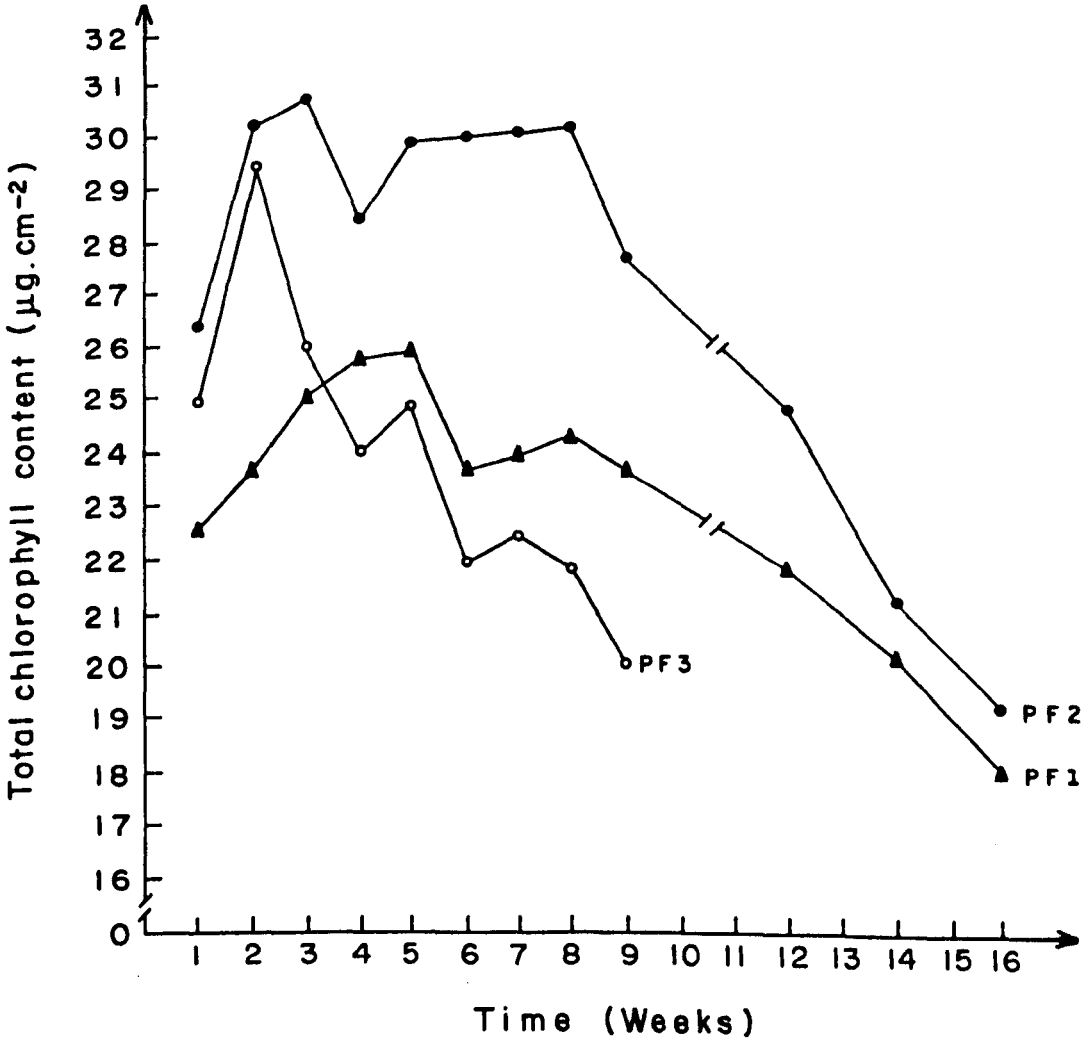
development of a new flush of leaves by each plant. It is observed that this second phase of rapid decrease was more pronounced than that during the fourth week. Probably, this is perhaps partly because the sampled leaves were now older and less vigorous, but also, and perhaps more significantly, at this time each plant produced more new flush leaves (four or five leaves) than in the first period of rapid protein loss around the fourth week. These changes in protein content in mature leaves are likely to be causally associated with the development of new leaves. It is appropriate to recall that a similar decrease also occurred in photosynthetic rates of mature leaves during development of a new flush (section 3.2.2 of this chapter). Clearly, these phenomena are associated. From this result it is difficult to speculate about the causes of the rapid drop in leaf protein during new flush growth. Nevertheless, an export of nitrogenous compounds, as amino acids, from mature leaves to developing leaves is a plausible explanation. As mentioned in section 3.2.2, total nitrogen level has been shown to decrease in mature cocoa leaves during the development of a new flush, and it was suggested to be due to a mobilization of this element by developing leaves which represent strong sinks for metabolites (Santana and Igue, 1979). If nitrogenous compounds, as amino acids, are translocated from mature leaves during the new flush growth, then reduction of the size of amino acid pools (in mature leaves) could cause lowered rates of protein resynthesis, and thus lowered levels of protein in these leaves. This probability is further strengthened by the finding of a recovery of protein levels in mature leaves during the later part of leaf expansion, when size

increase is due mainly to cell vacuolation and cell wall development not cytoplasmic increases. Generally, the decline in protein content of leaves has been suggested to result, primarily, from a reduced rate of synthesis as a consequence of amino acids being exported to other parts of the plant (Wareing and Phillips, 1978). The work of Thomas (1983) suggests that the remobilisation of nitrogen from older vegetative tissue is one of two possible sources of nitrogen for growth of the younger tissues.

Clearly, the progressive decrease in protein content beyond week 9 is related to changed metabolic activity associated with the normal leaf ageing process. It is well established for many other plants species that protein content declines gradually when the leaf is ageing (Friedrich and Huffaker, 1980; Hardwick and Woolhouse, 1967; Thomas and Stoddart, 1980; Wareing and Phillips, 1978). The reduction in protein content in senescing leaves has been demonstrated to result from the activity of proteolytic enzymes (Peterson and Huffaker, 1975).

Data in Figure 3.8 show changes in total chlorophyll content from the same leaves used for protein determinations. As for protein, the total chlorophyll also declined gradually with time. Slightly sharper decreases in chlorophyll were observed during the fourth week, especially in leaves from the PF_3 (oldest) and PF_2 flush (middle aged). From week 8 to 9 there was a further sharper fall in chlorophyll in PF_2 and PF_3 leaves. However the relative fall in chlorophyll at these times is much less than in the case of protein, and, unlike the situation with protein, there was no recovery in chlorophyll level after the eighth week. Clearly, changes in chlorophyll levels of leaves are not closely related to

Figure 3.8 - Changes in chlorophyll content per unit of leaf area of leaves from three mature flushes.


Total chlorophyll content (µg.cm-2)
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (Weeks)
PF3
PF2
PF1

production of new flush leaves. It is concluded therefore that changes in chlorophyll found here are the result of the normal leaf ageing process.

As discussed in section 3.2, decreased rates of photosynthesis were also observed in mature leaves when a new flush was developing. The associated reductions in leaf protein and photosynthesis, when a new flush develops does appear to indicate that a stress occurs within the seedling during the stage of higher demand by developing leaves. Indeed the reduction in photosynthesis may be a consequence of loss of protein in the form of photosynthetic enzymes. Although photosynthetic rates and protein content recovered, to some extent, they did not reach the values observed before the decline during the new flush development. It seems that the normal processes of senescence of mature leaves are accelerated each time a new flush develops. Thus, the contribution of a mature leaf, as provider of photosynthate for the seedling, may be sharply changed during the development of a new flush of leaves. However, due to problems of providing sufficient replications when estimation destroys leaf material, and the differences between plants in basic protein levels, it cannot be considered that a full understanding of protein composition of cocoa leaves during several flush cycles is yet available. A more comprehensive study of protein change in cocoa leaves and its relationships with change in photosynthesis is still required. The study should particularly include investigations of the changes in levels of individual proteins especially photosynthetic enzymes.

The assessment of photosynthetic capacities of leaves of all

ages on seedlings has thus been completed and it is now appropriate to move to examine the photosynthate export and import characteristics of leaves before finally rounding the discussion of the possible carbohydrate control of cocoa leaf development.

Chapter 4

Translocation of Carbohydrate and Carbohydrate Balance of a cocoa seedling through a flush cycle

4.1 Introduction

It was shown in Chapter 3, that mature cocoa leaves do not respond photosynthetically to increased utilization of photosynthates by a new developing flush of leaves. Developing cocoa leaves also have a very low photosynthetic capacity (Baker, 1974) which makes them net import organs for photosynthate during the major part of their developmental phase. It is possible, therefore, that it may become limiting to the several leaves which develop simultaneously in a flush at the apex. Work in Chapter 2 showed that smaller leaves which develop towards the end of a normal flush can grow larger without the competitive influence of early leaves. This is strongly suggestive of a stress developing throughout the development of one flush. Since no compensatory photosynthesis was observed in mature leaves during a new flush development, carbohydrate limitation may be the major candidate for the stress factor. In order to aid an understanding of how carbohydrate may control flush growth, this chapter is concerned with studies on patterns of carbohydrate translocation in cocoa seedlings throughout complete flush cycles.

The terms "sink" and "source" have been largely used to denote, respectively, the regions of consumption and production of assimilates. In this work, "sink" is used to describe,

specifically, developing leaves as regions of net carbohydrate consumption and "source" for mature leaves as regions of net carbohydrate production.

In general, the pattern of assimilate distribution in a plant is determined by characteristics of photosynthesis and by the relative proximity of the several sinks, although this may be modified by the source-size and pattern of vascular connections (Evans, 1975). In a situation where various sinks are competing for a limited supply of assimilates, each sink has a certain competitive power whereby it "pulls" or attracts assimilates against the effects of other sinks (Gifford and Evans, 1981; Starck and Ubysz, 1974; Wareing and Patrick, 1975). Thus, under conditions of limited supply, the relative magnitude of a sink may be exaggerated by its competitive strength (Evans, 1975). In accordance with the definition of Warren-Wilson (1972), "sink-strength" is the product of the sink size and "activity", "sink-activity" being the apparent power of the sink to utilise assimilates for its metabolism and growth (Wareing, 1972). It is known that a rapidly growing sink generates a steeper (than a weakly growing/competiting sink) gradient of assimilate concentration in adjacent sieve-tubes, which leads to flow from more distant sources (Gifford and Evans, 1981). This has been observed in some species (Robinson *et al.*, 1980) and also in cocoa seedlings (Sleigh, 1981), where developing leaves showed high sink strength while that of the roots became relatively weak. The metabolic activity of the sinks has therefore a great effect on the distribution pattern of assimilates (Shiroya *et al.*, 1961), but it is less clear how sinks can also control the rate of

export from the source leaf (Ismail and Sagar, 1981). Indeed, the outflow of assimilates from a source leaf depends also on factors within the leaf itself (Fondy and Geiger, 1982; Wardlaw, 1968). For example, export of sucrose from source leaf to sink can be controlled in part by: (a) photosynthetic rate and partition of fixed carbon between sucrose and starch; (b) the rate of sucrose synthesis; (c) intercellular transfer of assimilates to phloem regions and (d) phloem loading (Giaquinta, 1983). On the other hand, the relative level of import into young expanding leaves (sinks) depends on their stage of development, proximity to source leaves and, in some instances, on vascular connections between source and sink (Shiroya et al., 1961; Wardlaw, 1968; 1980). Young leaves undergoing the most rapid expansion are generally the most active importers (Larson and Dickson, 1973).

In a very young leaf, the tips of the lamina import more assimilate than the leaf base, but with continued expansion of the leaf-lamina, the pattern of transport of assimilates gradually changes becoming higher into the leaf-base compared with leaf-tips (Leffer, 1980). The leaf-tip is also normally the first portion of the lamina to acquire a CO_2-fixing capacity, with the parallel production of carbohydrate and finally loss of sink activity (Turgeon and Webb, 1975; Larson and Dickson, 1973). Eventually, this stage (developing of CO_2-fixing capacity), is marked by transition of the leaf from an importer to an exporter organ. In most species, developing leaves commence net export of assimilates at between one-third to one-half of their final size (Leffler, 1980;

Thrower, 1962; Wardlaw, 1968). However, this is not a universal pattern, since in cottonwood for example, the export capacity of the developing leaves does not plateau at half-maximum size, but continues to increase for some time thereafter (Leffler, 1980). In marked contrast, in cocoa leaves, net export does not commence until the leaves have reached their full size (Baker, 1974).

Despite the loading capacity, it has been postulated for some species that the active loading of the phloem occurs in minor veins creating a turgor pressure, and that unloading at the sink creates a region of reduced osmotic pressure, resulting in a concentration gradient (Fisher, 1978; Hanson and West, 1982; Housley and Fisher, 1977). Phloem unloading has received little attention. Some evidence has recently been presented indicating that phloem unloading does not occur by a passive leakage of solutes, but that it occurs by an energy-dependent and possibly carrier-mediated process (Bennett et al., 1984; Thorne and Rainbird, 1983; Wolswinkel and Ammerlaan, 1983).

In recent years, many studies involving ^{14}carbon-labelling have been used in attempts to determine and understand the patterns of distribution of assimilates and source-sink relationships with the aim of demonstrating the importance of sinks in the regulation of photosynthate distribution (Borchers-Zampini et al., 1980; Fondy and Geiger, 1980; Thorne and Koller, 1974). In some species the rate of assimilate transport does clearly respond to source-sink manipulation by defoliation or shading (Hanson and West, 1982; Wyse and Saftner, 1982); the general response being an increasing rate of assimilate translocation as the sinks are increased relative to the

sources (Fondy and Geiger, 1980). Many investigators have also attempted to identify points of restriction or regulation of photosynthate movement at the sink end of the translocation path (Koch, 1984), and phloem unloading has been considered as one point of possible control (Fondy and Geiger, 1980; Gifford and Evans, 1981; Thorne, 1982). However other mechanisms and regulatory processes have also been indicated in a number of species (Pharr et al., 1985; Fader and Koller, 1983). Factors such as age and position of source leaves, as well as vascular connections, have also been considered to play an important role in determination of photosynthate translocation (Lloyd, 1980; Murray et al., 1982; Koch and Avigne, 1984).

From considerations of species in general, it has been suggested that upper leaves predominantly supply photosynthate to the shoot apex; lower leaves to the roots and leaves at intermediate positions can supply in both directions (Ashley, 1972; Canny, 1984; Satoh, 1974; Shiroya, 1968; Wardlaw, 1968). For species showing flush behaviour, which have to support several simultaneously and rapidly developing leaves, and especially for cocoa in which developing leaves photosynthesise very little, there have been very few studies concerned with the movement of ^{14}carbon-photosynthate and yet understanding of such in this case will be particularly important to any understanding of the regulation of flush growth. The distribution of ^{14}carbon-photosynthate within young cocoa plants was followed by Adomako and Hutcheon (1974), but the study was aiming to determine the effect of infection with Cocoa Swollen Shoot

Virus (CSSV) on carbohydrate accumulation in the shoot, and the apical bud was previously removed to prevent flushing. Cannel and Huxley (1969) studying the distribution of ^{14}carbon-labelled assimilate in Coffea arabic, found that the developing leaves were a strong sink for carbohydrate. Some evidence also indicated that movement of assimilated ^{14}carbon into the shoot tip of tea plants was much lower in the dormant period than when the shoots were active in leaf production/expansion (Sanderson and Sivapalan, 1966).

Preliminary observations by Sleigh (1981) with cocoa, indicated that upper leaves, (younger mature leaves) did translocate predominantly to the developing flush leaves, and the oldest mature leaves mostly to the roots. Unfortunately however, Sleigh did not assess the situation for all stages of the flush cycle, nor for leaves at intermediate ages. It is therefore necessary to obtain detailed information about patterns of carbohydrate translocation: i) from differently positioned leaves on the plant and throughout the flush cycle to have an appreciation of the contribution of individual leaves as exporters of photosynthate to support the developing flush and; ii) from control and manipulated plants, during F-2 stage, to see whether leaves are operating below or at their maximum transport potential, during a "normal" flush development.

A new technique, using an external (to the leaf) monitoring system for radio-isotopes was developed to allow determination of continuous patterns of export over several days, both for individual mature leaves, and also for patterns of import into developing leaves. This procedure also permitted investigation of

export:import patterns when partial defoliation treatments were made. This allowed determination of whether maximal rates of loading and unloading were being utilized in normal flush development. In order to investigate whether the possible limitation in carbohydrate availability during new flush development was conditioned by limitations of export (source loading), or import (unloading) and/or by the competition effects of the several simultaneously developing leaves in a flush, and to quantify the source-sink relationship, several experimental approaches were followed involving investigations of:

1. **Determination of patterns of carbohydrate export** -(a) from leaves of all flush ages at each stage of the flush cycle, followed for 42 hours after $^{14}CO_2$ feeding to allow comparison of export rates during "day" and "night" conditions; (b) In view of the interesting results obtained from this study, patterns of export from leaves of plants maintained under extended dark and light periods were subsequently followed.
2. **Loading capacity in source leaf** - In order to investigate the hypothesis that limitation of carbohydrate supply was involved in the control of the flush cycle, it was necessary to examine the loading capacity for carbohydrate in the source leaves to see whether loading was operating at maximum rates under normal flush development. Loading capacity was inferred from rates of reduction in photosynthetically ^{14}carbon-fixed products in source

leaves. It was considered that removal of source leaves, except the fed one, would increase the carbohydrate demand from this leaf. Comparison of ^{14}carbon export from control and source-leaf-defoliated plants would show whether the source leaf had the potential to load (and export) ^{14}C-photosynthate faster than the rate shown under normal high demand during the F-2 stage.

3. **Unloading capacity in sink (developing) leaves** - for similar reasons to those given above, it was also necessary to determine whether flush leaf development was limited under normal development either by the unloading capacity (import) into individual leaves, or the competitive effect of other adjacent leaves. Comparison of import into one leaf of a new flush with other developing flush leaves intact and with a proportion of developing leaves removed was considered a suitable method to provide data to answer the question.

4. **A carbohydrate balance sheet** - Here the quantitative relationship between i) total current carbohydrate production capacity of seedlings, and ii) consumption of carbohydrate throughout a flush cycle were calculated. To enable this balance sheet to be calculated, data from Chapter 3 on photosynthetic capacity were combined with information from export and import characteristics of leaves as determined in this chapter.

4.2 Translocation of ^{14}Carbon-Photosynthate

4.2.1 Materials and Methods

All the plants used in these experiments were grown in a glasshouse as described in Chapter 2. To allow for adaptation from glasshouse to laboratory conditions, plants with three or four flushes were taken to the laboratory at least 24 hours before starting each translocation experiment. For studies on export patterns of photosynthate during the flush cycle, the third produced leaf from each of the three previous flushes (PF_1, PF_2, PF_3) was selected at each stage of the flush cycle (For nomenclature see Table 1.1 in Chapter 1). Previous flushes were denominated from the top to base of the seedlings, as PF_1, PF_2 and PF_3; referring to these as the youngest expanded flush, middle and oldest flush respectively (Diagram, Figure 2.1 in Chapter 2). Each selected leaf was exposed to ^{14}carbon-dioxide as described later in this section.

In order to provide information about loading capacity (export capacity), through an increased sink-source ratio treatment, plants were defoliated and exposed to ^{14}carbon-dioxide. Intact plants constituted a control. Plants for this series of experiments were selected for ^{14}carbon-labelling at the stage of the flush cyle where high photosynthate translocation to the new expanding flush was expected, i.e. mid-F.2 stage. In these experiments all the mature leaves (sources) were removed, except the one to be labelled with ^{14}carbon. One set of these plants was defoliated about two hours before the ^{14}carbon-labelling and another set, at six hours after

the ^{14}carbon-dioxide feeding. The ^{14}carbon-dioxide fed leaf was always the third leaf from a flush.

In experiments to characterise the basic patterns of carbohydrate translocation in dark and light periods, two sets of plants were exposed to ^{14}carbon-dioxide and then, one set was maintained in extended darkness and the other one in an extended light period. For these experiments, plants were selected at a stage when there was expected to be a relatively low demand for carbohydrate by the shoot apex, i.e. F-1-stage, so that any modifications to the normal light and dark export characteristics caused by leaf development did not complicate the picture.

In all the experiments, the petiole of the selected leaf was inserted through a slot in the wall of a perspex box, which was then closed, and made airtight with a sealing compound (Figure 4.1). A small Petri-dish within the box held 0.2 cm^3 of an aqueous solution of sodium (^{14}C)-bicarbonate, which provided a total radioactivity of 37 MBq. Three cubic centimetres of lactic-acid were injected through a "Suba-Seal" stopper and into the Petri-dish to release ^{14}carbon-dioxide from the bicarbonate solution. The plant was left under illumination for one hour to allow photosynthetic fixation, and then 5.0 cm^3 of 1 M sodium-hydroxide solution were injected through the "Suba-Seal" stopper into a second Petri-dish in the box to absorb any excess $^{14}CO_2$.

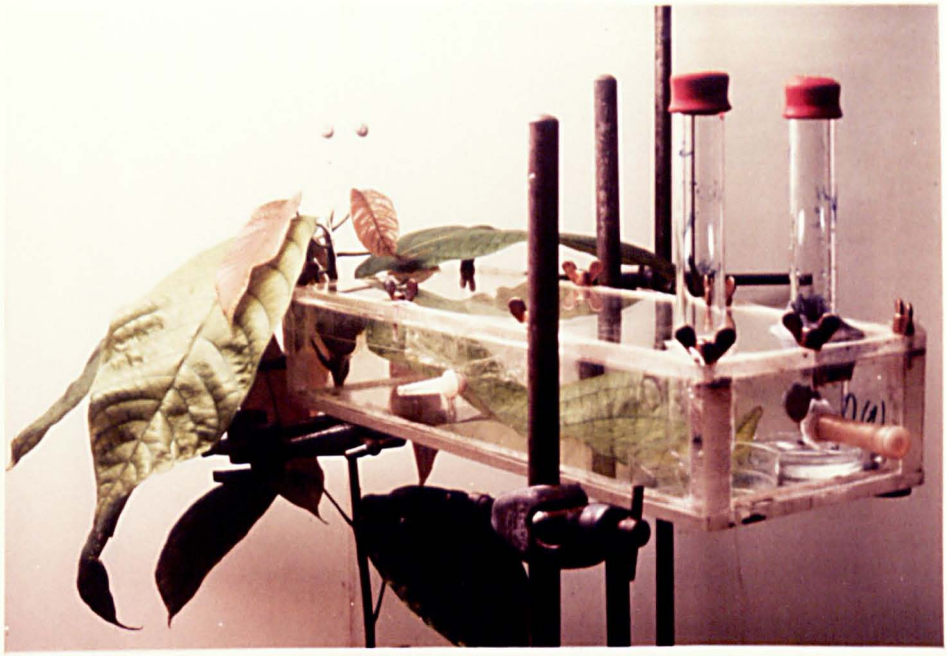
Throughout the period of ^{14}carbon-dioxide feeding and determination of translocation patterns, plants were sited in a fume-cupboard for radiation-safety reasons. They were illuminated by Warm-White fluorescent tubes which gave an irradiance at the fed

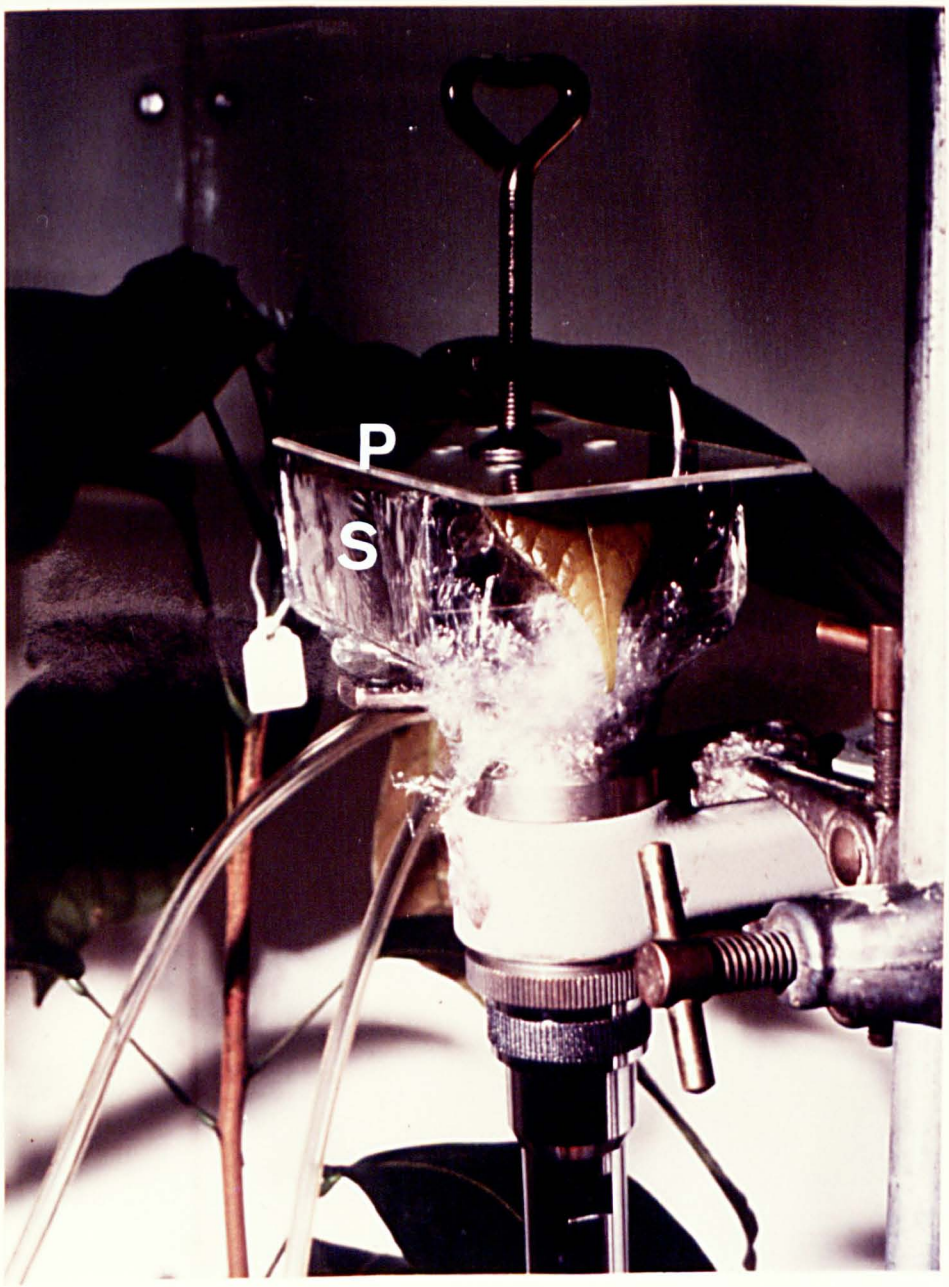
Figure 4.1 (a) - Leaf of cocoa seedling enclosed in a perspex box for exposure to ^{14}carbon-dioxide.

(b) - Monitoring of ^{14}carbon activity import into a developing leaf.

S = Head of gas flow ^{14}C counter, with cling-film covering.

P = Perspex retaining guide.




P
S

leaf of about 237 umol.m^{-2}.sec^{-1}. Temperature was 23±2°C. Unless otherwise stated a 12 hours day:12 hours night regime was maintained. Leaves were always fed with ^{14}carbon-dioxide at the same time of day to avoid any possible complications which might arise from circadian rhythms in photosynthesis and translocation within plants.

The movement of assimilated ^{14}carbon from leaves was estimated by continuously monitoring the surface of leaves attached on the plants with an external measuring system for radio-isotopes based on 2π gas-flow counters from a Berthold Radiochromatogram Scanning System attached to a rate-meter and chart recorder. The counting gas was methane with a flow rate through the counting heads of $5x10^{-3}$.cm^3.min^{-1}. The slit size (leaf area exposed to the counter) was approximately 3.0 cm^2 and it was separated from the leaf with a single layer of domestic "Cling-film". This monitoring was normally maintained on a leaf for up to 48 hours after feeding ^{14}carbon-dioxide to allow two light and dark cycles each of 12 hours duration.

Three replicate plants were used for each treatment in all the experiments. Data are expressed as percentages of the ^{14}carbon activity (c.p.m.) remaining in the fed leaf and the ^{14}carbon translocated out of the fed leaf was determined as:

$$^{14}C_t = {}^{14}C_f - {}^{14}C_r$$

where: $^{14}C_f$ = amount of ^{14}carbon fixed and detected immediately after the fed leaf was removed out of the box and attached to the

counter (100%).

$^{14}C_r$ = amount of remaining ^{14}carbon in the leaf at any time of the succeeding experimental period.

$^{14}C_t$ = amount of lost ^{14}carbon from the leaf at each time.

4.2.2 Results and Discussion

4.2.2.1 Carbohydrate export throughout the flush cycle

A general picture of the pattern of translocation of assimilated ^{14}carbon exported by the third leaf, in each stage of the flush cycle, is shown in Figure 4.2. Data represent average values for experiments from leaves of each flush PF_1, PF_2 and PF_3 with rates averaged over six hour periods. The general forms of the export curves, at each stage of the flush cycle, show that export of labelled carbon by the fed leaf was continuous throughout 42 hours after feeding, although export rates differed in this period. The fastest export rates occurred during the first 17 hours. Thereafter the rates declined considerably until the thirtieth hour, when there was an increase again. The second rapid phase was slower than the first one. Thus, the pattern of export throughout 42 hours shows three distinct phases of rates of movement of the labelled ^{14}carbon from the fed leaf. Distinct phases in the export of labelled ^{14}carbon from a treated leaf have also been observed in other species (Clauss et al., 1964; Farrar and Farrar, 1985; Moorby and Jarman, 1975). However, these latter observations refer to a shorter overall period than in this work. It is appropriate to note that, in cocoa leaves, the phases are determined by illumination

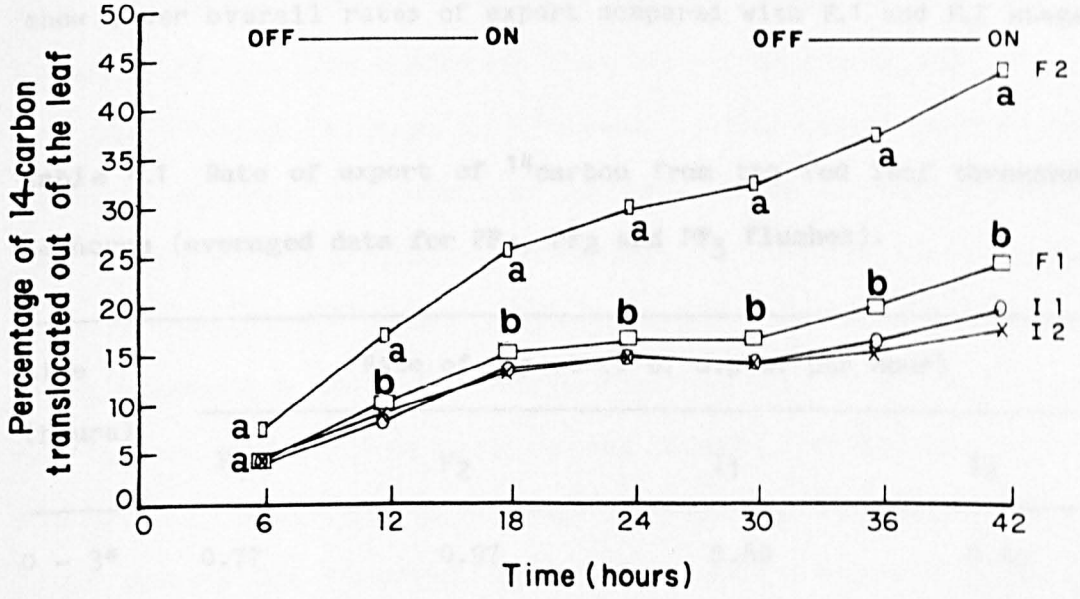
period (Figure 4.2 and 4.4 to 4.7). The highest rates of ^{14}carbon translocation out of the leaf were during the night at all the stages of the flush cycle. This high percentage of ^{14}carbon translocation overnight is strongly suggestive that much of the ^{14}carbon assimilated by the leaf, probably accumulated as leaf starch during the period of active photosynthesis and was then converted most probably into sucrose and subsequently transported during the night. This suggestion is supported by the finding of Adomako and Hutcheon (1974) in which a similar high rate of disappearance of ^{14}carbon from a $^{14}CO_2$ fed cocoa leaf was also observed during the night. These workers suggested that sucrose is the main transported sugar in cocoa, but there is no very clear evidence yet for this.

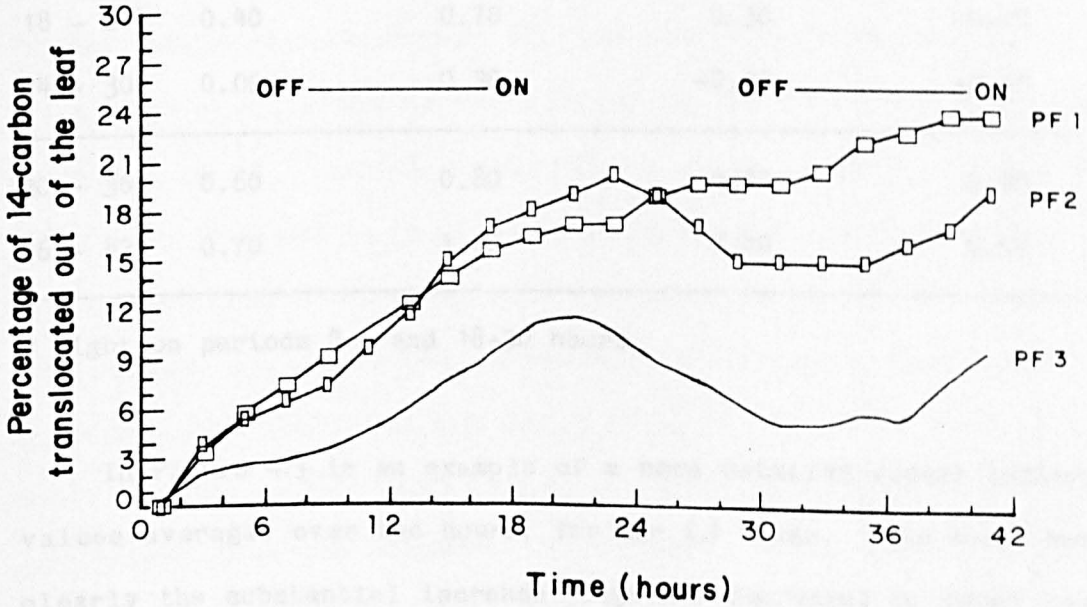
The highest rate of the ^{14}carbon translocation out of leaves was observed at the F.2 stage. This shows a rate which is significantly different statistically from the other stages of the flush cycle. No significant difference (at 0.05 of probability) was observed at six hours, although the actual percentage of export, at the F.2 stage, was higher. The higher translocation rate of ^{14}carbon out of the fed leaf at F.2 in relation to other stages, is likely to be due to the fact that during this stage the new leaves are rapidly growing, and growing cocoa leaves constitute a very strong sink for carbohydrate from the mature leaves (Sleigh, 1981).

When the results are expressed as rate of export (average percentage of reduction in ^{14}carbon per hour) very low rates are observed during the light period between 24 and 30 hours after the

Figure 4.2 - Pattern of translocation of ^{14}carbon out of mature leaves, at each stage of the flush cycle, throughout the 42 hours after ^{14}carbon-dioxide feeding. Means with different letters are significantly different at P = 0.05 (Tukey). There are no significant differences between F-1, I-1 and I-2 at any time. OFF and ON show change from light to darkness, line indicates dark period.

Figure 4.3 - An example (at I-1 stage) of "decline" in export of ^{14}carbon from the fed leaf over the greater part of the light period. OFF and ON as in Figure 4.2.


Percentage of 14-carbon translocated out of the leaf
OFF
ON
OFF
ON
F2
F1
I1
I2
a
b
Time (hours)


Percentage of 14-carbon translocated out of the leaf
OFF
ON
OFF
ON
PF1
PF2
PF3
Time (hours)

feeding (Table 4.1). These values in fact became "negative" (i.e. ^{14}carbon in leaves increased) in the I.1 and I.2 stages which do show lower overall rates of export compared with F.1 and F.2 stages.

Table 4.1 Rate of export of ^{14}carbon from the fed leaf throughout 42 hours (averaged data for PF_1, PF_2 and PF_3 flushes).

time (hours)	Rate of export (% of c.p.m. per hour)			
	F_1	F_2	I_1	I_2
0 - 3*	0.77	0.97	0.80	0.60
3 - 6	0.60	1.50	0.70	0.57
6 - 12	1.00	1.60	0.75	0.75
12 - 18	0.90	1.50	0.70	0.90
18 - 24*	0.40	0.70	0.30	0.20
24 - 30	0.00	0.30	-0.10	-0.10
30 - 36	0.60	0.80	0.20	0.40
36 - 42	0.70	1.00	0.40	0.50

* Light-on periods 0-6 and 18-30 hours

In Figure 4.3 is an example of a more detailed export pattern, values averaged over two hours, for the I.1 stage. This shows very clearly the substantial increase (negative decrease) in label over the greater part of the light period following the first dark

period. The increase in ^{14}carbon detected is greatest in the oldest PF_3 flush, much less in the mid-aged PF_2, and net export, (indicated by a continuous decrease over this period) is maintained from the youngest flush. Two phenomena thus need to be explained. First, why there is an increase in ^{14}carbon in the light period? and secondly why does the extent of increase differ so considerably in the different mature flushes? It might: i) Indicate that there is some re-import of ^{14}carbon products into older leaves, but this seems unlikely as the older leaves show very little general import of ^{14}carbon products from young leaves (Sleigh, 1981). Even if this was so it is difficult to see why it should be marked during the light period, ii) Represent import of secondary metabolites formed from the initial ^{14}carbon photosynthate, but again the magnitude, and the fact that it is greater in the oldest leaves, seems to make this also very unlikely. Maybe the explanation lies partly in the characteristics of the sensing technique. Not all of the ^{14}carbon in the monitored leaf segment will be counted with equal efficiency by a counter adjacent to one surface of the leaf; there will be "quenching" of ^{14}carbon occurring at sites of 'distant' to the counter. It might be that ^{14}carbon is originally "concentrated" into vein tissue, which may thus be sensed with relatively 'low' efficiency. If there was subsequently lateral movement of ^{14}C compounds from the veins this would cause the sensor to "see" radioactivity over a wider area and maybe produce a higher count from the "same" ^{14}carbon content. A different experiment where two sensors were placed on one leaf, one on the lower and the other directly opposite on the upper surface produced the same pattern (of

increase in ^{14}carbon in the light period). It is unlikely therefore that the finding is due to vertical rearrangement of the ^{14}carbon. It should be possible to resolve whether the increase is real by separating discs from a leaf at intervals and determining the absolute ^{14}carbon with scintillation counting. The problem still remains as to why the pattern of change is different in the flushes of different ages. Maybe export (loss) is maintained in the youngest flush because the "pull" of the adjacent sink (developing leaves) is greatest. Differences in pool sizes (current and stored) and thus accessibility of carbohydrates produced at different times for export from the different flush leaves could also be causal in these findings. The total component of carbohydrates translocated throughout the light period is, in part, formed by newly fixed carbon and in part from some stored carbohydrate which was formed during a preceding photosynthetic period. It has been suggested for other species that only a portion of the newly fixed carbon is exported immediately and that the carbon exported by a leaf consists partly of newly fixed carbon and partly of carbon from the leaf reserves (Ho, 1976; Hofstra and Nelson, 1969). Differences in the proportions of carbohydrate from the pools of current and stored fractions exported by different leaves could thus account for the findings. Clearly the pattern discovered (Figure 4.3) warrants further investigations.

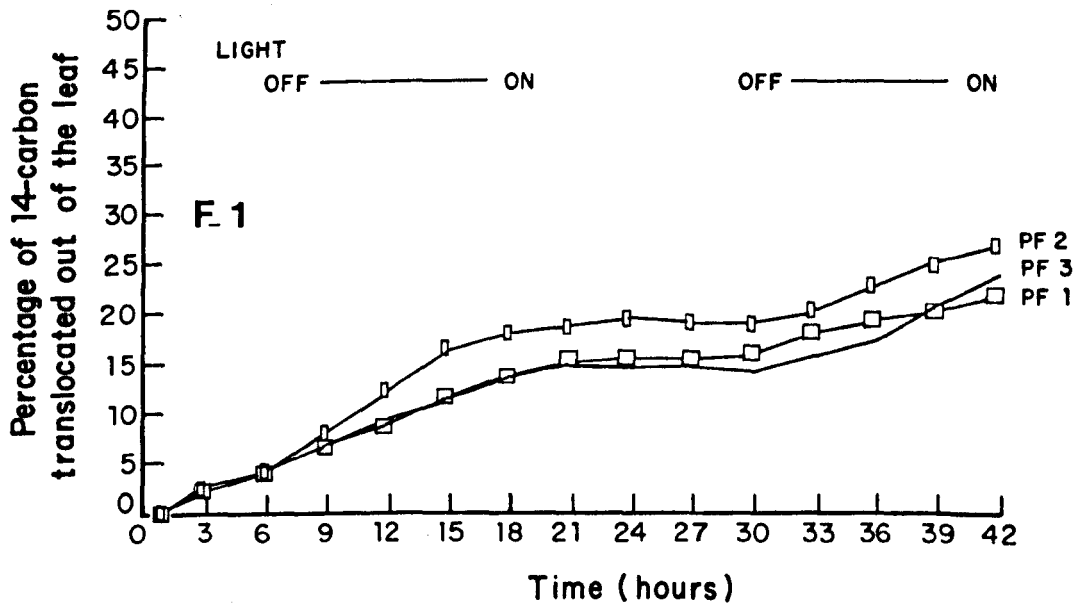
Figure 4.4 shows percentages of ^{14}carbon translocated out of the treated leaf from each of the previous flushes at the F.1 stage. The percentage of ^{14}carbon translocated out of the leaf followed a

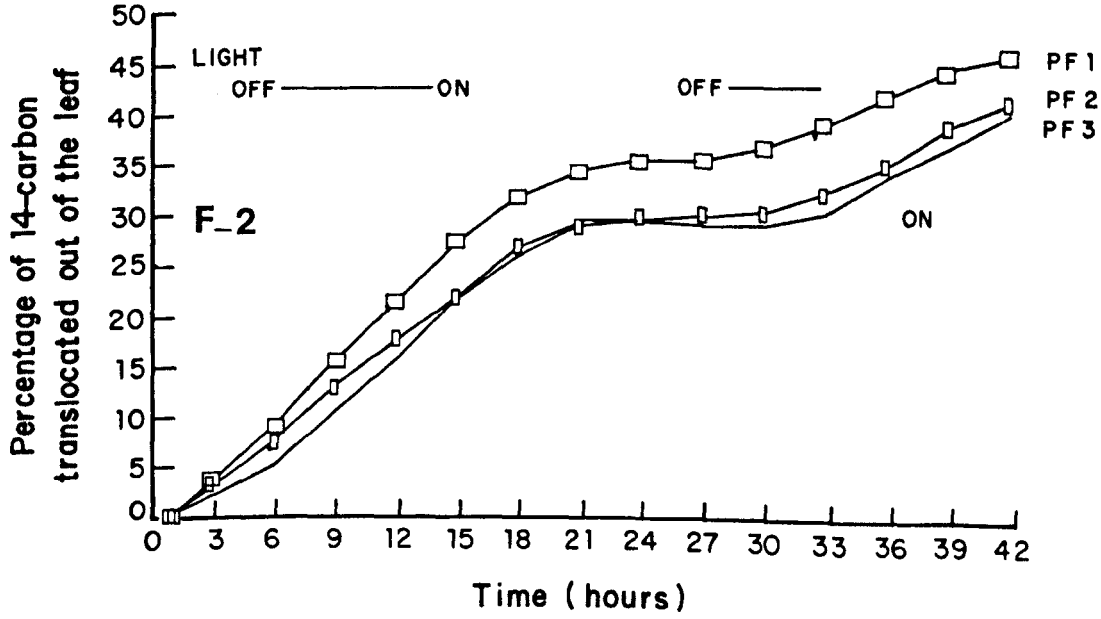
characteristic course as a function of time during the first 17 hours, when a high rate of export is observed (night period). In the following light period the rate of export from all the three flushes (PF_1, $PF_2$and PF_3) reduces. The export patterns of the labelled ^{14}carbon are very similar in all the flushes, but the middle-aged flush (PF_2) showed a slightly higher percentage of export in relation to PF_1 and PF_3 flushes. During the F-1 stage the root system is growing very rapidly (Sleigh, 1981) and the apical bud is opening to start a new flush of leaves. Thus there are two growing regions "demanding" assimilates for their growth at this stage. As mentioned in the introduction of this chapter, a general pattern of distribution of assimilates for other species establishes that the "middle leaves" of plants supply assimilates to both the shoot apex and roots (Ashley, 1972; Canny, 1984; Satoh, 1974; Wardlaw, 1968). The relatively higher export of ^{14}carbon from PF_2 flush may, therefore, be due to this flush supplying carbohydrate to two consumption sites whereas PF_1 will supply (mainly) the shoot apex and PF_3 just the roots.

The rate of fixed ^{14}carbon translocation out of the third leaf of each flush at the F.2 stages was much faster than in the F.1 stage (Figure 4.5). Curves show similar patterns to those of the F.1 stage, but with much steeper slopes. Continued rapid export raised the percentages of ^{14}carbon translocated out of the leaf in the first 18 hours, to over 60% of the total ^{14}carbon translocated in 42 hours. In F-2, higher percentages of the ^{14}carbon were moved out of all the three previous flushes, although the highest translocations were from the upper (youngest mature) flushes, which

Figure 4.4 - Percentage of ^{14}carbon translocated out of the third leaf of each flush at the F-1 stage of the flush cycle.
(Light off and on at times shown, giving 12 hours light and 12 hours dark).

Figure 4.5 - Percentage of ^{14}carbon translocated out of the third leaf of each flush at the F-2 stage of the flush cycle.
(Light off and on at times shown, giving 12 hours light and 12 hours dark).


LIGHT
OFF
ON
OFF
ON
F_1
PF 2
PF 3
PF 1
Percentage of 14-carbon translocated out of the leaf
Time (hours)
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
5
10
15
20
25
30
35
40
45
50


LIGHT
OFF
ON
OFF
ON
F_2
PF 1
PF 2
PF 3
Percentage of 14-carbon translocated out of the leaf
Time (hours)
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
5
10
15
20
25
30
35
40
45
50

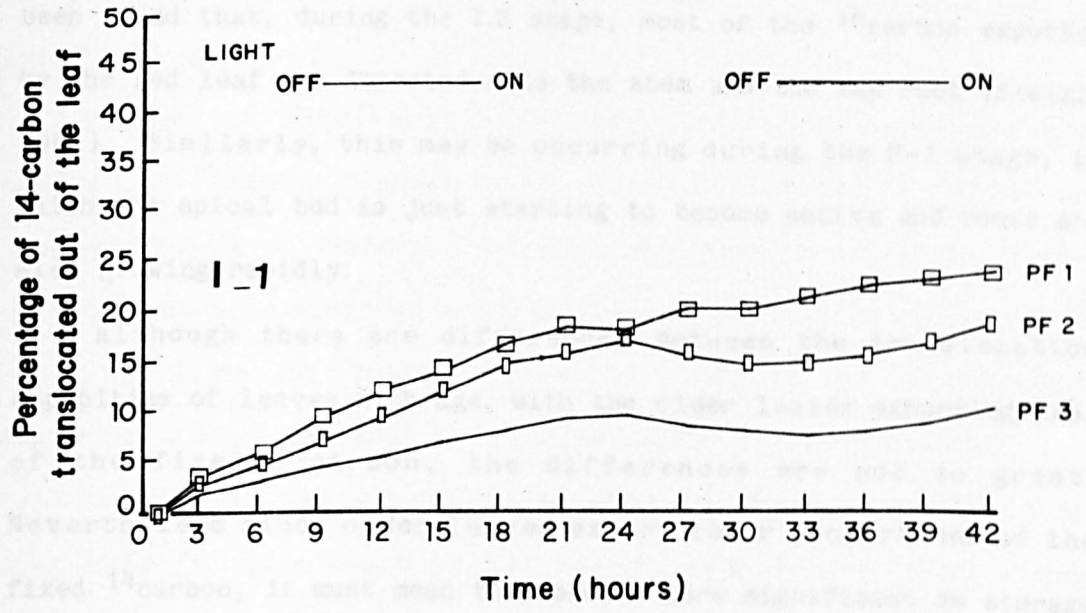
showed a (statistically) significantly greater export rate than the oldest flush (PF_3) from 12 hours onwards. This demonstrates that the youngest mature leaves are the major source of photosynthate supplied to the shoot apex during the new flush development. However, the previous flushes (PF_2 and PF_3) are also very important sources of carbohydrate for the developing flush.

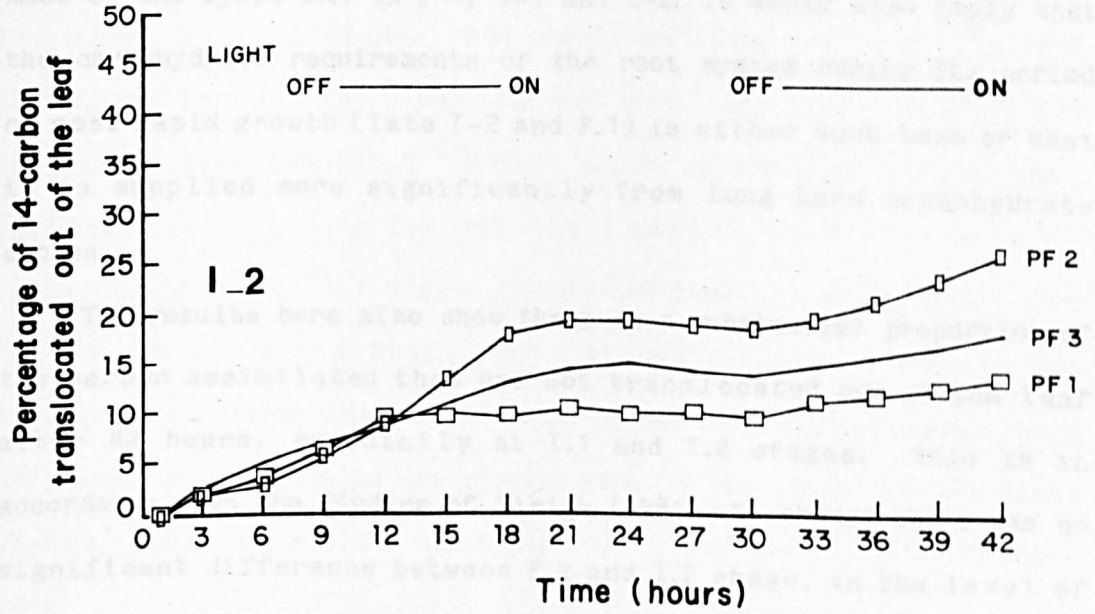
The patterns of export at I.1 stage are seen in Figure 4.6. The curves for the ^{14}carbon export rates show a very similar overall form to that at the F.1 stage. A more rapid export during darkness is observed for upper and middle flushes than for the lower flush. In general, lower levels of assimilated ^{14}carbon were exported from fed leaves during this stage, especially, from PF_3 flush. It has been suggested that the new flush leaves at the I.1 stage are still importing photosynthates from other leaves (Baker, 1974). Thus, the high rate of ^{14}carbon export from a fed leaf of the PF_1 flush, at the I.1 stage, may indicate that the flush nearest the new flush is the major supplier of carbohydrate to the new flush at this stage.

Figure 4.7 shows the patterns of export of the ^{14}carbon fixed at I.2 stage. As in the I.1 stage, low percentages of ^{14}carbon are exported out of leaves of all the flushes, but especially, from the upper flush (PF_1). A higher percentage of export was observed from the middle flush (PF_2). It is noted that the pattern of export from this flush is very similar to that of the corresponding positions in the F.1 stage (Figure 4.4). The F.1 is the subsequent stage to I.2 and it appears that the pattern of distribution of the ^{14}carbon

Figure 4.6 - Percentage of ^{14}carbon translocated out of the third leaf of each flush at end I-1 stage of the flush cycle.
(Light off and on at times shown giving 12 hours light and 12 hours dark).

Figure 4.7 - Percentage of ^{14}carbon translocated out of the third leaf of each flush at I-2 stage of the flush cycle.
(Light off and on at times shown giving 12 hours light and 12 hours dark).


LIGHT
OFF
ON
OFF
ON
I_1
PF 1
PF 2
PF 3
Percentage of 14-carbon translocated out of the leaf
Time (hours)
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
5
10
15
20
25
30
35
40
45
50


LIGHT
OFF
ON
OFF
ON
I_2
PF 2
PF 3
PF 1
Percentage of 14-carbon translocated out of the leaf
Time (hours)
0
3
6
9
12
15
18
21
24
27
30
33
36
39
42
5
10
15
20
25
30
35
40
45
50

established at I.2 was continued through to the F.1 stage. It has been found that, during the I.2 stage, most of the ^{14}carbon exported by the fed leaf was imported into the stem and the tap root (Sleigh, 1981). Similarly, this may be occurring during the F-1 stage, in which the apical bud is just starting to become active and roots are also growing rapidly.

Although there are differences between the translocation capacities of leaves with age, with the older leaves exporting less of the fixed ^{14}carbon, the differences are not so great. Nevertheless since older leaves export lower proportions of the fixed ^{14}carbon, it must mean they become more significant as storage sites.

The major finding in this section is the very major change in the total translocation at the different stages of the flush cycle; highest translocation being at F-2 when the new flush leaves will be consuming most carbohydrate. This shows that the export potential of cocoa leaves of all ages is much above that operating throughout most of the cycle i.e. in F-1, I-1 and I-2. It would also imply that the carbohydrate requirements of the root system during its period of most rapid growth (late I-2 and F.1) is either much less or that it is supplied more significantly from long term carbohydrate stores.

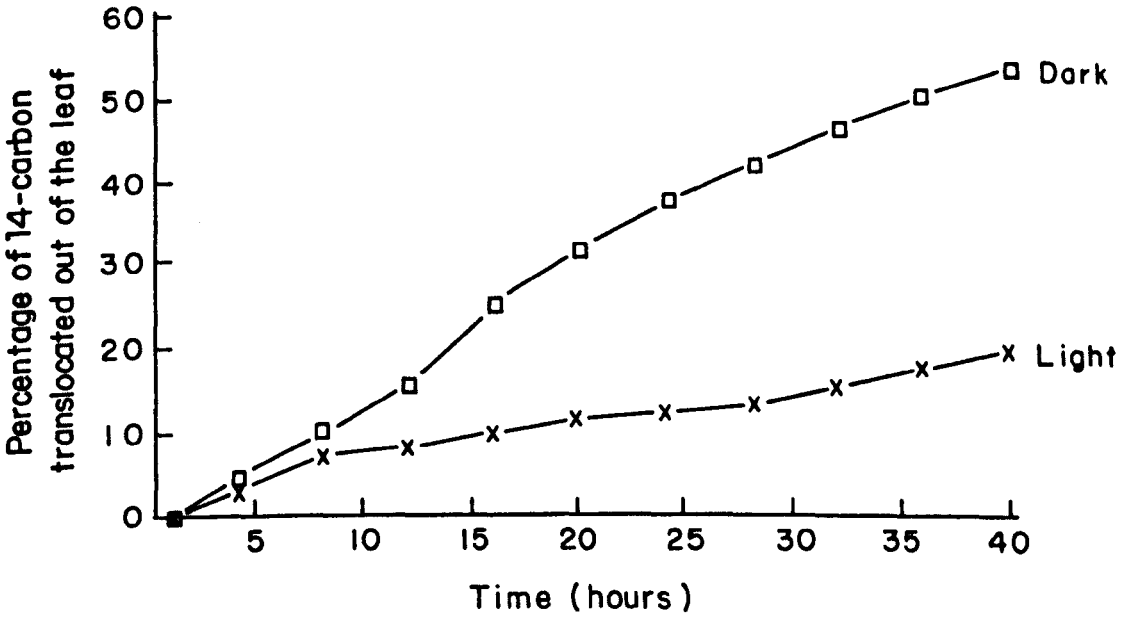
The results here also show there is a substantial proportion of the carbon assimilated that was not translocated out of the leaf after 42 hours, especially at I.1 and I.2 stages. This is in accordance with the finding of Sleigh (1981) who showed there was no significant difference between F.2 and I.2 stage, in the level of

non structural storage carbohydrate, although the level was lower at the F.2 stage.

Figure 4.8 shows results of experiments to determine the pattern of ^{14}carbon transport out of the fed leaves of plants in continuous dark or light for extended periods after feeding. These experiments were aimed at showing whether the differing rates of export in light and dark were directly caused by the illumination regime or a consequence of a circadian rhythm in translocation.

The fed leaves of plants maintained in extended darkness continued to show faster rates of ^{14}carbon export than those maintained in the light. During the period of extended darkness, about 53% of the fixed ^{14}carbon had been depleted by 40 hours, compared with only 20% in illuminated leaves. It is appropriate to say that these plants (in both light and dark treatments) were at the F.1 stage, at which it is found that fed leaves in normal light:dark rotations export around 24% of the fixed ^{14}carbon over 40 hours, representing a translocation response to a low sink demand of the shoot at this stage. The continued high rate of ^{14}carbon translocation by the leaf in extended darkness is strongly indicative that the fixed ^{14}carbon which accumulates in the leaf, probably as starch during the light period, is broken down and translocated out of the leaves in the darkness. This is clear from Figure 4.8 where the higher rate of export can be seen in the results from plants kept in darkness after the initial seven hours of light, in relation to the plants kept in the light for the whole period. The higher rate of export of ^{14}carbon in the dark than

Figure 4.8 - Percentage of ^{14}carbon translocated from the fed leaf of plants in extended light and dark periods.


Percentage of 14-carbon translocated out of the leaf
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
Time (hours)
Dark
Light

light is not, unlike the situation with defoliated plants discussed below, related to any difference in sink demand; since the compared plants were at the same developmental stage. This indicates that whilst the sink demand has a very important role in the regulation of translocation (Figure 4.5), carbon metabolism within source leaves also plays an important role in determining translocation patterns of mature cocoa leaves. If sucrose is the sugar translocated in cocoa, as mentioned above, presumably the partitioning of the fixed carbon between sucrose and leaf starch may be an important factor controlling translocation rates of mature leaves. It has been postulated that, in sucrose translocating plants, an increased export may be achieved by altered partitioning of fixed carbon between leaf starch and sucrose at the expense of the starch, without any alteration in photosynthetic rate (Pharr et al., 1985).

Since the rate of export of fixed ^{14}carbon was maintained at a high level throughout the extended dark period, this indicates that illumination status as well as sink demand at the apex can control export from a source leaf. In continuous dark, total ^{14}carbon export over 40 hours was around 53% of total ^{14}carbon fixed in the source leaf compared with around 20% export over the same period with normal light:dark alternation. The differences in export rate from a source leaf are thus due to the light:dark regime and not to an endogenous circadian rhythm. This is confirmed by the export in extended light which is again approximately linear over 40 hours but at a much lower rate, which is more typical of the light phase in the light:dark alternation situation.

To summarise, the results discussed above show that percentages of the labelled photosynthate translocated out of fed leaves varied considerably with the stage of the flush cycle. The highest rates of export were shown by all leaves at the F-2 stage, which corresponds to the phase of very high demand for carbohydrate by the shoot apex. It is valuable, however, to emphasise that even at the F.2 stage, when the percentage of export of current photosynthates has increased by 17% from interflush phases (I.1 and I.2) a high percentage of the ^{14}carbon fixed (around 68%) was still retained in the fed leaves 24 hours after the leaves were exposed to ^{14}carbon-dioxide. This percentage is much higher than that shown by other C_3 plants, which are considered to be low efficiency exporters of fixed ^{14}carbon, e.g. soyabean 46%; tobacco 50%; tomato 40% and castor bean 40% export within 24 hours after the fixing (Hofstra and Nelson, 1969). Cocoa leaves are therefore of low to very low efficiency in the translocation of the labelled photosynthates, at least, during the seedling phase.

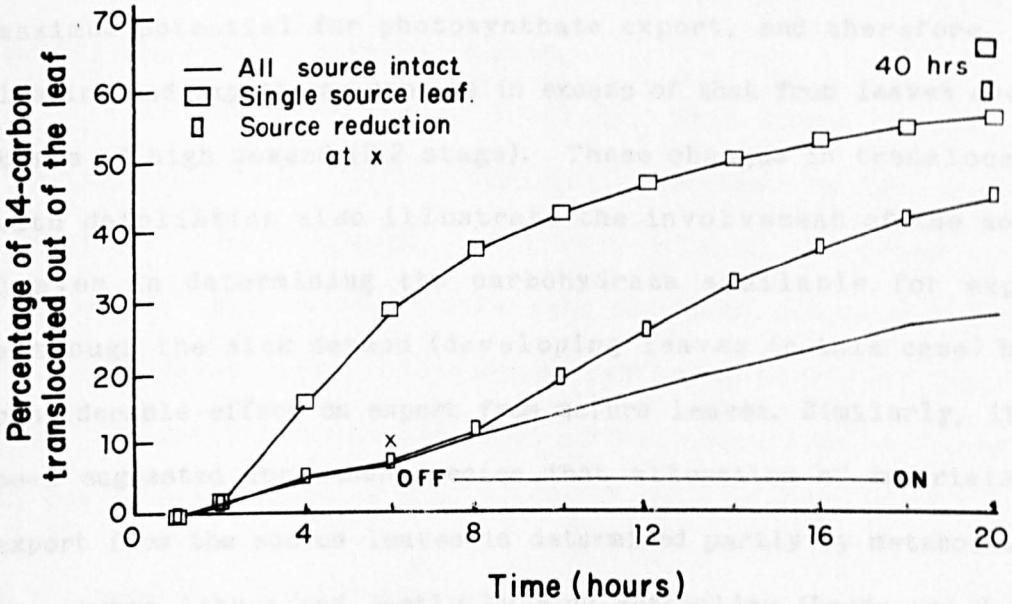
4.2.2.2 Loading capacity in source leaves

Figure 4.9 shows the export of assimilated ^{14}carbon from fed leaves when various other source leaves were removed. In these experiments the fed leaf was always the third of the PF_1 flush and all plants were at the F-2 stage. The control plants with no defoliation showed a very similar pattern of export to that from leaves of plants at the F.2 stage in earlier experiments, e.g. Figure 4.5 (Note that axes for Figure 4.9 are to different scales

from Fig. 4.5), where a rapid export rate is maintained throughout 17 hours after feeding. In the experiments with defoliation all plants were in a phase of high demand for carbohydrate by a rapidly growing new flush of leaves at the shoot apex. Defoliation of all mature source leaves except the fed one, six hours following fixation, produced a rapid change in pattern and rates of export of the assimilated ^{14}carbon very soon after defoliation. A distinctly different shape of export curve is observed in the plants with total defoliation two hours before feeding, to leave just the ^{14}carbon-dioxide fed leaf as the only source leaf. There is however a very similar export pattern of the fixed ^{14}carbon of both intact and defoliated (before feeding) plants between the first and second hours after the ^{14}carbon-dioxide fixing. This indicates that the defoliation treatment had no immediate effect on the export rates. It is therefore presumed that a similar pool of ^{14}carbohydrate was readily available in leaves of each treatment to support this early translocation. After the second hour export from the source leaf of the previously defoliated plants becomes extremely high.

The much higher rates of ^{14}carbon export from leaves of plants defoliated before $^{14}CO_2$ feeding demonstrate, clearly, that the remaining source leaf could significantly increase the export rate of the assimilated ^{14}carbon when the source "strength" was reduced relative to that of the sinks (which remained unchanged in the experiments). However, the plants with later defoliation (six hours after the ^{14}carbon-dioxide fixing) showed a slightly lower rate of export after defoliation than the plants with previous defoliation. Similar findings have been reported for a number of other species,

Figure 4.9 - Percentage of ^{14}carbon translocated from the fed leaf of intact and defoliated seedlings. Line above x-axis shows dark period. Separate points at upper right of graph show translocation values after 40 hours.


Percentage of 14-carbon translocated out of the leaf
70
60
50
40
30
20
10
0
All source intact
Single source leaf.
Source reduction at x
40 hrs
x
OFF
ON
0
4
8
12
16
20
Time (hours)

where defoliation produced enhanced rates of ^{14}carbon export from the remaining leaves (Starck and Ubysz, 1974), and the increased rate of export has been found to be related to decreases in leaf starch or in total carbohydrate levels in the remaining source leaves (Carlson and Brun, 1984; Hanson and West, 1982).

The enhanced ^{14}carbon translocation out of the remaining leaf following defoliation shows clearly that mature leaves of cocoa, in normal situations (intact seedlings) are operating much below their maximum potential for photosynthate export, and therefore, both loading and export can operate in excess of that from leaves even at times of high demand (F.2 stage). These changes in translocation with defoliation also illustrate the involvement of the source leaves in determining the carbohydrate available for export, although the sink demand (developing leaves in this case) has a considerable effect on export from mature leaves. Similarly, it has been suggested for other species that allocation of materials for export from the source leaves is determined partly by metabolism in the source leaves and partly by sink metabolism (Fondy and Geiger, 1982; Gifford and Evans, 1981; Ho, 1978; Thorne and Koller, 1974).

In plants defoliated two hours before the ^{14}carbon-dioxide feeding, the highest rates of ^{14}carbon export were observed during the first eight hours, while in intact and later defoliated plants the highest rates began to occur only after six hours following the $^{14}CO_2$ feeding. It is clear that leaves from intact and later defoliated seedlings exported more ^{14}carbon during the dark period (between 7 and 19 hours) while previously defoliated plants exported

the major proportion in the light period. This finding supports the observations referred to above that a considerable proportion of the fixed ^{14}carbon may have accumulated into a more stable pool during the light period in the intact and later-defoliated seedlings. The gradual decline in rates of ^{14}carbon export in previously defoliated seedlings, in darkness, indicates that the rapid removal of the ^{14}carbon in the light period could have prevented or, at least, very significantly reduced accumulation of recently produced photosynthate into storage compartments which are normally used to supply night translocation demands.

It is not possible to identify, from these results, the composition of the ^{14}carbon-labelled assimilates in the leaf, and any attempt, in this sense, could be artificial. Nevertheless, the hypothesis is consistent with what has been proposed for other species, where starch and sucrose synthesis represent competing sinks for photosynthetically fixed carbon, since increased formation of one product occurs at the expense of other (Huber, 1983).

4.3 Import of ^{14}carbon-photosynthate into expanding leaves

4.3.1 Materials and Methods

In these experiments all plants used were at the F-2 stage of the flush cycle. This stage was chosen since the highest rate (and amount) of export occurred at this stage. The source leaf chosen was the third leaf of the PF_1 flush, again chosen because the PF_1 flush showed the highest export. Accordingly source leaves were fed with ^{14}carbon-dioxide and photosynthetic fixation allowed. All

details of radioactivity levels used and exposure times were as described in section 4.2.1 for export studies. Two different techniques were used to measure import into developing leaves: a) liquid scintillation counting of leaf discs, and b) "external" monitoring with the Berthold Radiochromatogram Scanners, as detailed previously in section 4.2.1 and seen in Figure 4.1.

As mentioned in the introduction of this chapter (4.1) the level of import of photosynthate into a leaf at one time will depend upon its developmental stage at that time, leaves in most rapid growth being dominant importers. Further, the import into a developing leaf from an individual source leaf will also depend upon its phyllotactic relationship to the source leaf, reflecting vascular connections. This "preferential" transport from one source leaf to a particular sink leaf will be more exaggerated when the source leaf is in the previous flush nearest (PF_1) to the new flush. In any attempts to compare import into several developing leaves of one flush from a single source leaf, equivalent to where only one leaf is exporting ^{14}carbon labelled photosynthates, it is therefore necessary to consider phyllotactic features. In order to clarify the extent of phyllotactically directed transport, import into each of the developing leaves of a flush from a single source leaf for ^{14}carbon-photosynthate, is compared between a plant with all mature (source) leaves intact and one where the ^{14}carbon-dioxide fed leaf is the only source leaf, others having been removed prior to ^{14}carbon-dioxide feeding. For these determinations the technique of liquid scintillation counting was used. To determine the ^{14}carbon photosynthate imported into developing leaves, samples were taken

from both the source leaf at one and 23 hours after feeding and from expanding leaves 23 hours after the ^{14}carbon-dioxide fixation by the fed leaf, to allow sufficient time for significant import of labelled carbon into expanding leaves. Two or three replicate discs (depending on the size of the leaf) of 1.0 cm of diameter, were taken from the middle region of the leaf blade with a sharp cork borer. Each disc was accurately weighed fresh and placed in a separate glass scintillation vial and 2 cm^3 of absolute ethanol was added. After leaving this at room temperature for 72 hours (to allow the extraction of alcohol-soluble material) the alcohol was evaporated to dryness and then 10 cm^3 of a scintillation solution, consisting of 4 $g.dm^{-3}$ of PPO (2.5-diphenyl-oxazole) in scintillation-grade toluene, added into the vial containing one leaf disc. The samples were then counted in an LKB "Minibeta" liquid scintillation spectrometer (model 1211). Corrections were made for background and for quenching as assessed by the channels ratio method (Neame and Homewood, 1974). Three replicate countings of one minute duration each were made on each sample. The ^{14}carbon activity in each sample was obtained in counts per minute (c.p.m.) and calculated in disintegration per minute (d.p.m.). Data are presented as mean d.p.m. per milligram of leaf fresh weight. The radioactivity expressed per unit weight is indicative of the relative sink "strength" and independent of the physical size of the sink, i.e. total weight or size of a particular sink (Newell, 1982).

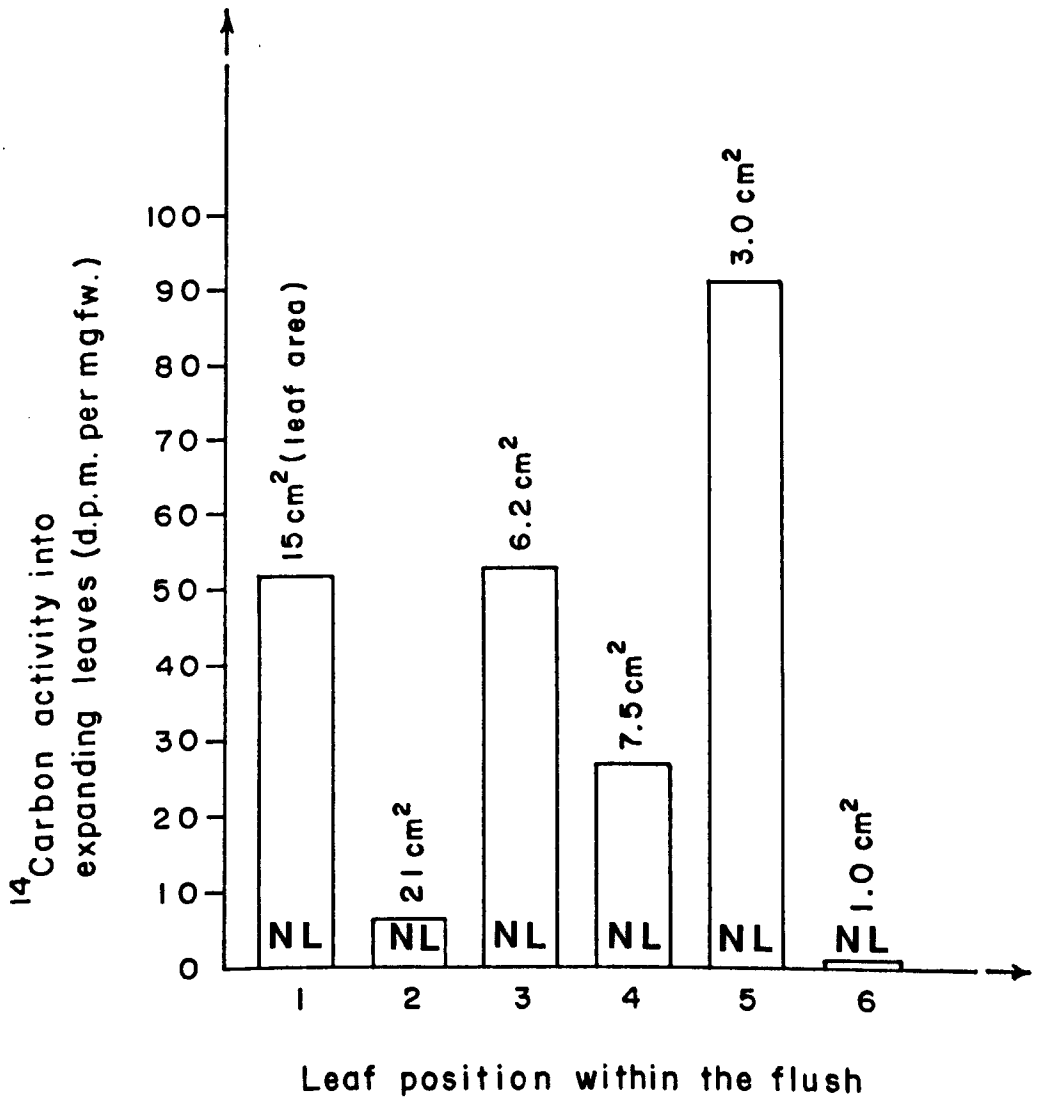
In order to provide information about the unloading capacity (import) of individual leaves within one flush, and to determine

whether there was any competition between the leaves for photosynthate, rates of import into one particular flush leaf were compared when all leaves of the flush were intact (competition possible - control situation) and when all leaves other, than the one being monitored, were removed (no competition) 23 hours after ^{14}carbon-dioxide feeding. Import into the remaining flush leaf was monitored for a further 24 hours and then a second feeding with ^{14}carbon-dioxide was made to the original source leaf. Import measurements into the remaining flush leaf were then continued for a further 24 hours. Import observations after the second feeding allowed investigation of the change in import capacity of the one remaining leaf when there was no competiton from the other leaves of the flush. These determinations were made by continuous monitoring of the ^{14}carbon labelled products arriving in the developing leaves with the Berthold Radiochromatogram Scanners attached as described in section 4.2.1 and shown in Figure 4.1b. The source leaf was fed with ^{14}carbon-dioxide in the way described previously. Import measurements were continued for a total of 45 hours.

4.3.2 Results and Discussion

Figure 4.10 shows the ^{14}carbon activity present in each leaf, from leaf 1 (oldest) to leaf 6 (youngest) within one flush, at the F.2 stage of the flush cycle, following feeding of ^{14}carbon-dioxide to the third leaf of the PF_1 flush. In this figure the data are expressed as disintegrations per minute (d.p.m.) per milligram of fresh weight of leaf tissue. It is observed that although all the leaves had received ^{14}carbon labelled products, there was no regular

Figure 4.10 - Activity of ^{14}carbon into each expanding leaf of a new flush 23 hours after the ^{14}carbon-dioxide fixation by the PF_1 source leaf. Leaf area values are given for each leaf.

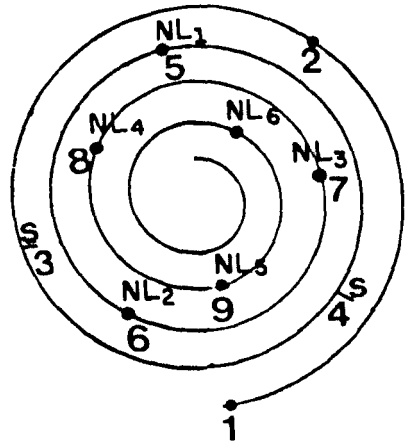

^{14}Carbon activity into expanding leaves (d.p.m. per mg fw.)
100
90
80
70
60
50
40
30
20
10
0
15 cm^2 (leaf area)
2.1 cm^2
6.2 cm^2
7.5 cm^2
3.0 cm^2
1.0 cm^2
NL
NL
NL
NL
NL
NL
1
2
3
4
5
6
Leaf position within the flush

pattern of distribution of the ^{14}carbon among the leaves, e.g. leaf 2 showed a very low ^{14}carbon activity while leaf 5 showed the highest activity within the flush. This pattern of ^{14}carbon distribution shows therefore that the source-sink relationships between cocoa leaves are complicated and there is interaction of many factors to determine an overall import capacity of one leaf. From this figure the effect on import of the position of the sink-leaf in relation to the source leaf is very clear. For example, leaf NL_5, which was in a "direct" position above the fed leaf, but separated from it by five leaves received the highest amount of ^{14}carbon labelled products, and leaf NL_2 (Figure 4.10), which was in a "lateral" position to the fed leaf, but separated from it by only one leaf, received a very small amount of ^{14}carbon products. No anatomical study on vascular connections was made in these experiments, but observations of the seedling shoots allowed identification of the positions of particular leaves in order from the fed leaf (numbered 1), those above in the production sequence being numbered 2, 3, 4 etc., on a spiral phyllotactic sequence of designation 3/8. Spiral phyllotaxy of designation 3/8 on cocoa seedlings, also observed previously by Greathouse and Laetsch (1969), was therefore confirmed.

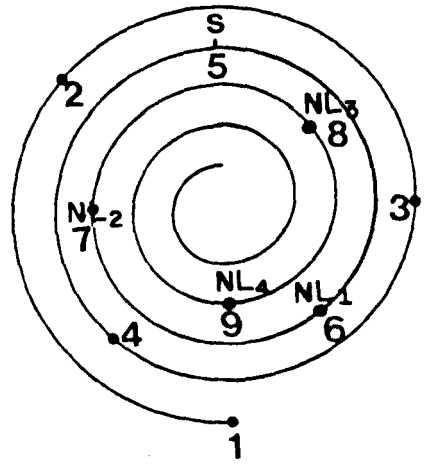
Relatively high amounts of ^{14}carbon products were imported by some leaves of Plant A in lateral positions to the fed leaf, e.g. leaf NL_3 and NL_4 (Table 4.2 and Fig. 4.11). The larger numbers on Figure 4.11 correspond to phyllotactic sequence from the fed leaf and smaller ones to position within the developing flush. Leaf NL_1

Figure 4.11 - Diagrammatic representation of spiral phyllotaxy (3/8) cocoa seedlings discussed in text.
NL numbers indicate leaf position within developing flushes, with NL_1 being the oldest/first produced leaf.
Scar (S) shows positions where spontaneous early abscision of leaves has occurred. Numbers indicate position of leaves from previous flush (PF_1).

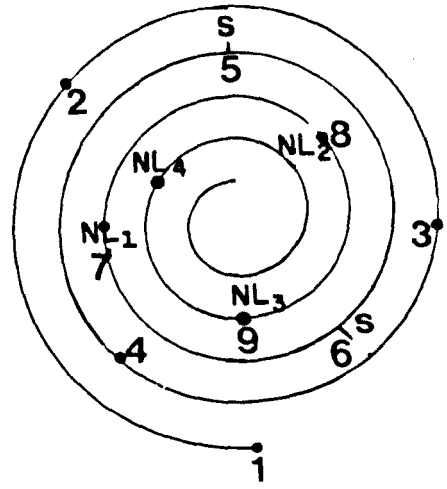
A


NL1
5
2
NL4
NL6
8
NL3
7
S
3
NL5
NL2
9
S
4
6
1

B


S
5
2
NL5
8
NL2
7
3
NL4
NL1
9
6
4
1

C


S
5
2
NL2
8
NL4
NL1
7
3
NL3
9
S
6
4
1

constitutes an exception to this pattern of import, because it was in an "opposite" position to the fed leaf, and it received a very similar amount of ^{14}carbon to that received by the NL_3 leaf which was in a lateral position. Since all developing leaves received ^{14}carbon products, it is evident that very complex conducting strands from the fed leaf may supply all the sink leaves, although it supplies preferentially that in most direct vascular connection with it, i.e. at the repeat position in the phyllotactic spiral. Similarly, effects of leaf position on pattern of assimilate distribution between developing leaves have been observed for other species (Joy, 1964; Larson and Dickson, 1973; Nelson, 1963).

Table 4.2 contains data on import of ^{14}carbon products into expanding leaves from: i) plants with all source leaves intact (Plants A and B) and ii) defoliated plants with only a single remaining source leaf (Plant C). Comparing data from columns 3 and 6, it is observed that a slightly different pattern of ^{14}carbon distribution occurs in A and B plants, i.e. the data from column 6 (Plant B) do not show such a close phyllotactic correlation between the sink leaf position and the fed leaf, as do the data in column 3 (Plant A). From column 6, higher activities of ^{14}carbon were observed in leaf NL_1 and NL_2 than in NL_4 which was in direct position above the fed leaf (Figure 4.11B). In this case (Plant B), however, leaf NL_1 and leaf NL_2 were at about 42 and 36% of their respective final areas, and thus in the very rapid blade expansion phase, while the leaves at corresponding positions on plant A (column 3 data) were still in the slow initial phase of expansion at 10 and 14% of final areas respectively. This indicates that

Table 4.2 **Leaf position, leaf area (cm^2) and ^{14}carbon activity (d.p.m. per mg f.w.) in expanding leaves 23 hours after photosynthetic ^{14}carbon-dioxide fixation by a source leaf (see also Figure 4.11).**

Position within the flush	Plant A			Plant B			Plant C		
	Position relative to fed leaf	leaf area cm^2	^{14}C	Position relative to fed leaf	leaf area cm^2	^{14}C	Position relative to fed leaf	leaf area cm^2	^{14}C
Column	1	2	3	4	5	6	7	8	9
NL_1	5	15.0	57.0	6	87.7	112.7	7	50.0	141.7
NL_2	6	21.0	6.8	7	92.8	130.3	8	31.4	96.1
NL_3	7	6.2	58.0	8	68.0	64.5	9	21.9	43.8
NL_4	8	7.5	27.0	9	27.8	80.3	10	7.0	21.9
NL_5	9	3.0	91.0	10	1.0	3.0	11	1.0	10.8
NL_6	10	1.0	1.6	None	-	-	None	-	-

destination of the ^{14}carbon products was also influenced by the developmental state of importing leaves. It appears, therefore, that leaf position has a more important role in determining distribution patterns of labelled photosynthate to developing leaves when leaves are in the early phase of development, and that predominance of sink the relative leaf:source leaf position as a determinant of distribution changes with time. When leaves in one flush reach the rapid expansion phase, they seem to predominate as importers of photosynthate from source leaves, although a clear

effect of position still remains as for the leaf NL_4 (Table 4.2). This leaf is in position nine (repeat position with the source leaf) and imports relatively more ^{14}carbon products than either NL_3 which is larger and therefore of a greater total sink size, or NL_5 which is smaller.

It is not possible from the few experiments carried out here, to characterize absolutely the relationships, in cocoa, between import of photosynthate to the different leaves of a flush and their size and position relative to a particular source leaf. This is due in part to the developmental differences already discussed between plants A and B, also because in plant A two leaves are "missing" have spontaneously abscissed leaving scars between the last PF_1 leaf and the first flush leaf, whilst there is only one miss on plant B. There are also three PF_1 leaves between the fed leaf in plant B and the first flush leaf (NL_1) but only one in plant A. Clearly to work out the absolute relationship between supply by an individual source leaf and import by particular sink leaves would require large numbers of uniform plants as well as detailed information on the system of vascular connection between leaves. Nevertheless, it is clear that import level to particular flush leaves from a single source leaf depends on its position and stage of development, and therefore, care must be taken in interpreting data on import into flush leaves from single source leaves.

The pattern of ^{14}carbon distribution among expanding leaves was completely changed by defoliation of leaves other than the fed one before the ^{14}carbon-dioxide fixation by the single remaining leaf (Plant C). Here, a regularly decreasing level of imported ^{14}carbon

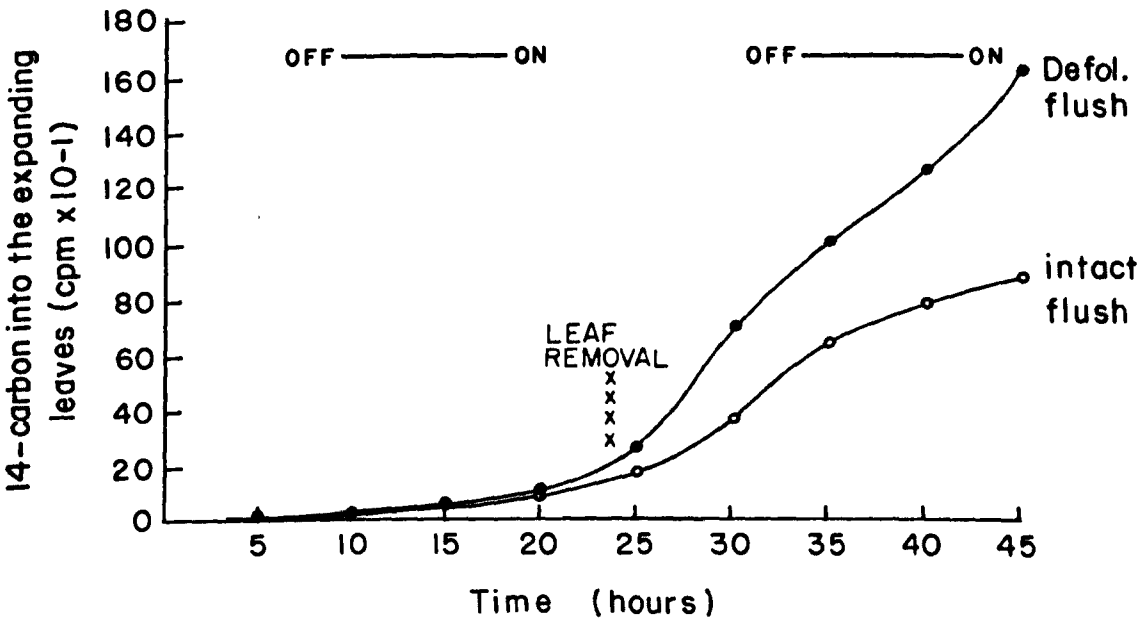
products is observed in successive expanding leaves towards the apex, leaf NL_5 being the youngest (Table 4.2). Thus, the pattern of distribution of the labelled photosynthate, among developing leaves, was clearly changed by the reduction of photosynthate supply to a single source leaf. In this case, more of the ^{14}carbon products were allocated to the earlier/nearer leaves in the flush. The finding here is supported by results found for other species, where reduced assimilate supply also affected the pattern of photosynthate distribution among sinks (Fondy and Geiger, 1980).

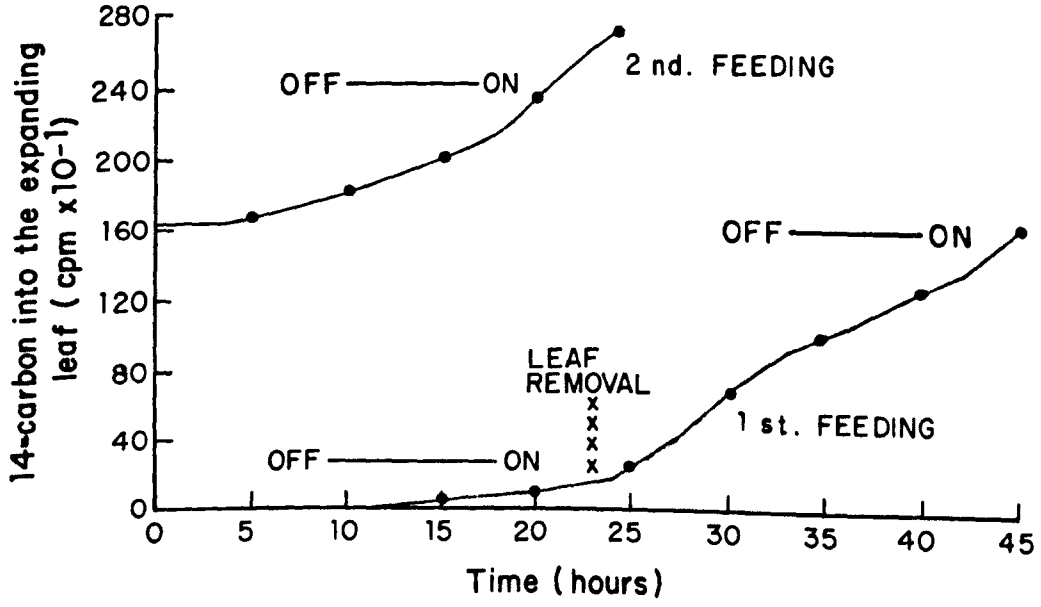
Patterns of import of the assimilated ^{14}carbon into expanding leaves, in a flush during 45 hours, are shown in Figure 4.12. From this figure (date for intact flush) it can be seen that the labelled ^{14}carbon arrived in the monitored sink-leaf (The first leaf within the flush) in detectable amounts about four to five hours after fixation of the ^{14}carbon-dioxide by the source leaf. This pattern of import, contrary to that for export, shows very low rates of change in level of label through the first 18 hours. The initial lag before import can be detected, is due to the transport time, through connecting stem etc, between source and sink leaves. Import continued throughout the remainder of the period, although slightly lower rates of import were observed during the night period. It is difficult to understand why the rates of import during the night are lower than during the day period when export trends during both the periods are the reverse, i.e. higher export at night.

Another interesting and significant feature shown in Figure 4.12 is the steeper slope of the import curve after all leaves other

Figure 4.12 - Activity of the ^{14}carbon imported into an expanding leaf from seedlings with intact and partially defoliated flush. Defoliation at 'x' after 23 hours from ^{14}carbon-dioxide fixation by a PF_1 leaf. (Light/dark changes indicated as previously described).

Figure 4.13 - Time course of the activity of the ^{14}carbon imported into the remaining developing leaf of a defoliated flush after two consecutive ^{14}carbon-dioxide feeding treatments to (24 hours apart) a PF_1 source leaf. Defoliation at 'x' after 23 hours from initial feeding. (Light/dark indicated as previously described).


14-carbon into the expanding leaves (cpm x10-1)
180
160
140
120
100
80
60
40
20
0
OFF
ON
OFF
ON
Defol. flush
intact flush
LEAF REMOVAL
5
10
15
20
25
30
35
40
45
Time (hours)


14-carbon into the expanding leaf (cpm x10-1)
280
240
200
160
120
80
40
0
OFF
ON
2 nd. FEEDING
OFF
ON
LEAF REMOVAL
1 st. FEEDING
OFF
ON
0
5
10
15
20
25
30
35
40
45
Time (hours)

than the monitored leaf, in the developing flush, have been removed (defoliated flush treatment). Comparison of the two curves of the Figure 4.12, shows the rate of accumulation of ^{14}carbon products is much faster in the one remaining leaf than in the corresponding leaf in an intact flush. The upper curve of Figure 4.13 shows that when a second ^{14}carbon dioxide feeding was made 24 hours later to the same source leaf of the plant with only one flush leaf remaining, the monitored one, the new ^{14}carbon products again arrived in the sink-leaf four to five hours after the (second) ^{14}carbon-dioxide fixation by the source leaf. (The lower curve is the same as in Figure 4.12 for defoliated flush and included here for ease of comparison with 2nd feeding curves). However the rate of import of the ^{14}carbon products into the leaf, through the following 18 hours, was much faster than that over the corresponding period after the first feeding. This higher rate of import is indicative that competition existed between leaves of the complete flush at the time of the first feeding. In the absence of inter-leaf competition, at the time of the second feeding, import into the one remaining leaf is consequently much faster. This also demonstrates clearly that a developing cocoa leaf has a greater unloading capacity than that being used when leaves of the flush are competing for (limited) photosynthate. The increased import of ^{14}carbon products into the remaining young leaf, in the defoliation treatment, is therefore strongly supportive of the suggestion that there is competition between leaves within one developing flush for carbohydrate and also that development of the flush leaves is not limited by a low photosynthate unloading capacity. The import of carbohydrate into

each leaf of a developing flush is, therefore, partially determined by the other leaves within the flush.

In summary the results presented here suggest that the photosynthate import chracteristics of sink leaves, in cocoa, are determined partly by vascular connections between particular source and sink leaves but are also strongly influenced by the state of development of the sink leaves. There is clear evidence of competition between the developing leaves of one flush for carbohydrate and that the carbohydrate unloading capacity of developing leaves exceeds that normally utilized. This means that the development of individual leaves is more likely to be limited by the amount of photosynthate available to it than by its ability to unload metabolites from the translocation system. A quantitative assessment of the sizes of carbohydrate sources and sinks in cocoa seedlings will be presented and discussed in the following section of this chapter.

4.4 Carbohydrate balance of cocoa seedlings throughout a flush cycle

4.4.1 Introduction - It has been described earlier in this thesis that developing cocoa leaves have very low photosynthetic capacity and do not become self-sufficient for carbohydrate until after leaf expansion is complete. Simultaneous development of several leaves within one flush will thus constitute a very major net consumption of carbohydrate over a period of around ten to fifteen days. It has been proposed that the cessation of leaf production in one flush may

be caused by a physiological stress developing within the seedling as a result of rapid shoot development and that carbohydrate could be a candidate for the stress component. Quite simply, during flush development, carbohydrate consumption might exceed production and leaf development be terminated by a negative carbohydrate balance within the seedling.

In earlier sections of the thesis detailed values have been determined for leaf growth rate (section 2.2.2), photosynthetic capacities of leaves of all ages on seedlings (section 3.2.2) and translocation capacities of leaves of all ages (PF_1, PF_2 and PF_3), throughout complete flush cycles. There is therefore sufficient data available to make effective estimates of carbohydrate balance, production versus consumption, for seedlings.

Averaged data from three seedlings with three mature flushes were used to construct a "typical" hypothetical seedling for balance calculations. The hypothetical seedling comprises three mature flushes PF_1, PF_2, PF_3 each with five leaves, and it is assumed to produce a new flush of five leaves with a final total leaf area of 0.09766 m^2, typical of the actual seedlings used throughout this study.

Values for carbon dioxide fixation and photosynthate translocation were used to calculate the daily carbohydrate input capacity into the new flush, and consumption was computed from leaf growth as increase in dry weight per day. Stages involved in the calculations are:

1. Carbohydrate production capacity of seedling.
2. Proportion of photosynthate exported from leaves.

3. Proportion of translocated photosynthate moving to shoot apex where new flush is developing.
4. Consumption of carbohydrate by the developing leaves.

Detailed calculations for each component are set out in Appendix 1.

The balance diagram presented in Figure 4.14 covers a 28 day period including early F-2, mid F-2 and I-1 stages of the flush cycle.

1. Carbohydrate production

To estimate carbohydrate production in the mature leaves, photosynthetic rates from figure 3.3 were utilized for the calculations. Effects of physiological ageing of leaves on net photosynthesis were taken into consideration by calculating net photosynthesis for leaves of each flush separately, and then the results were summed to give photosynthesis for the whole seedling. Daily photosynthate production was obtained from calculations of net photosynthesis during light period less night respiration.

Since leaves at different positions on the seedling stem are self-shaded to different degrees, the effect of mutual shading on photosynthesis of the whole seedling needed also some correction. Thus, light intensities were measured at several points within each flush of actual plants (PF_1, PF_2 and PF_3), with a PAR Quantum Sensor. Averaged values from several points and several plants were taken as the typical light intensity incident on each flush. From these average values of light intensity, photosynthetic rates

corresponding to a typical light intensity experienced by each leaf of each flush were determined from curves in Figure 3.3. Daily carbohydrate production in the seedling from each flush is thus corrected for leaf age and likely light intensity incident upon the flush.

From midway through the F-2 stage of the flush cycle, the new flush leaves (at approximately 77% of full expansion) produce significant amounts of carbohydrate. This is added to the production by the mature leaves in the mid and late F-2 stages to give the net total seedling carbohydrate production. Again calculations of net production per day are corrected for night respiration. The above calculations are considered to give a realistic estimate of total carbohydrate production per day of the hypothetical seedling. The values used in the balance were:

$PF_1 = 0.444$ g

$PF_2 = 0.165$ g

$PF_3 = 0.149$ g

It is now necessary to compute how much of the carbohydrate produced is exported from the leaves and what proportion of this is likely to move towards the shoot apex. Some will be likely to be retained in the stem and some transported to the roots.

2. Photosynthate export

In section 4.2, it was seen that the rate of export of the assimilated ^{14}carbon varied slightly with flush position on the

seedling. Thus export of photosynthate per day was calculated separately for each flush, and then summed for the whole seedling. Photosynthate export per day from:

PF_1 = 0.223 g

PF_2 = 0.073 g

PF_3 = 0.066 g

3. Translocation to shoot apex

This was taken to be 60% of the total exported from the mature leaves, and is based on: i) determinations made by Sleigh (1981) for distribution of ^{14}C-photosynthate from mature leaves to different parts of seedlings and ii) from results obtained earlier in this work on import into expanding leaves at the F-2 stage.

4. Carbohydrate consumption for growth

Daily amounts of carbohydrate consumed are calculated from detailed knowledge of growth rates of the flush leaves in the typical flush (Chapter 2). The increases in area have been converted to dry weight by use of a regression equation of leaf dry weight on leaf area (Appendix 1). It is then necessary to compute how much leaf dry matter will be produced by a given amount of photosynthate - assumed as carbohydrate. The value taken is that suggested by Penning de Vries (1983), and used by many workers in this type of calculation. The assumption is that 700g of (dry) vegetative tissue is formed from 1000 g of photosynthate, representing a conversion efficiency of 0.7.

4.4.2 Discussion

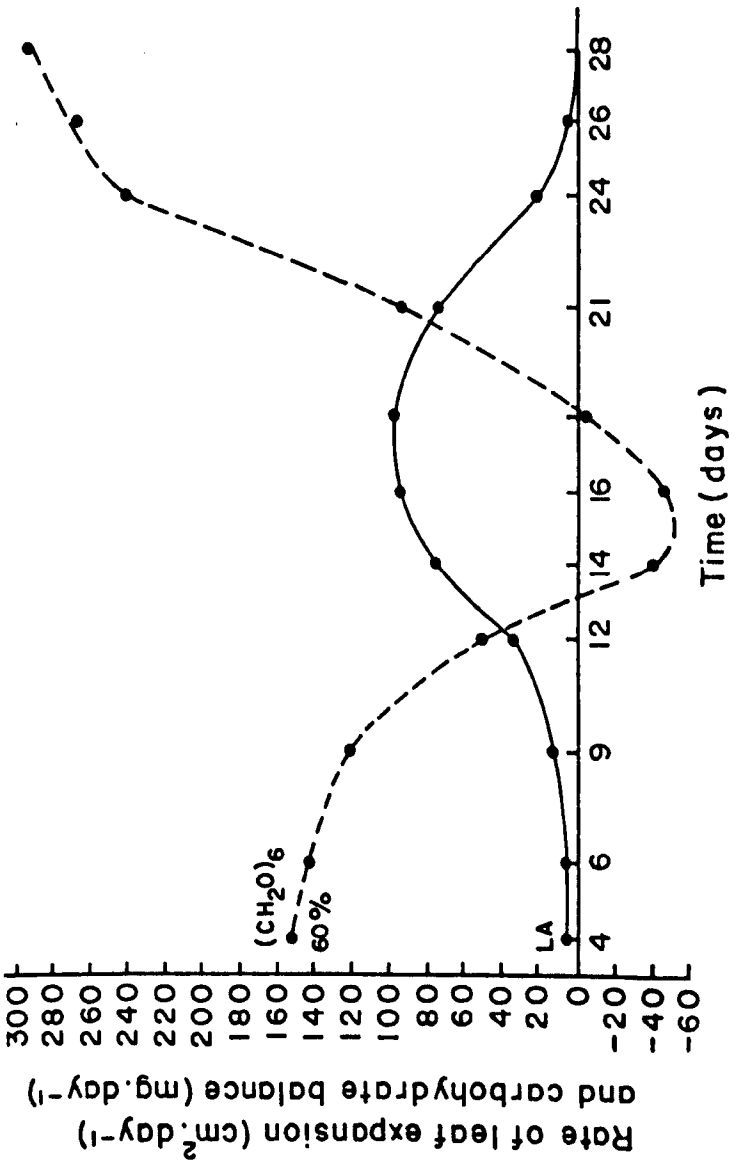
The estimated balance sheet for carbohydrate for early F.2, mid F.2 and I.1 stages of the flush cycle is presented in Figure 4.14. At the early F.2 stage the amount of carbohydrate translocated out of mature leaves to the developing flush exceeded that required for leaf growth. Thus, some material is probably stored in the upper stem and may be utilized for stem growth. During the mid-F.2 stage, when the flush leaves constitute a very strong sink for carbohydrate, the amount of carbohydrate required for leaf growth increased considerably. Although the percentage of fixed carbon translocated out of mature leaves had increased significantly during F.2 stage (section 4.2) the demand by the developing flush exceeds the calculated amount supplied by mature leaves to the apex and thus a negative balance of carbohydrate results at the shoot apex. Thus, the supply of carbohydrate from current photosynthate from mature leaves to shoot apex seems to be insufficient, at least for a short period to support the observed growth of the several simultaneously developing flush leaves. This suggests, therefore, that a stress of carbohydrate, at least temporarily, may occur and may be a factor responsible for limiting flush size and individual leaf size of cocoa seedlings.

A positive balance is again restored during the I.1 stage when the most rapid leaf growth has ceased, although some of the developing leaves are still net importing organs for carbohydrate. The consumption of carbohydrate by the shoot apex may be slightly

Figure 4.14 - Estimated carbohydrate $(CH_2O)_6$ balance for F-2 and I-1 stage of the flush cycle, with 60% of the current photosynthate production of the seedlings moved to support the expanding flush leaves.

Solid line (LA) shows total rate of expansion of all leaves of the new flush.

Time in days is time from initial measurement of leaf area at early F-2 stage.

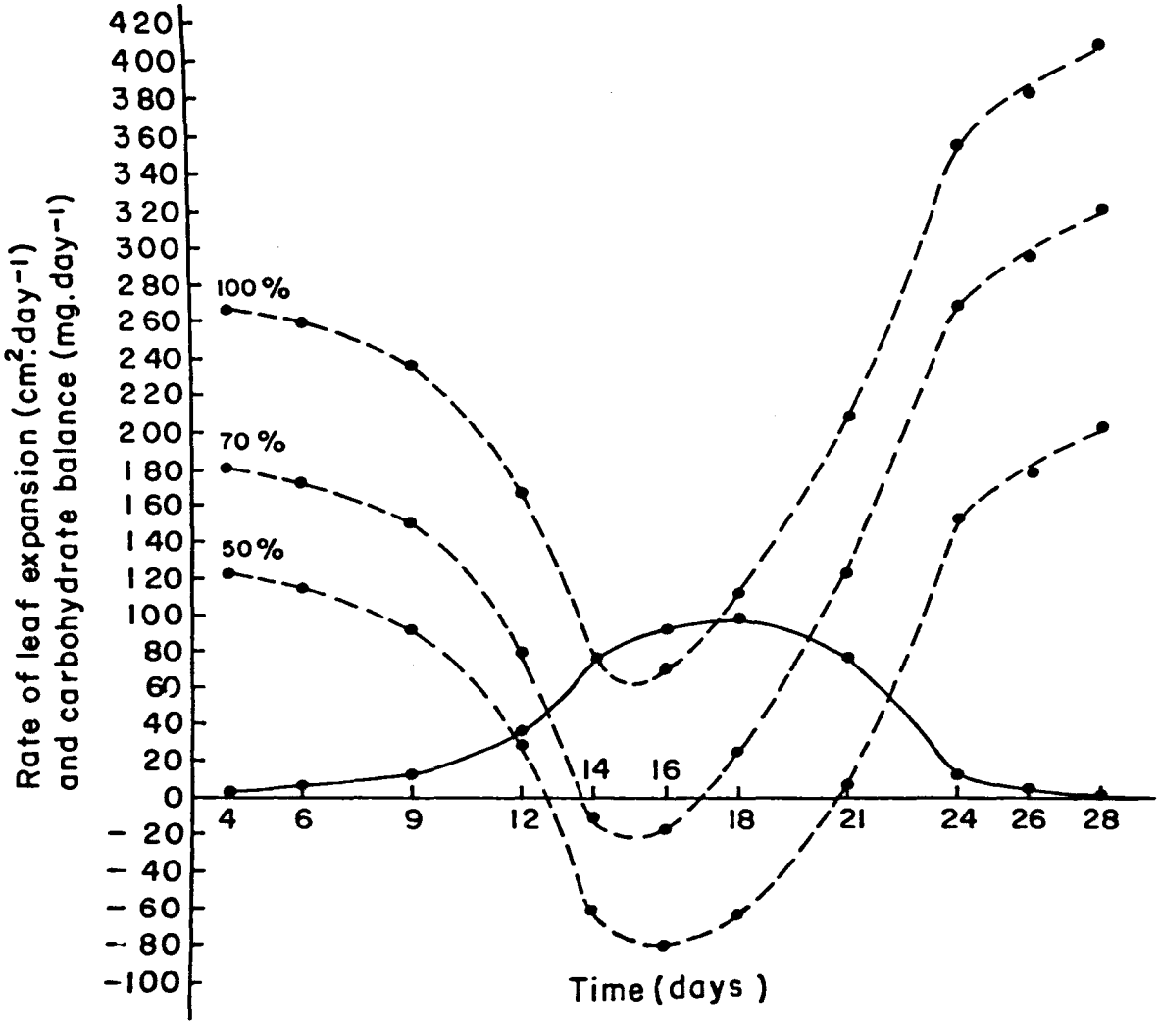

Rate of leaf expansion ($cm^2.day^{-1}$)
and carbohydrate balance ($mg.day^{-1}$)
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
4
6
9
12
14
16
21
24
26
28
Time (days)
LA
60%
$(CH_2O)_6$

under estimated because the demand for stem growth has not been included in calculations for the balance. Since the highest rate of stem elongation occurs during the late F.2 stage (Sleigh, 1981) it is likely that a significant part of the current carbohydrate available to the shoot apex region will be utilized for the stem growth at this time.

Although based on previous findings, there is the possibility that 60% is an under estimate of the proportion of photosynthate moving to apex region. To present a full range of possibilities Figure 4.15 shows calculations based on the movement of all current photosynthate (100%) to the apex, and in between values of 70% and 50%. Ignoring any import to any other regions of the plant these calculations show that the apex would be in slight positive balance for the support of leaf production if all photosynthate moved to the developing flush. However as explained above some significant amount of carbohydrate will be used for stem growth and at least maintenance of other tissues including the roots. This is part of the reason why the earlier assumption of 60% movement to the apex was used. Even taking the value of 60% some of the import to the apex region will certainly be used for stem production. If this is only 10% it will mean less will be available for leaf growth and therefore the effective negative balance of the shoot apex region would be greater than shown in Figure 4.14.

To be absolutely precise about the balance calculations it would be necessary to obtain much more information and refine the model. It is likely however that the assumptions used here have tended rather to under estimate what would be the actual consumption

Figure 4.15 - Estimated carbohydrate balance for the same period with other proportions of the current photosynthate production of the seedling moved to support the expanding flush leaves. Solid line shows total rate of expansion of all leaves of the new flush. Time in days is time from initial measurements of leaf area at early F-2.


Rate of leaf expansion (cm².day⁻¹)
and carbohydrate balance (mg.day⁻¹)
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
- 20
- 40
- 60
- 80
-100
100%
70%
50%
14
16
4
6
9
12
18
21
24
26
28
Time (days)

by the new leaves and the associated stem components. The calculation has thus ended on the safe side, and it is with confidence that the conclusion reached is that in an actual seedling the current carbohydrate available to the shoot apex will be insufficient to wholly support new flush production. Clearly, the "deficit" on current carbohydrate must be met by previously stored carbohydrate. Although the depletion of storage will not be too great in a flush with five leaves, this does mean that the seedling cannot support continuous leaf production, otherwise it would be in negative carbohydrate status which is an impossible situation. Nevertheless if it is a carbohydrate stress which limits the number of leaves produced in a flush, it is necessary to ask how the plant senses such a stress. Since it seems unlikely that in normal circumstance reserves are depleted too much, does the plant in some way "measure" the mobilisation of stored carbohydrate, and is there a pre-set level of safe reduction of reserves? It may be that under adverse natural conditions, or artificially induced adverse situations such as removal of newly formed flush leaves which cause continuous leaf production (Sleigh, 1981; Vogel, 1975), the plant is employing a survival mechanism, for it must have established leaves to survive, and in such situations greater depletion of storage reserves are allowed. Furthermore this discussion has considered only carbohydrate, but as will be discussed later, growth control may be likely to include balances of other components.

Chapter 5

Apical Bud Activity in Cocoa

5.1 Introduction

The cocoa apex has been described as active when new leaves are being produced and dormant when there are no signs of leaf production visible to the naked eye. Descriptions of the shoot apex have been made on the basis of external morphological features of the shoot tips and developing leaves (Greathouse et al., 1971; Orchard, 1977). Different phases of the cycle of shoot apex activity and dormancy (the flush cycle) have been distinguished and termed: i) Flush-one (F-1), when the apex breaks dormancy, judged as apical bud swelling and opening of outer stipules (which sheath the bud) and the earlier leaves in the flush emerge from the bud: ii) Flush-two (F-2), new leaves expand rapidly; the phase ending when the last formed leaf is at aproximately 80% of its final size; iii) Inter-flush-one (I-1), the phase when the last produced leaf completes expansion and rapid greening of the leaves occurs; iv) Interflush-two (I-2), a leaf "maturation" phase, the leaves become dark green, the colour typical of mature cocoa leaves. Phase I-2 is terminated when the bud swells and bursts to start a further flush cycle. In terms of the whole cycle the shoot apex has been described as "active" in F-1 and "dormant" in later F-2, I-1 and I-2 stages. The flush development has also been described by other methods which establish arbitrary stages of development for individual leaves by describing the developmental stage of each leaf between its emergence from the bud and complete blade development

(Vogel, 1975).

As discussed previously, in the general introduction to this thesis, there has been increasing interest in the physiological mechanism which controls the events of the flush cycle, and in particular, the changes in state of the shoot apex from dormant to active and back to dormancy (Bird and Hardwick, 1982; Greathouse et al., 1971). Consideration has also been given to the effects of the developmental stage of other developing leaves in the flush on the determination of the rhythmic growth of the shoot apex (Vogel, 1975). A number of shoot apex studies have paid attention to changes in levels of growth promotors and inhibitors throughout the cycle in leaves near the apex and the apex itself (Alvim et al., 1974; Hardwick et al., 1982; Orchard, 1977) these being considered likely to be related to the rhythm control.

To make the distinction between active and dormant status of the apex from naked-eye observations would seem to be superficial for a full understanding of the internal morphological (and physiological) changes occurring in the apex during the course of a flush cycle. Many of the changes in the apex which are necessary as a prelude to leaf expansion presumably occur before any externally visible changes are observed. It is, for example, well established for deciduous plants that many changes, including the formation of new leaf primordia, take place in terminal buds when they are covered in bud-scales or stipules during winter and early spring. In this case other complex developmental stages take place, such as the formation of bud-scales from the leaf primordia, which occurs in the early stage of dormancy. These events cannot be seen by the naked-

eye, but yet the apex is not truly dormant, if developmental changes are taking place (Smith and Kefford, 1964). In cocoa, the shoot apex has been considered to be dormant during Interflush (I-1 and I-2) stages, because there is no apparent structural development of the terminal bud. However, before being able to say with certainty the stage when the apex is truly dormant, and successfully interpret the significance of any changes in hormonal and carbohydrate status of plants, it is imperative that the apex is examined microscopically throughout the flush cycle to obtain information on the timing of leaf primordia production.

Detailed descriptions of shoot apices have been made for some other species showing growth periodicity (Bond, 1945; Parke, 1959; Purohit and Nanda, 1968; Tolbert, 1961). For cocoa, however, the only published report was limited to shoot apices of branches with plagiotropic leaf arrangement (Greathouse et al., 1971). These latter authors did not present any photomicrographs and their observations covered only Interflush and F-1 stages. Their data are difficult to interpret, but seem to show that the number of leaf primordia increased by three or four either in the period from Interflush to F-1 stages, or, may be including F-1 if the development was early in the phase and the sampling later. Conclusions from results of Greathouse et al. (1971) are essentially that the number of primordia remains constant throughout Interflush, i.e. the apical meristem is considered inactive during Interflush stages. It is however difficult to understand their findings, since whilst only three or four primordia were initiated during the primordial development stage in F-1, an average of 10.7 leaves was expanded in a flush cycle. The problem is to understand when the

other primordia are formed, or it may be that their observations are not complete.

In other species which show flushing, Purohit and Nanda (1968) found that new leaf primordia were formed in only the dormant/interflush phase in Callestemom viminalis, but Bond (1945) showed a 'fairly constant' rate of primordia formation throughout the full growth cycle of tea. There seems therefore to be no general similarity amongst flushing species as to the timing of leaf primordia formation, and the situation for cocoa is unclear. In an attempt to clarify the situation, and determine at which, if any, period of the flush cycle the cocoa shoot apex can be truly described as either active or dormant, the experimental work of this chapter comprised of a series of examinations of terminal buds of cocoa seedlings by light and scanning electron microscopy (SEM) at each stage of the flush cycle.

5.2 Materials and methods

Plants used for terminal bud dissections were grown in the glasshouse as described in previous chapters. Four seedlings approximately ten months old were sampled at each stage of the flush cycle. Terminal buds were excised 1.0cm below the base of the lowest stipules and the bud-base embedded in paraffin wax to allow micro-surgery during handling. Stipules and associated blades were sequentially detached and measured. When stipules and young blades below around 1 mm in length were reached, the remains of the apices were prepared for scanning electron microscopy (SEM) examination. In view of the sheathing nature of the remaining structures and

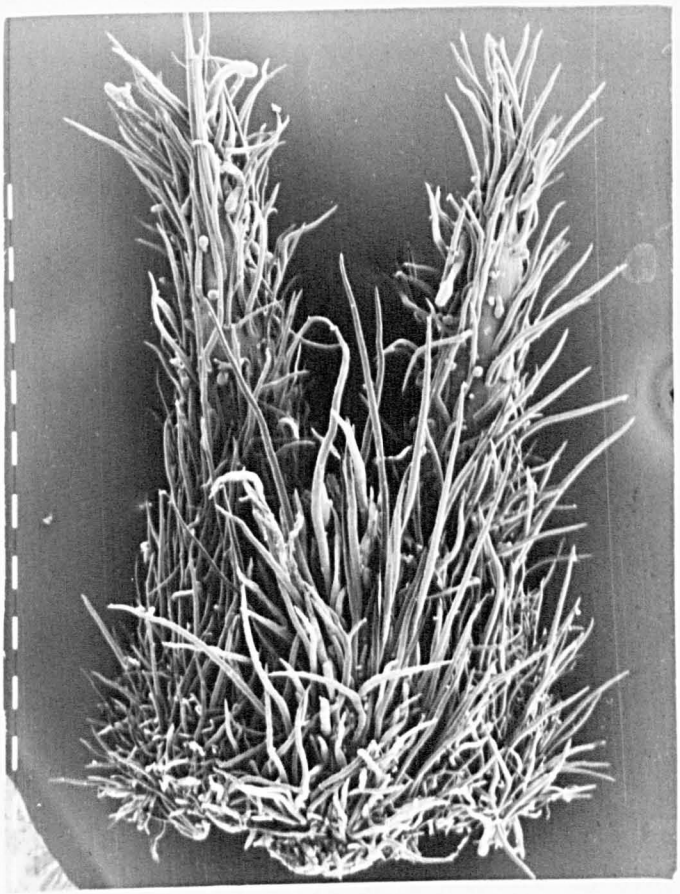
large number of hairs the meristematic dome could not be seen at this stage. Initial SEM and photography was however carried out on the alloy-coated specimens and then the coated apices returned to the light microscope where further stipules and leaf removal was carried out so as to eventually reveal the dome. Removal of structures below 1.0 mm on fresh (uncoated) material inevitably resulted in damage and/or release of mucilage which obscured the features of the apical dome. For SEM specimen preparation, dissected apices were dehydrated in a series of solutions of ethyl alchol in water, for 15 minutes in each of: 5, 10, 15, 25, 40, 50, 60, 75, 85, 90 and 100% x 2 changes, then the alcohol was removed in a Polaron Critical Point Dryer. Dried apices were vacuum coated with gold: palladium alloy before observation in a Philips Scanning Electron Microscope (Model 501 B).

5.3 Results and discussion

Plates 1-12 show a series of micrographs from SEM studies of cocoa apices representative of sequential stages of the flush cycle. Plate 1 is a low magnification view of an apical bud at the F-1 stage, with outer stipules removed to reveal the larger bud leaves/primordia. In strict botanical terminology a leaf comprises petiole, blade and stipules, if present, as in this case. To simplify expression here however the term leaf will be used to mean presumptive petiole and blade only, and stipules identified separately. The main features in Plate 1 are the range of size of leaves, reducing progressively from outmost (oldest) towards the central dome, and copious hairs on the young leaves. The high degree of hairiness, which is seen in more detail on Plate 2, is unusual

Plate 1 - Single apical bud at F-1 stage, stipules removed. (Each dash on scale = 100 μm).

Plate 2 - Young leaf and associated pair of stipules from an apical bud at F-1 stage (Each dash on scale = 77 μm).

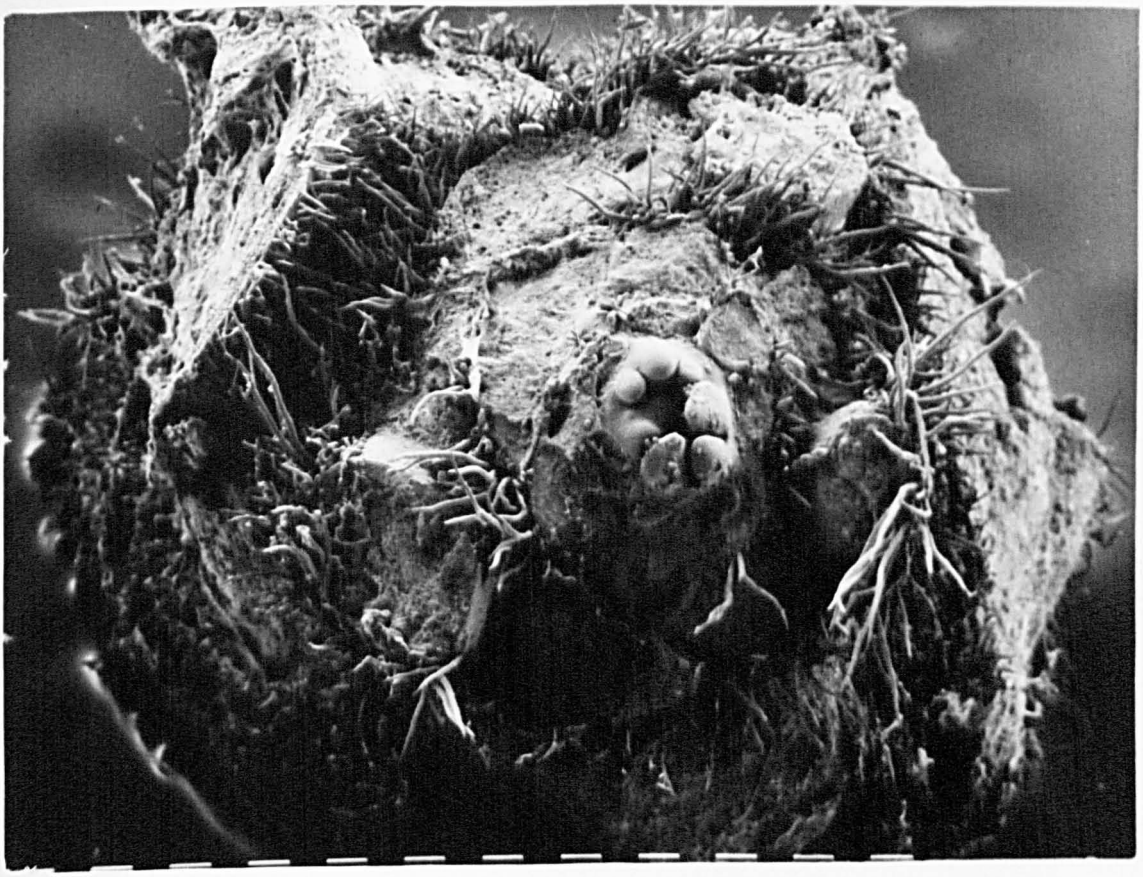


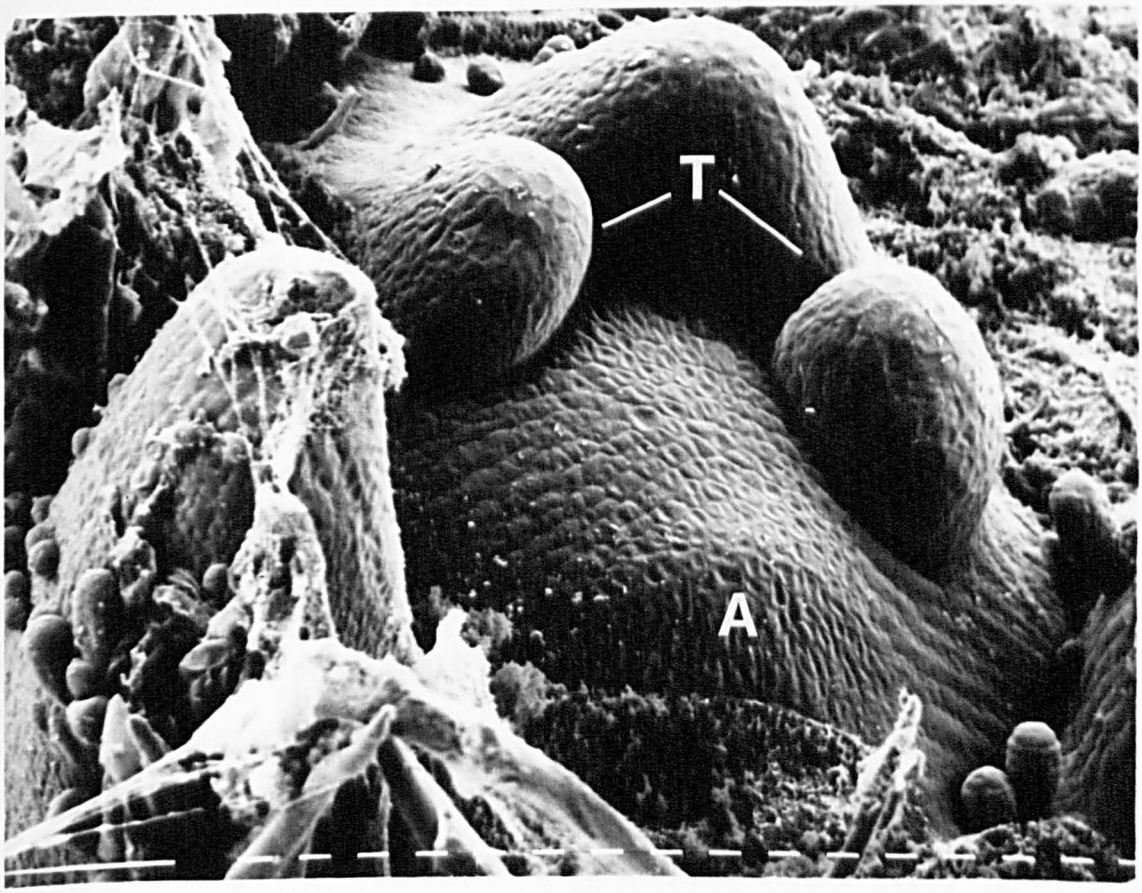
for young leaves sheathed within an apical bud. Different types of hairs can be recognised on the two stipules and leaf. The leaf is between the stipules and about 40% of their length. Four different types of hairs, developed only from epidermal cells above veins, were previously observed by Hardwick et al., (1983) on leaves which had expanded to around 30% of their final size. Hair density on the present structures is very much greater. This reduction in density of hairs as leaves expand is likely to be due to the increase in leaf area, (hairs become more widely spaced) and there may even be a loss of some hairs with leaf development. These authors do not comprehend the function of these hairs, other than to suggest a possible protective function for them towards the young leaves. Certainly from the appearance it would seem very likely than any potential predative insects would find it very difficult to make direct contact with the main surfaces of the delicate young structures. It will be readily appreciated now just how such a dense covering of hairs on young leaves and stipules, together with the sheathing arrangement of the outer structures obscurring the younger leaves/primordia, make it difficult to observe the apical dome. Hence the necessity to remove outer structures in order to reveal the apical dome. Plate 3 shows a whole apical bud with outermost leaves and stipules removed to reveal the youngest primordia and apical dome. Leaf and stipule bases can be seen together with many hairs between bases and successive leaf positions.

Plates 4-12 show details of young primordia at distinct stages of the flush cycle, especially at F-1 and F-2 stages. On Plates 4-6

Plate 3 - Whole apical bud with outer leaves and stipules removed. Apical dome and youngest primordia in centre (scale 100 μm).

Plate 4 - Apical dome. A: very early leaf primordium; T: tripartite primordial arrangement of a leaf and associated stipules (scale = 10 μm).



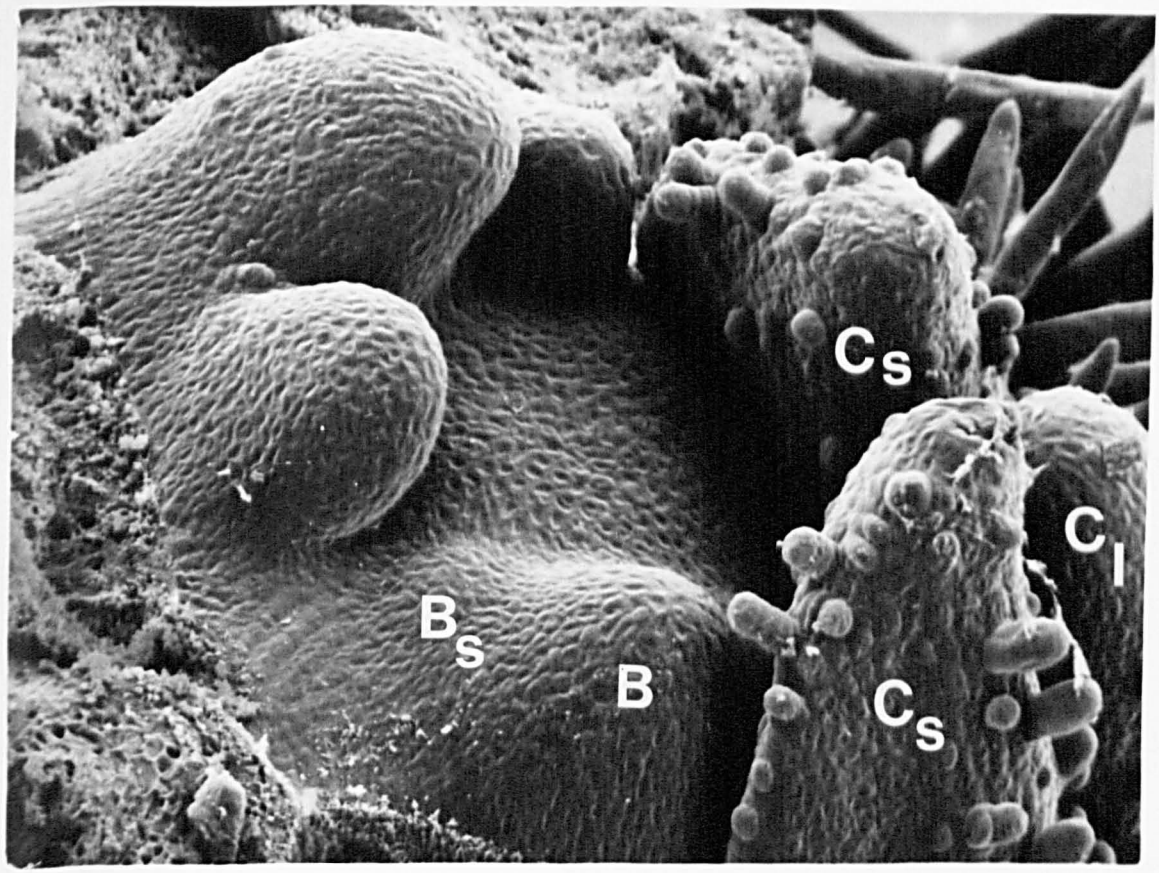

T
A

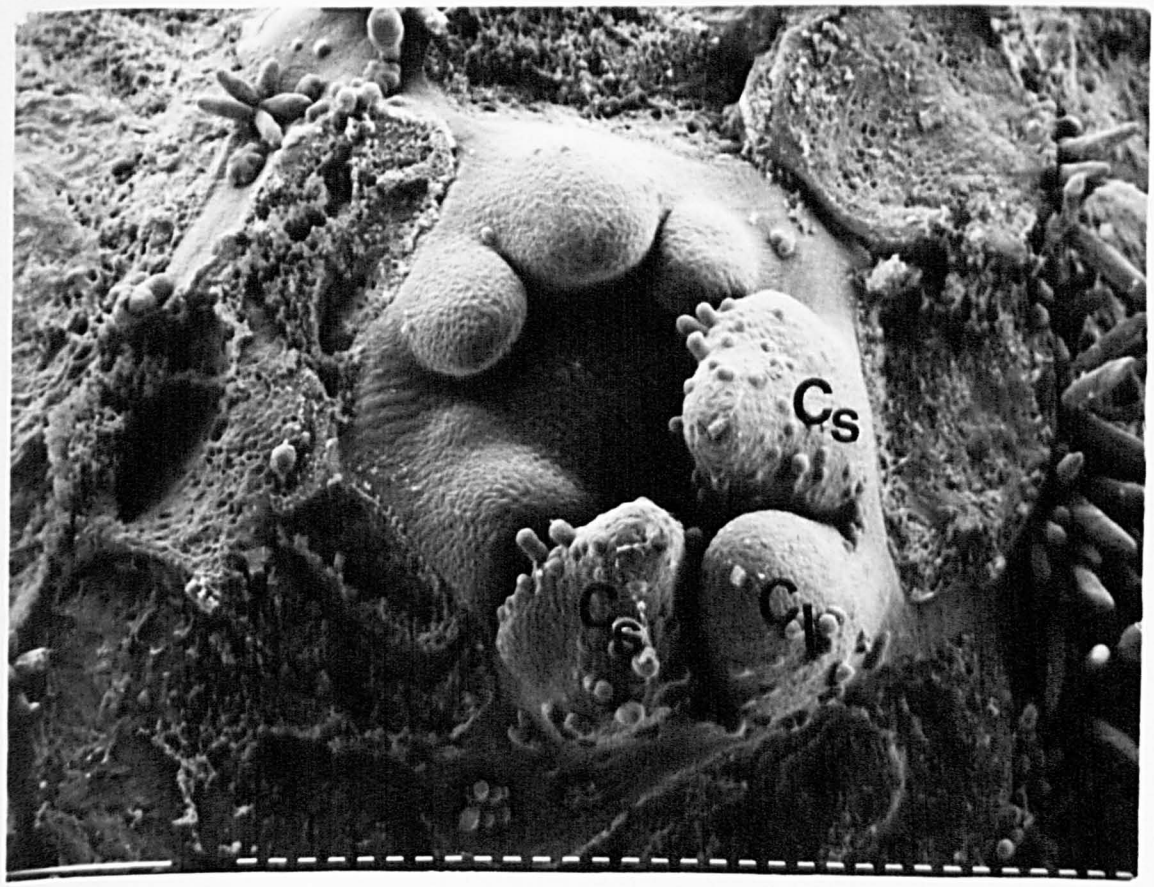
major features of these structures at the F-1 stage are revealed. Structure A on Plate 4 is the youngest primordium and appears as a single dome of the presumptive leaf blade and petiole. No clear formation is noted at this initial stage of the two stipule primordia which will be associated with this leaf. A slightly older stage, B on Plate 5, shows a leaf primordium B and the small primordium (Bs) of one of its two associated stipules. At a more advanced stage of development it is easy to recognise the tripartite nature, T on Plate 4, of the primordia for each 'complete' leaf (leaf and two associated stipules). Growth of the leaf initialy exceeds the stipules, but by stage C on Plate 6, stipule growth accelerates (or that of the leaf slows), so that stipules become the larger structures.

The apical dome and the three or four smallest primordial groups are not hairy, but by the time stipules are around 150μm long, Cs on Plate 6, Ds on Plates 7 and 8, hair formation is beginning; first on the stipules and then later on the leaf itself when it reaches around 100 μm long, C and D on Plates 6 and 8. Plate 9 is an additional observation, at F-2 stage, showing that hair formation appears to start from the base and progresses towards the tips of the primordia, especially in the case of leaf primordia.

Plates 7, 8 and 10 show the same apex of a plant at F-2 stage at three successive stages of dissection, with the smallest primordia only revealed on Plate 10, when the largest sheathing structures were removed. For orientation, E is the same leaf on Plates 7, 8 and 10. These plates show more clearly the phyllotaxy of the primordia and the triangular bases of the stipules. Leaf bases are more circular, as is seen for G and H on Plates 11 and 12.

Plates 5 and 6 - Two different views, at higher magnification, of the apical dome shown on Plate 3. Letter B shows the youngest primordium; C indicates the same primordia on both plates; s, stipules; l, leaf. (Each dash on scale = 10 μm).

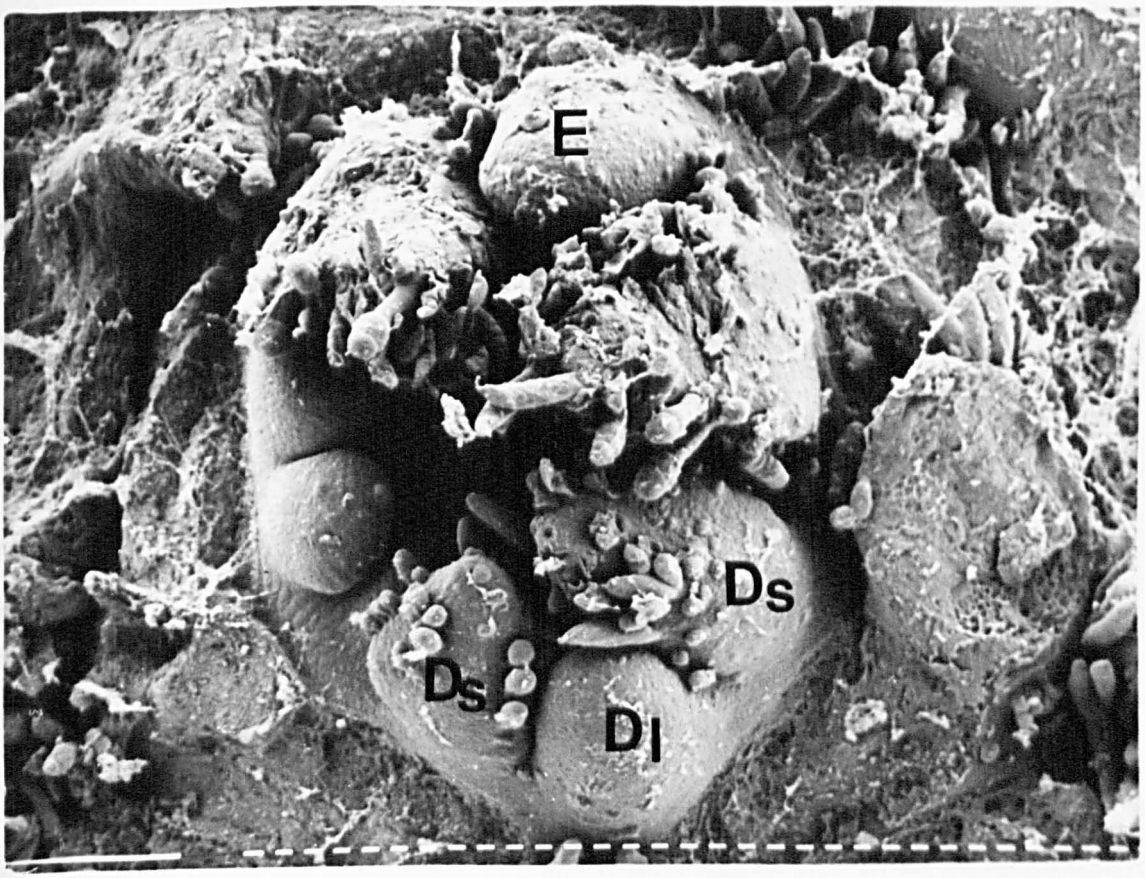

Cs
Ci
Bs
B
Cs

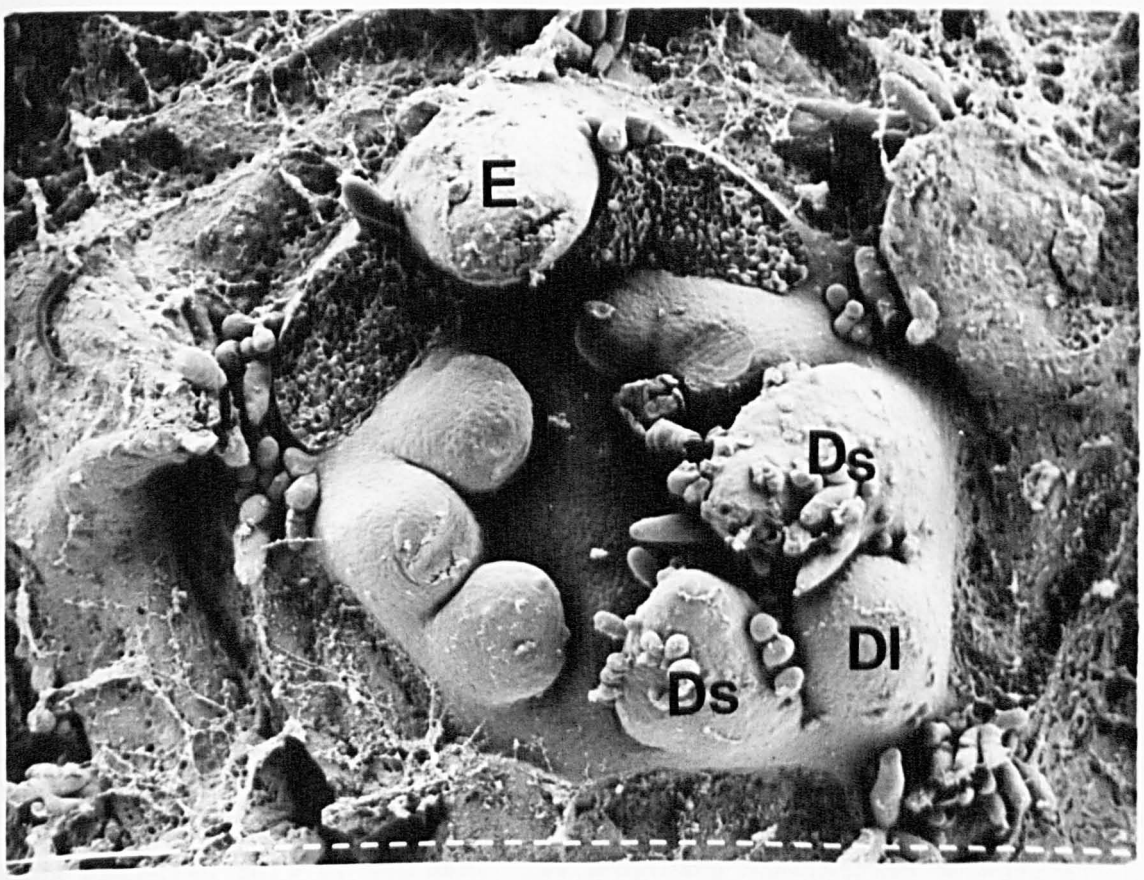

Cs
Cs
Ci

Plates 7, 8 and 10 (overleaf). Series of three micrographs of the bud, at the F-2 stage of the flush cycle, at three levels of dissection, E and D identify same primordia on each plate; s, stipules; l, leaf (Each dash on scale = 10 μm).

Plate 7 - initial stage of dissection;

Plate 8 - second stage of dissection.


E
Ds
Ds
Dl


E
Ds
Dl
Ds

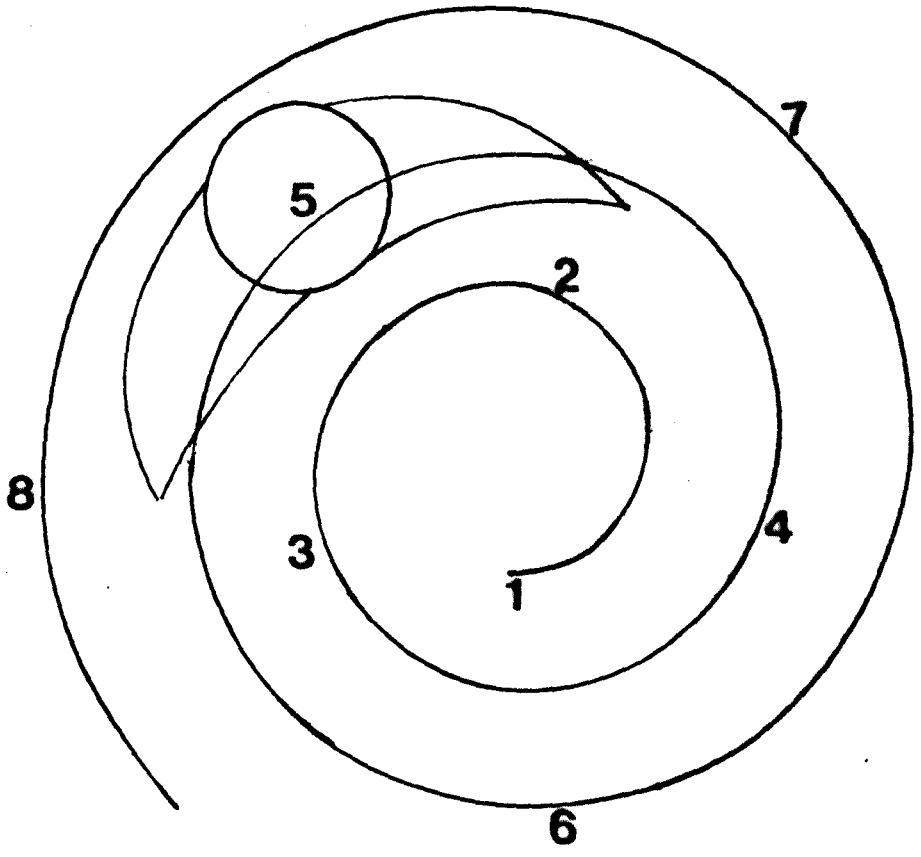
The transparent overlay for Plate 10 shows the phyllotactic spiral superimposed onto the apex with leaf positions numbered from one, the smallest (youngest) primordium until the eighth. The ninth primordium, which is unfortunately not visible on the Plate, would complete the repeat position in a spiral, with primordium one being three complete turns of the stem axis removed and with eight leaves (primordia) between primordium one and nine, producing the 3/8 phyllotaxy. Also outlined on the overlay are the triangular shapes of the stipule bases and more circular section of the leaf base. The phyllotaxy of the apex on Plate 10 is right-handed (anti clockwise), but on Plate 6 the phyllotaxy is left-handed (clockwise). It is quite common for different individuals of one species to show either left or right-hand spirals for leaf arrangement.

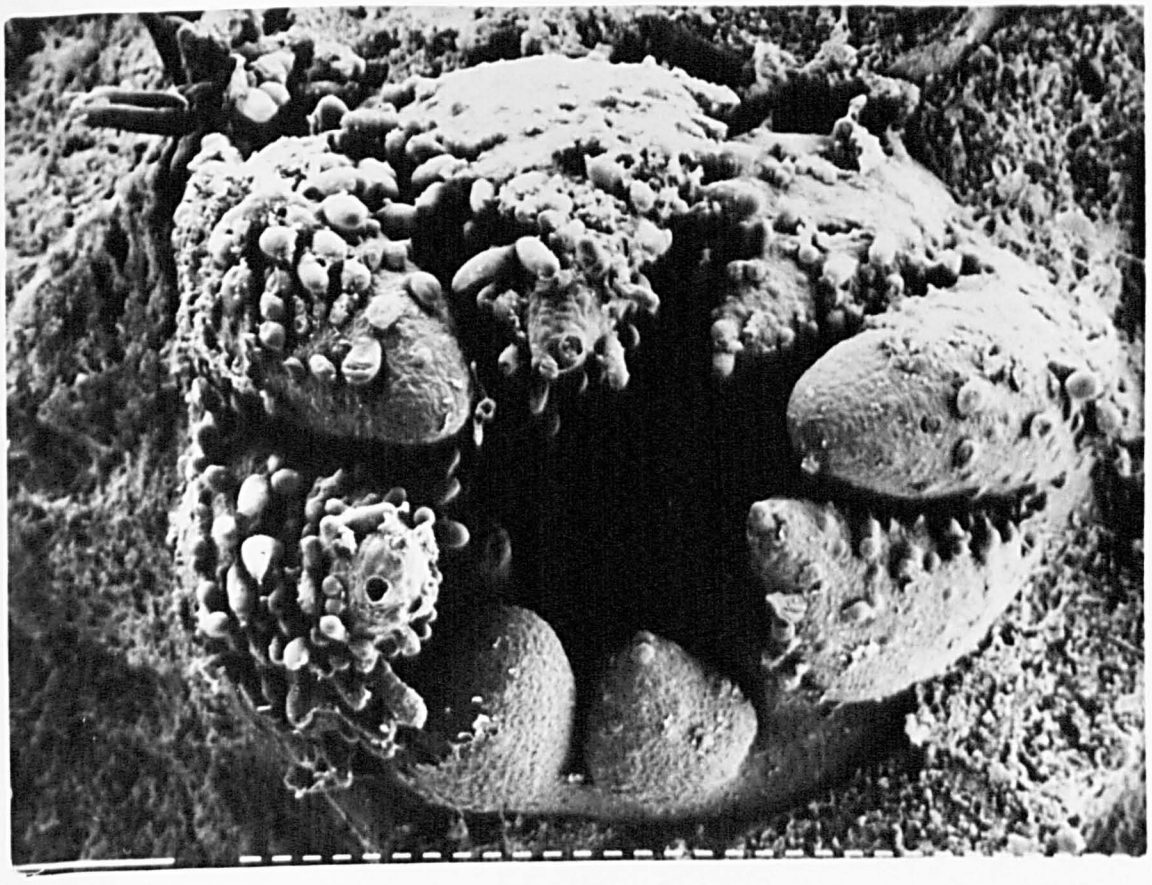
Plates 7 and 10 are of a bud at the F-2 stage, and 11 and 12 form a bud at the F-1 stage. There are no obvious differences in the bud structures at these stages. Plates 11 and 12 also emphasise the 'hair problem'. Stipule and leaf primordia H on Plate 11 were removed to show bases more clearly on Plate 12. The low-angle view of Plate 12 also shows clearly the tripartite nature of each complete unit of leaf and two stipule primordia, and the earliest formation of a hair on a stipule (J) ahead of the adjacent leaf.

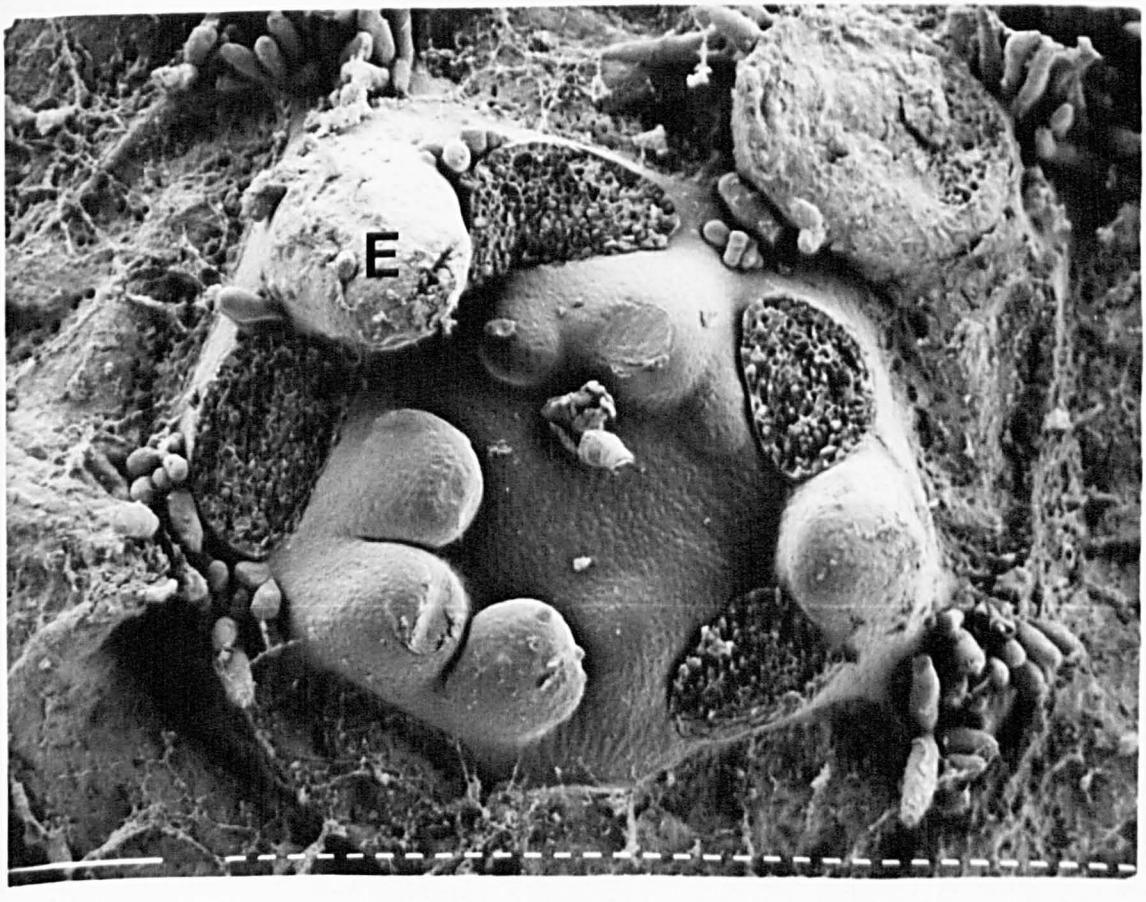
Table 5.1 shows counts of leaves and primordia in buds at different stages of the flush cycle. The structures are separated into size categories as follows: i) above 9 mm long; ii) between 1 and 9 mm long, iii) below 1 mm but light microscope visible, and iv) those revealed only by the SEM. The arbitrary distinction between a

Plate 9 - Additional observation of a bud at the F-2 stage showing initial stage of hair formation. (Each dash on scale = 10 μm).

Plate 10 - Same bud seen on Plates 7 and 8 in the third stage of dissection showing the youngest primordia and the phyllotaxy of the leaf arrangement in cocoa seedlings highlighted on the transparent overlay. (Each dash on scale = 10 μm).


1
2
3
4
5
6
7
8

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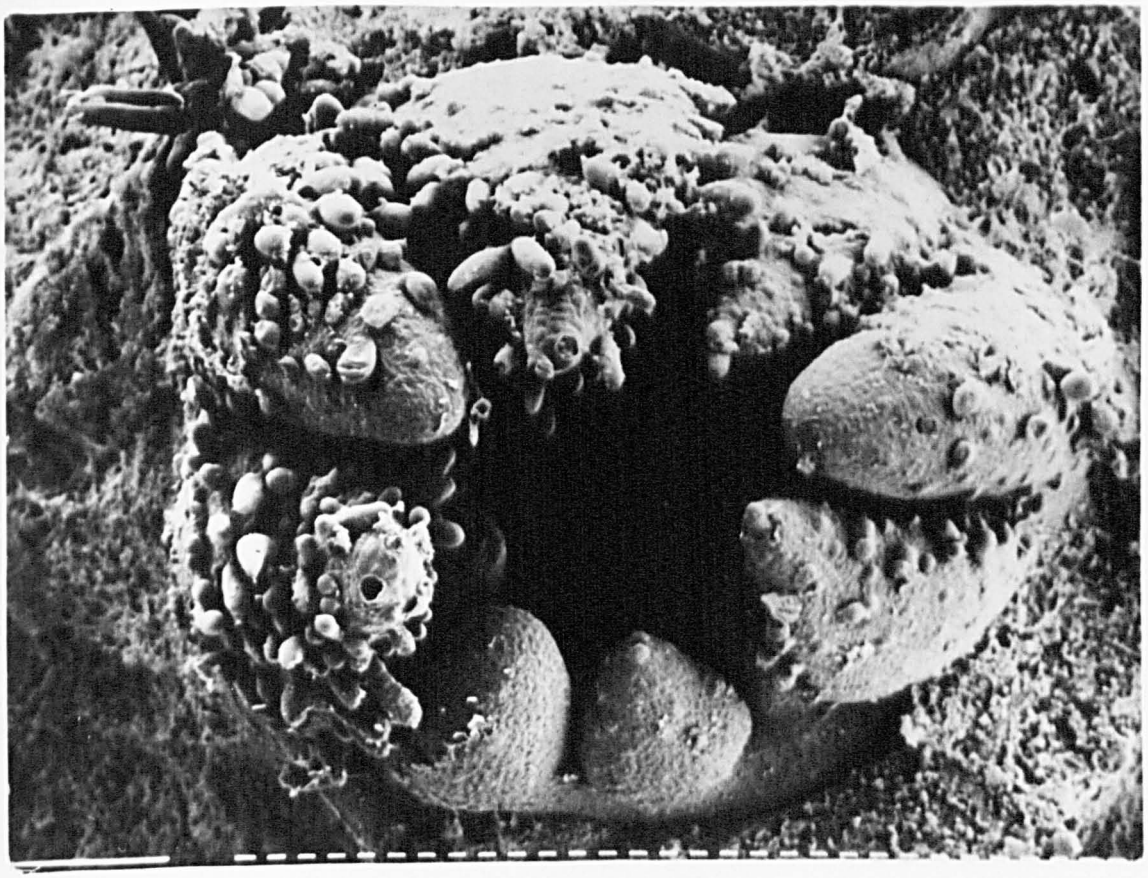
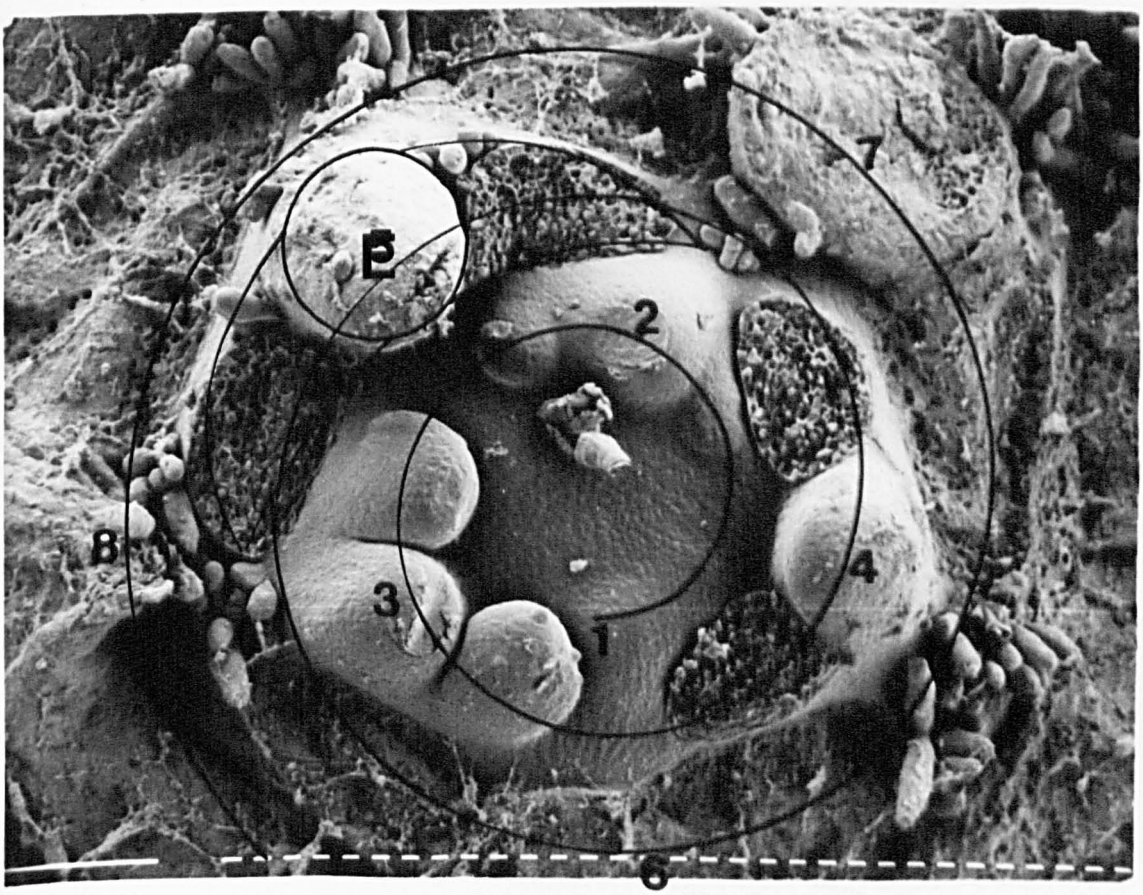
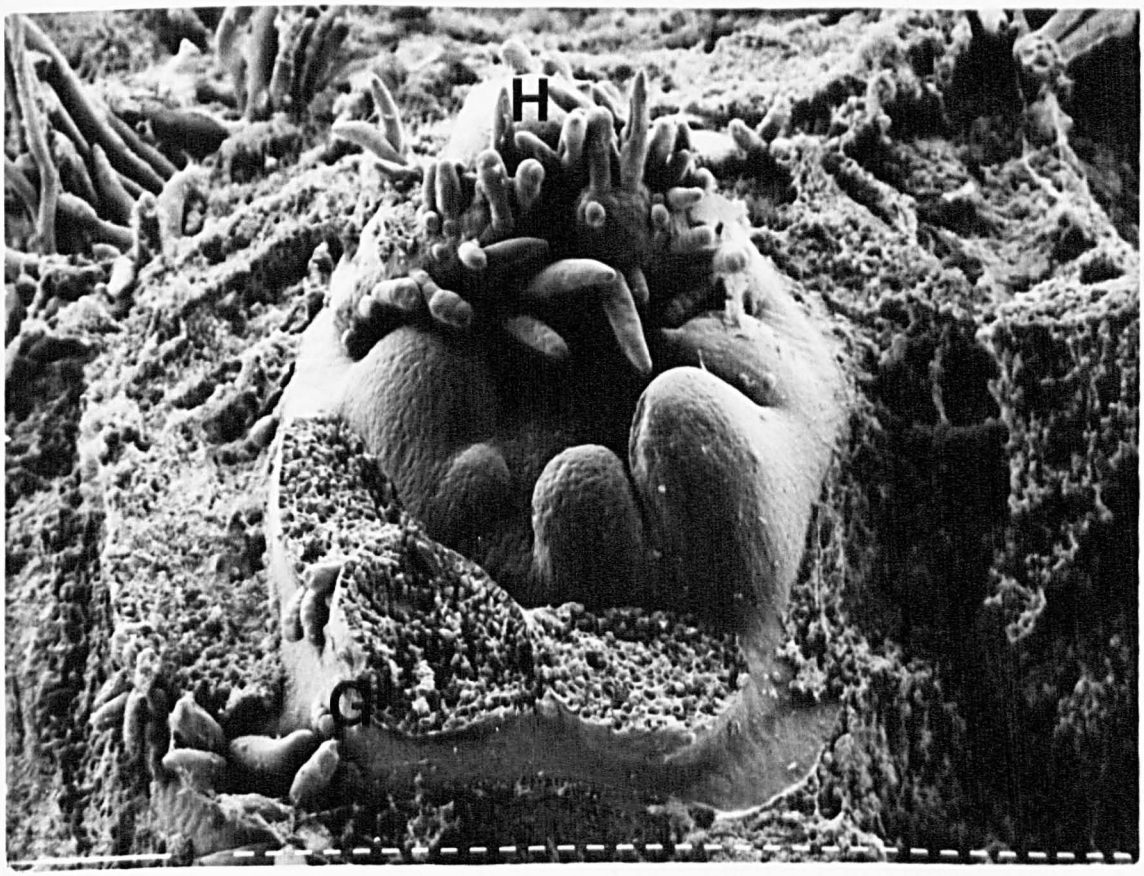
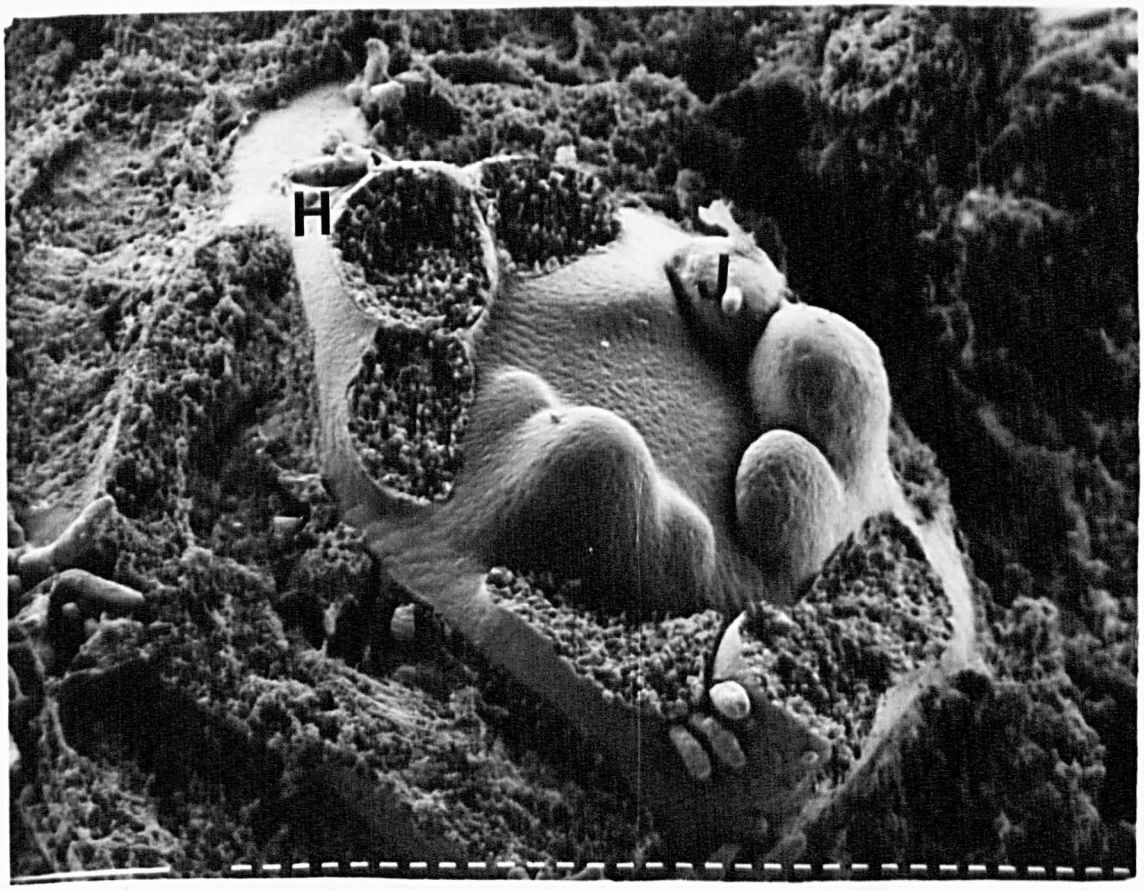



E
2
7
B
3
1
4
6

Plates 11 and 12 - Photomicrographs of the same bud at the F-1 stage of the cycle, at different levels of dissection. G indicates the circular base of a leaf primordium; H indicates the same primordial group on each plate, but with most of the structures dissected away on Plate 12, to emphasise the circular leaf base and more triangular stipule bases. J indicates a stipule primordium with the first signal of hair formation. (Each dash on scale = 10 μm).


H
G


H
I

leaf and primordium is taken at 1 mm. From 1 mm in length the structure is termed a leaf and below 1 mm a primordium. Data represent an average for eight plants at each stage of the flush cycle. Developing leaves at F-1 and F-2 are included in the count of total leaves at these stages because they constitute part of the developing flush. Around five to seven of the small leaves in the bud, and above 1mm long at the F-1 stage, form the new flush at F-2 stage.

Table 5.1 - Average numbers of leaves and primordia revealed in the buds at different stages of the flush cycle; i) above 9 mm; ii) between 1 and 9 mm; iii) below 1 mm and light microscope revealed; iv) SEM revealed, and mean totals per bud. Values for leaves and primordia are means ± standard deviation.

Stages	Leaves		Primordia		Mean
	above 9 mm	between 1 and 9 mm	below 1 mm (light micr) revealed	SEM revealed	total
F-1	1.5	8.125±1.12	4.75±1.83	4.25±0.95	18.6
F-2	5.7	6.250±0.83	4.37±0.92	5.50±2.12	21.8
I-1	none	5.875±0.65	4.25±0.71	3.00±1.0	13.1
I-2	none	5.875±0.65	4.57±0.53	5.00±1.4	15.4

The number of leaves expanding at the F-2 stage is included in the count of the total number of leaves at this stage because the small leaves from which these leaves expanded were present and would

be counted at the F-1 stage. Since the total number of leaves plus primordia is greater at F-2 than F-1 stage (21.8 compared with 18.6) this must mean new primordia are initiated from F-1 to F-2, or in very early phases of the F-2 stage. The number of leaves (between 1 and 9 mm) is however significantly greater at F-1 than in other stages. This evidently reflects the start of accelerated growth of the older primordia within the bud which will form the leaves of the new flush at the F-2 stage. The average total number of structures shows a trend of increase from I-1 through I-2 to F-1 stages (13.1 to 15.4 to 18.6). It is thus clear that primordia are also formed throughout the I-1 and F-1 period, although the major increase occurs between the F-1 and the F-2 stages. It is evident from these results that primordia are formed throughout the whole flush cycle, i.e. including the so called dormant stages of the apex.

Although all the information presented is based on morphological features of the apices (no apices are sectioned), it gives a general picture of primordia formation within the apical bud. Apart from the information on the stage of primordia formation, these data also show that the terminal bud contains a 'store' of leaves and primordia sufficient for more than one and at least two or three flushes with a mean number of six to eight leaves per flush. In view of the large number of leaves and primordia in store within the apex, it follows that the number of leaves which expand in one flush is not limited by mere availability of primordia.

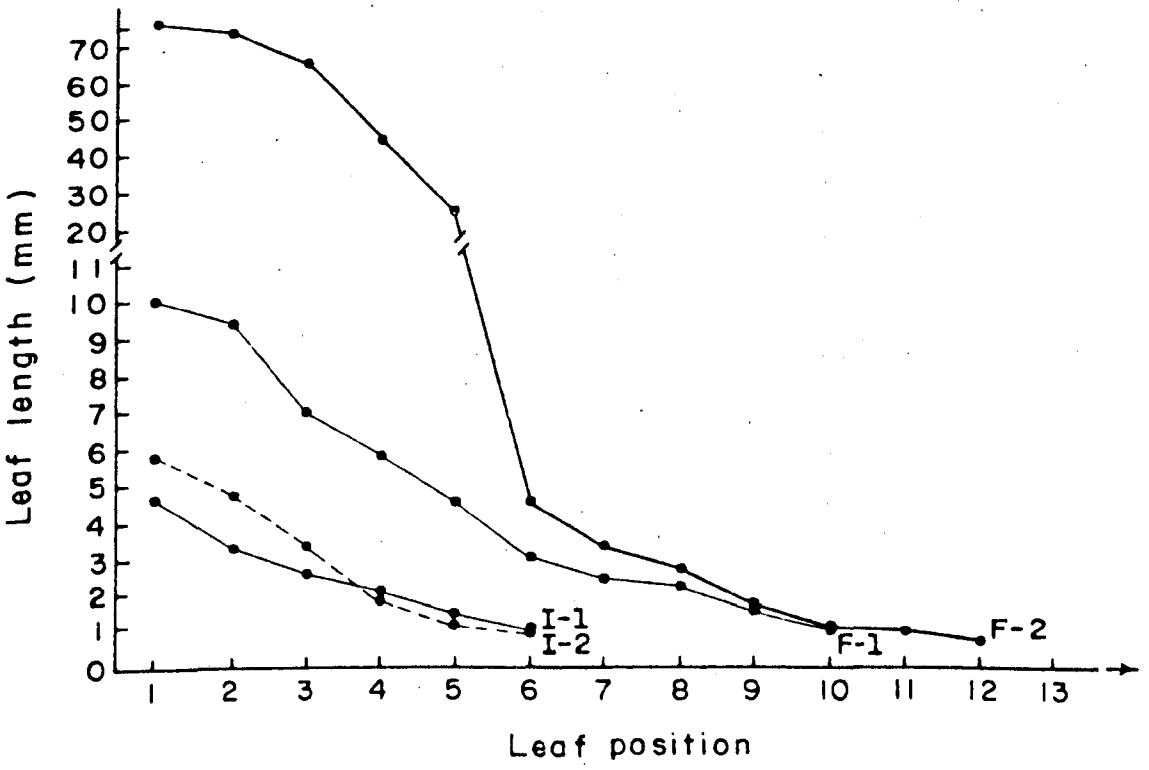
Figures 5.1 and 5.2 show the lengths of leaves above approximately 1 mm, and the mean length of the two stipules associated with each leaf of five apices at each stage of the flush

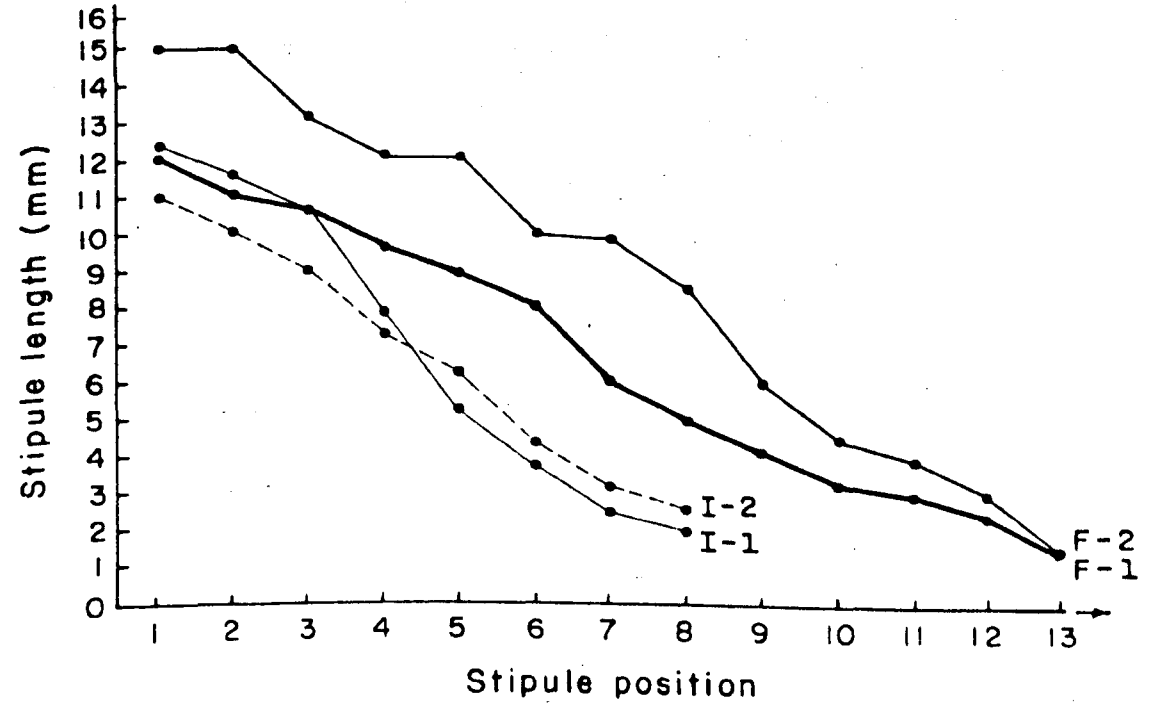
cycle. Leaves and stipules at position 1 are the outermost, and largest, structures in the bud. At F-2 leaves at positions 1 to 5 have expanded from the size at F-1 when within the bud and are now technically out of the bud. There are within the complete apices many primordia smaller than approximately 1 mm, but it is not possible to measure these accurately from the SEM study. From these data it is clear that the growth of leaves and stipules within the bud takes place throughout the flush cycle. It is not possible to be definitive about comparing growth rates at different phases of the flush cyle, since the x-axis, leaf position, is not strictly linear with respect to time. Nevertheless it seems that in the case of leaf primordia there is more rapid growth between I-2 and F-1, as the flushing phase of the cycle begins, than between I-1 and I-2, i.e. in the inter-flush period. For stipules this is only more visible from position 4 to 8. Growth of the largest leaves within the bud at positions 1 to 5 is very rapid between F-1 and F-2 since these are the new flush leaves beginning their major expansion to mature leaves. Stipule growth between F-1 and F-2 is also greater than between other phases, but that of the stipules of the new flush leaves (positions 1 to 5) does not, unlike for leaves, accelerate proportionally more than structures at positions 6 and higher numbered positions (younger). Whilst emphasising again that the horizontal axis is not strictly linear, it does seem safe to state that there is no abrupt discontinuity in size between adjacent leaves and stipules within the bud, at least down to a size of 1 mm. This is important because it means that the development of leaves and stipules within the bud is not synchronised in groups

Figure 5.1 - Leaf length within individual buds at different stages of the flush cycle.

Figure 5.2 - Average length of the pair of stipules associated with each leaf within individual buds at different stages of the flush cycle.

N.B. For both Figures - Position 1 is the outermost (oldest) leaf or stipule pair in the buds, upward numbered positions are in sequence towards the apical dome and represent increasingly younger structures.


Leaf length (mm)
70
60
50
40
30
20
11
10
9
8
7
6
5
4
3
2
1
0
I-1
I-2
F-1
F-2
1 2 3 4 5 6 7 8 9 10 11 12 13
Leaf position


Stipule length (mm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
I-2
I-1
F-2
F-1
1 2 3 4 5 6 7 8 9 10 11 12 13
Stipule position

representing pre-determined flushes of leaves. As an example of this conclusion consider the relative sizes of leaves at positions 4, 5 and 6 at F-1 and F-2. Size differences are very similar at F-1, but when the new flush expansion begins, leaf 5 expands very rapidly whilst leaf 6 does not, and yet at F-1 the size difference between leaves 4 and 5 and, 5 and 6 is almost identical. This finding together with the fact that only a proportion of the total leaves and primordia within the bud do develop at the start of a flush cycle strengthens the hypothesis that it is events within the bud at the time of F-1/F-2 which determine the number of leaves which forms the new flush and not previous events.

As shown in Chapter 4, during the development of a new flush the current carbohydrate supply by mature leaves was not sufficient to fully supply the high demand by expanding leaves. So that, it is possible that succeeding primordia within the bud are receiving insufficient carbohydrate to allow their successful growth and development into leaves and thus the number of leaves in the new flush is delimited. That first leaves developing compete with leaf primordia in the bud was previously claimed for tomatoes by Hussey (1963), where he suggested that carbon assimilates were the material for which they competed. Also Ballard and Wildman (1964) have suggested that the dormancy of buds in other species, as measured by the occurrence of mitotic activity, may be controlled by carbohydrate levels.

Regarding comparisons between previously published work on primordia formation in species which flush, it is now clear that primordia in cocoa are formed throughout the growth cycle, at least in seedlings, and not as previously suggested by Greathouse *et al.*,

(1971), confined to the active growth phase at the shoot apex. Cocoa is thus very similar to tea (Bond, 1945) but unlike C. viminalis (Purohit and Nanda, 1968). The apex cannot therefore be considered totally dormant in the phases when leaf expansion does not occur and any theories which attempt to explain the control of shoot apex activity will have to accommodate the finding of different degrees of shoot apex activity throughout the growth cycle rather than a sharp distinction between active and dormant (non-active) periods.

Chapter 6

General Discussion

From the results and discussion presented in previous chapters it is clear that the complete flush cycle of growth in cocoa is complex, with changes not only in leaf but also stem and root development rates throughout the cycle. Nevertheless although there is likely to be interaction between growth centres, it is possible, and particularly useful for initial discussion purposes, to recognise three distinct episodes in the shoot growth during one flush cycle. There are, in order following a period of leaf production; i) cessation of leaf production, i.e. establishment of dormancy at the shoot apex, ii) maintenance of shoot apex dormancy and iii) breaking of shoot dormancy. It is possible that the control of each of these three episodes may be via different mechanisms.

Changes in the state of the shoot apex from dormant to active and back to dormant have been considered to be mediated by changes in the balance of plant growth regulators, particularly changes in abscisic acid (ABA) and cytokinins. Evidence for the involvement of these growth regulators in controlling the leaf production in cocoa, have been reported by Alvim et al. (1974); Hardwick et al. (1982) and Orchard (1977). These authors interpreted that the observed increases in ABA levels which are correlated with the onset of dormancy are related to the development of a water stress in the plant, and that later decreases in ABA and increases in cytokinin levels are due to the relief of the water stress. No systematic explanation has however been given for the control of these changes

in hormone levels in plants grown in optimal environmental conditions as used by the last two authors.

Borchert (1973) elaborated a hypothesis, based on a computer simulation, and some data from cocoa growth statistics, which proposed that the flush cycle was indeed controlled by changes in plant water status and that such changes resulted in turn from changes in root:shoot ratios throughout the cycle. However, Hardwick et al. (1985) have demonstrated that during a complete flush cycle of growth in seedlings growing in optimal conditions there was no indication of a water stress developing at any stage of the cycle. They thus suggested that the theory of water stress control (Borchert, 1973) is not valid to explain the endogenous control of leaf production in cocoa seedlings.

Although the above theories, based on plant growth regulator changes, have attempted to explain the alternation between dormancy and activity periods of the apical bud, no satisfactory explanation has yet been produced. Apart from considering the clear cut difference between leaf production and no leaf production phases of the cycle it was considered important that data should be collected on the details of leaf development rates etc., during the leaf production phase because this detail may give more clues to the actual control mechanism. This work is reported in Chapter 2. Two significant findings were firstly the progressive decrease in final leaf area in the sequence of leaves within one flush, and secondly, the usual abscision of one or two leaves "between" two leaf production phases, usually just at the end of leaf production. As mentioned above the events of activity and dormancy of the apical

bud have been suggested to be driven by hormonal control. However there are reasons to believe that any hormonal control may be derived from changes in growth activity of parts of the plants themselves, caused either by general changes in amounts of carbohydrate within the plant or by changes in distribution pattern. For example increased carbohydrate supply to root system which thus stimulates root growth and, more rapidly growing roots increase hormonal output (largely cytokinins) which when exported may subsequently control events at the shoot apex. An interaction between shoot and root growth would therefore occur. More indications that carbohydrate may control leaf production come from the observations that: i) the several simultaneously developing leaves in one flush have very low photosynthetic capacity (Baker, 1974) they will therefore cause a very major consumption of carbohydrate, and ii) that root and shoot development alternate, (Sleigh, 1981) which may be because there is not sufficient carbohydrate to maintain continuous growth of both root and shoot systems. Recently, Bird and Hardwick (1982) proposed the hypothesis that plant carbohydrate status may be the major factor determining the events of the flush cycle in cocoa. The major part of this thesis has thus been concerned with a detailed investigation of the characteristics of leaf growth, and the production and translocation of photosynthate/carbohydrate in order to evaluate this hypothesis for cocoa seedlings, and see whether the three episodes outlined in the first paragraph of this chapter can be explained on the basis of carbohydrate control.

Results presented in Chapter 2 showed that the progressive decrease in final area of successive leaves within a flush was

associated with a decreasing rate of leaf growth and also with lower cell numbers per leaf. Differences between large and small leaves were observed to originate at a very early phase of blade expansion, between commencement of unfolding and the start of the initial phase of rapid expansion, a phase during which the majority of cells in the upper epidermis are produced. Since the major proportion of cells are formed after unfolding commences, this suggests that events occurring after leaf emergence are more important in determining the final size of the cocoa leaves than events when leaves are within the bud. Similarly it has been reported for other species that about 90% of the cells in a mature leaf are produced after unfolding commences (Dale, 1982; 1985; Hannan, 1968; Sunderland, 1960) and it was also suggested that in tobacco the events occurring after leaf unfolding are more important for the control of final leaf size than those before unfolding (Hannan, 1968). From observed patterns of individual leaf development (see Figure 2.2) one can see that when later leaves (ones which are smaller at full expansion) were still at the unfolding phase, the earlier leaves in the flush were already at the phase of rapid expansion, which is a phase of very high shoot demand for carbohydrate. Since later leaves were at the phase of most cell formation when earlier leaves constituted a strong sink for carbohydrate, clearly the significantly lower cell number shown by later leaves could be the result of sub-optimal supply of a growth supportive factor(s) such as carbohydrate, to achieve the total potential of cell division (production) in these leaves. It is indeed known for other species that the final cell number of a leaf

is governed by the amount of metabolites that the leaf can import from mature leaves (Dale, 1976; 1982). It has however been suggested (Borchert, 1973) that water stress, presumed to develop during the flush development, is responsible for the halting of leaf expansion resulting in the gradually decreasing size of the leaves within a flush. This suggestion cannot be true since the results obtained here show that differences in leaf size within a flush are determined by cell number and not by cell size. In addition the differences in leaf development originate at an early phase of expansion, (phase of greatest cell formation), when there is no significant increase in transpiration demand, since the young leaves do not yet have functional stomata. Furthermore, it has been shown previously (Hsiao, 1973; Michelena and Boyer, 1982) that the effect of water stress on leaf development is on cell expansion rather than on cell division.

Further evidence that there are competitive effects between leaves of one flush for carbohydrate, i.e. earlier leaves in the flush compete with later ones, was evidenced by the significantly larger areas produced by the later (remaining) leaves in one flush where the first and second leaves were removed when the later leaves were at an early stage of development. This demonstrates that later leaves have a higher potential for growth than that shown in a normally developed flush. Although the areas of remaining leaves were significantly increased (compared with the same leaves on control plants), when relieved from competitive effects of earlier leaves, these remaining leaves in the pruned flush still showed progressively reduced sizes with advancing position. Clearly these latter leaves still have their development affected by the earlier

remaining leaves which also had faster growth rates. These findings and interpretation are compatible with observations for other dicotyledons where leaves with higher growth rates dominate as sinks in the share of photosynthate from mature leaves (Starck and Ubysz, 1974).

From results presented in section 2.3.2 it is shown that a greater number of leaves and larger individual leaves (with correspondingly greater cell number per leaf) were produced in the flush following a period of continuous excision of young leaves, as compared with control plants. Such responses to continuous defoliation may reflect a reaction by the plant to restore the shoot:root ratios, like the compensatory growth found for other species when plants were partially defoliated (Humphries, 1958; 1960; Jacobs and Bullwinkel, 1953; Njoku, 1956). For cocoa, Sleigh (1981) showed that extended periods of defoliation resulted in continued (compensatory) root growth during the leaf excision period. It has been reported (Ericsson et al., 1985) that one of the mechanisms involved in the control of compensatory growth is an enhanced photosynthate level in the plant. It is therefore suggested that the larger areas and greater number of leaves shown by cocoa in a flush following defoliation may be a response to an enhanced availability of carbohydrate as the result of extended sink leaf removal. During extended defoliation, carbohydrate consumption for leaf growth will be reduced and continued photosynthesis by mature leaves will have ensured a higher supply for the root growth (which is moderate in amount), and likely also created a build up of storage carbohydrate above the level which occurs in a normal

interflush period. It is probable that the continuous leaf production during the excision periods is a result of an increase in cytokinin supply to the shoot apex from the increased root growth, whilst the larger than normal flush (more and larger leaves) following the defoliation results from increased amounts of carbohydrate available to support leaf growth. Production of flushes with more leaves than normal following a defoliation treatment, and the continuous production of leaves during extended periods of defoliation, also show that the apical bud is capable of continuously producing and expanding leaves, but during normal flush development the leaf production is brought to a halt at mid F-2 stage. It has been suggested (Orchard, 1977) that this cessation is the result of a competition between the apical meristem and the young expanding leaves for carbohydrate and/or cytokinins. This hypothesis was later supported by Sleigh (1981) when he showed that the expanding flush leaves constitute strong sinks for carbohydrate from mature source leaves and is now further strengthened by results discussed above which indicate that a competitive effect of the earlier leaves can limit the expansion of later leaves within the flush. This would also provide an explanation for the frequent curtailment of the development of one or two leaves at the end of one flush which fail to expand beyond about 1 cm, and abscise.

From results presented in Chapter 5 it was seen that leaf primordia are formed throughout the flush cycle, including the so called dormant period (Interflush) of the shoot apex, and that the apical bud contains a "store" of primordia. The fact that only a proportion of them develop to form one flush, clearly demonstrates that the number of leaves which form one flush is not limited by the

availability of primordia within the bud, but rather by some factor which prevents some of the "waiting" primordia from commencing further development. It is distinctly possible that this factor is insufficient carbohydrate to allow cell division, expansion and successful leaf development to occur. It is therefore suggested that the cessation of leaf production at the F-2 stage is the result of insufficient carbohydrate as a consequence of the high demand and competition among expanding leaves for a limited supply from current photosynthate. It is now appropriate to consider in more detail the availability and supply of photosynthate to the shoot apex.

As shown in section 3.2.2, the overall results on photosynthetic capacity of mature leaves, with particular respect to rates during the development of a new flush, demonstrate two features. Firstly, the photosynthetic capacity of leaves reduces with age, the reduction being partly due to increased stomatal resistance and partly to the fixation process. Secondly, cocoa leaves do not have any ability to increase their photosynthetic capacity during a phase of major increase in carbohydrate consumption by the seedlings. The simultaneous development of several leaves, together with stem growth, must create a strong drain for carbohydrate from mature leaves, and as there is no compensatory change in photosynthetic capacity of these leaves in response to the increased demand, it is therefore more likely that a carbohydrate stress will originate at the shoot apex.

It is also shown in section 3.2.2 that reduction of the number of mature leaves (source leaves) during F-2 stage, which will cause even greater demand for the photosynthate products from the

remaining leaf, still failed to stimulate any increase in photosynthesis of the remaining leaf. It is therefore evident that, unlike for some other species (Harold, 1980; Ho, 1979; Mondal et al., 1978), cocoa leaves have no capacity to increase photosynthetic production even under conditions of very major increase in sink demand. It is therefore suggested that, in cocoa, carbon requirements for growth, at least for leaf growth, exert no direct control on the photosynthetic capacity of mature leaves.

Data in Chapter 3, also show there were changes in the protein content of mature leaves during the new flush growth. It is difficult to speculate from these results about the mechanisms controlling the leaf protein change. However, it is possible that this protein fall is a result of the enhanced translocation of nitrogenous compounds, such as amino acids, from mature to expanding leaves at these times. It has been reported that, in a number of plants, nitrogenous compounds are translocated from mature leaves to supply the nitrogen demand of young leaves during the initial phase of expansion (Bray, 1983) and that growing leaves are heavy consumers of organic nitrogen from older leaves (Trewavas, 1985). If nitrogenous compounds are translocated from mature cocoa leaves, then this in turn, will contribute to lower rates of protein resynthesis in these leaves, and consequently the lowered leaf protein levels.

As shown in the same section (3.2.2) a decline in net photosynthesis was also observed in mature leaves during the development of a new flush. The decreases in leaf protein and net photosynthesis at the same time may emphasise that a stress does occur within the seedlings during the stage of higher demand for

organic compounds by the shoot apex. In fact, the reduction in photosynthesis may be the result of loss of leaf protein as photosynthetic enzymes. Similarly it has been reported that in tomato leaves of all ages, photosynthetic rate was limited by the activity of RUBISCO, determined mainly by the amount of RUBISCO protein (Besford et al., 1985). It is therefore suggested that lowered photosynthetic performance of mature cocoa leaves during the flush development may be the result of changes (reductions) in RUBISCO levels as a consequence of the translocation of nitrogenous compounds to expanding leaves. However, further investigation on photosynthetic enzyme levels in mature cocoa leaves, and their interactions with leaf nitrogen status throughout the flush cycle, are required for a better understanding of the photosynthetic response of mature leaves to increased sink demand in cocoa plants. Such a study would be important to an understanding of the control of cocoa pod production as well as leaf production.

To this point it is clear that some carbohydrate stress is indicated during flush leaf development and that mature leaves are not able to increase their photosynthetic performance to cope with increased demand. In fact their photosynthetic ability even declines from normal during the phase of leaf production. However, carbohydrate production is only one component of the carbohydrate supply position within a plant. In order to understand further the possible regulation of leaf growth by carbohydrate it is necessary to investigate carbohydrate distribution between source leaves and the various sinks, and also any role played by storage carbohydrate in meeting demands. The following part of the discussion therefore

concerns the questions of carbohydrate loading in sources leaves; transport from source to sink and unloading in sink leaves, all factors which could control the availability of carbohydrate for the developing flush.

As shown in section 4.3.3 the rates of translocation of ^{14}carbon products from leaves fed with ^{14}carbon-dioxide varies with the stage of the flush cycle. The highest rates of ^{14}carbon translocation were observed at the F-2 stage, which represents the stage of higher demand for carbohydrate by the shoot apex. Independently of the stage of the flush cycle, higher translocation rates were found to occur during the night than at day time. Similarly Hutcheon and Adomako (1974) studying the effect of infection with Cocoa Swollen Shoot Virus (CSSV) on accumulation of photosynthetically fixed ^{14}carbon in young plants of cocoa, (with the apical bud previously removed to prevent flushing), showed that higher rates of the ^{14} carbon translocation also occurred during the night. When cocoa seedlings were kept in extended (42 hours) dark and light periods the higher rates of ^{14}carbon translocation out of the plants in darkness was maintained. The higher rates of ^{14}carbon translocation at night indicates that the major part of the ^{14}carbon fixed during the light period was stored (temporarily) before being mobilised and translocated out of the leaves in the dark periods. It has been suggested (Farrar and Farrar, 1985) that in barley plants the relatively high rate of translocation in darkness is maintained by mobilization of leaf starch and vacuolar sucrose, the more important contributor to translocation being sucrose mobilization from the vacuole. Whether this is so in cocoa plants cannot be stated since there is a lack of information. Further

studies are needed to clarify this point. Nevertheless, if sucrose is the major sugar translocated in cocoa, as previously suggested (Hutcheon and Adomako, 1974) then the control of partitioning of the recently fixed carbon between sucrose and leaf starch, or even vacuolar sucrose, may be the basis of the factor controlling the translocation patterns of photosynthate from cocoa leaves. It has been suggested for other species (Fondy and Geiger, 1982; Ho, 1978; Thorne and Koller, 1974) that the sink demand has a very important role in regulating photosynthate translocation, although the carbon metabolism within the source leaves may also play a role in determining translocation patterns of these leaves. Evidence to support this suggestion can be seen from the rapid compensatory changes in export of the ^{14}carbon fixed by a single remaining source leaf when all the other source leaves were previously removed (section 4.2.2.2). In this case a higher rate of ^{14}carbon translocation was observed during the light period, soon after the ^{14}carbon-dioxide fixation, and it was followed by a gradual decline in translocation during the night. This gradual decline overnight, clearly shows that the more rapid removal of the ^{14}carbon-photosynthate from the single source leaf during the light period, could have prevented or, at least, considerably reduced the accumulation of recently produced photosynthate in storage compartments which would normally be used to supply night translocation demand. On the other hand the higher rates of the ^{14}carbon translocation, in darkness, from leaves of intact and later defoliated plants (six hours after the feeding) show that a considerable proportion of the newly fixed photosynthate has

accumulated in a more stable pool during the light period and is being subsequently translocated during the night. It does seem therefore that diurnal changes in carbohydrate metabolism in source leaves are decisive factors controlling the translocation patterns of leaves in cocoa seedlings, although it is recognised that differences in sink:source relationships may have triggered the changes in source leaf metabolism. Whether these diurnal changes in source leaf metabolism also determine the partitioning of fixed carbon among different sinks in cocoa is not clear from the results presented here, but it has been suggested for other species that: i) partitioning of exported carbon among sinks appears to differ between day and night (Fondy and Geiger, 1985; Huber, 1983; Kerr et al., 1985); and ii) that the diurnal carbon allocation between starch and sucrose can also affect partitioning among sinks (Geiger and Fondy, 1985). Huber (1983) showed that for soybean, tobacco, wheat, peanut and red-beet there were clear associations between leaf starch formation and allocation of carbon between shoot and root, the leaf starch being primarily utilized for growth of the shoot in the dark. It is difficult to see that this explanation applies totally to cocoa since export at night is higher at all stages of the flush cycle even when there is no leaf growth (e.g. I-1 and I-2 stage). However, it is suggested that the higher translocation overnight may reflect allocation of sucrose from leaf starch breakdown to other sinks. Nevertheless night export is greater at F-2 and this may represent the export of photosynthate for leaf growth mentioned by Huber (1983).

The rapid compensatory changes in the ^{14}carbon-export from the remaining source leaf of defoliated plants also show that the fed

leaf (source) can increase its export capacity when the sink demand upon it is increased. This clearly demonstrates that mature cocoa leaves in normal situations (intact seedlings) are operating much below of their maximum potential for photosynthate export and hence, both loading into the phloem and transport out from the leaf can operate faster than that which normally occurs from leaves, even at times of high demand (F-2 stage). It is interesting to note the comparatively low rates of export shown by mature cocoa leaves, even at the F-2 stage where only about 30 to 40% of the fixed ^{14}carbon was moved out of the leaf in the 24 hours following fixation. Other C_3 plants, which are generally considered to have low efficiency in the export of fixed ^{14}carbon, translocate around 40 to 50% in 24 hours (Hofstra and Nelson, 1969).

It has been reported for other species that defoliation treatments resulted in rapid compensatory changes in export and allocation patterns of photosynthate without changes in photosynthetic rates of source leaves (Fondy and Geiger, 1980; Wyse and Saftner, 1982) and that compensatory changes in photosynthetic rates apparently require changes in the photosynthetic apparatus (Thorne and Koller, 1974). In the present work, as mentioned above, there was no detected change (from infra-red gas analysis studies) in net photosynthesis of the one remaining source leaf following defoliation of all the other mature leaves of cocoa seedlings when new flushes were developing. Since source leaf defoliation resulted in increased export rates but no increase in photosynthetic rates, it suggests that the sink demand, at least in seedlings, does not directly control the photosynthetic capacity of mature leaves.

Furthermore, source leaf defoliation also appeared to reduce photosynthate accumulation during the light period, since the export rate was very much higher than normal (in intact plants). In some other species such changes have been shown to result in increased photosynthetic rates (Neales and Incoll, 1968; Upmeyer and Koller, 1973), but as this is not so for cocoa it seems that neither changes in export rates of photosynthate, nor assimilate accumulation, modify photosynthetic rates of cocoa leaves. It is therefore suggested that other mechanisms, such as interactions between nitrogen metabolism and levels of photosynthetic enzymes (and/or activity) are involved in the control of photosynthetic activity of cocoa leaves. It has been reported for soybean that much of the initial decline in RUBISCO activity was accounted for by the decline in the amount of RUBISCO protein (Boon-Long et al., 1983) and that leaves having higher carbon exchange rates per unit leaf area (CER_A) also contain more photosynthetic enzymes per unit leaf area than leaves with lower CER_A (Silvius et al., 1978). It is therefore suggested that further studies on photosynthetic enzymes in mature cocoa leaves are required before we can have a complete understanding of photosynthetic performance of these leaves throughout the flush cycle.

Results in section 4.3.2 showed that the photosynthate import relationships of sink leaves in cocoa are partly determined by vascular connections between particular source and sink leaves, but are also strongly influenced by the state of development of the sink leaves. The influence of vascular connections on photosynthate translocation pattern between source and sink leaves has been studied in a number of species (Larson and Dickson, 1986; Shiroya et

al., 1961; Thaine et al., 1959; Wardlaw, 1968), and it has also been suggested that the relative level of import into a developing leaf depends on its developmental stage and on vascular connections with the source leaf (Larson and Dickson, 1973; Nelson, 1963; Shiroya et al., 1961; Wardlaw, 1968). More detailed studies have shown that in some plants there are highly specific vascular connections whereby a source leaf can only supply the "aligned compartment" of a particular sink (Koch, 1984; Koch and Avigne, 1984; Murray et al., 1982). In the case of a cocoa seedling, where one source can supply all the leaves within one flush, (although the supply is not equal to all leaves), the vascular connection system is complex, but not so highly specific. It is, however difficult to characterise absolutely the relationship between any particular source leaf and different sink leaves within one flush due to the lack of basic information on the vascular organisation in the cocoa plant. Unfortunately, this is a point that has not yet received research attention. This topic is of particular concern since the products required for the general growth of cocoa, and also the ultimate production of cocoa pods, must move through the vascular pathways and yet without more detailed knowledge concerning the vascular system, we will not be able to understand basic aspects of cocoa growth control and pod production.

Since the earlier leaves of a flush, in more rapid expansion, imported a greater amount of the ^{14}carbon products than later leaves, which were in the initial phase of expansion, there clearly exists at this stage a stronger demand by earlier leaves for photosynthate for their growth. This supports the hypothesis

introduced previously that there is interaction and competition among expanding leaves for photosynthate within one flush, which can determine the individual size and number of leaves in the flush. As also demonstrated elsewhere in the same section, there was a faster rate of import of the ^{14}carbon products into a monitored sink leaf (developing flush) when all the other sink leaves of the flush were removed. Clearly, this confirms that one leaf in the flush is in competition with others for photosynthate and also that a developing cocoa leaf had a greater unloading capacity than that normally utilized during its development in a normal flush (intact flush). It is therefore suggested that development of a leaf within one flush is not limited by its metabolite unloading capacity, but by the limited availability of photosynthate to it as a result of limited supply to the whole flush and competition between the leaves in the flush. This also provides strong support for the hypothesis that the development of leaves within one flush is linked. The results from the ^{14}carbon import studies fully complement the results of growth measurements made when partial defoliation of one flush was carried out. In the former there is increased photosynthate supply to the remaining leaves, when competition is reduced, and in the latter case there is increased growth.

Further evidence that the supply of carbohydrate from current photosynthate becomes limited at the shoot apex, was shown by a quantitative assessment of the sizes of carbohydrate sources and sinks of a hypothetical cocoa seedling (shown in section 4.4). The calculated carbohydrate balance for the seedling was found to be very low positive or even negative during the phase when the earlier leaves in the flush were in the phase of rapid expansion. It was

therefore demonstrated (based on findings in the thesis and certain assumptions taken from data in the literature) by calculations, that the supply of carbohydrate from daily photosynthesis of mature leaves to the shoot apex was insufficient, at least for a short period during F-2, to fully support the expansion of the several simultaneously developing flush leaves. Although this constitutes a very strong support for the hypothesis that a carbohydrate stress develops in the shoot apex during the flush growth, the shoot should not be considered in isolation and ultimately carbohydrate availability calculations should also involve considerations of storage carbohydrate mobilisation. Although growth may be restricted by a transient carbohydrate stress, leaf development does continue in a negative carbohydrate balance situation and thus some storage material must be mobilised to make up the balance.It is however straightforward to understand that growth cannot continue at a rate which causes continuous depletion of reserves, otherwise the plant would soon become exhausted of reserve and death could follow. There must be within the plant an effective sensing mechanism detecting storage depletion and having a feedback effect on growth to prevent excessive depletion of reserves by halting growth. Following a period of relative growth dormancy during which current photosynthate production will exceed consumption, stores will be replenished to a point where further growth is permitted.

The continued production of new leaves when there is excision of young immature leaves around 2.0 cm long; stimulation of early flushing by removal of new flush leaves at F-2 (period of reversible dormancy, Vogel, 1975) and production of a larger than usual flush

when extended prunning is stopped, may all be seen as "special" cases of development where the normal mechanism protecting excessive depletion of carbohydrate reserves is overridden by the attempt of the plant to maintain the root:shoot equilibrium as normally as possible and the plant is able to deplete reserves to a lower than normal level. To be successful in a range of natural environmental stress situations and even natural plant damage (equivalent to pruning) a successful species will need to have some inbuilt capacity for flexibility regarding utilisation of reserve components.

As already mentioned, in completing a description of shoot growth control, the root (and stem) must not be ignored. In the case of cocoa it is clear from work by Sleigh (1981) that shoot and root growth of cocoa seedlings interact, with alternating periods of growth of each organ. This in itself is seen as further confirmation of limited carbohydrate availability in the plant which means that simultaneous growth of both structures is not possible. Further development of hypotheses which attempt to explain the control of growth of the whole plant, and of its different parts, can be seen in work of Reynolds and Thornley (1982). They propose, for several species, a model which suggests an explanation for the partitioning of growth between shoot and root in terms of i) balancing between carbohydrate synthesis by the shoot and nitrogen uptake by the root, and ii) subsequent distribution and utilisation of the components in each growth centre. It would now be very correct to extend studies on growth control in cocoa seedlings to include a comprehensive examination of nitrogen uptake, distribution and metabolism within the plants.

Summary of discussion and hypothesis for growth control - Summarily the information which has emerged from this work suggests that the growth pattern of the cocoa seedling shoot apex is mainly controlled by the carbohydrate status within the seedlings, although it is not possible to rule out completely that hormonal control is also involved. As mentioned early in this chapter, changes in the shoot apex during the flush cycle involve three different events, i.e. i) cessation of leaf production (at the F-2 stage), ii) maintenance of bud dormancy (at I-1 and I-2 stages) and iii) breaking of bud dormancy at F-1 stage. It is sugested that the carbohydrate control on these events is as follows. During the phase of rapid growth of the new leaves (in the F-2 stage) the demand for carbohydrate is very considerably increased at the shoot apex. Since the carbohydrate supply from mature leaves is not able to be increased to meet the large requirement of the strong sink of several simultaneously developing leaves, and because they have low photosynthetic capacity of their own (Baker and Hardwick, 1973), carbohydrate stress develops within the seedlings. An early indication of carbohydrate limitation is very major reduction of root growth during mid F-2, this system submitting to the stronger sink of the shoot apex. Afterwards growth of the new later leaves in the flush is particularly restricted (they are smaller at maturity) due to competition between leaves in the flush for carbohydrate. Leaf growth and production of more leaves thus stops. Some carbohydrate reseves are mobilised during the phase of negative current carbohydrate balance to complete leaf development (Sleigh, 1981). Enhanced new primordia formation is also possible during early F-2 because of the

favourable carbohydrate availability, but the rate reduces to low as carbohydrate stress develops later in F-2. Another feature of the reduction in root growth caused by shoot competition for carbohydrate is that the output of growth stimulating plant growth regulators would be reduced (Orchard, 1977), and thus stimulation of shoot growth would also be lost, causing shoot dormancy by linked carbohydrate stress and low growth regulator promotion. During I-1 stage the newly expanded leaves are still not self-sufficient in terms of carbohydrate (Baker and Hardwick, 1973) and thus their contribution to the carbohydrate supply for the seedling will then be low. It is only during I-2, after the new leaves are fully photosynthetic, that the carbohydrate status of the plant is returned to normal with a strongly positive carbohydrate balance. Dormancy is thus largely maintained in interflush phases because of the need to replenish carbohydrate reserves. The breaking of dormancy at F-1 can take place when carbohydrate levels are high again. Under the now favourable carbohydrate conditions the root system is the first to show a detectable growth increase.

(This therefore may in part be an "accident" because it is easy (with root boxes) to see initial changes in growth rate of roots against a glass "window" whereas the initial changes in growth of the shoot apex will be "hidden" within the sheathing stipules of the terminal bud. Earlier growth stimulation of roots may also occur when the sink strength of the new flush leaves declines as they mature and become photosynthetically active since the declining sink activity of the new flush will affect the source furthest away first i.e. the older (PF_2, PF_3) leaves, relieving them from the role of supplying the shoot. They will then be able to supply the root system and stimulate its growth. Indeed export from the PF_3 and PF_2 leaves is seen to increase at this time (I-2), before the increase from PF_1 leaves when the shoot apex growth increased in F-1 and F-2).

The increase in root growth will increase production of root-originating plant growth regulators, such as cytokinins and

gibberellins (Orchard, 1977) which when exported will reinforce the growth stimulation caused by increased carbohydrate availability, leading to bud break at the F-1 stage and the sequence of events described at the start of the section will begin again. The growth cycle is thus complete and continued largely through alternating carbohydrate status of the seedlings.

This study constitutes a fundamental contribution to the understanding of the growth pattern of cocoa seedlings and provides basic information for further studies on carbohydrate partitioning in cocoa plants. It would now be possible to extend the investigation into the following aspects: i) compartmental analysis of photosynthate assimilates in cocoa leaves; ii) relationships between leaf nitrogen level, photosynthetic enzymes and photosynthetic capacity of leaves, and iii) examination of the phloem connections between leaves in order to provide a more complete understanding of photosynthate translocation in cocoa plants.

Chapter 4 - Appendix 1

Estimate of carbohydrate balance in cocoa seedlings.

1. Carbohydrate production

Determination of total leaf area per seedling, maximum photosynthetic and night respiration rates (using infra-red gas analyser) were made for mature leaves from three flushes. Data from these determinations are shown in table 4.3

Table 4.3. Components for carbohydrate production calculation of mature leaves.

Flush	Light intensity at flush level (μ mol.$m^{-2}.s^{-1}$)	Net Photosynthesis ($g.CO_2.m^{-2}.s^{-1}$)	Night respiration ($g.CO_2.m^{-2}.s^{-1}$)	Leaf area (m^2)
PF_1	300	15.3×10^{-5}	0.99×10^{-5}	0.12308
PF_2	204	11.7×10^{-5}	0.82×10^{-5}	0.06065
PF_3	122	8.0×10^{-5}	0.62×10^{-5}	0.08148

1. Production of PF_1 flush

Since photosynthetic rates of leaves were not constant throughout the light period, it decreased from 13:00 h onwards, total photosynthesis per day calculations included a consideration for the decrease from 13:00 to 17:00 h. So, from 13:00 to 14;00 h photosynthesis represents 93.8% of the maximum and:

14 : 15 hrs = 79.8% of maximum

15 : 16 hrs = 62.6% of maximum

16 : 17 hrs = 53.8% of maximum

17 : 18 hrs = 49.2% of maximum

For PF_1 flush the maximum net photosynthesis was 15.3 x 10^{-5}g $CO_2.m^{-2},s^{-1}$. So it has for each hour starting from 13:00 to 17:00h:

13:00 = 14.3 x 10^{-5}g $CO_2.m^{-2}s^{-1}$

14:00 = 12.16 x 10^{-5}g $CO_2.m^{-2}.s^{-1}$

15:00 = 9.58 x 10^{-5}g $CO_2.m^{-2}.s^{-1}$

16:00 = 8.23 x 10^{-5}g $CO_2.m^{-2}.s^{-1}$

17:00 = 7.53 x 10^{-5}g $CO_2.m^{-2}.s^{-1}$

Total net photosynthesis per each hour period.

13:00 = 0.5148 g $CO_2.m^{-2}$

14:00 = 0.4377 g $CO_2.m^{-2}$

15:00 = 0.3449 g $CO_2.m^{-2}$

16:00 = 0.296 g $CO_2.m^{-2}$

17:00 = 0.271 g $CO_2.m^{-2}$

Total net photosynthesis from 13:00 to 18:00 h is = 1.8648 g $CO_2.m^{-2}$.

Total net photosynthesis from 6:00 to 13:00 h is = 0.5508 x 7 hours = 3.8556 g $CO_2.m^{-2}$.

Total net photosynthesis in the 12 hours 6.00 to 18.00 is: = 5.720 $g.CO_2.m^{-2}.day^{-1}$

Total net photosynthesis (PHS) for PF_1 flush is therefore 5.720 x 0.12308 m^2 = 0.704 $g.CO_2.day^{-1}$.

Total net PHS for PF_1 = 0.704 $g.CO_2.day^{-1}$.

Daily net PHS for PF_1 = Daytime net PHS - night respiration.

Night respiration for PF_1: 0.99 x 10^{-5} $g.CO_2.m^{-2}.s^{-1}$.

Total Respiration in 12 hours = 0.42768 $g.CO_2.m^{-2}$.

Total respiration for PF_1 = 0.0526 $g.CO_2.night^{-1}$

with a leaf area of 0.12308.m^2.

So, daily net PHS for PF_1 flush is:

0.704 - 0.0526 $g.CO_2.day^{-1}$

Daily net photosynthesis for PF_1 = 0.6513 $g.CO_2.day^{-1}$

For PF_2 flush, with PHS calculated on an identical basis as for PF_1, but using a light intensity of 204 $\mu.mol.m^{-2}.s^{-1}$.

Total net PHS for PF_2 = 4.373 $g.CO_2.m^{-2}.day^{-1}$

Total net PHS for PF_2 = 0.264 $g.CO_2.day^{-1}$

Night respiration for PF_2

Night respiration = 0.82 x 10^{-5} $g.CO_2.m^{-2}.s^{-1}$

Night respiration = 0.354 $g.CO_2.m^2.day^{-1}$

Total night respiration for PF_2 = 0.0214 $g.CO_2.day^{-1}$.

So Daily Net PHS for PF_2 is

0.264 - 0.0214 $g.CO_2.day^{-1}$ which is

Daily net PHS for PF_2 = 0.2426 $g.CO_2.day^{-1}$

Total PHS for PF_3 flush, calculated as above, but using a light intensity of 122 $\mu mol.m^{-2}.s^{-1}$ and a total leaf area of 0.08148 m^2 gives a daily net photosynthesis:

Daily net PHS for PF_3 = 0.219 $g.CO_2.day^{-1}$

Thus the total daily net fixation of carbon dioxide by the hypothetical seedling is formed from:

Daily net PHS PF_1 = 0.6513 $g.CO_2.day^{-1}$

Daily net PHS PF_2 = 0.2426 $g.CO_2.day^{-1}$

Daily net PHS PF_3 = 0.219 $g.CO_2.day^{-1}$

The stoichiometry of carbon reduction shows that six molecules of CO_2 must be fixed to produce one molecule of hexose carbohydrate, i.e. 264 g of CO_2 form 180 g of hexose. Therefore daily production of carbohydrate for seedlings is the sum of:

PF_1 = 0.444 g $(CH_2O)_6$

PF_2 = 0.165 g $(CH_2O)_6$

PF_3 = 0.149 g $(CH_2O)_6$

2. Photosynthate export

It is not easy from the ^{14}carbon-dioxide feeding experiments to obtain highly accurate estimates of the proportion of photosynthate produced by a leaf in 24 hours (day) which is exported. Carbon-dioxide fixed will go into different pools, some representing short-term storage and other longer term pools (Farrar & Farrar, 1985). From the ^{14}carbon feeding experiments it is clear that ^{14}carbon fixation products remain in the leaf for several days and some will be permanently incorporated into leaf tissue and thus not available for export and some will be respired and lost as gaseous CO_2. Less than 100% of the photosynthate produced by a leaf will thus be

available for export.

Results on ^{14}carbon export, discussed in section 4.2.2, showed that on average the leaves from PF_1, PF_2 and PF_3 flushes exported respectively 35.3, 29.6 and 29.4% of the fixed ^{14}carbon from the source leaf at F-2 stage in 24 hours. The continuing pattern of export for 48 hours also showed that about a further 10 to 15% of the fixed ^{14}carbon was translocated out of the fed leaf between 24 and 48 hours after the ^{14}carbon-dioxide fixation. So, the total export of fixed carbon in each day was considered as, for example as the sume of 35.3% (the current day photosynthate in 24 hours) plus a further 15% representing export of the previous day's photosynthate. Any contribution of photosynthate produced two or more days previous to the day in consideration will be less than 5% and can be safely ignored.

So, the proportion of photosynthate translocated at F-2 stage for

PF_1 = 35.3% + 15% = 50.3%

PF_2 = 29.6% + 15% = 44.6%

PF_3 = 29.4% + 15% = 44.4%

The amount of carbohydrate translocated from PF_1 = 50.3% of net production, which is 50.3% of 0.444 g = 0.223 g per day.

Similarly the calculation for PF_2 is:

Production = 0.165 g

Total export at 44.6% of production,

So amount translocated = 0.0736 g $(CH_2O)_6$

For PF_3 calculation is:

Production = 0.149 g

Total export at 44.4% of production is = 0.0662 g $(CH_2O)_6$

Total carbohydrate exported per day is thus the sum of that from each flush.

PF_1 = 0.2230 g

PF_2 = 0.0736 g

PF_3 = 0.0662 g

Total = 0.3628 g

3. Translocation to shoot apex and developing leaves

From results showing i) carbohydrate distribution in cocoa seedlings (Sleigh, 1981), and ii) results obtained earlier on import into expanding leaves, it was found that on average, at the F-2 stage, about 60% of the carbohydrate translocated out of the fed leaf was imported into developing leaves. Thus the carbohydrate availability to developing leaves from current photosynthate is calculated as 60% of 0.3628 g = 0.2177 g.

4. Carbohydrate consumption for leaf growth

As explained in section 4.4.1 the daily amount of carbohydrate consumed is calculated from a detailed knowledge of growth rates of the flush leaves in a typical flush (Chapter 2). Increase in leaf area is converted to a dry weight equivalent from the regression equation of the line produced by plotting $\log_{10}$ dry weight against $\log_{10}$ leaf area, which is: $\text{Log}_{10}\, y = 0.77 + 0.73 \log_{10} x$, where: x = leaf area; y = dry weight; n = 25; $r^2 = 0.98$.

To originate the equation, 25 leaves of several sizes from 2.5

cm long to fully expanded leaves, were randomly collected from 15 plants comprising plants from early F.2 to I.1 stages of the flush cycle. Length and width were then recorded for leaf area calculation, and the leaves were separately oven dried at 65°C for 72 hours for dry weight determinations. Leaf area was calculated by the equation shown in Chapter 2.

Dry weight calculated from the equation was highly correlated with that from the dried leaves (r = 0.99). So, leaf area increase for each day was converted to an equivalent dry weight and then the carbohydrate required to produce the dry weight increase was calculated. The carbohydrate requirement was then calculated assuming that 700 g of vegetative tissue can be formed from 1000 g of carbohydrate photosynthates (Penning de Vries, 1983).

Finally, the daily balance for carbohydrate for the seedlings was obtained through: **Carbohydrate availability - carbohydrate consumption = carbohydrate balance.**

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