THE DISTRIBUTION OF GROWTH IN A MAIZE LEAF

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ABSTRACT

The extension in a maize leaf phytomer was studied in terms of the one dimensional displacement of its fabric relative to its base as a function of position and of age. The relationship between displacement, position and age was termed the displacement field. The displacements, during a 24 hour interval, of a series of points spaced along the length of a particular phytomer was recorded. This provided an Eulerian evaluation of the 24 hour displacement field. A population of plants each representing a different age were used and the average displacement field of the phytomer during its period of growth was established.

Extension was localised in the region named the growth zone at the base of the phytomer. The length of the growth zone rose and fell following a wave shaped curve with respect to age. It was never constant. The relationship between the displacement of and position of points within the growth zone formed a smooth sigmoid curve. The presence of the ligule and of the node of leaf insertion did not give rise to any local reduction in extension.

A method of tracing the movement of a point in the leaf fabric, through the growth zone, over a period of time is explained. Such Lagrangian pathlines are described using referential notation, $x=x(t;x_o)$. The pathlines of points were constructed by a stepwise addition of the relevant displacement values read from the displacement field for each successive position and age.

The pathlines of two chosen points provided positions, as a function of age, for the boundaries of a growing and moving segment. The segment was in mid lamina. Its length increased from 0.025mm when its lower boundary was at 3.6mm from the leaf base to 2mm at maturity. The numbers by types of cells in the lower epidermis of the segment were surveyed at a series of plant ages. There was an initial decrease and then a considerable increase in the average length of the non stomatal cells during the period of the experiment. During the same period the segment increased in length and this was accompanied by an increase in the number of cells in the sample rows. This increase in cell numbers was continuing when the segment reached a position 40mm from the base of the leaf within the then 67mm long growth zone. The associated cell division was occurring in all of the cell categories, not the stomatal complexes alone. The development of stomata with respect to plant age is discussed. Stomatal initiation in the leaf segment was not restricted to a single occasion or unique position in the growth zone.

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CHAPTER 1

INTRODUCTION

Growth refers to the increase in size of a structure as a function of time. If the growth in length is observed and the length is subdivided, each of the subdivisions will contribute to the overall extension. The rate of increase in the subdivisions may differ. For example in a structure such as a plant root there is a mature region, showing no extension, and a growth zone. Within the growth zone the rate of extension is greater than zero and at any given time varies with the distance from the root tip. Structural and biochemical changes associated with tissue development take place in growth zones. The levels of these characters can be estimated at specified times and positions in a growth zone but if they are to be used as illustrations of the changes that occur during development it is necessary also to know how far the material at a particular site will travel during the time interval involved. This allows the observed differences in level to be discussed as a function of the age of the tissue and not merely as a function of position on a length of an axis.

The growth zone studied was that of a maize leaf. The species *Zea mays* L. is a member of the Gramineae and the leaves of this family are typically strap shaped. A strap shaped structure is built when expansion in a single dimension predominates. In the Gramineae this polarisation of expansion is associated with the occurrence of rows of cells running parallel to the long axis of the leaf. It is also associated with a growth zone restricted to the base of the leaf during the greater part of the leaf growth period. The Gramineae includes some of the most important crop species and this coupled with the simple geometry of the developing leaves has led to their being used as subjects for studies of foliar growth in a large number of experiments. As a result there is a considerable body of information about leaf development in the Gramineae. This includes Knowledge of the effect of environmental factors on the overall increase in length of the leaves as a function of age and of the levels of chemical and morphological characters of leaf tissues as a function of position. Only a small amount of attention has been paid to the distribution of growth through the length of the leaf however and a true age function is lacking in the majority of the structural and chemical studies. This thesis describes a method of determining the distribution of growth by measuring the displacement of marks in the growth zone of a maize leaf. It also demonstrates the use of this information to establish the relationship between the position of a particular point in the leaf structure and time, and shows that this allows changes in the structure of the developing epidermis to be discussed as a function of age.

CHAPTER 11

REVIEW: DESCRIPTION AND LOCATION OF THE MAIZE PHYTOMER GROWTH ZONE

EXTENSION OF THE PHYTOMER.

The formation of leaf primordia at the stem apex in maize was described by Sharman (1942a). The leaf initial is first recognised as a protrusion at the side of the stem apical dome (Fig. 1). The protrusion increases in length to form a pointed leaf tip and extends laterally as two ridges which proceed to encircle the stem apex. The ridges overlap where they meet at the far side of the apex. At this stage the leaf primordium resembles a collar. The lower margin of the collar adheres to and surrounds a disc of stem tissue, the node. The leaf initials are in two vertical series and their tips alternate on either side of the stem apex. In the primordial condition the interval between the nodes, the supporting internodal stem, is very short. Poethig (1984) was able to demonstrate that the initial protrusion of the leaf was the result of altered activity in a group of cells in the apical dome. This group included cells from the two outer layers of the dome and as the primordium increased in size cells were recruited from a volume of tissue two cell layers deep, several cells in vertical length and occupying a complete horizontal ring round the stem apex. The predominant activity during the growth of the leaf consists of vertical extension of the collar parallel to and above the stem apex. This converts the collar into a cowl and the upper part of the cowl eventually unrolls to form the leaf lamina. The primordial ligule appears near the base of the cowl. The supporting internode also extends vertically. The composite structure of a leaf with its node and the supporting stem internode is called a phytomer (fig. 2).

Leaf expansion follows a pattern. The increase in size of the primordium is gradual but builds up at a virtually exponential rate (Williams, 1975). This is not however sustained; a maximum rate is achieved and is followed by a decline in rate until increase ceases. Leaf growth is thus represented by a sigmoid curve. A number of leaf characteristics have been studied as a function of time and the relationships follow the same pattern. For example Kaufman (1959) measured blade, sheath and whole leaf length in rice. Williams and Rijven (1965) also measured lengths, and Williams and

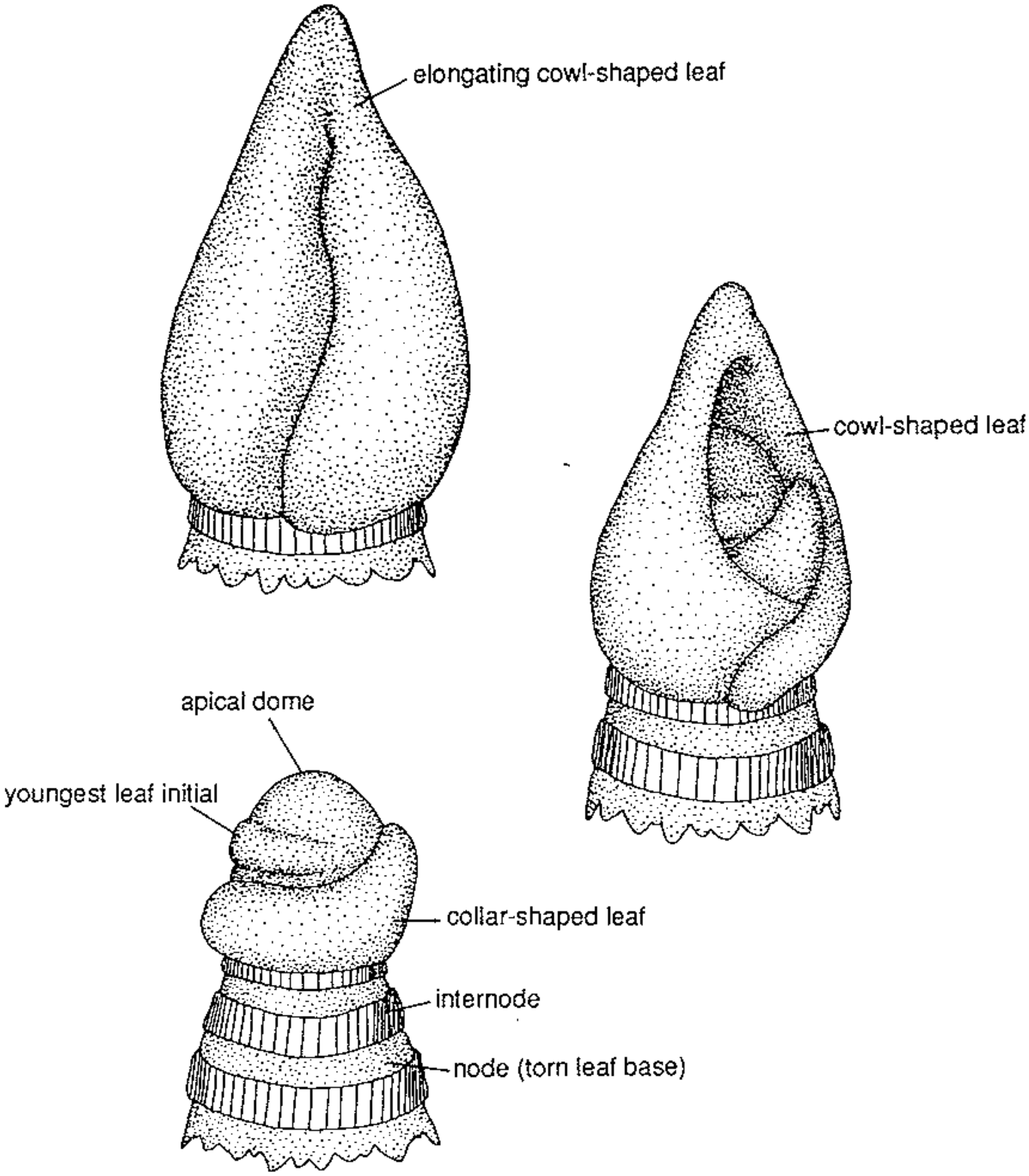


FIG. 2.1. Appearance of the stem apex of a maize plant. The leaf primordia were dissected away in sequence. Magnification: x160. Redrawn by I. McGee.

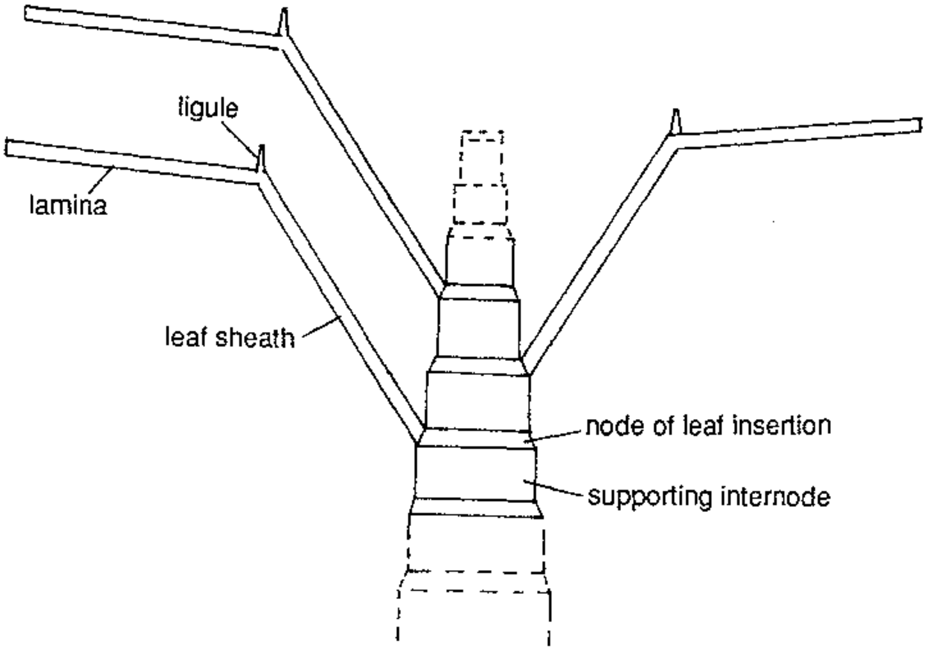


FIG. 2.2. Diagram to show the structure and arrangement of phytomers on a member of the Gramineae.

Williams (1968) volume and dry weights; they worked with a series of leaves on wheat plants. McCree and Davis (1974) examined leaf area in sorghum. In each case when the cereals were grown in constant conditions the levels of each of the characters as a function of age yielded a sigmoid curve.

These records describe the integrated expansion of the whole leaf but the expansion does not proceed at a uniform rate throughout the length of the leaf, or of the phytomer. In their studies on the effect of defoliation Begg and Wright (1962) showed that there was no capacity for regrowth in a fully exposed leaf lamina of reed canary grass. They argued that growth was occurring in that part of the leaf which was enclosed within the sheath of older leaf bases and that extension of the distal part of the lamina must have occurred in this basal length at an earlier time. In their experiments on Faris banding Taylor Halligan and Rowley (1975) found that the Faris bands, which form on a leaf as a result of wide temperature differences during growth, could not be seen for several days. This was the time it took for the affected part of the leaf to emerge from within the sheaf of the older leaves. These experiments showed that there is a section of mature leaf subtended by a length in which extension is still proceeding.

The region involved in extension is termed the growth zone. The various parts of the phytomer emerge from the growth zone in sequence commencing with the leaf tip. Sontag (1887, cited by Kaufman, 1959) found that the tip of the grass leaf ceased to extend when the leaf was in the region of .5 mm long. Kammerling (1904, cited by Ruinard 1966) observed that the lamina, sheath and supporting internode in a sugar cane phytomer extend and mature in that order. Kaufman (1959) showed that the leaf sheath entered a phase of appreciable extension a plastochron later than did the leaf blade. Malvoisin (1984) carried out an extensive study of phytomer extension as a function of age in wheat . He measured age in terms of the numbers of fully expanded leaves. Under constant temperature he obtained typical sigmoid curves for each of the three parts of the phytomer: leaf lamina, leaf sheath and internode. The timing of these curves showed that whereas the lamina was affected by a high rate of extension at a time when extension of the sheath and the supporting internode was extremely small or zero; extension in the two latter built up, and in fact was proceeding relatively rapidly during the period of maximum lamina extension. Extension of the leaf sheath and internode reached their highest levels however when the lamina growth rate had fallen prior to its cessation. Approximately 0.98 and 0.4 of the total length of the sheath and internode respectively were formed while the lamina growth rate was falling. Cessation of growth in the lamina preceeded that in the sheath and internode by approximately one plastochron and extension ceased in the leaf sheath slightly earlier than in the internode.

These observations tend to suggest that the grass leaf is extruded by a region of production in the growth zone. Research in which growing grass leaves are subjected to irradiation shows that this is not a satisfactory model. If maize plants which are heterozygous for genes involved in the production of chlorophyll are irradiated some of the cells lose the capacity to produce chlorophyll and they and their descendant cells are yellow. Poethig (1984) irradiated maize seedlings with leaf primordia .6 and 1.2mm long, and Stein and Steffenson (1959) irradiated maize seed. They grew the plants and located and measured the yellow sectors on the leaves. Their work showed that the cells present in the primordia were ancestral to different parts of the leaf. Poethig (1984) also showed that while the increase in the more distal parts of the leaf was much more rapid and obvious, extension at its base occurred throughout the period of its growth and eventually the basal part of the primordium gave rise to a substantial part of the mature leaf. The various sections of a grass leaf are present in the primordium. They are in a diminutive form but are in their correct relative positions, and leaf growth consists of their expansion.

Sharman (1942) described the growth of maize phytomers as occurring in a wave of extension followed by maturation. The effect of the wave commenced at the tip of the lamina and moved through to the base of the supporting internode. The wave shape represented the pattern of variation in the rate of extension as a function of position within the growth zone at a given time. The movement of the wave reflected the observation that different sections of the leaf were involved in their major expansion in a sequence commencing at the tip and proceeding towards the base.

The length of and the rate of expansion within the growth zone alters during leaf growth. Yamazaki (1963) examined the relationship between the lengths of the whole leaf, the leaf sheath and the growth zone in rice leaves. She prepared a detailed description of the six youngest leaves on a stem apex. She expected the growth of the leaves to follow an exactly repeating pattern and used leaf number as a time scale. She took the most proximal position of mature stomata as an indicator of the distal limit of the growth zone. She found that the length of the growth zone increased very rapidly, reached a maximum and fell away quite suddenly. The ligule was distinguished near, but not at, the base of the growth zone shortly before the latter reached its maximum length. The rate of increase in length of the leaf was maintained for a short period after the appearance of the ligule. This indicated very high rates of expansion in the lower lamina and sheath at this time. As the growth zone became restricted to the immediate vicinity of the ligule in the lamina, and to the leaf sheath, the overall extension rate fell back. After the ligule had left the growth zone the length of the latter became rapidly smaller until it reached zero. The rice leaf sheath was formed in the primordial leaf; it lay within the growth zone throughout leaf growth. The rate of extension of the leaf sheath was slow, it evidently increased as leaf extension continued but it never compared with that of the more distal parts of the structure. Malvoisin (1984) also showed that the relative growth rate reached a higher maximum in the lamina than it did in either the sheath or the internode and that the internode showed the lowest relative growth rate.

The leaf or phytomer growth zone includes a meristem or region in which cell division is taking place. Denne (1960) carried out a study of the frequency of mitoses and the distribution of cell size as a function of position in the growing leaves of *Narcissus pseudonarcissus*. The species is not a grass but has a similar strap shaped leaf with a morphological division into blade and sheath. Denne showed that whereas they were less frequent near the leaf tip, cell divisions occurred throughout the length of leaves which were less than 15mm long. In these leaves the position where the highest number of cell divisions was recorded moved out from the base as the leaf became longer. This outward movement was limited however and the position of most abundant cell division was in the vicinity of 10mm from the base of the blade in leaves which were 100 or 250mm long as well as in the 15mm long leaf. No cell divisions were found more than 50 mm from the base in any of the leaves. When the leaf was 500mm long and almost full grown the very few cell divisions that occurred in the blade were found at its very base. In leaves of this size the leaf sheath had extended to at least 15mm and divisions were found throughout the sheath but their frequency rose towards its base. Even the highest level in the sheath however was not equivalent to that found in the lamina during the central part of the growth period. Thus Denne showed that there was a meristem near the base of the narcissus leaf. Its length increased during early leaf growth and in the palisade tissue came to extend 50mm into the leaf. It apparently retained this length during the greater part of the leaf's growth although there is evidence that it was shorter when its activity was restricted to the sheath cells alone. It occupied 20mm when the leaf was almost mature.

At its inception the growth zone of the phytomer is continuous with that of the apex and it remains so until the mature region has extended to include the whole leaf and the its node of insertion. In this work however I have found it convenient to consider the phytomer growth zone as a separate entity with boundaries drawn at the bases of two sequential internodes. I have described the phytomer growth zone as intercalary because it lies between two regions of mature tissue: that advancing from the leaf tip and that established in the lower stem.

HISTOLOGICAL STUDIES OF TISSUE DEVELOPMENT

Histologists involved in the study of cell development have found the growth zone of grass leaves useful and a number of studies of cell structure as a function of position in the leaf have been carried out. The work on leaf extensiom which I have described above shows that a set of data collected at a single time and recorded in terms of position will include information about all of the longitudinal parts of the leaf. The tissues occupying increasingly distal positions in the growth zone are therefore not the basal tissues in a more mature state. Miranda, Baker and Long (1980) pointed out the need for caution in comparing immature tissues from one part of a leaf with those which are mature but lie in another part. They investigated a number of cell characters as a function of position on a maize leaf. Their leaves were 17cm long and had a ligule at 5cm. They regarded the basal 5cm as being immature but they found considerable variation along the length of the mature section. For example the lengths of the epidermal cells varied as a function of position. Their mean lengths ranged from approximately 230µm 3cm beyond the ligule to 160µm near the tip of the blade on the lower surface and from 130µm to 170µm on the upper surface. There was an erratic rise and fall of epidermal cell lengths along the blade but an overall fall towards the tip. As opposed to these limitations the leaf growth zones of the Gramineae have a simple geometry with the cell rows parallel to the leaf axis and in them, the average cell in a more distal piece of tissue shows a greater degree of differentiation than does one in a proximal piece. The growth zones have been used to great advantage in a number of studies of cell differentiation, particularly those of stomatal development.

I shall review some of the studies which have used relative positions in the growth zone to describe the process of stomatal differentiation.

Campbell (1981) described the sequence of morphological events during stomatal differentiation in maize. He observed that asymmetric divisions took place in certain of the rows of cells in the immature epidermis. These prestomatal cells gave rise to pairs of cells and the more distal of the pair occupied a very short section of the cell row. These were the guard mother cells. The lengths of these cells increased until they they were approximately square. At this stage two small cells were formed adjacent to the guard mother cell but originating as part of cells in the adjoining epidermal cell rows. These became the subsidiary cells. The guard mother cell became rounded in outline and divided with the new wall parallel to the leaf axis. The rounded outline gave way to an elongated shape. The subsidiary cells also extended parallel to the line of the cell rows and acquired a triangular shape. Campbell described the size of the subsidiary cells as dominating the appearance of the mature stomata in which the guard cells were very long and narrow and the pore a dumb-bell shape. Campbell also pointed out that whereas when the stomata were first formed (he was referring to the initials closest to the base of the growth zone) they alternated in easily identified rows, and this regularity was not apparent in the mature epidermis.

Porterfield (1937) studied the cytology of the basal sections of cell rows in growing internodes and the sheath leaves of bamboo stems. He found very short cells in the cell rows at the base of the young stem internodes. These cells were broader than long, their cytoplasm was dense, and their nuclei large. He anticipated that some of these cells would be the guard mother cells of stomata. He found other stomatal guard mother cells formed following asymmetric division in protodermal cells which had travelled further from the base of the organ and had become elongated and might be vacuolate. In this part of the meristem alternate cells of the protodermal row were involved in asymmetric division and the nucleus or unusually dense cytoplasm occupied the distal end of these cells prior to division. He noted the presence of vacuoles in the proximal parts of these cells. Porterfield cites Pfitzer (1870) as having pointed out that the expansion of the developing guard mother cells is slower than that of the surrounding cells and suggested that the intrusion of the subsidiary cells as extensions of cells in neighbouring rows is a consequence of this. Porterfield's observation that stomatal guardmother cells do not all originate at the same time in the developmental sequence is interesting and it could help to explain the difference in the arrangement of stomata in the mature tissue as compared to that of the initials at the base of the growth zone noted by Cambell. If additional stomatal initials are formed they are never located adjacent to an earlier guard mother cell. Bunning (1956) suggested that a developing stoma inhibits the formation of others adjacent to it but that, since the tissue was expanding, the distance between the initials would be increasing and this would eventually lead to there being so little inhibition in sites halfway between the earlier initials that new stomata would form in them. Porterfield observed that the alternating pattern of protodermal and stomatal cells is maintained during the formation of the additional stomatal initials.

Stebbins and Shah (1960) also observed that the stomatal complexes usually alternated with single intervening epidermal cells. Stebbins and Shah described the stomata as occurring in cell rows parallel to the leaf axis and found that these rows were in a pattern which was peculiar to the species or a group of species. They found that in maize there were usually two or three stomatal rows adjacent to each other and these sets were separated by a contrasting set of cell rows bearing no stomata. The stomatal rows were above or below mesophyll tissue between the vascular bundles in the leaf. Stebbins and Shah pointed out that stomatal rows could be recognised before there were any guard mother cells present because the cells in these particular rows were relatively short. Working along the rows which included stomata Stebbins and Shah studied the variation in the developmental stage of the complexes as a function of position. They found considerable variation and in some regions this took the form of alternation between a less and a more mature stage in successive complexes. In a few instances a guard mother cell was found in a region where the majority of the stomatal complexes were mature.

EXTENSION AS A FUNCTION OF POSITION WITHIN THE GROWTH ZONE

In order to extend the body of information provided by these histological studies it is necessary to be able to describe the extension and resulting change in position of the tissues involved. This would allow changes in tissue length and in cell number and stucture to be studied as a function of time as opposed to as a function of position along the leaf. Extension in a growing leaf in the Gramineae is not uniformly distributed. It varies with the position in, and also the age of, the leaf.

In order to study the distribution of extension along the leaf a set of reference points is needed. These must be placed at intervals through the length of the growth zone. In a grass leaf the natural features (tip, ligule, leaf insertion and lower node) are available but they do not provide sufficient subdivision. Avery (1933) followed the divergence of a set of marks painted onto the surface of an expanding leaf and a number of workers have used his method to study growth in Dicotyledonous leaves. These painted marks can only be applied to an exposed organ however and the expanding region of a grass leaf is enclosed within a sheaf of older leaf bases. These outer leaves must either be cut away or marks must be inserted through them.

Kuiper (1915) experimented with a set of holes made with a fine needle which was thrust through the leaf sheaf surrounding the stem apex. The holes were inserted at 10 or 20mm intervals. After several days he harvested and dissected the plants. He located the holes and calculated the extension that had taken place between them. His method was used by Van Dillewijn (1939) who recorded the position of the holes on all of the leaves in the sheaf in order. He showed that at a given plastochron age extension as a function of position took the form of a curve. Extension was low near the base of the phytomer, it rose, reached a maximum, dropped, and finally fell to zero as the position became more remote from the base of a leaf. In young leaves the maximum was in the central part of the growth zone. As the plastochron age of the leaf became greater the part of the phytomer affected by maximum extension moved basipetally. The shape of the extension curve was not altered by the position of the ligule in the growth zone but both length and maximum extension were smaller by the time internode extension became measurable. The graphs indicate zero extension at the node of leaf insertion. This is in contrast to Malvoisin (1984) who measured nodal growth rates. Van Dillewijn's diagrams however show substantial extension in the segments of phytomer immediately above and below the node and I suggest that the lack of extension at the node was assumed rather than measured.

Davidson and Milthorpe (1966) used pinholes to locate the growth zone in coxfoot *Dactylis glomerata*. Their interest was in the effect of defoliation on leaf growth and their measurement of divergence in the growth zone was minimal, but they estimated that the punching had caused a 22% reduction in growth. Kemp (1980) carried out a more extensive study in order to locate the growth zone and to establish a relationship between its length and that of the encircling leaf sheath. He inserted the pinholes at 10mm intervals and harvested the plants after 24 hours. He calculated the relative extension of the length of leaf between pairs of holes in the series. All segments for which the natural log of the ratio was greater than 0.05 were included in the growth zone. He worked with partly to fully emerged leaves from a population of variously sized wheat plants and found a wide variation in their lengths of growth zone. This variation was related to the length of the sheath of the leaf in question; as the sheath became longer the growth zone became shorter. Kemp expected to find two separate sheath and blade growth zones but found little evidence for the separation. His growth zones exhibited a similar profile of extension as a function of distance from the leaf base to those of Van Dillewijn. Extension was low at the leaf base rose acropetally, reached a maximum, and fell rapidly at first and then more gradually to the end of the growth zone. The region showing high extension moved towards the leaf base as the ligule was displaced outward. This region reached the base of the leaf when the ligule lay a short distance beyond the end of the growth zone. In this experiment no measurements were made of extension in the internode.

During the course of their experiments on the water potentials in a growing maize plant Michelena and Boyer (1982) and Westgate and Boyer (1984) used the expansion of tissue between pin holes to locate elongating regions in the plant. Michelena and Boyer found that 20 days from planting there was an elongating region in the fifth leaf. Their pin holes were inserted at 1cm intervals when the leaf was a little more than twice the length of the enclosing sheaths and they measured expansion over a 10 or 14 hour period. In leaf five at this age the growth zone extended 6cm from the base, the region of maximum extension coincided with the ligule and the decrease from this maximum was sharper proximal than it was distal to the ligule. Westgate and Boyer measured the elongation rate as a function of position on the fifth leaf on a 16 day old maize plant. In this leaf the region of elongation extended a little over 5cm from the leaf base. The extension of the internodes was examined when the plant was 42 days old. The highest rate of extension was found in internode twelve and it was concentrated in the basal third of the organ. Westgate and Boyer followed the changes in water potential as a function of position in the maize plants for a considerable number of days but apparently did not repeat their experiments to locate the regions of elongation. Therefore they had no data on any changes that occurred in the growth zone during organ development.

As opposed to the method of locating the elongating region or growth zone in a grass leaf by marking it with pin holes, Volenec and Nelson (1981) evolved two alternative approaches in their study of cell dynamics in the leaves of tall fescue. They used safranin in ethanol to dye selected elongating leaves and located the nonlignified region. This gave no measure of the rate of elongation within the region however. Their second technique therefore involved following the movement of marks. The outer leaf bases were dissected away from a growing tiller. Marks were painted at 1mm intervals on the surface of the chosen leaf. The protecting leaf sheaths were replaced by a cylinder of moist green paper. Extension between the marks was measured after 24 hours. They also located the end of the growth zone as the position beyond which the mean epidermal cell size did not increase. The leaves used in these experiments were all the same selected size. The length of their exposed laminae was less than half the length of the previous fully expanded leaf. A slow and a faster growing genotype of tall fescue was studied and these exhibited different lengths of and extension activities within their respective growth zones. In both genotypes the rate of extension in the growth zone as a function of distance from the leaf base followed the pattern described for other species of Gramineae with rapid lead up to, and fall away from, a more or less centrally placed maximum. The maximum was lower but occupied a greater length of the leaf in the genotype with a higher rate of leaf elongation. Nelson and Volenec did not consider changes in the distribution of extension which might occur as a function of the stage of development of the leaf.

MOVEMENT OF TISSUE THROUGH THE GROWTH ZONE.

The intercalary growth zone of the grass leaf has been used in a number of experiments on the biochemical changes that occur during tissue development. In particular the group led by Professor Leech has studied changes in the morphology and chemistry of the photosynthetic system as a function of position in the growth zones of cereals and ryegrass leaves. The group worked with a population of equal aged young seedlings which were undergoing uniform leaf extension (Boffey, Ellis, Sellden and Leech, 1979). They discussed their findings in terms of a basifugal gradient of maturity which extended through the growth zone at the sampling time. In their report on a study of chlorophyll formation Boffey, Sellden and Leech (1980) pointed out that cell age was not directly proportional to position; and they described and used a method of determining the rate of movement of cells through the growth zone.

They made an initial assumption that there was a meristematic region at the base of the growth zone and that it was extremely short. Cells moved out from this region and increased in age as they did so. The experiment involved the first leaves of a population of seven day old wheat seedlings. The growth of the first leaf on a set of similar plants was measured over a period of 2.5 days, and mean daily leaf growth rates were calculated. A strip of the coleoptile was cut away to expose the first leaf for marking. Marking was at 5mm intervals and was carried out with ink. Plants whose growth subsequent to this was abnormal were culled. Boffey, Selden and Leech expressed the upward displacement of each of the marks as a fraction of the total leaf growth rate during the same time interval. They named the ratio the relative growth rate. Thus they accumulated several data sets. Each was referenced to a particular time and the values within it were recorded as a function of position. The sets were drawn as graphs and values for relative growth rate at 0.1cm intervals were read from the graphs into a computer. These values were than available for the calculation of the time required for a given point to move 0.1cm. In the calculation the time was taken as equal to 0.1 divided by the product of the relative growth rate and leaf growth rate. Taking a cell or a point in the tissue which was initially at the base of the leaf they calculated the time taken for it to move outward in 0.1cm steps. Where there was no data set available, linear interpolation between neighbouring sets was used to supply a value at the correct time. Thus they achieved a sequence of coordinates for cell age as a function of position on the first leaf of a seven day old seedling.

In this experiment the values for rates of growth are recorded as being those of particular positions at a specified time. They were apparently calculated from the difference in position of a mark and the interval between the times of its location. The size of these length and time spans was not recorded but must have been substantial for practical reasons. The report does not say whether the position and time values used as references for the growth rate were those found at the beginning, at the end, or midway through the intervals of time and length involved. This is an important consideration since the rate of movement of tissue as a function of time is not linear and use of a single figure to represent a changing rate of displacement must lead to distortion. If for example the reference position was that at the beginning of the measured time span its calculated rate of movement would have been too high and using inflated values in sequence would lead to an underestimate of the age of the tissue. If on the other hand the reference positions lie at the end of the span the reverse will be the case. The effect of referencing the rate of growth to a median position would depend on the exact relationship between rate of movement, position and time within the interval but it would rarely represent the true value.

The streak photographs of growth in a root tip which were obtained by Erickson and Sax (1956) show the movement of marks placed on the surface of the root over a period of time. Position was represented by the vertical axis of the picture and time by the horizontal axis. Erickson and Sax showed that the marks give rise to the streaks and the slopes of the latter measured at a given point on the time axis represented the velocity with which they are receding from the root tip. If Boffey, Sellden and Leech had plotted the positions of the individual marks on their barley leaves as a function of time they would have had curves similar to the streak photograph and could have obtained values for what they termed, absolute growth, from them. Erickson and Sax (1956) had recorded the velocity field of the root tip. Their velocities were in sets each of which gave the values for velocity as a function of position relative to the root tip at a given time. Gandar (1980; 1983) explains the importance of distinguishing between sets of data which describe the characters of a growth zone in terms of the values obtained at set of positions along it and those which describe the characters of a piece of developing tissue which moves through it. He points out that the former, for example the sets of velocity data in Erickson and Sax's experiment, is a spatial or Eulerian description; while the latter, represented by the streaks in Erickson and Sax's experiment, is referential or Lagrangian. He used the term referential because the data is that which refers to a moving point. A point is normally distinguished by a statement which describes its position but when the position changes as a function of time it is necessary to identify it as the point which occupies a position X, at a particular time t_0. This is its reference position. ~~and it~~ The point moves along a pathline (x(t)) which may then be described by a referential statement x=x(X,t) (Gandar 1983a). If the change in level of a character which occurrs during development is to be considered the relevant values are those which are found at positions along the referential pathline, but the measurement of characters in a growth zone can usually only be carried out on a harvested plant and levels are recorded in terms of a position at the time of harvest. In other words they are recorded in a spatial distribution. Erickson and Sax recorded the spatial distribution of the velocity of movement away from the root tip through the length of the root. The velocity was that of the mark but it was also an attribute of the position occupied by the mark at the given point in time. At a different time another piece of the growing tissue will occupy the same position. The velocity of this piece is attributed to the position at the new time. Thus the velocities at different points on a body are functions of position and of time. The body is described as having a spatial velocity field. If the total growth of the body is constant as a function of time the spatial velocity field will be in a steady state and the velocities at individual positions throughout its length will be constant. The root studied by Erickson and Sax was in a steady state of growth and Gandar (1980) made use of this fact in his calculation of cell production from Erickson and Sax's data. He based this calculation on the fact that when there is a flow of material through a section of a body such as a growing root any change in the quantity of material within the section as a function of time will result from the rate of movement through it and from the input of additional material into it. He expressed this statement in terms of a continuity equation for a segment whose length tends to zero. In the continuity equation, the rate of input equals the product of the spatial change in level of material and of velocity across the length of the root included in the segment, plus any secular change in level attributed to the position. He argued that since the root was in a steady state there would be no secular change in this case, in the level of, wall density, as a function of time and used the spatial distribution of the quantity, and of the velocity, measured by Erickson and Sax, to calculate the rate of cell production at a series of points through the growth zone.

Thus Gandar showed that the continuity argument could be used to calculate rates of accumulation of materials as a function of position in organs which are involved in a steady rate of growth. It may not be possible to apply this method to a leaf growth zone. When growth is not proceeding steadily the factor which represents the change in the inflow to the section over time will be present in the continuity equation and its effect cannot be separated from the production rate (Gandar, 1980). Growth of a root is indeterminate and could be expected to proceed steadily unless environmental conditions changed. Leaves on the other hand grow for a limited period of time and the studies which I have reviewed have indicated that even in the strap shaped grass leaf the rate of growth rises, reaches a maximum and then falls back. Possibly the growth might be regarded as steady for the period which extends for a short time before and after the achievement of the maximum growth rate. An alternative is to follow the movement of a particular part of the leaf as Boffey, Sellden and Leech (1980) did and to study growth activities in terms of that portion. The position of such a portion as a function of time would be represented by two referential pathlines. Gandar's work shows that these can be obtained if the spatial velocity field has been evaluated.

CHAPTER III

THE LOCATION AND CONTINUITY OF THE GROWTH ZONE IN RELATION TO PHYTOMER AGE

3.1. INTRODUCTION

Most of the investigations into the vegetative growth of grass have concentrated upon measuring changes in whole leaf size as a function of time (e.g. Abbe et al, 1941; Williams, 1960 and Jain, 1970). Such measurements provide no information about the differences in growth rate that occur along the length of the extending structure or the associated differences in tissue maturity. These aspects are of considerable interest when growth and the effect of environment on growth are being considered. Some authors have set out to differentiate between the elongating and mature tissue (Kuiper, 1915; Van Dillewijn, 1939; Davidson and Milthorpe, 1965; Kemp, 1979; Boffey, 1980; Volenec and Nelson, 1981; Michelena and Boyer, 1982; and Westgate and Boyer, 1983), but in general the leaf has been treated as a unit with regards to age. Information about the distribution of extension along the growing leaf is not easily obtained and that which is available is incomplete, ambiguous and has sometimes been misinterpreted.

Grass leaf growth is described as being intercalary (Sharman, 1942; Esau, 1943). The tip of the leaf matures first and maturation proceeds in a basipetal direction until leaf blade, leaf sheath and also the subtending internode are fully formed. The unit consisting of the three structures, leaf blade, leaf sheath and internode, may be referred to as a phytomer (Fig. 2.2) and I use the term in this work. Sharman (1942) suggested that the growth of the phytomer should be considered as a wave which moved throughout this phytomer. The vertical component of Sharman's wave is the rate of extension of the tissue, and the horizontal component is the position at which the extension occurs. Sharman was aware of the ontogenetic variation to be found through the length of the phytomer. She described the anatomy of the ligule region and node in detail but she did not suggest discontinuity in the wave associated with these structures. Her work has been misinterpreted in this regard and has been quoted (Barnard, 1964;

Langer, 1972; Kemp, 1979; Dale, 1982) as supporting the opinion that grass leaf growth occurs with two separate meristems, one in the blade, and the other in the sheath.

It is necessary to understand precisely what is meant by the term meristem. In his discussion of the terms used in describing growth and cell pattern formation in plant axes Green(1976) defines the meristem as being a region in the growing axis in which cell divisions take place. The growing axis may also contain a region, adjacent to the meristem, of expansion without cell division, conveniently termed the ameristem. Green names the whole expanding section the growth zone. I will use this term here. It is synonymous with the "zone of elongation" discussed by Davidson and Milthorpe(1965) and by Kemp(1979). The word meristem has in the past been used to refer to either the whole of the growth zone or to a region where cell division can be found. Regions of small undifferentiated cells have also been named meristems. While these are anatomically recognisable, their behaviour with regard to expansion and cell division is usually not known. Thus authors using the term may write as though they have identified the growth zone when in fact they have observed only the meristem or a part of it and this leads to misunderstanding.

If a coherent picture of the extension growth in a grass phytomer is to be obtained it is necessary to have a set of information which describes the distribution of extension activity within it and which records the changes in this activity that occur during the time span of the growth. I have limited this study to the extension on a line between the base of the phytomer and the tip of the leaf. Positions are found by measuring along the line, taking the position of the base of the internode as zero. The displacement of a point away from the base over a period of time represents the extension of the section of the phytomer proximal to it. Th extension between two points equals the difference between the displacements of the points.

The experimental procedure for studying growth involves measuring the displacement of marker points, relative to one another. Davidson and Milthorpe(1965) showed that the growth zone of grass leaves is totally enclosed within the bases of older leaves and is therefore not easily marked. The growth zone can be exposed for marking by dissecting away the older leaves (Boffey, 1980; Volenec, and Nelson 1981) but the resulting damage is severe. The technique of marking with pins was used by Kuiper(1915), Van Dillewijn(1939), Davidson and Milthorpe(1966), Kemp(1979), Michelena and Boyer(1982) and Westgate and Boyer(1983). This is more acceptable than dissecting away the outer leaves but it does involve destruction of the plant being studied in order to record the growth. This means that when the pin hole method is used there tends to be a lack of information about changes related to age. Continuous measurements on an individual plant can, however, be replaced by a study which uses a set of sample plants as representative of a population.

This chapter shows how the extension growth in a maize phytomer can be investigated using the pin hole approach, how it can be described in terms of displacement as a function of position, and how the length of the growth zone in which the displacement occurs changes as a function of age. The length and the capacity for extension of the growth zone at different times is discussed in comparison with the outward movement of the leaf tip, ligule and upper node during the growth of the phytomer.

3.2. MATERIALS AND METHODS

CULTURAL.

The plants for the experiment were grown in a controlled environment chamber in the Climate Laboratory of the Plant Physiology Division of the Department of Scientific and Industrial Research (Brooking, 1976; Warrington et al, 1978). The light during the 24 hour photoperiod delivered by four 1000W Sylvania "metal-arc" high pressure discharge lamps, and four 1000W Philips tungsten iodide lamps provided a mean photon flux density of 710 μe m^{-2}sec^{-1}and mean irradiance of 149 W m^{-2}at trolley height. Temperature, carbon dioxide and relative humidity were maintained at: 23±0.5 C, 340±40ppm and 75 5% respectively. North Carolina State University nutrient solution was delivered automatically at the rate of 120ml per pot three times each day for the first four days and six times each day thereafter.

Plants of maize variety FP74 were grown from seed. The grains were screened by eye, and abnormal large, small or damaged ones were rejected. A 15mm depth of North Carolina potting mix was placed in each of 54 plastic 'planta' bags. 90 grains were pressed, scar down, into the surface of the mix in each pot using a template to ensure uniform distribution of the grains. Grains were covered to a depth of 3cm with vermiculite. The 'planta' bags were placed in hard plastic one gallon pots (fig. 1).

The seedlings were thinned on days 7, 17 and 26 to leave eight, four and one uniform plants respectively in each pot.

SAMPLING.

Each day six experimental subject plants were chosen at random under the constraint that uniform numbers be maintained in each pot as far as possible. The group of six plants was harvested after three days. During the intervening days the heights for each plant of its leaf tips above the rim of the gallon pot were recorded. Twenty four hours before harvest three of the plants were marked using the punching apparatus.

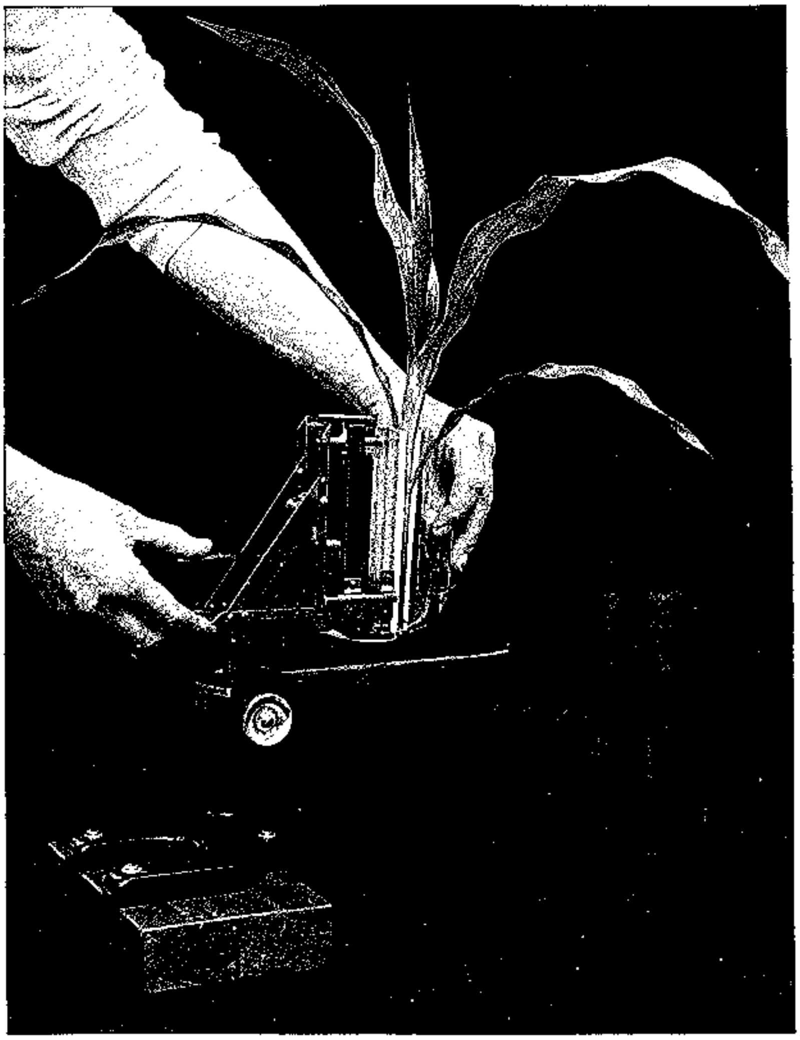


Fig. 3.1. Punching apparatus in use. The vertical row of 0.025 mm diameter pins set up at 2mm intervals, slides through guides, entering and being withdrawn from the plant with a horizontal motion when the lever is operated.

PUNCHING.

Punching was carried out using a punch consisting of a set of sixty four, 0.25mm diameter entomological pins which ran through fixed guides and were mounted at 2mm intervals in a vertical row on a block (fig. 1). Operation of a lever moved the block with the pins in a horizontal direction. This punching apparatus was carried on a head whose vertical position could be adjusted. When a plant was to be punched the apparatus was arranged beside it, so that the set of pins was directed towards the midrib of phytomer six and was positioned so that the line of holes would extend upward from, and immediately below, the insertion of leaf five (fig. 1). The line of holes formed in a plant by punching and the line as it might appear when the plant was harvested twenty four hours later are shown in figure 2 a and b.

HARVESTING.

At harvest the older leaves on the plants were dissected to expose phytomer six. The leaf insertion occupies a length of the plant stem. The midpoint of this length, at the insertion of leaf five, was taken as the base of phytomer six (fig. 2).

The lengths of the leaf blade, the leaf sheath and the internode of phytomer six were measured.

Leaf six was partially unrolled to expose a line of holes adjacent to the midrib (fig. 2). The positions of each of the holes in the line, and of the base of phytomer six relative to them were recorded. A specially adapted dissecting microscope with a sliding caliper fastened to its stage was used to hold the plant and locate the holes.

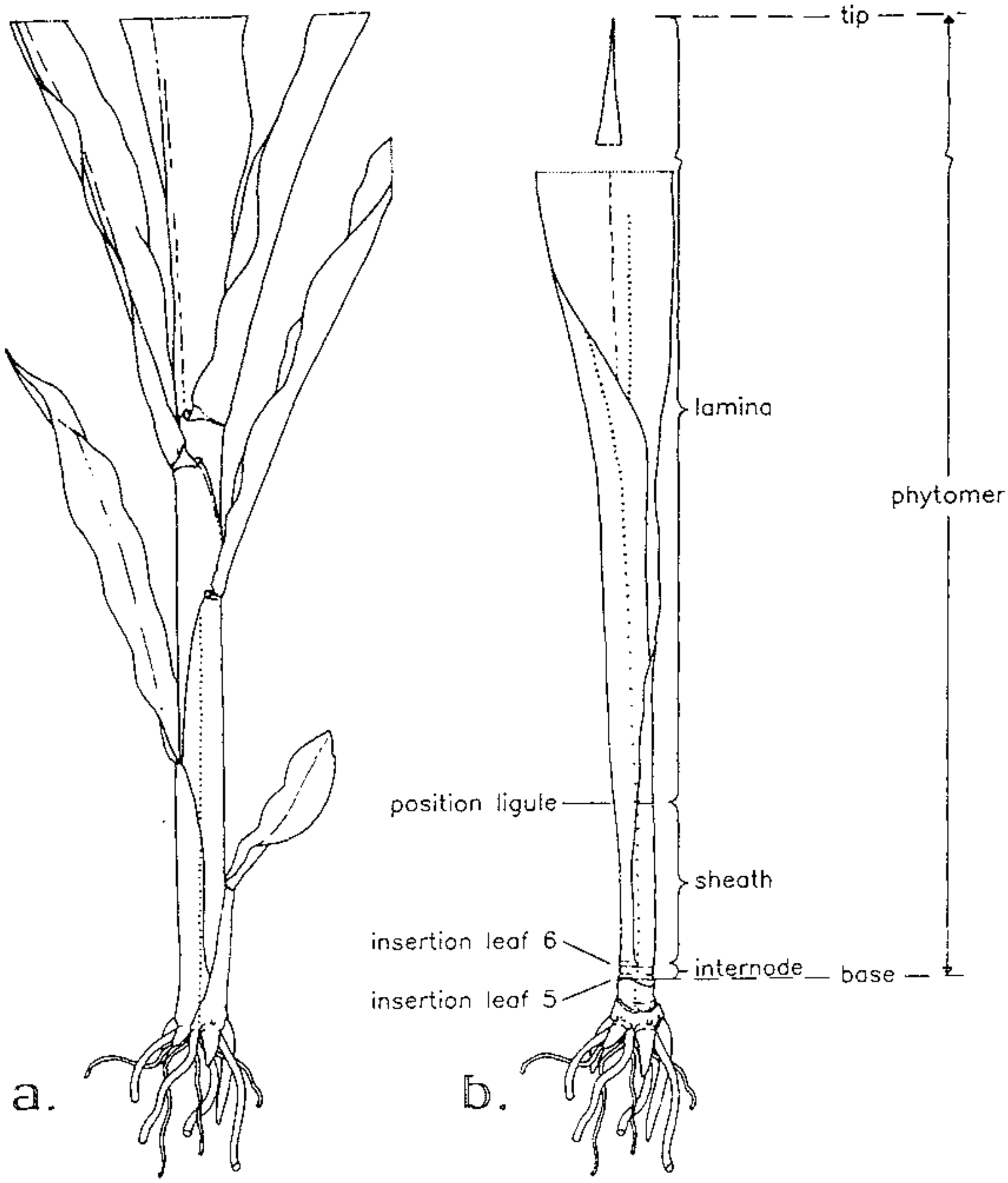


Fig. 3.2. Set of punched holes as they appeared on a plant immediately after punching (a), and twenty four hours later (b). In (b) the outer leaves have been removed to expose the base of leaf six. The base of phytomer six is established at the point of insertion of leaf 5. Scale: x 0.75.

RESULTS AND DISCUSSION

THE EXPERIMENTAL PLANTS AS A UNIFORM POPULATION.

The destructive harvest that was necessary during this experiment precluded continued observation of an individual plant. Information about earlier or subsequent growth could only be obtained by using new individuals. These had to be similar in all characters other than age. The conditions of this experiment were arranged to give rise to as little variability due to genetic or environmental differences as possible. Mean values of the population parameters that varied as a function of age were obtained by fitting constrained B-splines (Spriggs, 1985) through the time indexed data sets. Examples of the curves obtained using this technique are shown (figs. 7, 8a).

A study of the use of a plastochron index as proposed by Erickson and Michelini(1957) instead of time as a scale for the age function was carried out but showed that time was a more useful measurement.

A condition recognised as "onion tip" (Warrington pers. comm.) occurred among the plants in the experiment. In "onion tip" the blade of the leaf which is emerging from the sheaf of older leaves is damaged, and eventually withers. The damage appears to occur as a result of there not being sufficient room inside the base of the preceding leaf to accommodate the expanding lamina. The surface of the lamina appears to be damaged by friction. The plants most affected by the condition were culled. "Onion tip" is the result of excessively rapid elongation of the leaves, the plants available for sampling subsequent to this cull represent the slower growing section of the original population. Examination of the behavior during growth of these remaining plants is still valid but the values obtained apply only to this particular subpopulation.

DAMAGE CAUSED BY PUNCHING.

The effect on growth of the damage caused by punching was estimated by comparing the upward movement of the tips of the leaves on punched plants with that on unpunched plants of similar age. Daily means of leaf tip movement were prepared and those for the punched plants were considered as a function of those for the unpunched plants (fig. 3). A regression line through the means and origin was fitted and drawn, together with a line to represent equal growth in punched and unpunched plants. These indicate a 24% reduction in overall growth during the ensuing twenty four hours as a consequence of punching. This is similar to the figure of 22% found by Davidson and Milthorpe (1966). It was not possible in this work to detect any variation in the effect of punching on different parts of the growth zone.

The appearance of the epidermis twenty four hours after insertion of a pin hole is shown (Fig. 4). Cells adjacent to the hole are extended at right angles to its perimeter. The size and shapes of cells more than 100u from the break seem unaffected.

THE EFFECT OF HANDLING

Jaffe(1973) demonstrated a substantial reduction in growth in a number of species including the cereal *Avena sativa* when they were repeatedly rubbed and Beardsell (1977) showed that handling maize plants in order to measure them was sufficient to reduce growth. Handling the plants was therefore likely to reduce growth in this experiment. All plants were given as far as possible the same amount of handling so that comparisons between them would remain valid. The reduction due to punching would be superimposed on this background thigmomorphogenic effect.

THE 24 HOUR CHANGE IN POSITION OF PIN HOLES

The positionof a point is recorded in terms of its distance from the base of the phytomer. The appearance of a typical set of holes at the time of punching and 24 hours later is shown in fig. 2. The holes were numbered: from zero upward in such a way that the base of the phytomer lay between hole 0 and hole 1. This was done at harvest when the structure had been exposed by dissection. The distance (x') of each hole from the base at harvest was calculated from the records of the positions of the holes and of the base. This was done at harvest when the structure had been exposed by dissection. (x;x',b ,x' ,x' ,x'...x').

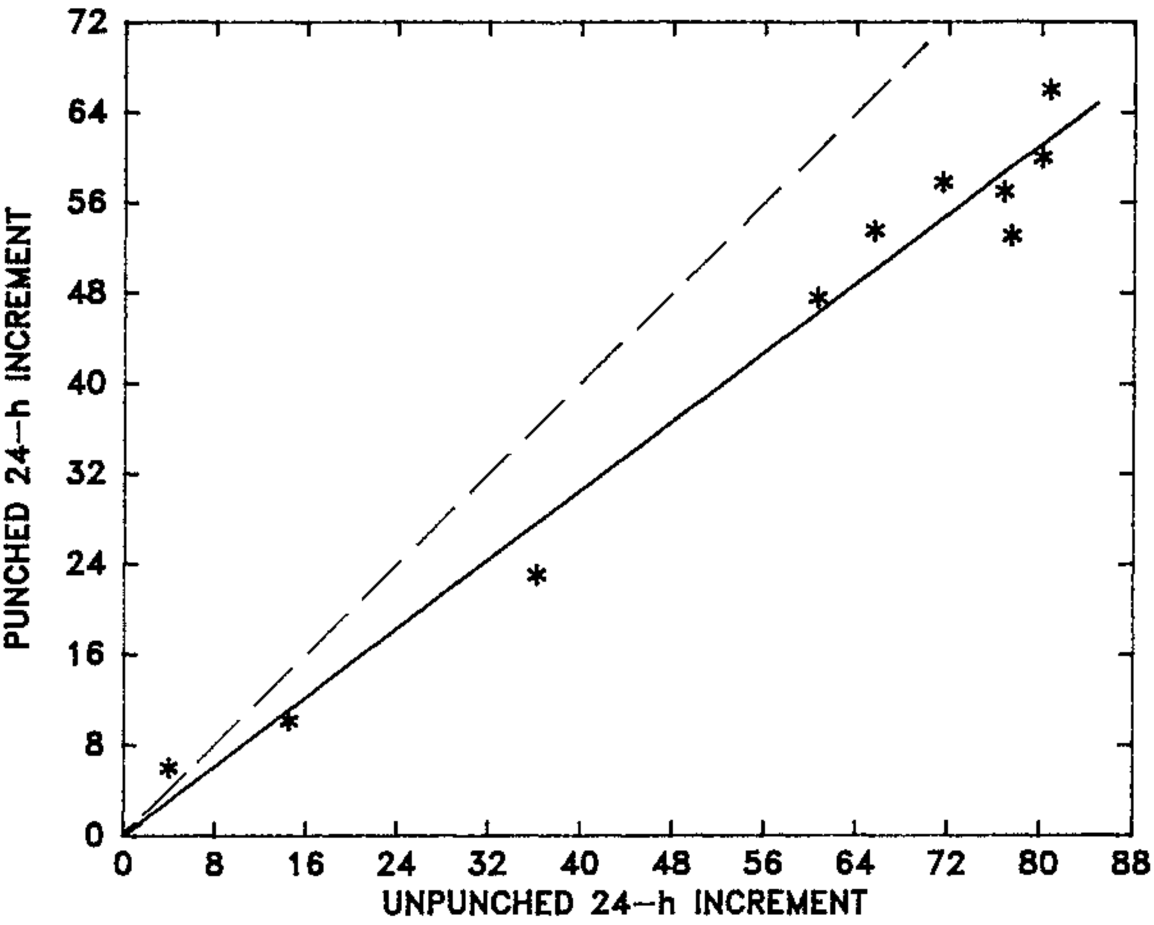
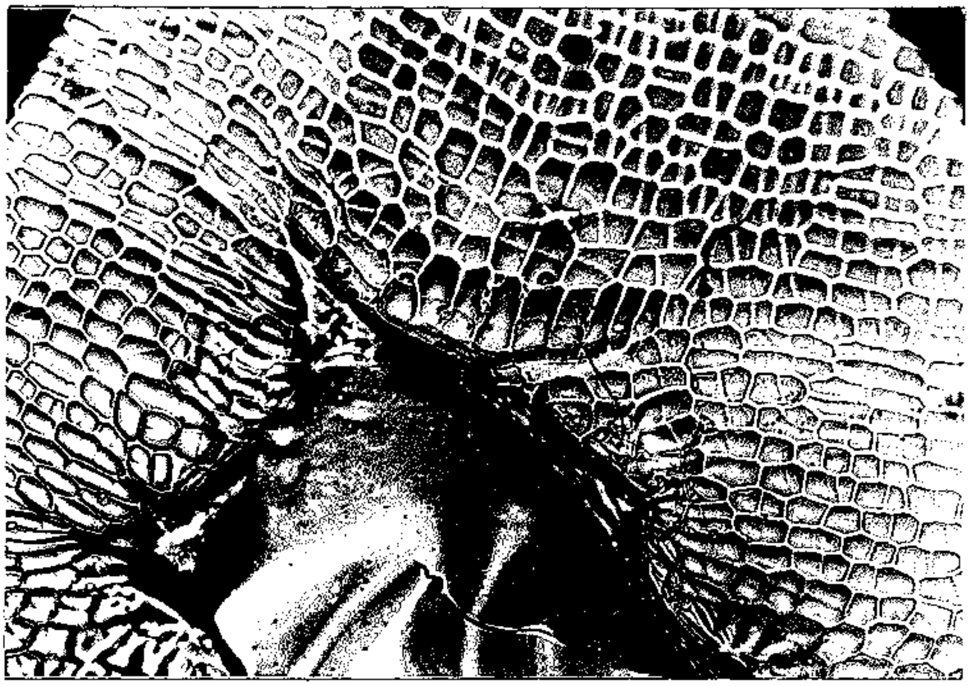


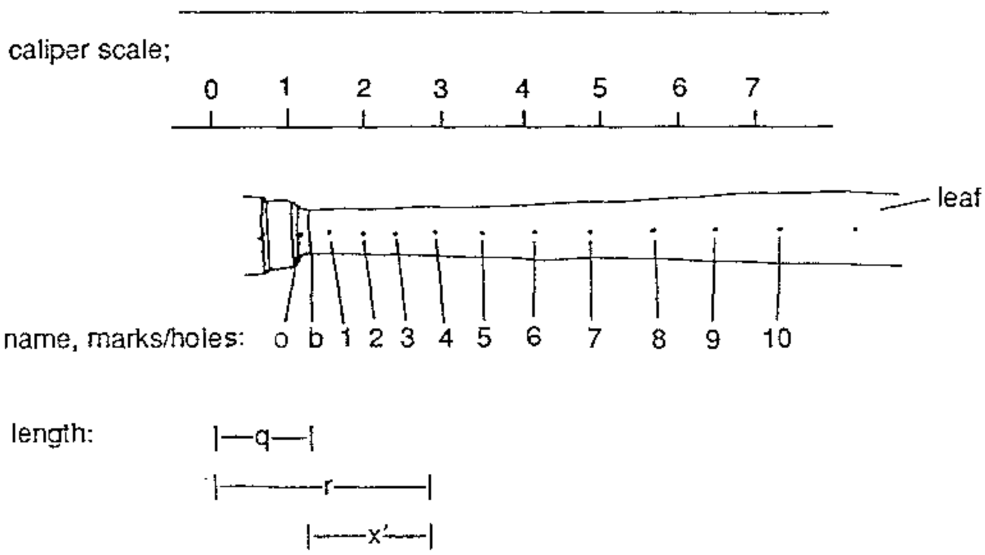
Fig. 3.3. The reduction of growth due to punching. Mean daily extension of exposed leaves on punched as compared with unpunched plants, (*). The line expected if punching had no effect, (- - -). The fitted regression, (——). The regression coeficient = 0.76 and the standard deviation = 0.02.



base ⟵ ⟶ tip

Fig. 3.4. Pinhole damage to the abaxial epidermis. The hole was punched into a region undergoing active extension, 5.6 mm from base on a 17 day old plant, the plant was allowed to grow for twenty four hours, before the replica of the surface was prepared. Magnification, x350.

Diagram to show the method of finding hole positions on the leaf



$x'=r-q$

The distance (x) of each hole from the base at punching was also calculated. The holes inserted into the plant were initially 2mm apart, and therefore, the distance from hole 1 to the nth hole at punching was 2(n-1)mm. Since it was fortuitous and uncommon for a pin hole to coincide with the base of the phytomer, to establish the position of hole 1 relative to the base at punching, I assumed that the extension in the segment between the holes 0 and 1 ,(the pair that spanned the base) was uniform and calculated the distance between hole 1 and base at the time of punching by linear interpolation:

distance of base to hole 1 at punching =

$$\frac{\text{base to hole 1 at harvest}}{\text{hole 0 to hole 1 at harvest}} \times 2\text{mm.}$$

The distance (x) of a hole from the base at the time of punching was the sum of its distance from hole 1, and the distance of hole 1 from the base. Thus x_1, x_2, x_3,...x_n

were calculated. Figure 5 is the plot of x' versus x together with the graph illustrating no change in position.

DISPLACEMENT

I will refer to the basifugal movement of a point during a specified period as its displacement (d). The displacement of a hole during the twenty four hours between punching and harvesting was the difference between its position in mm at punching and at harvesting (d =x'-x).

A set of representative curves in which the displacements of the holes in the phytomer are plotted as a function of their initial positions is shown in figure 6. The graphs included in this set were selected to illustrate the changes that occur in the extent and distribution of displacement during the growth of the phytomer. The length and age of the phytomer are shown to the left of each curve and the position of the end of the growth zone, ligule and node of leaf insertion are indicated on each. The 24 hour displacements of marks form smooth sigmoid curves through the growth zone. The curves increased and decreased in size during the period of growth. The overall extension of the phytomer was represented by the displacement of the distal extreme of the growth zone. The curves therefore suggest that when the greatest rate of extension was proceding, approximately day 23.4, the phytomer had reached rather more than half of its ultimate length and the length of the growth zone was at least 80mm. Both the base of the lamina and the leaf sheath were involved in substantial extension at this time. The region in which maximum extension was taking place moved basifugally until the phytomer reached its greatest rate of extension, and basipetally thereafter. It is interesting to note that there is no discontinuity in the displacement as a function of position curves associated with either the ligule or the node of leaf insertion. Slower growth, or zero growth in a particular region resulted in little or no additional displacement in that region, creating a plateau on the curve, and there was a reduction in the displacement of all points beyond this section. There is thus no evidence from the displacement measurements for the existance of discrete blade and sheath "meristems" in maize phytomer growth. Barnard(1964), Langer(1972), Kemp(1979) and Dale(1982) describe the development of two "meristems" separated by the ligule. It is not clear whether what they referred to as a meristem can be equated in Green's terminology to

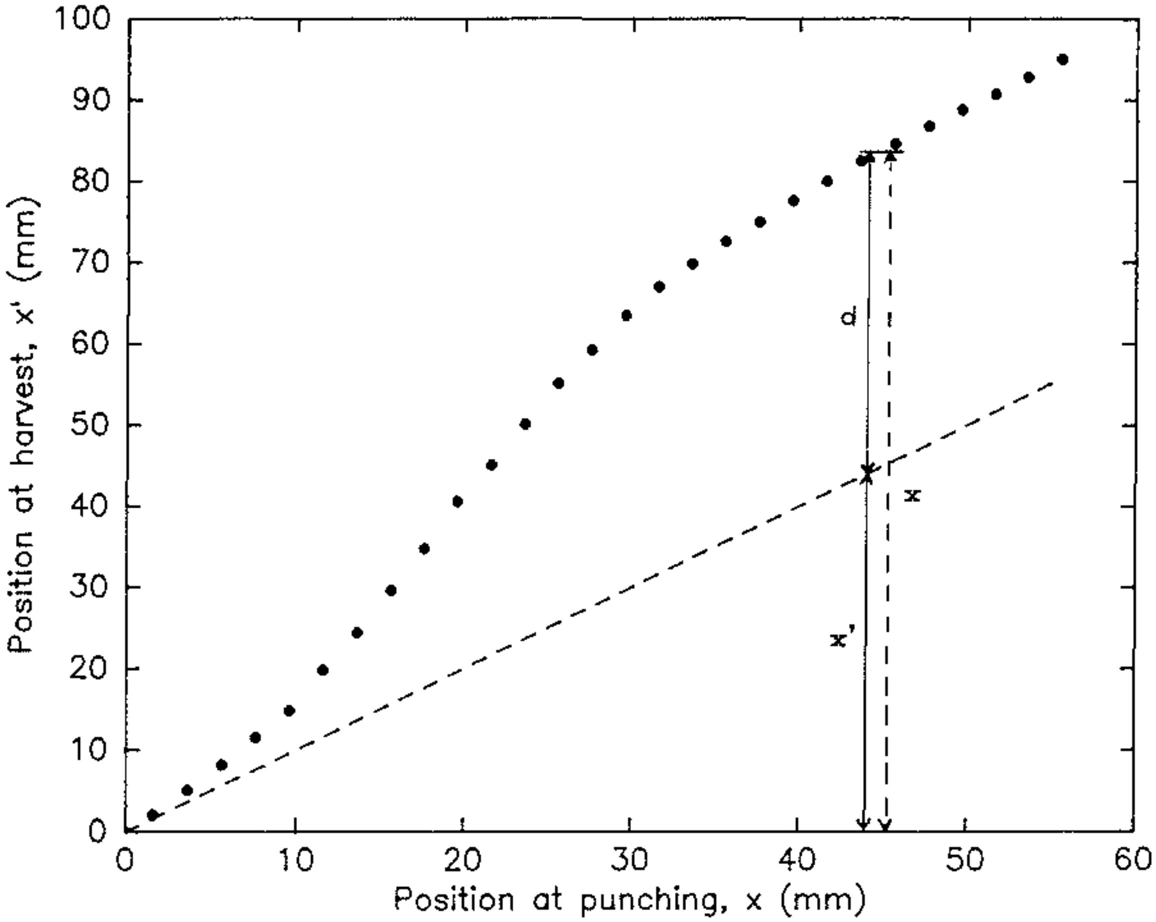


Fig. 3.5. The position of a set of pin holes following 24 hours of growth compared with their initial position in the plant at punching. The position is recorded in terms of the distance on a line following the phytomer from the base of its supporting internode to the leaf tip. The origin is at the base. The y = x line represents the position of the holes in the absence of growth. If the initial position of a hole is x and its position after 24 hours is x' then its displacement (d) during growth, is x'-x.

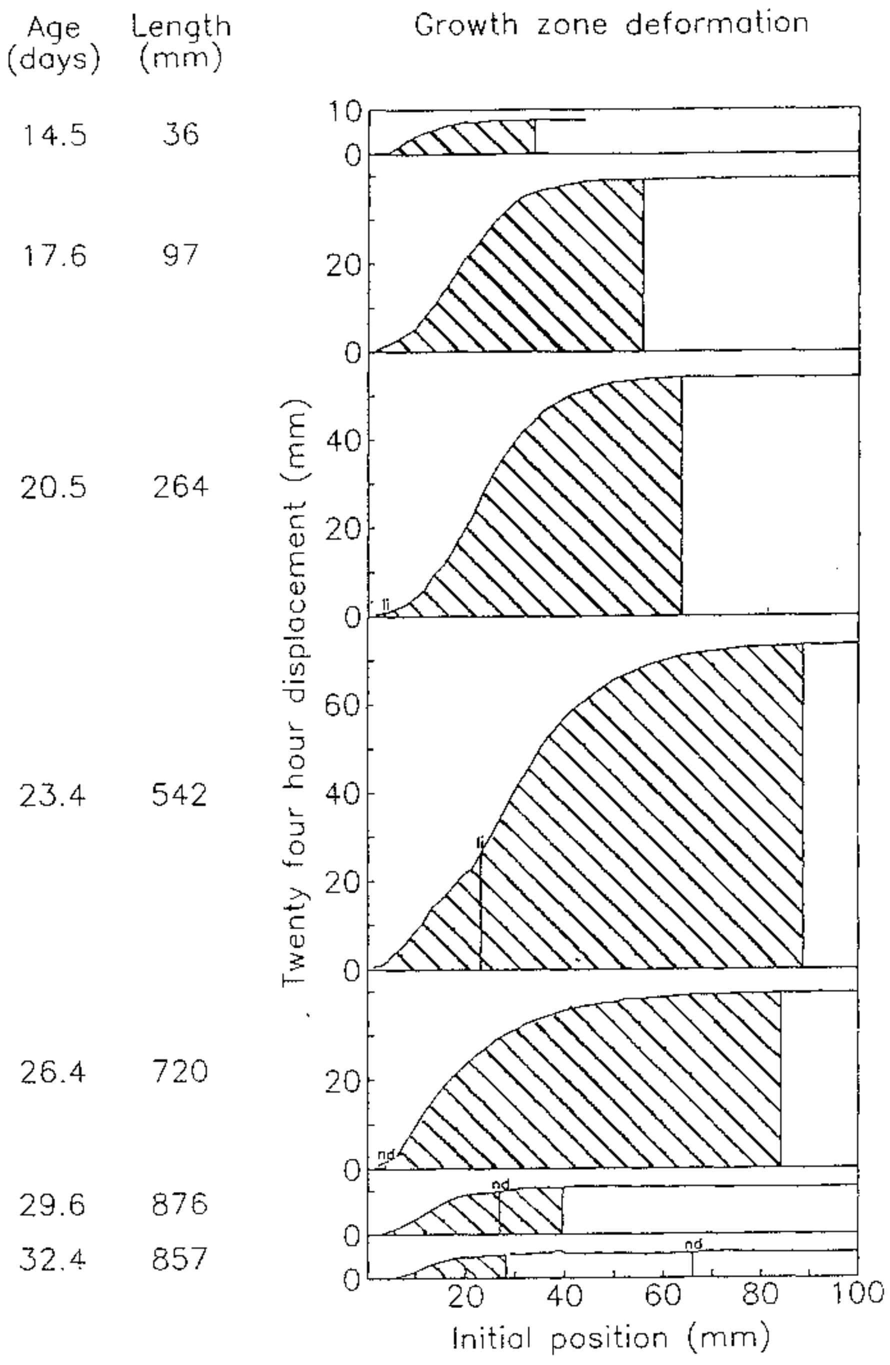


Fig. 3.6. The twenty four hour displacements of pin holes as a function of their initial position. Representative curves at several different stages in the extension of phytomer six. Age (=time from sowing to punching) and the composite length of the phytomer are displayed in days and millimetres to the left of the curve, in each case. Extent of the growth zone is shown (hatched). The positions of the node of insertion, (nd) and of the ligule, (li) in phytomer six are also shown where they are within range.

the growth zone or whether they intended to identify a region of cell division where there was little extension but which was associated with an ameristem or region of cell extension without division. In either case they suggest a change in the rate of extension where the ligule is inserted. My results demonstrate a single uninterrupted growth zone. In the Poaceae there is a band of small parenchyma-like cells located opposite the ligule in the abaxial epidermis at the junction of blade and sheath (Soper, 1955). Such a tissue was observed in this work but its presence could not be associated with any lack of capacity for extension during growth as would have been necessary if the cells were to remain ameristematic and small while extension was occurring in "meristems" on either side of them.

LOCATION OF THE GROWTH ZONE

The growth zone was taken as extending from the base of the phytomer to the point beyond which there was no further sustained increase in the displacement of the pin holes (cross hatched region, fig. 6). By definition there can be no expansion beyond the end of the growth zone, so that the rate of displacement of its distal extreme parallels that of the leaf tip. The position of the latter is normally used when obtaining figures for leaf extension.

AGE-RELATED CHANGES IN LENGTH OF THE GROWTH ZONE

In figure 6 the graphs show the considerable change in length of the growth zone during the expansion of the phytomer. The complete set of growth zone lengths found in this experiment was plotted as a function of age and a constrained B-spline (Spriggs,1985) was fitted through them (fig. 7).

Data for displacement could not be obtained before age 14 days because attempts to insert a continuous set of pin holes into rolled leaves which were less than 1.5mm in diameter at this time, proved unsuccessful. At age 14 days the growth zone was about 17mm long; it was extending rapidly; and the rate of extension was increasing. After day 17 the rate of increase in length began to fall off until on day 24 a maximum length of approximately 80mm was reached. The maximum length of this *Zea*

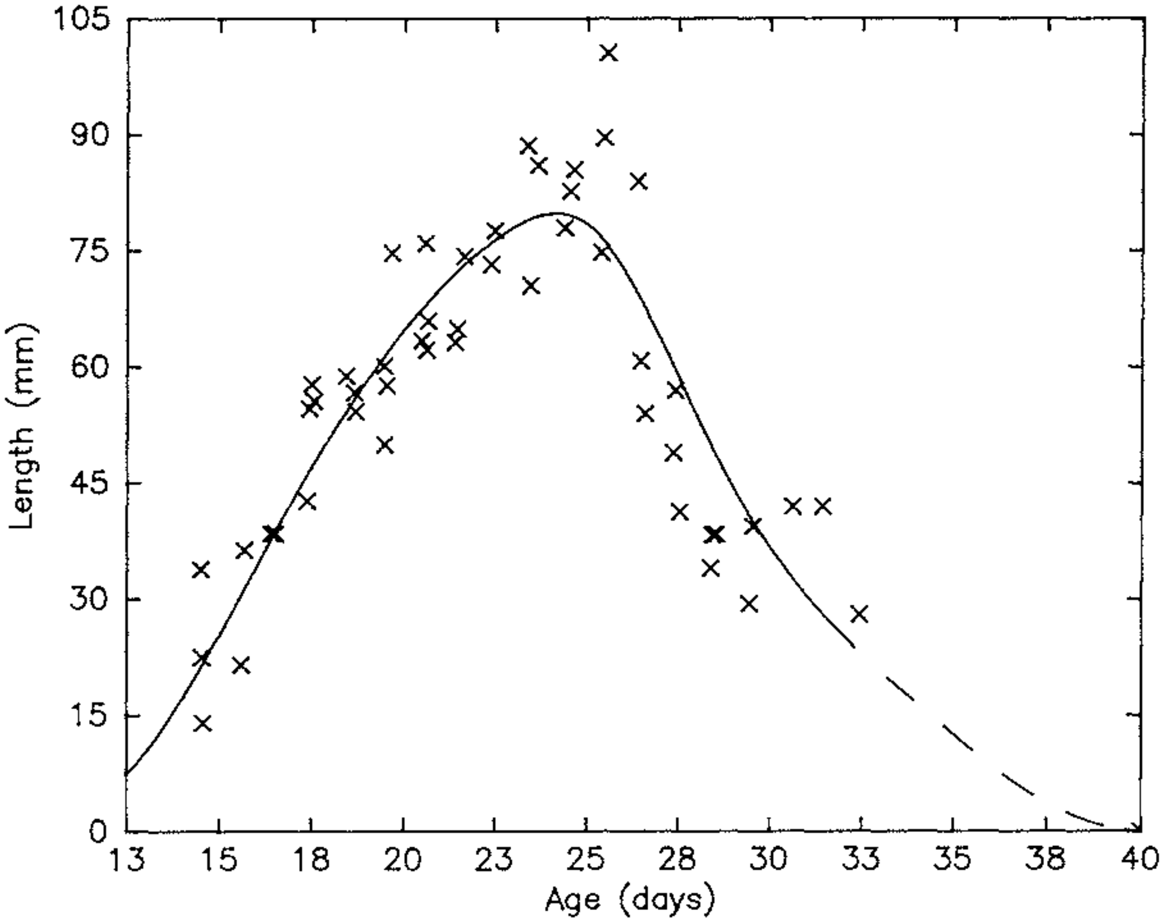


Fig. 3.7 The length of the phytomer growth zone as a function of age as the time of punching. The values for lengths of growth zone found on individual plants are plotted (×). A constrained B-spline has been fitted through these values and the resultant curve is shown (—). The growth zone includes all parts of a plant axis where extension is taking place. The proximal limit of the growth zone (=zero length) is at the base of the phytomer.

mays growth zone was not sustained and there was no indication of its being a constant for any period of time, as was assumed by Davidson and Milthorpe (1966) for *Dactylis glomerata*. Boffey and Leach (1979), working on *Triticum aestivum*, showed a rise followed by a fall in their figures for the length of the extension zone as a function of age but decided to consider it constant during a period that spanned the time of maximum length. In the maize phytomer there was a rapid decline between days 25 and 29 followed by a more gradual fall to day 33. After day 33 the supply of plants in the experiment was exhausted. It is likely that this fall would have continued and the length of the growth zone would have reached zero at perhaps day 40. Kemp(1979) working on *Triticum aestivum* found a similar decline in his data which represented the growth zone length following the emergence of the tip of the blade from the surrounding leaves.

EXTENSION OF THE PHYTOMER IN RELATION TO THE LENGTH OF THE GROWTH ZONE

The length of the growth zone is compared with the growth of the phytomer as a function of age in figure 8. The length of the phytomer components are plotted in section (a) of the figure. Constrained B-splines have been fitted through these data and the derivatives, describing rate of displacement of tip, ligule and node, are shown on the same age scale in the section (b) below.

The extension of the leaf blade proceeded very gradually before age 14days, and the rate increased to reach its highest levels between age 21 and 23days. The maximum extension rate of the phytomer was in the region of 82mm/day at age 22.5 days. After day 22 lamina extension began to decrease and had reached zero by day 28.5. The tip of the leaf emerged from the encircling lamina of leaf 5 when the plant was approximately 18 days old. The ligule was sufficiently well defined for the leaf sheath to be recognised from day 19. The remaining two thirds of the lamina extension occurred between ages 19 and 25.5days when the ligule moved out of the growth zone.

The movement of the ligule away from the base showed a gradual increase in rate to about age 22.5days when there was a sharp upturn carrying the maximum extension rate of the sheath to 41.mm/day at age 26days. The fall in the extension rate of the sheath following age 26.5 days was rapid but had not reached zero by the end of the experiment.

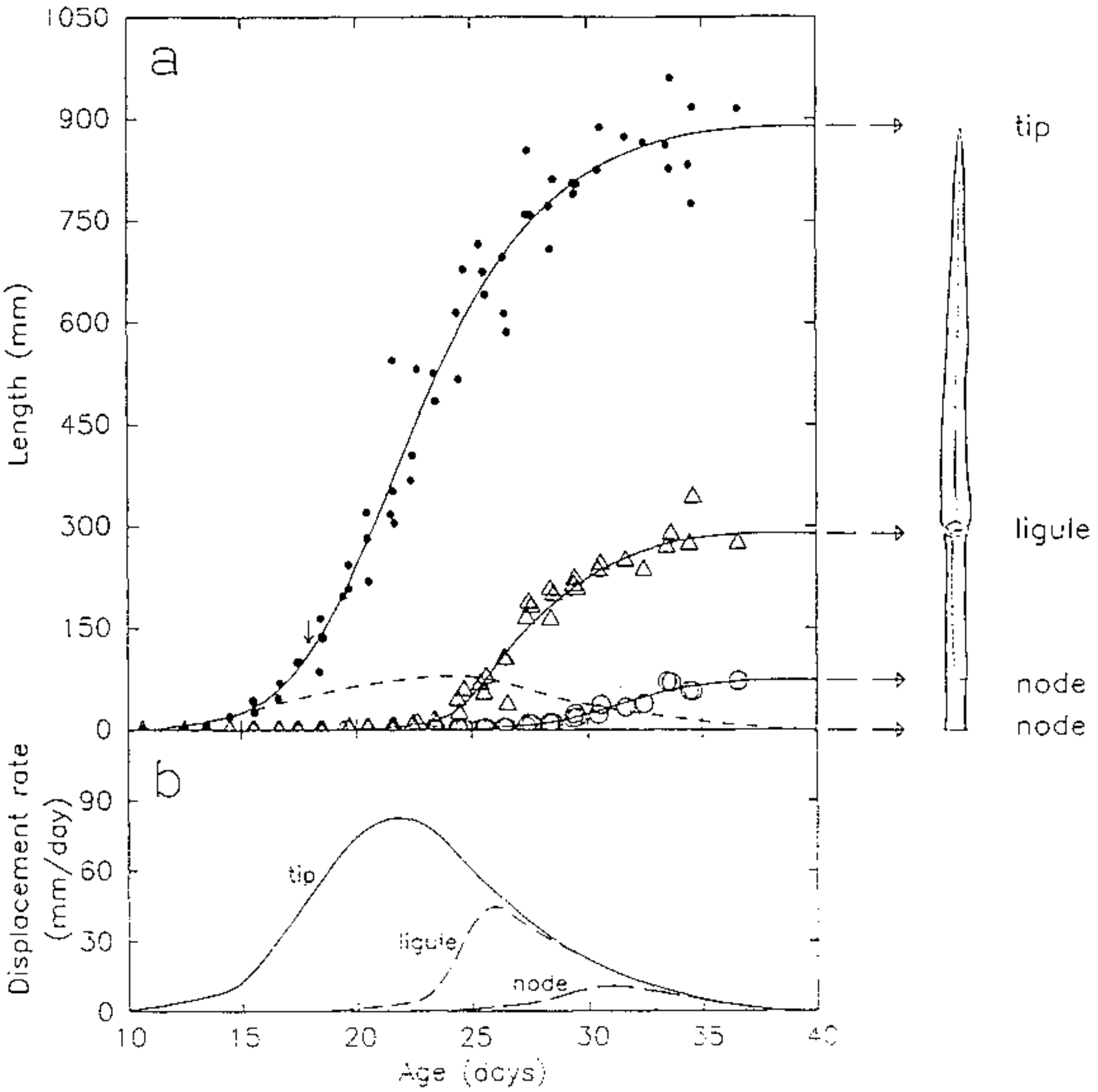


Fig. 3.8. Extension of phytomer six. (a) Shows the length of the whole phytomer and of its components: blade, sheath and internode, together with that of the growth zone as a function of age. Zero age is taken as being the day of sowing, and the lower node as zero length on the line following stem and leaf axes. Positions of leaf tip, • ; ligule, △ ; node of insertion, ○ . B-spline through these data sets, ——. B-spline through distal limit of the growth zone, – –. Time of emergence of the leaf tip, ↓ . (b) Rates of displacement of the tip, ——, ligule, - - and node,— -, from the base of the phytomer as a function of age and plotted on the same age axis as (a).

There was measurable extension of the phytomer internode from age 22days onwards. The rate of increase in internode length was very slow throughout its growth but within this restraint its extension became more rapid after day 26, and reached its greatest rate between days 31 and 33, after which it decreased and was approaching zero at the end of the experiment.

Expansion of the three morphological sections of the phytomer - the lamina, sheath and internode - follow in sequence. Expansion is slow when the section is small, and lies close to the base of the phytomer in its primordial state. Expansion is most rapid while the greater part of the section passes through the central region of the growth zone and it is again slower during the final extension of its base in the distal part of the growth zone. This could be anticipated from the shape of the curves (fig.5) where the greatest change in 24 hour displacement occurs midway through the growth zone. The variation in growth rate within the length of the growth zone and in relation to age will be considered in more detail in Chapter 4.

RATE OF GROWTH COMPARED WITH GROWTH ZONE LENGTH

Changes in the rate of extension of the phytomer are not simply related to changes in the length of the growth zone. When phytomer expansion rate is expressed as a function of growth zone length (fig. 9) the result is a loop, not a line. The time at which certain of the pairs of values were found are marked on the graph. During the early part of the phytomer expansion the ratio of the extension rate to the length of the growth zone was rising. The extension rate was increasing more rapidly than was the length of the growth zone. The ratio (mm/day/mm) was 0.44 on day 14, and 1.1 on day 18. The greatest ratio of extension rate to growth zone length, 1.16, was reached during day 20 and although continued increase in the length of the growth zone carried the highest total extension rate to a maximum of approximately 82.5 on day 21 the growth rate to growth zone length ratio was falling at this time. It had fallen to 0.8 when the growth zone reached its maximum length during day 24 and to 0.67 during day 26 when the length of the growth zone became the factor which diminished more rapidly. From day 28 to day 32 both extension rate and length of the growth zone fell steadily and exhibited a more or less linear relationship. The ratio of extension rate to growth zone

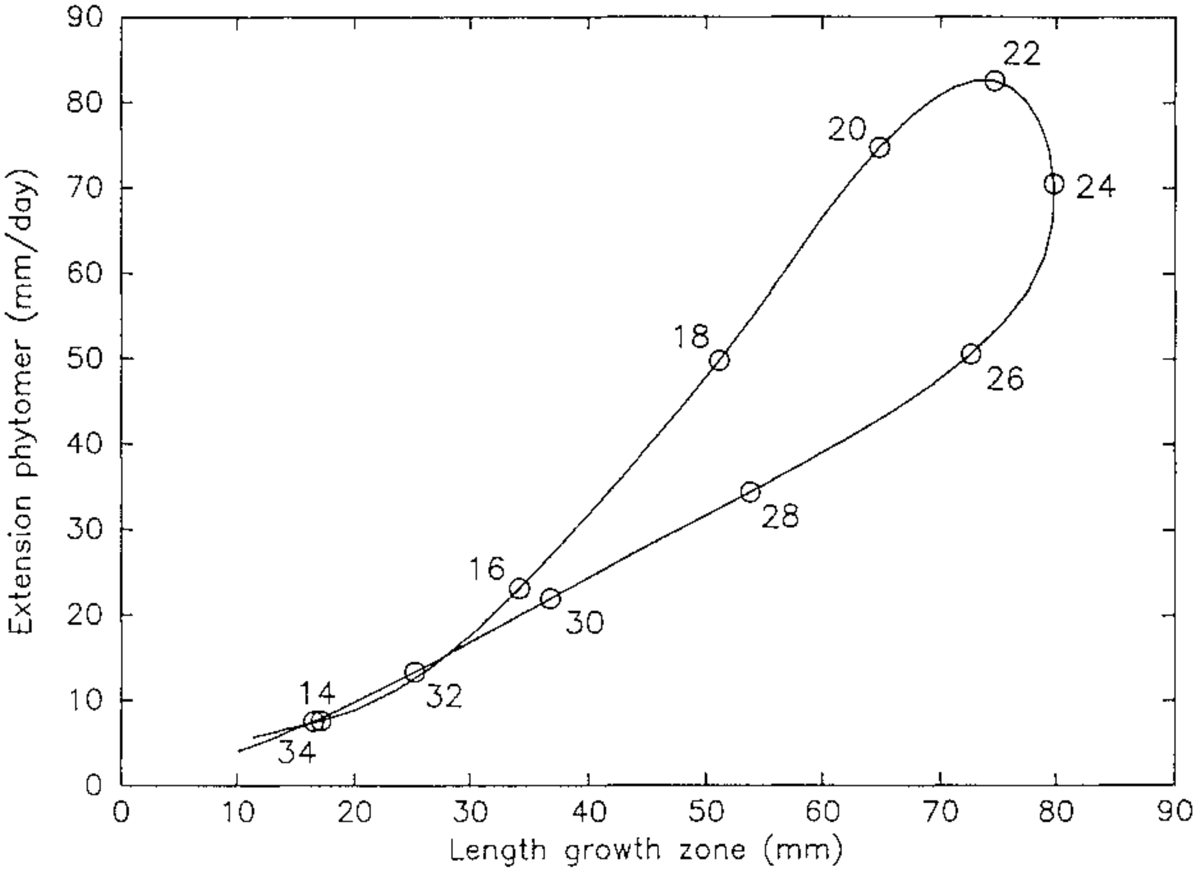


Fig. 3.9. Rate of extension of phytomer six compared with the length of the growth zone. The function at 0.0 hr on alternate days is indicated: ○. The length of the growth zone was obtained by inserting and examining the displacement of a set of pin holes. The extension was that which occured during the 24 hours following insertion of the pin holes. The figures used to obtain this graph were read from constrained B-splines fitted through the experimental data on growth zone length and culminant displacement.

length had fallen to 0.45 by day 34; a figure very close to that of the young, 14 day, growth zone.

The greatest rate of extension occurred during the elongation of the central portion, of the blade. When the growth zone was at its maximum length, extension was proceeding in the sheath and the lower part of the lamina. At this stage extension, rate per unit length of the growth zone had fallen and it fell still further when the extension was of the internode only. If the growth were considered in terms of production of volume of tissue as opposed to extension the fall off might not appear so substantial. The more massive structures of sheath and particularly internode must require a greater inflow of substrate for their manufacture. Also when they are being built, a greater proportion of the growth capacity must be involved with expansion in a direction perpendicular to extension and would therefore not be measured in this experiment. It is to be noted that the highest extension rate occurs while the parallel sided part of the lamina is extending. With regard to the demand for substrate, that of other expanding organs may affect the supply available to phytomer six. Phytomer seven was reaching its maximum extension rate between days 23 and 25, the time during which the ratio of leaf extension to growth zone length was falling most rapidly. The phytomers higher on the stem were also beginning to grow vigorously during the later part of the extension of phytomer six: the tip of leaf eight had emerged by day 25 and that of leaf 9 by day 28.

CONCLUSION

The results of this experiment have provided figures which support the description offered by Sharman(1942). She describes a coherent, basal wave of growth, which proceeds through the maize phytomer with the leaf blade, leaf sheath, associated internode, and finally nodal roots expanding in sequence within it. It seems wisest to consider the phytomer in this way, as a single expanding unit. The difference in extension rate which are associated with the morphological components of the phytomer are not separated by sudden changes. They are part of a continuum and this includes the anatomical boundaries.

CHAPTER IV

TRACING PATHLINES THROUGH THE GROWTH ZONE

INTRODUCTION

In grass leaves when samples of the tissues are taken in a linear series from the leaf base outwards towards the tip they show ordered increase in maturity.

Grass leaves have therefore been chosen as a source of material for the study of developmental processes in for example the work of Stebbins and Shah (1960), Obendorf and Huffaker (1970), Leach, Rumsby and Thompson (1973), Hawk, Rumsby and Leech (1974) Robertson and Laetch (1974), Taylor and Mackender (1977), Chapman and Leech (1979), Boffey, Ellis, Sellden and Leech (1979), Wellburn, Robinson and Wellburn (1982), and Ellis, Jellings and Leech (1983). Samples were taken at regular intervals along the leaf and the differentiation of tissues was described in terms of the positions. Either an assumption that the age of the tissues is linearly related to the distance from the leaf base was implicit in such work, or the lack of a time scale was perforce accepted.

Boffey, Sellden and Leech (1980) pointed out that the distribution of cell age through the length of the growth zone is not linear and a method for establishing a relationship between age and position is needed. They developed a method for computing cell age as a function of position on the first leaf of a wheat seedling. However, while it provides an estimate of age in the experiment in which it was used, this method has limitations. It was developed for use on very young seedlings; a relatively small number of measurements were used to characterise a complex system; and it included some unsatisfactory assumptions (chap. 2).

However, while the use of the ratio of the growth rate at points along the leaf to the growth rate of the whole leaf at a given time in Boffey et al's work is cumbersome, their approach shows that if the rate of basifugal movement at points in the growth zone can be obtained, as a function of time, then it should be possible to follow the track or pathline of a cell during growth. Gandar (1980 and 1983) emphasises the value of identifying particular moving points which can be located in the structure of the tissue, such as cell walls, and following them through the growth zone. He clarifies the relationship between points in space, and particles moving past such points. In particular he discusses velocity and shows that the velocity of a moving point can be calculated if the spatial velocity field is known.

The spatial velocity field describes the variation of velocity with position, along, and time ,with respect to, an organ. Consider a set of recognisable structures, cell walls for example, distributed along an extending organ such as a leaf. The structures occupy a particular set of positions at a given time. This current set of positions temporarily describes the arrangement of the structures in space, it is a spatial description. Each structure is moving and its movement with respect to a fixed point, the base of the leaf for example, is described as its velocity. At a given instant in time the structures occupy a certain set of positions. Their velocities are attributes of the positions and can be described spatially as a function of position. Thus at a given time velocity varies with position and at a given position velocity varies with time. This two-dimensional variation is a described as the spatial velocity field.

In terms of movement the velocity field represents the one dimensional rate of displacement of particles in an organ away from a chosen point, such as the base of the meristem, as a function of position and of time. Erickson and Sax (1956) obtained values for the velocity field in their classic experiments with maize root apices. They demonstrated the movement of points away from the root tip by photographing marks placed on the surface of the growing apex at a continuous series of short time intervals. The result was a picture showing a set of curved streaks. The slopes of the curves provided values for the rates of displacement away from the root tip at a chosen set of distances and at a series of different times. Thus they obtained a spatial description of the velocity field but they did not analyse their data in Lagrangian terms or consider the changes as functions of the route of the moving material labelled by the marks. Gandar (1980) used figures published by Erickson and Sax to demonstrate the validity of the Lagrangian approach but his results were restricted by the available data.

In order to be able to describe the change in position of a moving point in the growth zone, the point has to be identified, which can be done using a statement of its position at a specified time. The pathline of travel before or after that time can then be recorded in terms of values for the time and position coordinates. This is a referential description; the sets of coordinates are referenced to the identifying position and time. Bartaud and Gander (1985) describe referential pathlines in terms of functional notation, I will use the same symbols in this work.

A fragment centred on a referential point will follow the same route but the fragment will expand at the same time as it moves in the flow of growth. The movement of two marks one at either boundary will describe the expansion, as well as the position, of a piece of leaf fabric.

In my experiment I have worked with a single aspect of velocity, that of displacement. I have defined displacement as the one-dimensional movement away from a point of origin during a period of time. In chapter 3 (Figs. 3.5 and 3.6) I show curves which display displacement as a function of initial position, each of which describes the travel of all points on the leaf during a specified 24hour time interval. It is apparent (fig. 3.6) that displacement varies with age as well as with position. The relationship between displacement, position and time has been named the displacement field (Gandar, 1980).

In this chapter I will show how the sets of pin hole movements obtained (chap. 3) at a sequence of ages establish the form of a displacement field, and how this latter can be used to reconstruct the referential pathlines of points travelling through the growth zone to become part of the mature maize leaf.

THEORY

Displacement of a material element is the difference between its position within the growth zone, before and after a specified period of time. In Chapter 3, I showed that the displacement, d, of points on a growing phytomer varies both with initial position in the growth zone, x, and with the age, t, of the plant (Fig. 3.6, Chap. 3). The relationship between displacement, position and age can be symbolised as $d(x,t)$.

In the experiment described in Chapter 3 the displacement of points were measured over a fixed 24 hour period. If the period over which displacement is measured is maintained as a constant then $d(x,t)$ is the displacement field for that time interval (Fig. 4.1). In this experiment therefore $d(x,t)$ is the 24 hour displacement field.

The displacement field, $d(x,t)$, describes the movement, d, that will occur during the ensuing time interval, of any point, nominated by its present position, x, at current time, t. This is a spatial representation of the movement within the growth zone but it can be used to calculate the referential pathline of points in the leaf fabric that are travelling past spatial positions.

When values have been obtained for the displacement field of a particular growth zone the movement, d, of a point during the chosen time interval can be read from it. This is a small part of the total movement or pathline, $x=x(t;x_0,t_0)$, of the point through the growth zone but the point can be followed through a sequence of displacement periods (fig. 4.2). The point is at x in the growth zone at time t. These coordinates are named x_0 and t_0, the starting position and time. The starting position, x_0, can be any point on the line between the base and the tip of the leaf; and similarly the starting time, t_0 can be any time within the span of the growth period. The material element is moved forward in steps. Each step occupies the time interval of the displacement field. The value for $d=d(x_n,t_n)$ is obtained from the displacement field at each step.

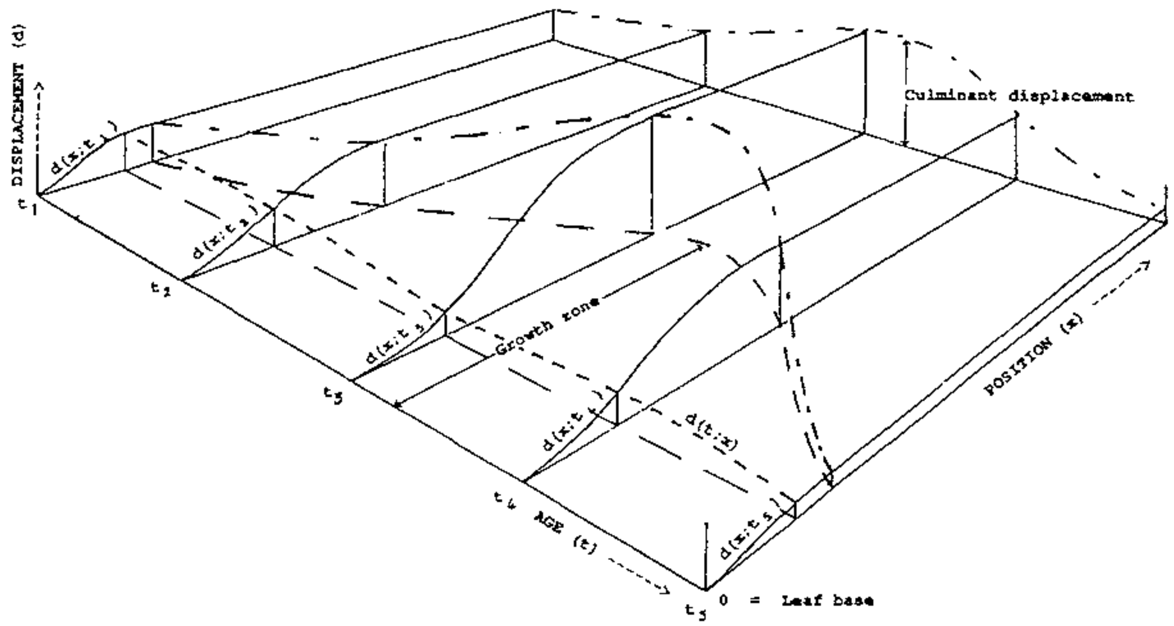


Fig. 4.1. An idealized three dimensional graph to show the displacement (d) in the growth zone of leaf six as a function of position (x) and age (t). The displacement is the difference between the positions of a material element before and after a constant period of time during growth. Displacement as a function of position d(x;t) is shown at five representative ages; t_1, t_2, t_3, t_4, t_5. It increases through the length of the growth zone, it reaches a maximum or culminant displacement at the distal limit of the growth zone and sustains it to the tip of the leaf. The distal limit of the growth zone as a function of time is shown – –. The culminant displacement as a function of time at a selected position beyond, and at the distal limit of, the growth zone is shown – - . Displacement at a given distance (x) from base as a function of time d(t;x) is shown as - - -.

Fig. 4.2. The steps in the construction of the pathline of a point moving through the leaf growth zone. The point is a material element of the fabric of the leaf. It can be visualized as an infinitely small fragment, an atom in a cell wall for example.

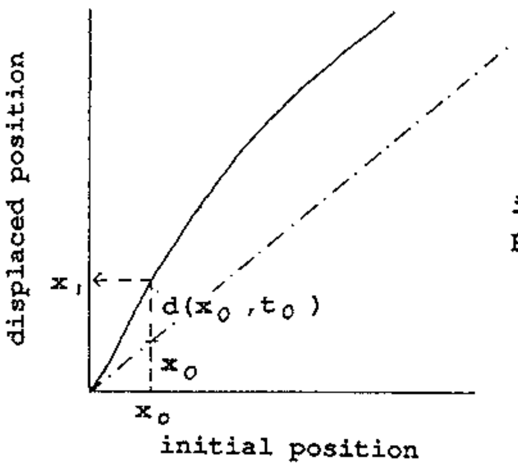
The point which is at position x_0 at time t_0 is followed through this set of diagrams. Displacement parallel to the leaf axis is being considered (Chap. 3). Curve a, shows the displaced position (——) of the leaf fabric as a function of its initial position in the growth zone following the time period t between t_0 and t_1. The displacement d(x) is the difference between the initial position (– - –) and the displaced position.

Thus x_0 is displaced $d(t_0,x_0)$ during the period, and,

at time $t_0+t=t_1$, has arrived at $x_0+d(t_0,x_0)=x_1$.

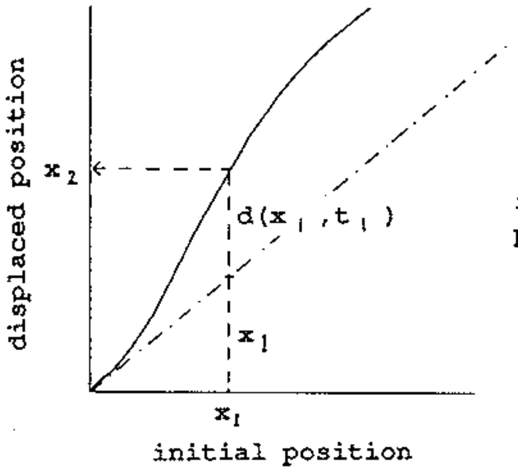
The argument is repeated with respect to the time periods between t_1 and t_2 (graph b), t_2 and t_3 (graph c). When a pathline is being constructed the position $x(t;x_0,t_0)$ is followed in this manner throughout the period of interest.

A.



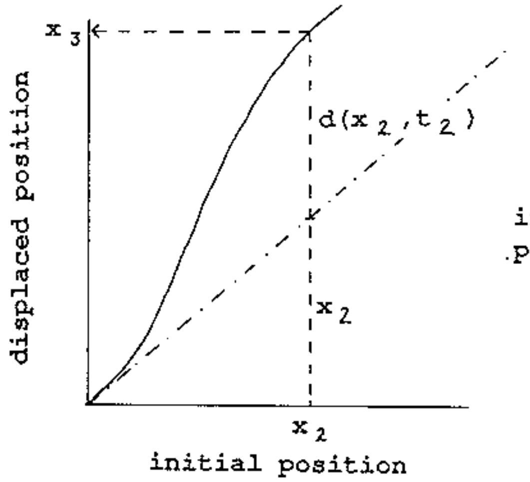
initial time = t_0
initial position = x_0
period of displacement = t
new time = $t_1+t = t_2$
new position = $x_0+d(x_0, t_0) = x_1$

B.



initial time = t_1
initial position = x_1
period of displacement = t
new time = $t_1+t = t$
new position = $x_1+d(x_1, t_1) = x_2$

C.



initial time = t_2
initial position = x_2
period of displacement = t
new time = $t_2+t = t$
new position = $x_2+d(x_2, t_2) = x_3$

(d=displacement during a tday period)

Between time t_0 and time t_0+1days the point will move
$d(x_0,t_0)$mm.

Therefore,

at time $t_1 = t_0 + 1$days, the point will have reached position $x_1 = x_0 + d(x_0,t_0)$mm
at time $t_2 = t_1 + 1$days, the point will have reached position $x_2 = x_1 + d(x_1,t_1)$mm
at time $t_3 = t_2 + 1$days, the point will have reached position $x_3 = x_2 + d(x_2,t_2)$mm,
etc.

The procedure is repeated throughout the growth period. During each step the arrival position coordinates, $x_n+1 = x_n + d(x_n,t_n)$, and time, $t_n+1 = t_n + 1$days, dictate the displacement to be added in the subsequent time interval. Thus the position and time coordinates which describe the pathline, $x(t;t_0,x_0)$ of a point travelling through the growth zone are calculated. The procedure can be repeated for as many different points in the leaf fabric, labeled $(x^1,x^2,x^3...x^n)$, and identified by differing reference position and time statements $[(t_0^1,x_0^1),(t_0^2,x_0^2),(t_0^3,x_0^3)...(t_0^n,x_0^n)]$, as are of interest.

SOURCE OF DATA

In Chapter 3, the displacement occurring in the growth zone of the whole phytomer was examined. In this chapter attention is restricted to the leaf alone.

The base of the leaf is taken as being the point of insertion of its sheath on the stem and this is the origin for position measurements.

The experimental procedure was described in Chapter 3. A set of pin holes was punched into the base of the growing phytomer. Harvest took place 24 hours later. During harvest the positions of the leaf base and of the ligule were recorded in relation to the positions of the line of displaced pin holes. The distance from the leaf base to each of the pin holes at the time of harvest and at the time of punching was calculated following the same procedure as was used in the phytomer growth zone study (Chap. 3). Displacement was the difference between the position at harvest and that at punching and a profile of the displacement as a function of initial position was prepared for each plant similar to those shown in Figure 3.6, Chapter 3.

The values for displacement obtained in Chapter 3 could not be used directly to obtain pathlines (Theory, Chap. 4) both because they showed considerable scatter (figs. 4.3, 4.4) and because they were not uniformly distributed (fig. 4.6) in terms of age and position. With regard to age the harvest of the plants to measure displacement occurred exactly 24 hours after punching but the punching ages were staggered within each day. In terms of position, while the holes on a given plant at punching were equidistant from one another, their positions with regard to the leaf bases varied from plant to plant. Thus it was necessary to carry out the following data analysis to scale and smooth the data and to obtain a grid of coordinates representative of the displacement field preparatory to calculating pathlines for points.

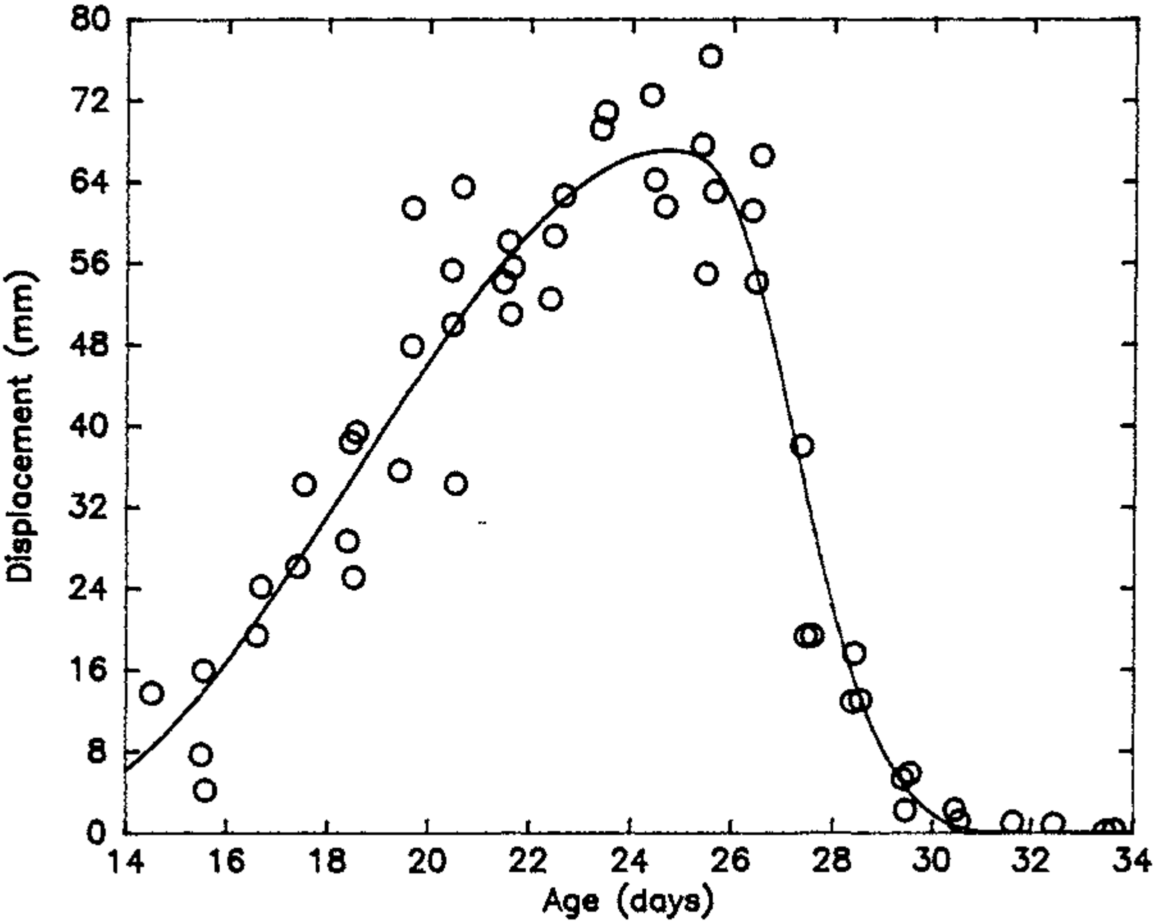


Fig. 4.3. Culminant displacement in the leaf during a twenty four hour period as a function of plant age at the time of harvest. Plant age was taken as zero at the time of planting. Recorded data points,o. Constrained B-spline fitted through the data (——). The culminant displacement is that which occurs at, and beyond, the distal end of the growth zone.

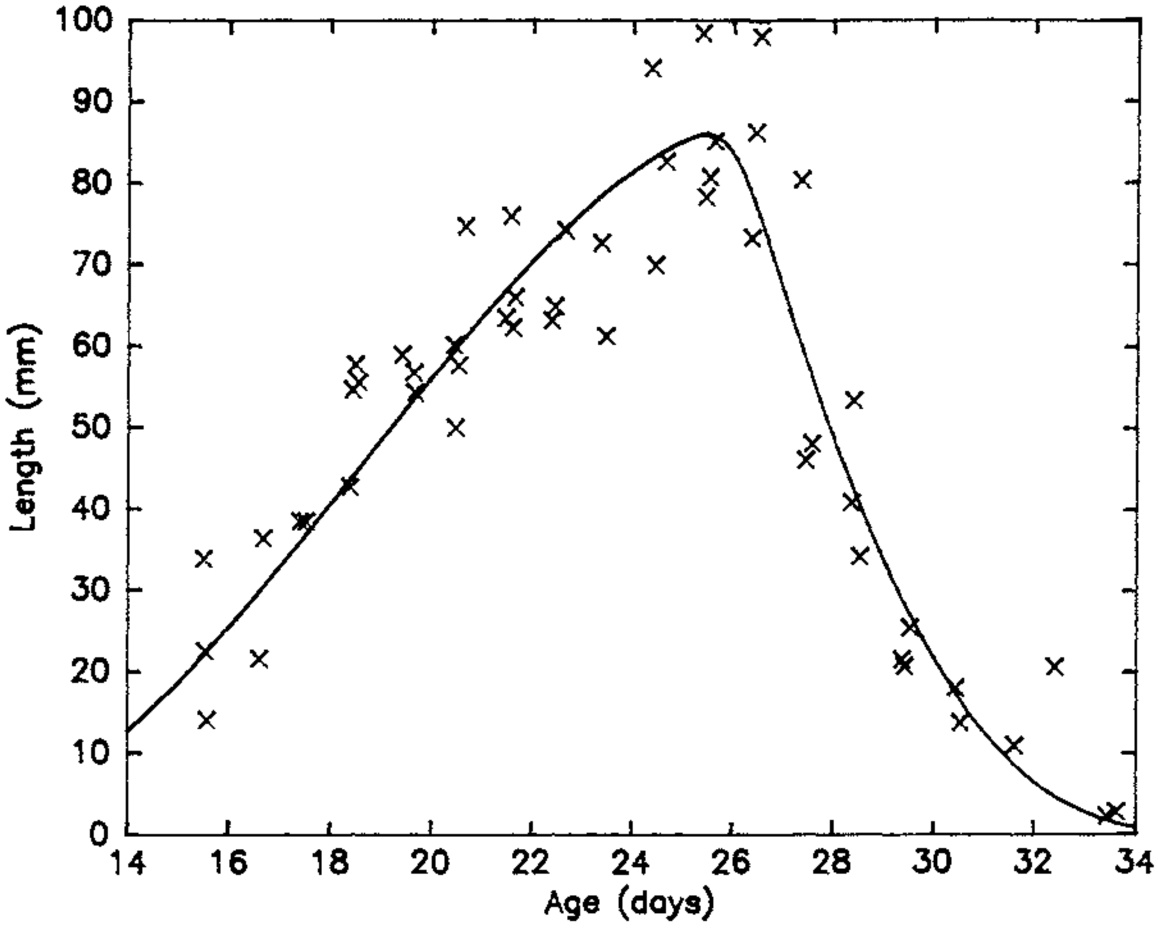


Fig. 4.4. Length of the leaf growth zone as a function of plant age (0=time of planting) at the time of harvest. Recorded data points, X. Constrained B-spline fitted through the data (——). The distal limit of the growth zone is the point on the leaf beyond which there is no further sustained increase in displacement.

DATA ANALYSIS

CULMINANT DISPLACEMENT

The culminant displacement was defined as that sustained by the leaf at or beyond the end of the growth zone. Tip displacement, therefore, equalled culminant displacement. Displacement as a function of position has been measured and discussed (Chap. 3). The values for 24 hour culminant displacement are plotted as a function of age in figure 4.3. The constrained B-spline fitted through the data points represents the mean culminant displacement as a function of age for the population of experimental plants. Culminant displacement value was read from the B-spline at the age of punching of each of the plants. The set of values representing displacement as a function of initial position on an average plant (Fig. 4.5) of a particular age would include the mean culminant displacement indicated for that age. Each set of plant data was adjusted so that its culminant displacement coincided with the mean culminant displacement for its age (Fig. 4.3) It was assumed that the difference in expansion that had yielded a culminant displacement greater or smaller than that shown by the mean curve was proportionately distributed through the growth zone and the data set for each plant was adjusted accordingly.

$$\text{adjusted displacement value} = \text{recorded value} \times \frac{\text{culminant displacement from spline}}{\text{recorded culminant displacement}}$$

GROWTH ZONE LENGTH

The individual plant data sets were also adjusted to coincide with the growth zone length-to-age function within a population. The reasoning and technique was similar to that used for the culminant displacement. A constrained B-spline was fitted which described growth zone length as a function of age (Fig. 4.4). Growth zone length values were read from it at ages corresponding to those of the experimental plants. The displacement data sets were adjusted to include these coordinates.

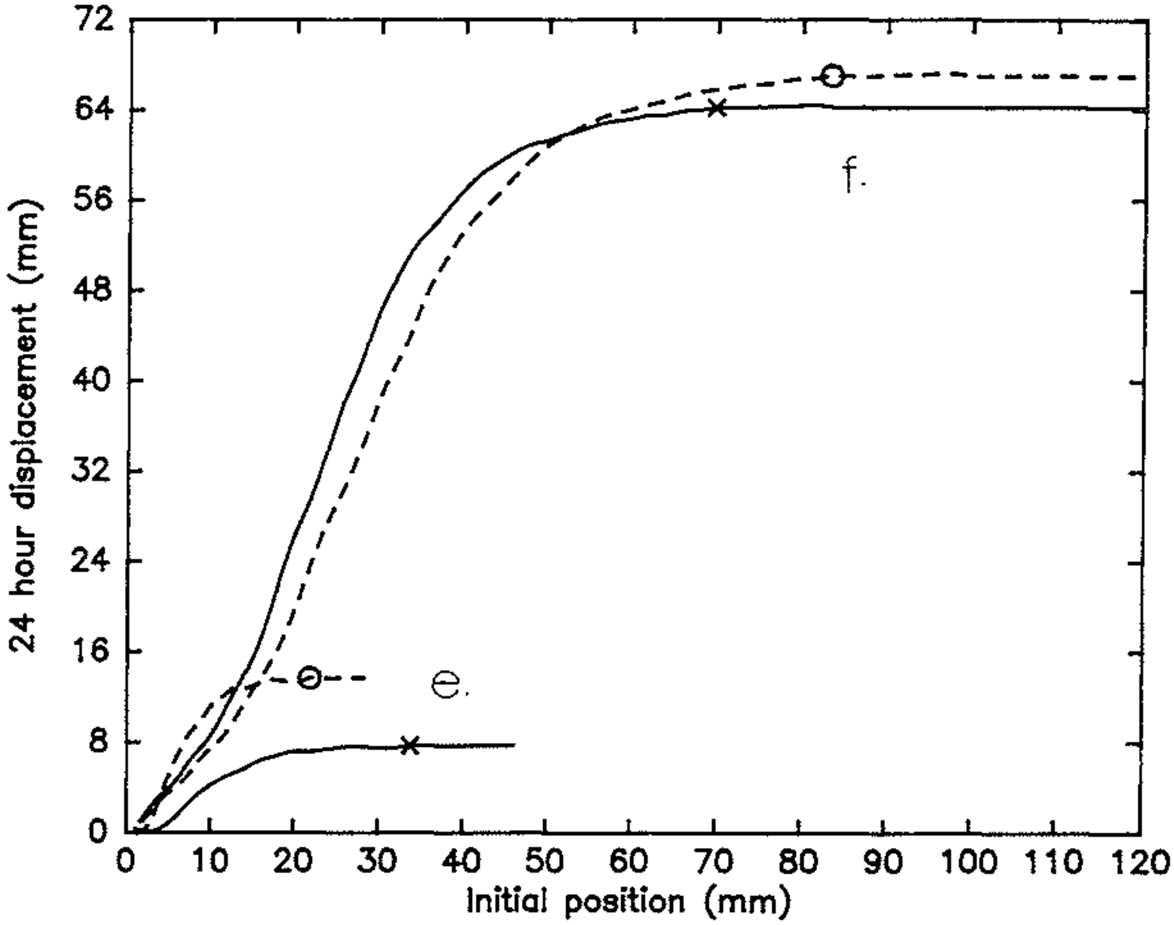


Fig. 4.5. The effect of adjusting displacement as a function of initial position in the growth zone for two plants aged: e, 15.5days and f, 24.5 days. Recorded values for displacement as a function of initial position (——). The distal limit of the growth zone is the point on the leaf at which the culminant displacement is reached and, therefore, the length of the growth zone is the distance between leaf base and this point. Culminant displacement and length of the growth zone are coordinates of a point on the displacement curves. This point is marked ,X, on the recorded 24hour displacement curves of the two plants used as illustrations. The coordinates for points ,O, on the adjusted curves are the culminant displacement and growth zone length read at the appropriate age from the population culminant displacement (Fig. 4.3) and growth zone length (fig. 4.4) curves. The displacement values d(x;t) were adjusted to include these population representative coordinates.

$$\text{adjusted initial position} = \text{recorded initial position} \times \frac{\text{growth zone length from spline}}{\text{recorded growth zone length}}$$

The effect of the combined adjustment is shown in Figure 4.5.

THE GRID OF DISPLACEMENT FIELD VALUES.

A representative portion of a map of the positions and ages at which displacement was measured is shown in figure. 4.6. The data are in sets of positions at fixed times d(x;t). The times are the ages at punching of the experimental plants and the positions are those of the pinholes as they were inserted. A set of distances from the base was chosen. These were multiples of 5mm from the base and rarely coincided with the position of the pin hole marks. The displacement at each of the chosen positions, at the ages represented by the experimental plants, was obtained by linear interpolation between the two sample positions immediately basifugal and basipetal to that chosen. This yielded sets of values for d(t;x), where x=(5,10,15,...100mm). A constrained B-spline was fitted through the scatter of each of these sets (Fig. 4.7) and the displacement as a function of position at chosen times, d(x;t) values, where t=(14.5,15.5,16.5,...30.5days), were read from the set of B-splines.

THE DAMAGE FACTOR

The maize plants were damaged by the punching procedure and there was a consistent reduction in the growth of the leaves (Chap. 3). The reduction in total length of the leaf which is punched on a single occasion is not great, because the time involved is only a short part of the overall growth period. The method proposed for the construction of pathlines, however, involves the addition of displacement values, each of which was measured during the period immediately following the infliction of damage, and the resulting pathline will only represent normal growth if each of the displacement values used is increased to compensate for the effect of damage. It was assumed that the reduction in displacement was distributed in proportion throughout the length of the

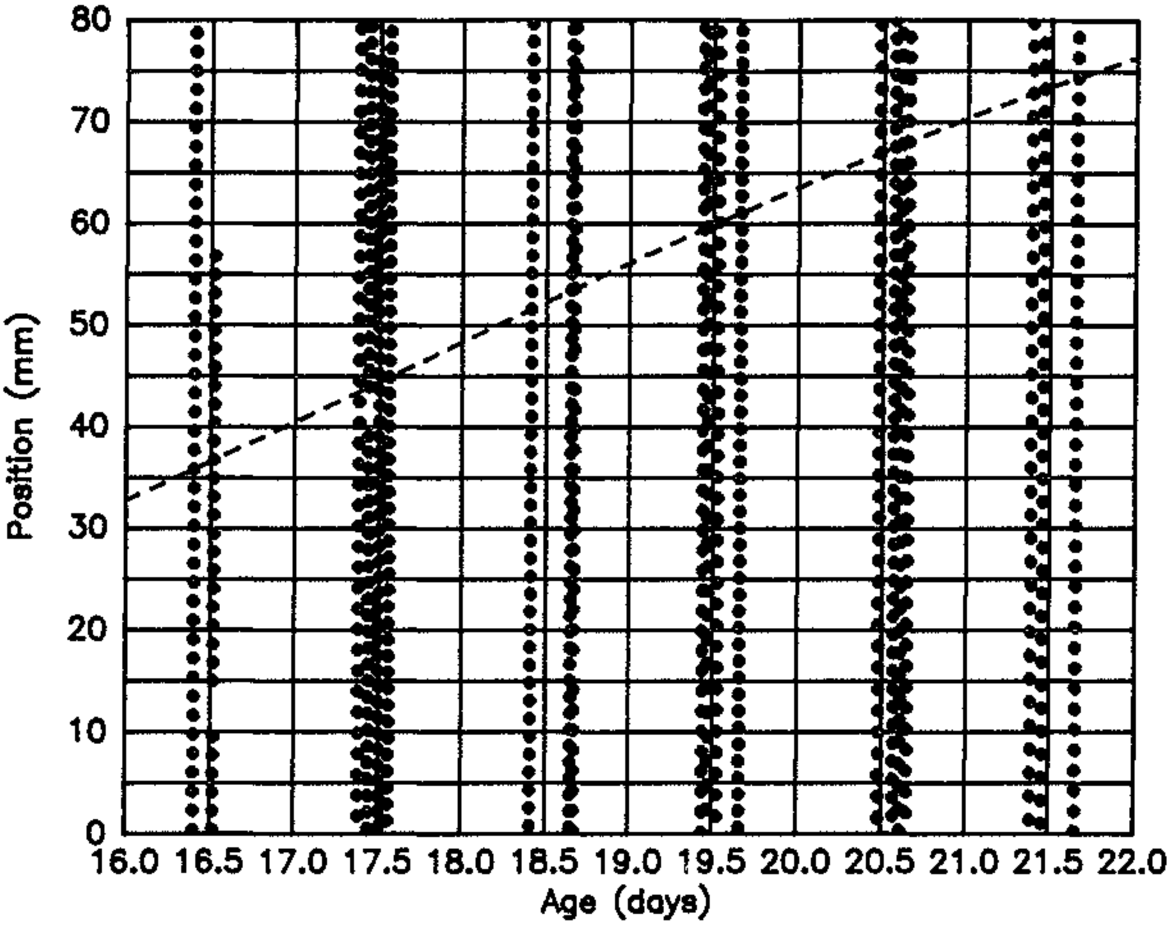


Fig. 4.6. Map to show a representative part of the array of positions and ages at which 24hour displacement was evaluated. The positions are the initial positions of the pin holes and the sets for twenty plants are plotted as a function of the age (0=time of planting) at which each plant was punched. The 24hour displacements at a uniform set of ages and positions were calculated from this array as follows. Values of d(x;t) where x={5,10,15...100}mm were obtained for each plant by linear interpolation. The sets of values for displacement as a function of time at these 5mm interval positions were taken. A constrained B-spline was fitted through each of these d(t;x) sets, and the values on these at t={14.5,15.5,16.5...33.5}days were recorded. The distal limit of the growth zone is shown - - -.

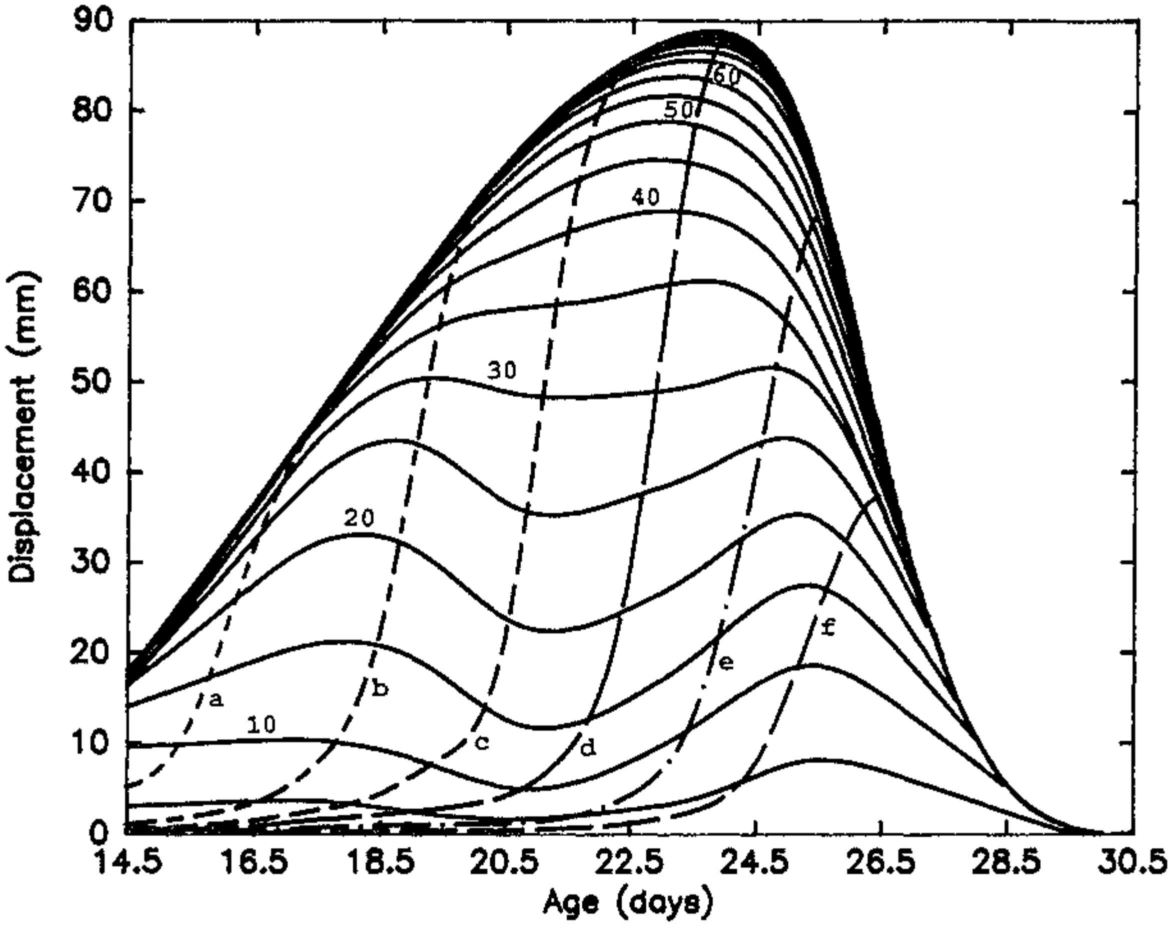


Fig. 4.7. 24hour displacement as a function of age at punching d(t;x), at selected intervals along the axis of leaf six(——). The numbers on the lines represent the distance from base in mm at which the curve for displacement as a function of time was located, the lines are at 5mm intervals between zero and 90mm.

The 24hour displacements $d(t;x_0,t_0)$ on a set (a,b,c...f) of referential pathlines is also shown. The reference coordinates of the pathlines, in mm and days, and the position arrived at in the mature leaf in mm are;

code	x	t	in mature leaf.
a	6.700	14.5	775, near tip.
b	1.700	14.5	650, upper lamina.
c	0.793	14.5	506, mid lamina.
d	0.470	14.5	355, lower lamina
e	0.205	14.5	200, ligule.
f	0.067	14.5	100, mid sheath.

growth zone and therefore all values for displacement should be increased by a factor of 0.24 (Chap. 3). Such increased values were used at each step in the pathline calculation.

RESULTS AND DISCUSSION

THE DISPLACEMENT FIELD

The experiment yielded a matrix of values for the 24 hour displacement in a leaf growth zone. The matrix shows the displacement for a grid of positions and times. The positions are at five millimeter intervals between the leaf base and 90mm. The times are at 24 hour intervals commencing on day 14.5. The displacement field is shown as a graph (fig. 4.7).

At a given age displacement varies with spatial position within the growth zone. This statement is represented as, d(x;t) and at all ages forms a sigmoid curve (Chap. 3, Fig. 6). Similar curves are found for other organs, for example the work on root tips by Goodwin and Sepka (1945) and Erickson and Sax (1956). Other workers,for example Van Dillewijn (1939), Volenec and Nelson (1981), Michelina and Boyer (1982), Westgate and Boyer (1984) measured the extension between marks, their results were plotted as absolute or relative growth rates, but their observations would also yield a sigmoid curve for displacement from a point of origin.

The displacement at a given distance from the base of the leaf varies with age. This statement is represented as d(t;x), and examples of d(t;x) curves are used to demonstrate the displacement field (fig. 4.7). In figure 4.7 the positions (x) are at five millemeter intervals between the base and 90mm along the leaf. These d(t;x) graphs show that displacement at a given position tends to rise from a low level at the start of the experiment and to fall towards zero when the leaf is nearly mature. During the interval between these periods of early and late leaf growth in the proximal part of the growth zone, the initial rise in displacement is followed by a period of lower displacement and then a second rise, but in the distal part the initial rise is sustained, a maximum is reached and the subsequent fall is a smooth one.

The length of the growth zone rises and then falls during the growth period (fig. 4.3). The set of positions used to show the displacement field (fig. 4.7) extend beyond the distal limit of the growth zone and where they do so they display the current culminant displacement. The culminant displacement as a function of age takes the form of a wave (figs. 4.1, 4.7). Since the displacement in this experiment was measured over a constant age interval and by definition there could be no extension beyond the end of the growth zone, culminant displacement was equivalent to the rate of extension of the whole leaf. Other investigators have obtained similar wave shaped curves for the rates of extension as a function of age of leaves of the Gramineae, for example Williams and Williams (1968), McCree and Davis (1974) and Watts (1974).

If the displacement at a particular position (x) on the d(x;t) curve is considered it represents the sum of the expansion of all the material between it and base, and the slope of the curve at that point, or the first derivative of d(x;t) at x, describes the contribution to extension made at that point. Thus the difference between the displacements found at two positions at a given age represents the extension taking place in the intervening length and the separation of the d(x;t) curves on the displacement field graph (fig. 4.7) shows the extension capacity or stretch in the various segments of the growth zone. The capacity for extension in each of the intervals varies with age. The part of the growth zone which shows the greatest capacity for extension changes position during growth. If the position of that portion of the growth zone which shows an extension of 10mm or more in the five millemeter segment is followed. It is seen to be in the segment between five and ten millimeters of base at the start of the experiment. It moved outward and became a broad band during the first two thirds of the experimental period. It occupied a position between 15 and 30mm on day 22, and one between 30 and 35mm on day 24; day 24 was just prior to the time of maximum leaf extension. After this the region of high stretch shrank and retired swiftly toward the leaf base. It lay between 5 and 10mm on day 26. In the days leading up to maximum leaf growth, coincident with the increase in the length of the region of high stretch, there was also a smaller increase in the length near the base of the leaf where very little stretch was occurring and this lead to a fall and subsequent rise in the graphs showing displacement as a function of time, d(t;x), at the zero through 35mm positions (fig. 4.7). This means that there was a lower rate of outward movement of such developing tissue elements as temporarily occupied that basal spatial zone. At all times the tissue was moving from a region of zero displacement at the base of the leaf to one of greater rate of movement further out. The change in displacement at a given position with respect to time is a comparison between the movement of different tissue elements which passed through the zone in sequence and although each of these had a steadily increasing basifugal movement they were not travelling at the same speed when they occupied the same spatial position. This will be clearer when it is discussed in terms of the pathlines of these elements.

THE RECONSTRUCTION OF PATHLINES

The pathlines were reconsructed using the stepwise calculations described in the theory section of this chapter.

Where a displacement value was required for either a position other than at one of the five millemeter interval set that had been evaluated, or for a time other than midday, it was obtained by linear interpolation between values that were available at adjacent positions and times. This will have produced values which are slightly high where the linear interpolation was used to bridge a concave, and slightly low where it spanned a convex section in the curves. The distortion which has resulted is however very small and is insignificant by comparison with the other possible sources of error in the experiment.

A curve was drawn through the set of calculated values. It represented the pathline of a material element. Several of these pathline curves (x^1,x^2;x^3,...x^{14}) are shown (fig. 4.8). Each of the material elements (x^n) is identified by a statement of its position (x_0) at a particular time (t_0). These are the reference position and time of the material element. The pathline that an element (x^n) follows can now be described by values for position and time. This is expressed in symbols as $x=x(t;x^n,t^n)$, (Bertaud and Gandar; 1985). The reference position and time may be anywhere on the pathline since each pair of coordinates is unique and lies on only one pathline. For example the reference pair for x^6 was 0.778mm from base at time 14.5days from planting; but the material element moved on its pathline to position 10.44mm at time 19.5days and these two values could have been used as reference coordinates with equal validity.

It would be possible to follow the pathline back from a more remote position towards the base of the leaf by setting up a displacement field in which the displacement was referenced to its arrival or "harvest" position and time, as opposed to its departure or "punching" position and time.

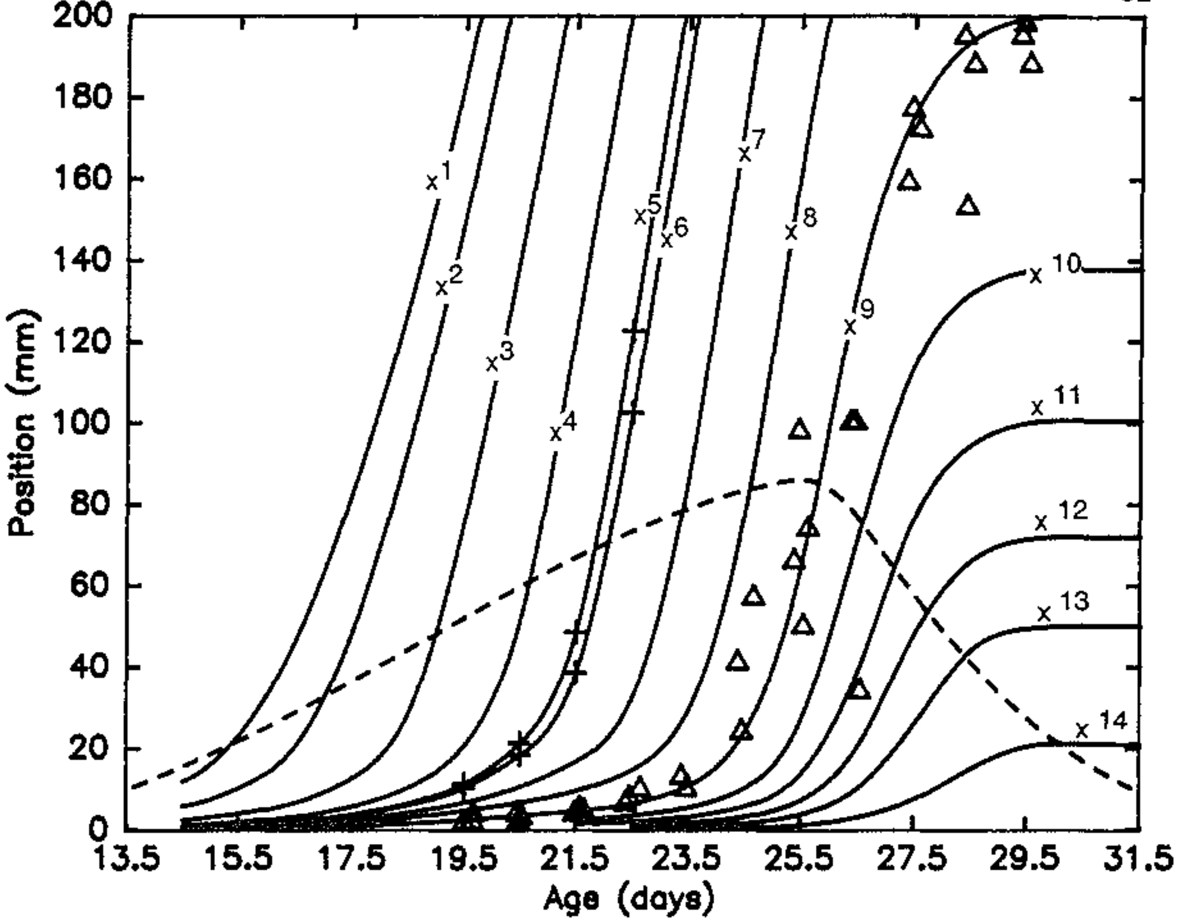


Fig. 4.8. Pathlines, $x=x(t;x_o^n, t_o^n)$, of referential points in the base of leaf six during growth. The reference coordinates in mm and days, and the position of the points in the mature leaf are;

$(x_o^1, t_o^1)=(14.5, 12.00)$, on mature leaf, $x=802$mm
$(x_o^2, t_o^2)=(14.5, 6.000)$, on mature leaf, $x=767$mm
$(x_o^3, t_o^3)=(14.5, 2.550)$, on mature leaf, $x=694$mm
$(x_o^4, t_o^4)=(14.5, 1.270)$, on mature leaf, $x=601$mm
$(x_o^5, t_o^5)=(14.5, 0.8325)$, on mature leaf, $x=519$mm
$(x_o^6, t_o^6)=(14.5, 0.7781)$, on mature leaf, $x=500$mm
$(x_o^7, t_o^7)=(14.5, 0.550)$, on mature leaf, $x=398$mm
$(x_o^8, t_o^8)=(14.5, 0.390)$, on mature leaf, $x=300$mm
$(x_o^9, t_o^9)=(14.5, 0.205)$, on mature leaf, $x=200$mm
$(x_o^{10}, t_o^{10})=(21.5, 2.600)$, on mature leaf, $x=138$mm
$(x_o^{11}, t_o^{11})=(21.5, 1.550)$, on mature leaf, $x=100$mm
$(x_o^{12}, t_o^{12})=(22.5, 1.200)$, on mature leaf, $x=72$mm
$(x_o^{13}, t_o^{13})=(22.5, 0.635)$, on mature leaf, $x=50$mm
$(x_o^{14}, t_o^{14})=(23.5, 0.300)$, on mature leaf, $x=21$mm

The distal limit of the growth zone is shown (- - -). The increasing length of the region lying between x^5 and x^6 is shown, ‡ .

At age 19.5 the length is 0.97
At age 20.5 the length is 2.93
At age 21.5 the length is 9.89
At age 22.5 the length is 20.0

THE EXTENSION OF A REGION

The pathline describes the movement of an infinitely small piece of material It does not describe the expansion of a finite length of tissue such as that which would necessarily be included in an experimental sample. It is therefore more useful to construct two pathlines and to use the length of material which lies between them as the sample. The two pathlines, as for example x^5 and x^6 (fig. 4.8) will diverge and the region between will become longer but at each sampling time it represents the same section of the leaf fabric.

The reference coordinates t_0 and x_0 for x^5 and x^6 are day 14.5 with 0.832mm and 0.778mm respectively. At maturity the region lies between 520mm and 500mm which is midway along the length of the leaf lamina.

The divergence of the two pathlines is indicated by the vertical lines which span the length between them at daily intervals. The span is the difference between the position, $x=x(t;x_0,t_0)$, on one pathline, x^5 and that on the second, x^6, at the given age. This difference provides values for the extension of the region as a function of time.

Rapid increase in length of the region, x^5-x^6, occurs from day 19. Prior to day 19 the region increases by a little less than twice its initial length during each twenty four hour period, between day 19.5 and day 21.5 the increase is a factor of 3, and between day 20.5 and 21.5 the factor is 3.4. The upper pathline leaves the growth zone between day 21.5 and 22.5. The length of the region between the two pathlines outside the growth zone is 20mm. Its length has multiplied by 20 during the five days between day 19.5 and day 24.5.

MOVEMENT OF THE LIGULE AND LEAF TIP

The ligule is a natural mark visible on the expanding leaf. It could be recognised from day 19 onwards and its position in relation to the base of the leaf was recorded during the harvest of the maize plants. The position of the ligule during leaf growth can be compared with a constructed pathline (fig. 4.9). The pathline of x^7 which lay at

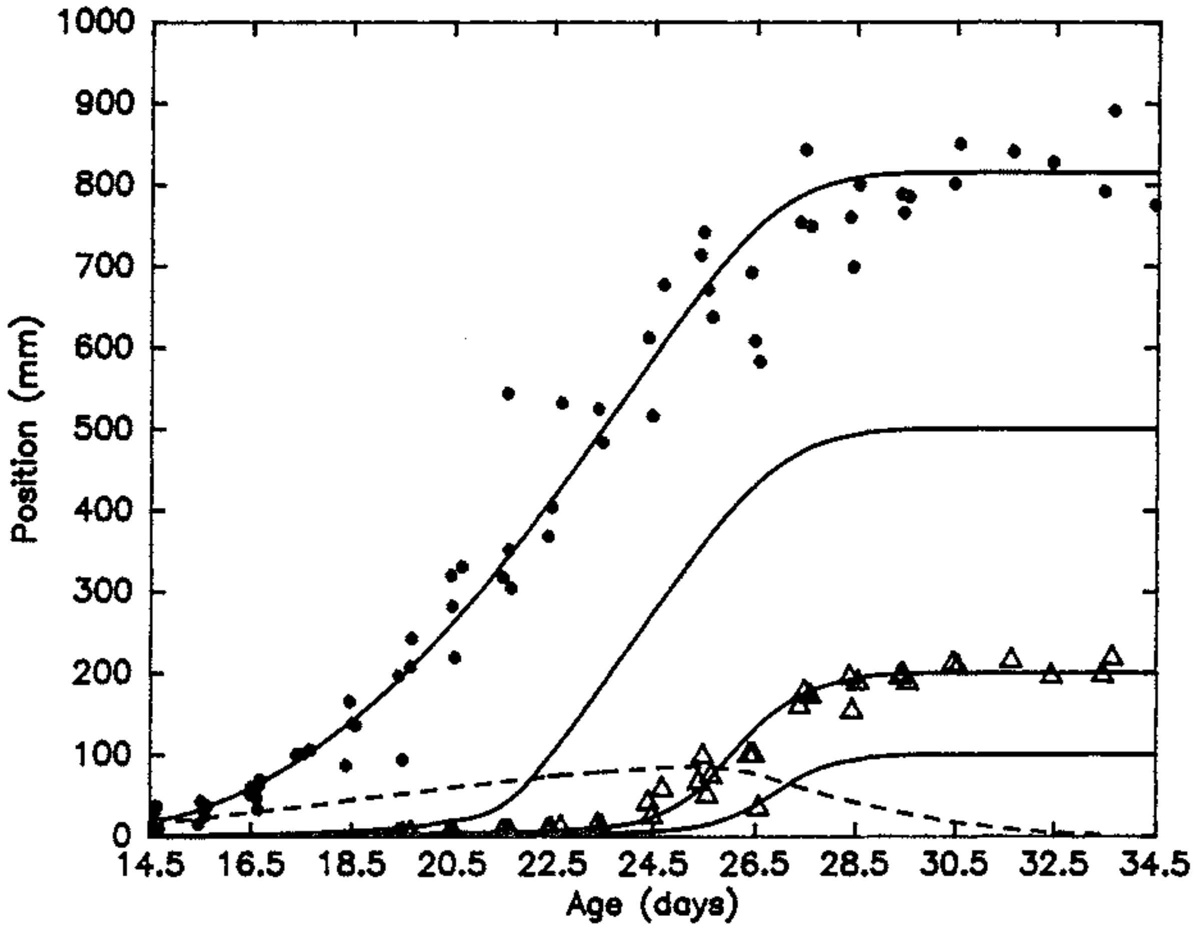


Fig. 4.9. Graph to show the growth of the leaf from 18mm to maturity. The relative positions as a function of age of the tip (•), the ligule (Δ) and the distal limit of the growth zone (– –) are shown; with for comparison, a set of pathlines (——). The reference age (t_o), and position (x_o) of the pathlines are;

x_o mm	t_o days	position in mature leaf mm
18	14.5	815
0.8325	14.5	520
0.7781	14.5	500
0.205	14.5	200
0.067	14.5	100

0.205mm on day 14.5 follows a route in terms of time and position among the actual plant records which must be close to that of the ligule on the notional population average leaf. This provides an effective check and indicates that the method used, to calculate the pathlines is essentially correct.

The tip of the leaf had moved out of the growth zone before the leaves were big enough to be punched. The movement of the leaf tip can however be calculated from a knowledge of the displacement field. Since there can be no increase in displacement outside the growth zone all parts of the leaf that lie beyond it must move parallel to one another and the culminant displacement describes their movement. Thus if the average length of a leaf on day 14.5 is 18mm the pathline x^1 calculated with these as its reference coordinates should, and infact does (fig. 4.9) produce a pathline of position as a function of time which fits through the measured values for leaf length.

EXTENSION IN THE GROWTH ZONE IN RELATION TO POSITION ON THE MATURE LEAF

The pathline curves (Figs. 4.8 and 4.9) which display the positions of material elements as a function of time are all basicaly sigmoid in shape with slow outward movement in the base of the growth zone giving way to an increasing rate as the tissue elements they represent move outwards. The rate of displacement is shown by the slope of the pathline. Each pathline shows a different shape within the growth zone This is consistent with the lack of any period of steady state in the growth of the maize leaf (Chap. 3).

When a given pathline arrives at the limit of the growth zone, its displacement is the culminant displacement which I have shown varies as a function of age (fig. 4.3). Thus the rate of travel of tissue leaving the growth zone, represented by the slope of the pathlines (fig. 4.8), increases from day 14.5 until the maximum culminant displacement is reached on day 24 after which the rate of travel decreases reaching zero at leaf maturity. Beyond the growth zone the rate of displacement on all the pathlines equals the culminant displacement in the growth zone beneath them and the pathlines run parallel (figs. 4.7, 4.8, 4.9).

The displacement as a function of initial age on a selected set of pathlines is plotted for comparison with the displacement field (fig. 4.7). This provides an indication of which parts of the leaf are involved in the displacement shown. The pathlines of points immediately behind the leaf tip were in a high stretch region in the first few days of the experiment but they were also close to maturity in the relatively short growth zone and did not achieve a high total growth (Figs. 4.8 and 4.10). The extended zone of low displacement found between 5mm and 10mm at at the base of the growth zone between days 19 and 24 was occupied by the pathlines of lower leaf sheath tissues. Beyond them the pathlines of upper sheath and lower and mid lamina showed a somewhat higher, although still relatively low, rate of displacement between day 14 and day 21. These pathlines were however diverging from one another and the more distal of them were approaching the zone of high extension more rapidly than those below. By day 19 the midlamina pathlines were beyond 15mm and had entered the region of high extension capacity. The ligule was in this region by day 22. The lower leaf sheath tissues showed a low rate of expansion and remained close to the base until day 24 after which they entered the zone of maximum stretch and their period of high expansion in it, but by this time the zone was narrow lying within 5mm of the base of the now short growth zone, and the rate of increase of length within it was small.

The passage through the high extension region of the growth zone of the major part of the lamina and upper sheath, coincided with the time of the growth zone's greatest length. From day 14.5 until they left the growth zone the divergence of the tissues from midlamina to upper sheath was thus greater than that of tissues in the leaf tip and lower sheath. This greater divergence of midleaf tissues as compared with the others can be demonstrated by calculating the width as a function of time, of regions bounded by pathlines and sited in different parts of the leaf (fig. 4.10).

LEAF AND PHYTOMER GROWTH ZONES

The leaf growth zone is part of the phytomer growth zone. Before the extension of the internode commences it equates to the phytomer growth zone and while the internode is extending it is the outer part of it, that part distal to the the upper node. The leaf growth zone is reduced to zero when the upper node of the phytomer moves out of the phytomer growth zone . My discussion (Chap. 4, Theory) of the displacement field

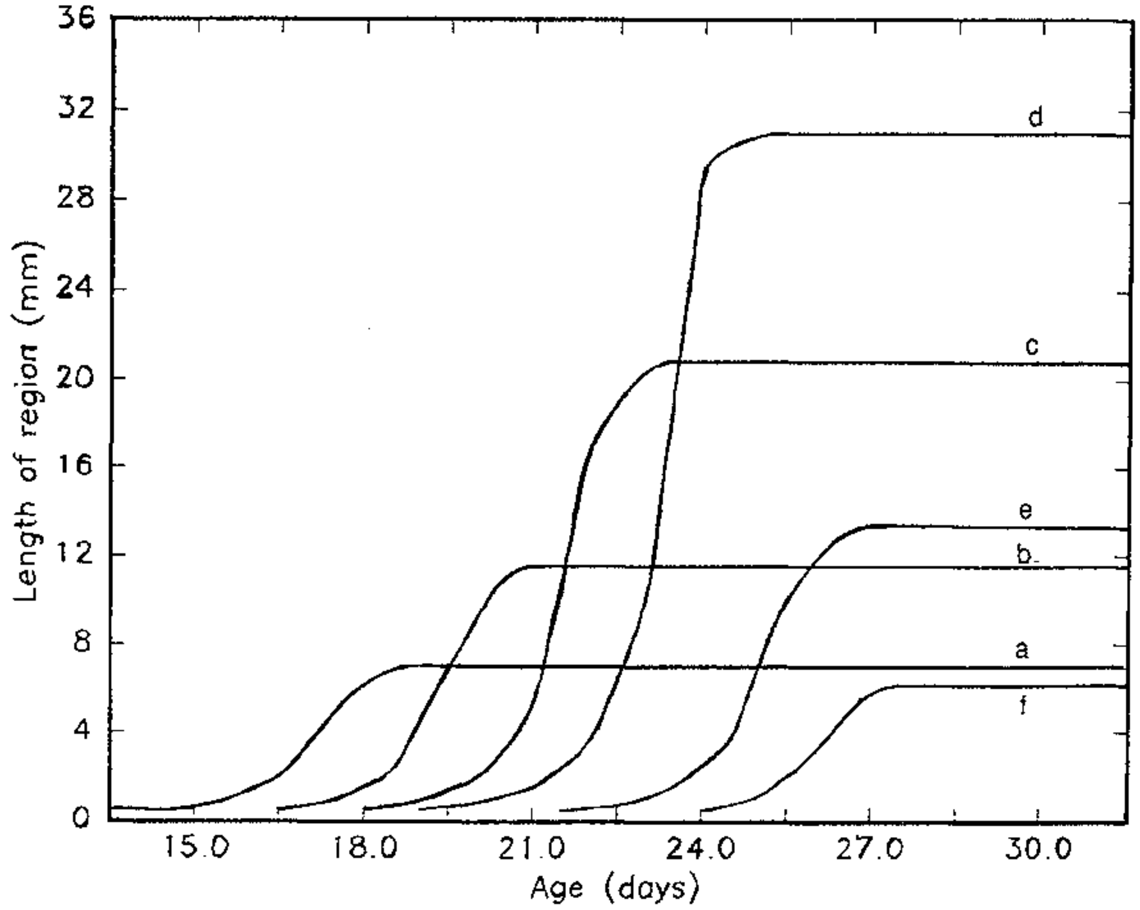


Fig. 4.10. Length of regions bounded by pairs of pathlines. Each segment of leaf tissue was 0.5mm long when the position on the distal pathline was 5mm. The region of the leaf of which each region was a part is indicated. The initial position and age, and the position at maturity on the pathlines, in mm and days were;

Region	Distal pathline			Proximal pathline		
	x	t	mature	x	t	mature
a. near tip	5.0	13.5	752	4.5	13.5	746
b. upper lamina	5.0	16.5	655	4.5	16.5	644
c. mid lamina	5.0	18.0	508	4.5	18.0	488
d. lower lamina	5.0	19.0	373	4.5	19.0	342
e. ligule	5.0	21.5	207	4.5	21.5	194
f. mid sheath	5.0	24.0	113	4.5	24.0	107

applies equally to a leaf or to a phytomer growth zone. The only difference is in the changed values for position which results from the shift in the site named as base, and taken as the origin, for the position measurements.

CONCLUSION

Development in the maize leaf can now be studied in terms of a region, which represents a chosen piece of tissue, travelling through the growth zone. The piece of tissue can be located at chosen sampling times and rates of change during development can be established. Further since the change in length of the piece of tissue under investigation is also available, quantitative changes in developing properties which are measured on a per unit length basis can be considered in terms of the concurrent increase in size of the piece of tissue. I have made use of a sampling system based on regions moving through the growth zone as a function of time in my work on the rate of development of the stomatal complex on a maize leaf. I will describe this work and discuss the sampling system in more detail in Chapter five

The grass type leaf has been selected for development studies by a number of workers because of the ordered arrangement of the growing tissues. Its value in this respect is enhanced if the observations can be set to an actual time scale. This can be done by the use of the pathline reconstruction method that I have described.

CHAPTER V.

DEVELOPMENT IN AN EPIDERMAL SEGMENT IN THE GROWTH ZONE OF A MAIZE LEAF

INTRODUCTION

While studies of tissue development in Monocotyledonous leaves have revealed very useful facts in the past, the value of the information has been limited by the lack of a capacity to measure the rate of expansion and consequent rate of displacement of the tissue in which the changing characters are observed. In its absence the importance of this information has not been appreciated and as a result some of the conclusions drawn from the studies are open to question. Because workers in the field were limited to discussing their observations in terms of distances from the leaf base this led to the virtual assumption of a linear relation between position and time. Further, since the only available information about local changes in length was that which was reflected in differences in cell length in the absence of cell division, it was convenient to consider the growth zone as divided into two parts. Extension was ignored in a small basal region, named the "zone of cell division". In the longer, more remote section, named the "zone of cell extension", cell division was considered absent, and increases in cell length could therefore be taken as reflecting increases in tissue length.

Boffey et al (1980) described a method of relating cell age to the distance from the base of the leaf in cereal seedlings. Several intracellular and biochemical studies have been carried out, for example by Dean and Leach (1982), in which this method has been used to establish the time scale; but the method has limitations. I have discussed these (Chap. 2). Boffey assumes that all cell divisions take place in an infinitely small meristem at the base of the leaf and then follows the movement and increase in age of what is termed "a cell". She does not allow any discussion of the length of the meristem. I have chosen to examine development in terms of a segment of tissue. In a segment I can study expansion and cell number as well as differentiation as a function of age.

Leaves are determinate structures each of which has a finite growth period. This growth is most usefully described in terms of expansion accompanied by cell division (Haber, 1962; Haber and Foard, 1963). In work on Dicotyledons expansion and cell division have been shown to be concurrent during the greater part of leaf growth, but it is generally accepted that there is a final period of expansion without cell division (Avery, 1933; Sunderland, 1960; Milthorpe and Newton, 1963; Dale, 1964, Denne, 1966; Steer, 1971). Cessation of cell division is apparently asynchronous in the various tissues in a leaf. In particular cell division is considered to cease at an earlier age in the epidermis as compared with the mesophyll layers (Avery, 1933; Heslop-Harrison, 1962; Haber and Foard, 1963; Dale 1964, Maksymovych and Blum, 1966; Denne, 1966; Steer, 1971), but divisions have been observed in the stomatal complexes after they have apparently ceased in the rest of the epidermis (Stebbins and Shah, 1960; Shanks, 1965; Denne, 1966).

In the leaves of Dicotyledons the expanding area or growth zone encompasses virtually the whole leaf during the greater part of the growth period. This has been shown for example in tobacco (Avery, 1933; Poethig and Sussex, 1985); cocklebur (Maksymovych, 1963) and spinach (Saurer and Possingham, 1970). Avery however found that the tip of a tobacco leaf was mature when the leaf was only 2mm long. He showed that maturation proceeded basipetally but that the rate at which the end of the growth zone retired down the length of the leaf was initially very slow. Maxymowych (1973) and Saurer and Possingham (1970) described a similar sequence of events in Xanthium and spinach. The same sequence occurs in monocotyledonous leaves but the increase in length of the expanding portion, or growth zone, is limited. The growth zone in the grass leaf remains short and occupies a relatively smaller proportion of the leaf length during the greater part of the leaf's growth period (Sharman, 1942; Denne; 1960, Ritchie, Chap 3). The difference in growth pattern between dicotyledonous and monocotyledonous leaves is therefore one of degree rather than fundamental character.

The grass leaf meristem encircles the shoot apical meristem. This ring involves a large number of cells from its initiation and the number increases with the increase in the circumference of the stem (Poethig, 1984). The greater part of the leaf growth is perpendicular to the initial ring and as a result cell division is largely polarised and the cells are arranged in rows which lie more or less parallel to the leaf axis. A small number of additional rows are formed by nonpolarized divisions within the meristem. They are only important in the adding to the width of the blade near its base (Poethig, 1984).

The development of the stomata in the epidermis was of particular interest in this work. Stomata and their mother cells can be readily identified. The sequence of events that occurs during stomatal formation has been described for several grasses. For example Porterfield (1937) worked on bamboo, Stebbins and Shah (1960) on barley, and Yamazaki (1963) on rice. In surface view the epidermal cells in a grass meristem are basically rectangular and are arranged in parallel longitudinal files or rows; guard mother cells are formed in certain of these rows. A guard mother cell is the product of an asymmetric division in a meristematic cell. During the division there is an initial polarisation of the cytoplasm, such that the distal part of the cell appears more dense. When cytokinesis occurs the new guard mother cell is smaller than its sister cell and occupies the distal end (Stebbins and Shah, 1960). The guard mother cell enlarges and its walls become convex, to give it the appearance of having a comparatively lower water potential than its neighbours. It becomes elongated parallel to the leaf axis and finally divides to give rise to two guard cells with the stoma between them. During its expansion the guard mother cell becomes flanked by two subsidiary cells. These appear initially as bays extending from the cells in the adjacent rows which abut onto the guard mother cell. The bays fill the otherwise unoccupied space beside the guard mother cell whose lateral expansion does not equal that of the intervening cells, later these bays are cut off by cell division. The whole stomatal complex is thus a four celled structure which lies within the central of three parental epidermal rows. This description was obtained when the character of the stomatal initials where recorded in order as they were encountered in the cell rows which lead up the leaf from its base. The spatial arrangement was taken as presenting a developmental sequence. The possibility that extension of the tissue in the outer growth zone might be very great and additional cell divisions might occur in the outer growth zone was not considered. Finally a time scale was not available.

I have shown (chap 4) that the position of a referential point in the elongating tissue of a maize leaf growth zone can be established as a function of age. I will now describe how two such points were used to identify a particular moving and expanding segment in the midlamina of the leaf. The morphological characters in the lower epidermis of this segment were recorded as a function of time. In particular records were made of numbers of cells in a span of the segment, and the appearance of the developing stomatal complex and hairs. I will show that this allows me to discuss developmental changes in morphological characters of the epidermis in relation to both time and the concurrent extension in the tissue.

MATERIALS AND METHODS

PLANT MATERIAL

The cultural conditions, punching treatment and method of harvesting applied to the experimental set of maize plants has been described (Chap 1). The epidermal samples were from the same set of leaves. They were obtained during each harvest immediately after the positions of the punch holes had been established. A replica was prepared of a strip of the lower surface of the leaf which was 5mm wide and lay midway between the midrib and the margin (fig. 5.1).

REPLICATION OF THE EPIDERMIS

Replication was chosen as the simplest means of obtaining an image of the epidermis which would both show the position of the cell walls clearly, and could be stored for a length of time. Several replicating media were tested but did not produce satisfactory images of the very fragile, moist surface on the immature cells at the base of the growth zone. Selley's plastic glue diluted with 20% methyl ethyl ketone did however form a useful replica. The glue was run onto the surface of the leaf with a single stroke of a paint brush. The replicating medium was applied to the lower epidermis from the leaf base to a point beyond 100mm. It followed a line midway between margin and midrib and to the right of the midrib when the leaf was vertical and observed from outside the plant.

Before the replica was removed from the leaf, marks were painted on it at 25mm intervals. The first of these marks was 25mm from the leaf base. A strip of cellotape was applied to the outer surface of the replica and used to lift it off the leaf and attach it to a microscope slide. Test measurements showed that there was no change in length of the replica during this procedure or during the subsequent months of storage.

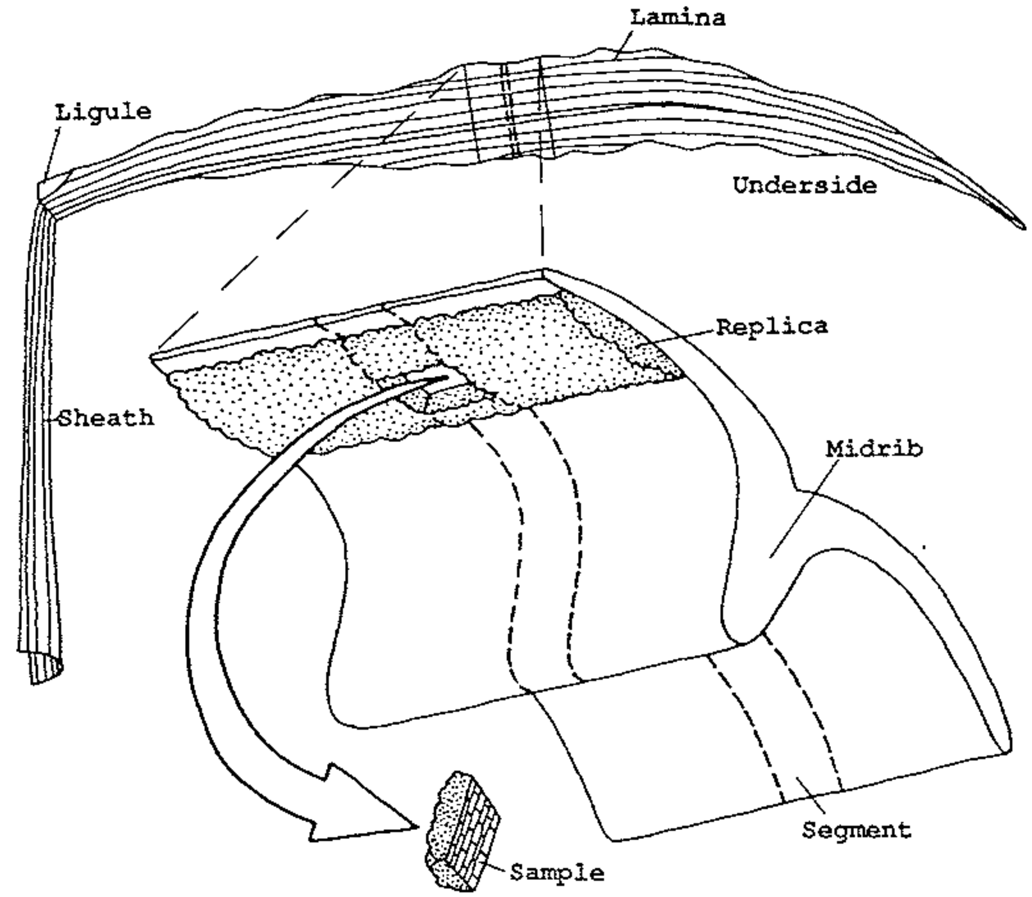


FIG. 5.1. Portion of a mature leaf to show the arrangement of the replica, the chosen leaf segment and the sample of cell rows in the segment.

THE LOCATION OF THE PROXIMAL AND DISTAL BOUNDARIES OF THE SEGMENT

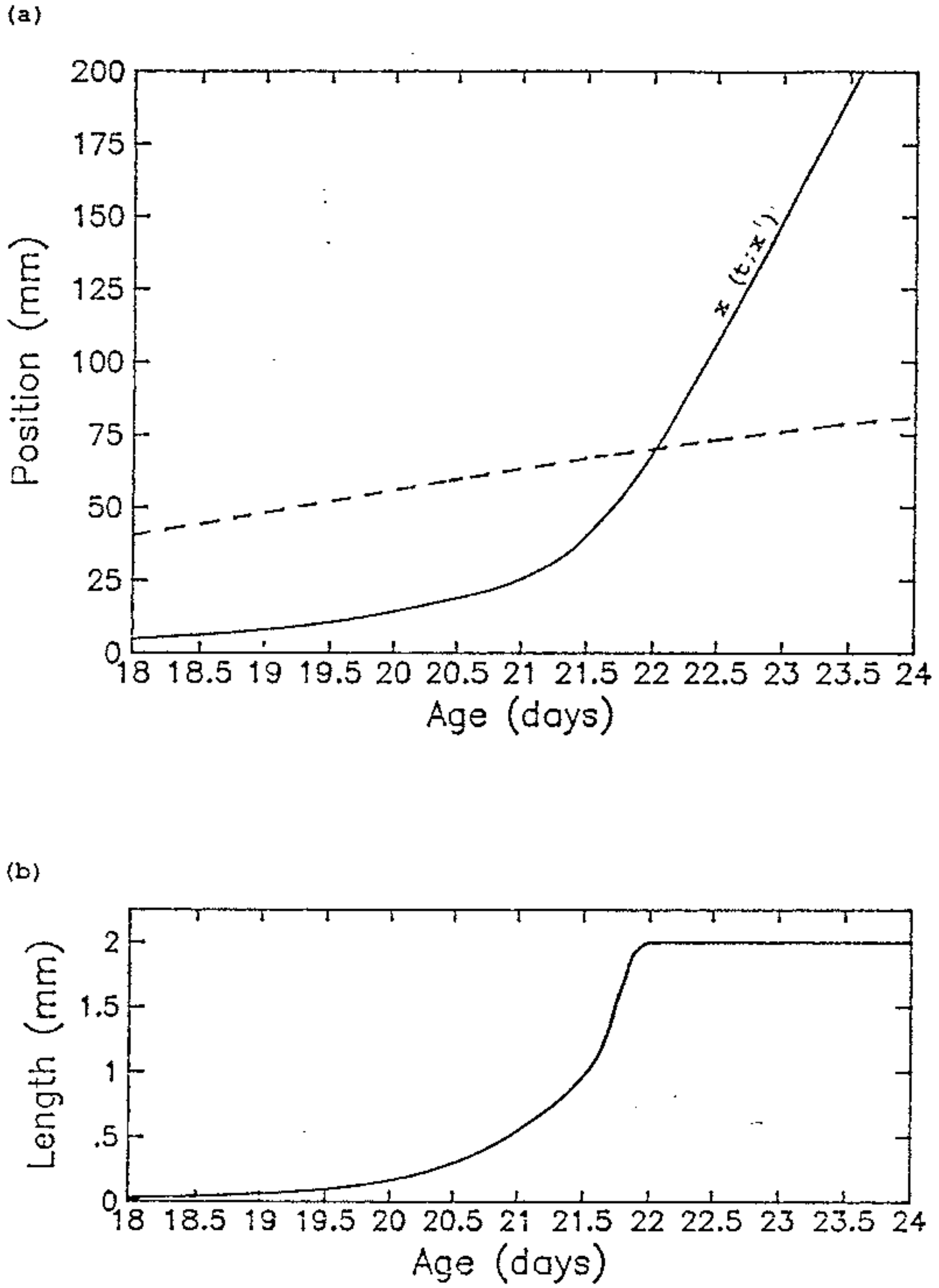
The segment of leaf which in the mature leaf would occupy the two millimetres midway between the tip and the base of the lamina was chosen as the sample for study. In this work x equals the position of a transverse line across the leaf described in terms of its distance from the leaf base. The pathline $x=x(t;x_0)$ for each of the boundaries of the segment was established using the method described in Chapter IV. The movement and length of the segment are shown (Fig. 5.2 a, b). At the initial time, t, day 14.5, the proximal and distal boundaries were at, $x^1=0.7874791$mm and $x^2=0.792935$mm. The length of the segment at this time was 0.00558mm. At maturity the boundaries were at: $x^1=504$mm and $x^2=506$mm. Thus the segment represented a 2mm length of the leaf at maturity.

The time interval of the pathline calculation was 24hours and it provided values for positions at midday between day 14.5 and day 34. The times of harvest of the plants were grouped about, but did not coincide with, midday. B-splines (Spriggs, 1984) were therefore fitted through the pathline figures; and position values for the segment, at the time of harvest of each individual plant, were read from the these splines.

The position of the proximal boundary of the sample segment on the replica was located to within 2mm at a macroscopic level and to an exact point under a microscope. A Reichert Visopan microscope was used with the image displayed on a screen. The proximal and distal boundaries of the segment were set up as vertical lines on this screen.

THE EPIDERMAL SAMPLE.

The segment extended across the width of the leaf. A rectangular sample portion of the segment centred on the midline of the replica was selected for detailed examination. The length of the sample was that of the segment and its width was basically 16 adjacent cell rows.



LENGTHS: 0.04 0.1 0.3 0.97 2.0 2.0

FIG. 5.2. Position (a) and length (b) of the segment as a function of the age of the plant. The segment lies between the moving points x^1 and x^2. Where t =14.5, x^2=0.793mm and x^1=0.787mm, and the position of x as a function of age is $x(t;x_0)$.
(a) $x^1(t;x_0^1)$, ———; distal limit of the growth zone, - - -.
(b) $x^2(t;x_0^2)-x^1(t;x_0^1)$, ———.

There is no further extension of the segment after it has left the growth zone, when x =74mm and t=22days.

The epidermis of the maize leaf consists of rows of predominantly rectangular cells with their longer sides aligned and parallel to the leaf axis (Figs. 5.3 and 5.4). Examination and scoring of the sample rows proceeded from the proximal to the distal boundary of the segment along each row in turn. The rows were studied in order, taking the most axial first. They were numbered where they cut the proximal boundary of the segment. A selection of samples of different ages is shown (Fig. 5.5 a,b,c,d,e,f).

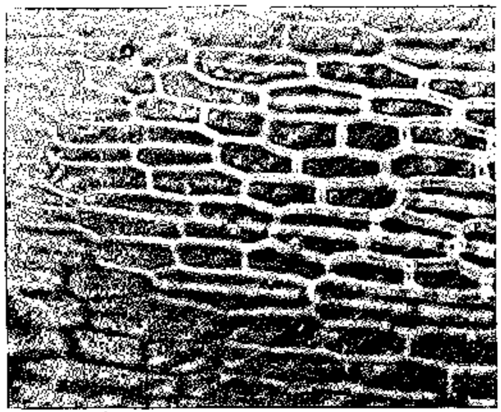
Units were recognised and counted within the rows. A unit is defined in this work as the section of tissue which lies between two sequential transverse cell walls in a row (fig. 5.4). A transverse wall is any wall which links the two sides of the row. Its shape may vary but its orientation always includes components prpendicular to the leaf axis. The position of a diagonal or curved wall was taken as being midway between the two extreme positions touched by the wall.

Cell rows are not continuous throughout the leaf (Fig. 5.3). During counting, where two rows were found butted to the end of a single row within the segment the row nearer to the midrib of the leaf was regarded as the continuation of the proximal row, and the more marginal was recorded as a discontinuous row. This was numbered and included as an additional row in the sample. An example of this occurs in the sample at 18.55days (Fig5.5 a), where numbers 1-12 and 14-17 are complete rows but row 13 is marked as incomplete. This sample contained 16 complete rows plus 1 incomplete row. On the other hand where, as in the day 19.64 sample (Fig. 5.5 b), two rows numbered 1 and 2 at the proximal margin butted onto a single row part way across the segment, the row nearer the midrib, row 1, was considered to continue through the single row, and the more marginal, 2, was recorded as incomplete. The sample thus contained 15 complete rows and one incomplete row.

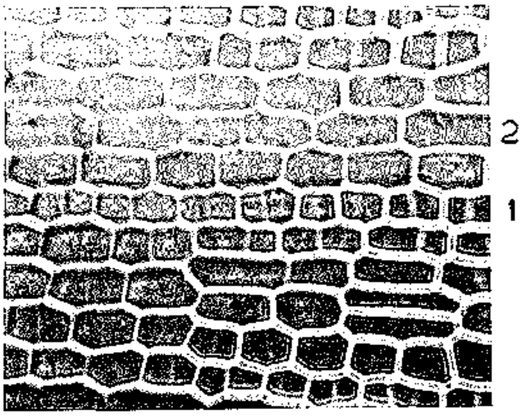
The boundaries of the segment rarely coincided with transverse walls in the rows. Where units extended across the proximal boundary they were excluded, where they extended across the distal boundary they were included in the sample record.

UNIT TYPES IN THE EPIDERMIS.

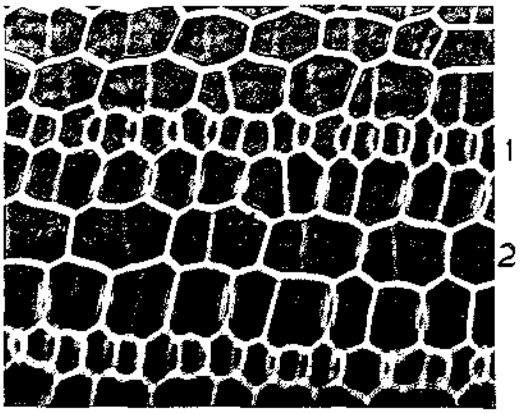
Each of the units encountered within the sample was assigned to a type category. A description of the categories is given (Fig. 5.6 a,b,c).



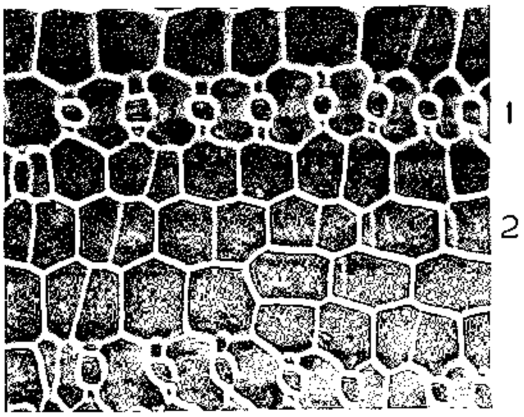
(a) x^1=3.485mm, t=17.41dy
x^2-x^1=0.025mm



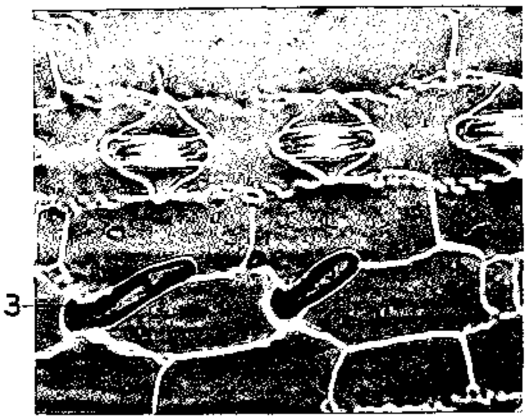
(b) x^1=6.639mm, t=18.55dy
x^2-x^1=0.046mm



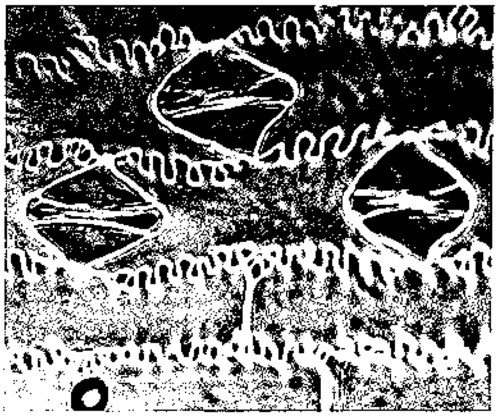
(c) x^1=11.503mm, t=19.64dy
x^2-x^1=0.114mm



(d) x^1=18.640mm, t=20.47dy
x^2-x^1=0.114mm



(e) x^1=38.089mm, t=21.47dy
x^2-x^1=0.902mm



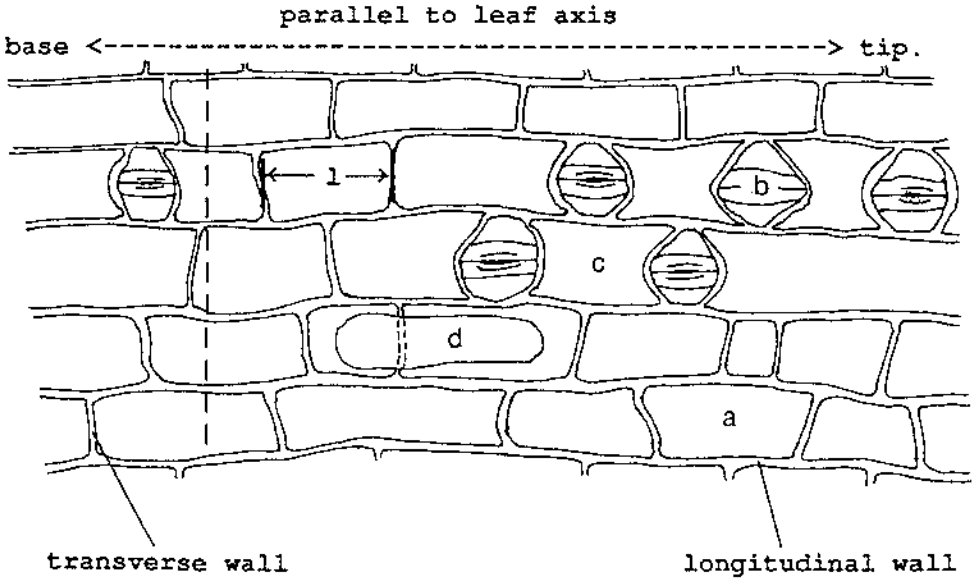
(f) x^1=98.045mm, t=22.39dy
x^2-x^1=1.928mm

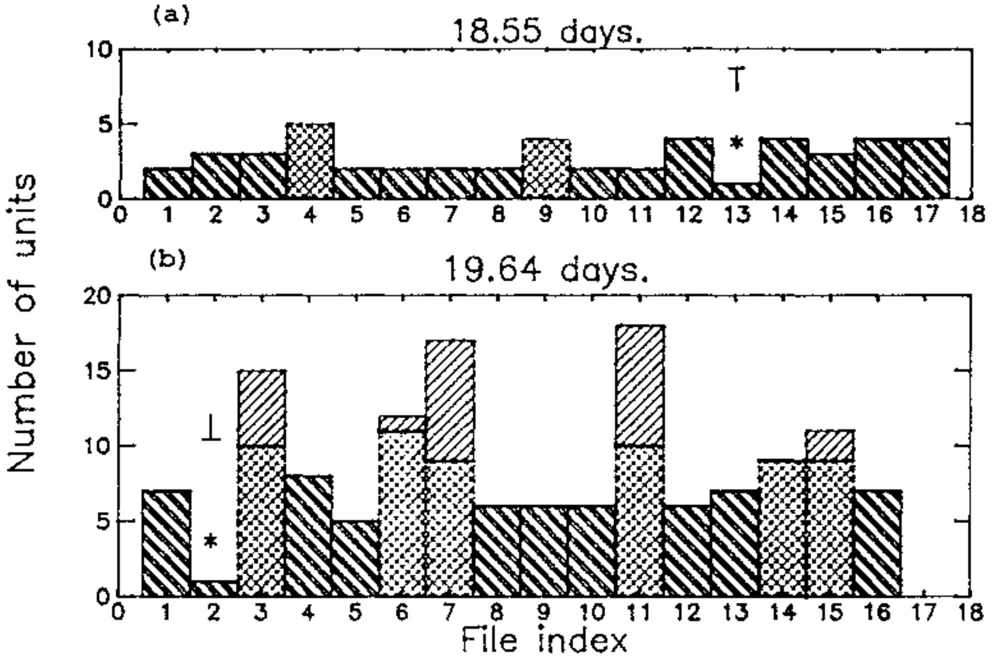
Magnification: X35.0

100μ

FIG. 5.3. Replicas of the lower leaf epidermes. The tissue shown includes or lies within the length of the chosen segment. x=position of the tissue with reference to leaf base, t=age of plant at sampling. x^2-x^1=length of chosen leaf segment at age t. "Major stomatal" row (1), "astomatal row" (2), hair (3).

FIG. 5.4. Diagram to illustrate the definition of the term unit as it is used in this study. Representative units are shown: a, simple; b, stomatal; c, intervening simple; d, hair. The unit is a subdivision of a cell row, it occupies the length, l, between two neighbouring cell walls. The walls involved are those which are, or have a component perpendicular to the leaf axis. The position of the cell wall relative to the base of the leaf is taken as being midway between the two extreme positions touched by the wall



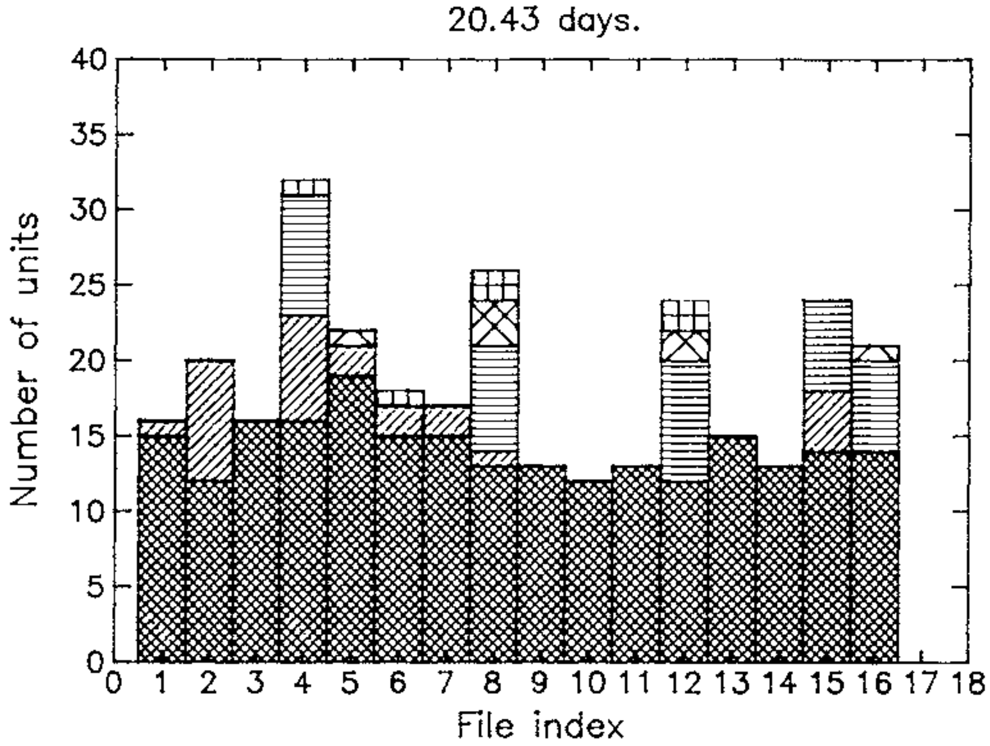


Proportion of unit type:

simple,	1.00	1.0	.00	1.0	1.0	.00	.00	1.0	1.0	1.0	.00	1.0	1.0	.00	.00	1.0
short,	.00	.00	.67	.00	.00	.90	.53	.00	.00	.00	.56	.00	.00	1.0	.82	.00
elliptic,	.00	.00	.33	.00	.00	.10	.47	.00	.00	.00	.44	.00	.00	.00	.18	.00

▧ 1, simple. ▦ 2, short. ▨ 3, elliptic.

FIG. 5.5. The number and type of unit found in the cell rows in a representative set of samples. The sample consisted of 16 rows which were adjacent to one another at the proximal boundary of the segment. The rows are displayed in the positions in which they were found. The file index numbering is on a line from the midrib towards the leaf margin. Where rows are discontinuous within the segment they are marked (*). Where two proximal rows abut a single distal row they are marked (⊥). Where a single proximal row abuts two distal rows they are marked (T).

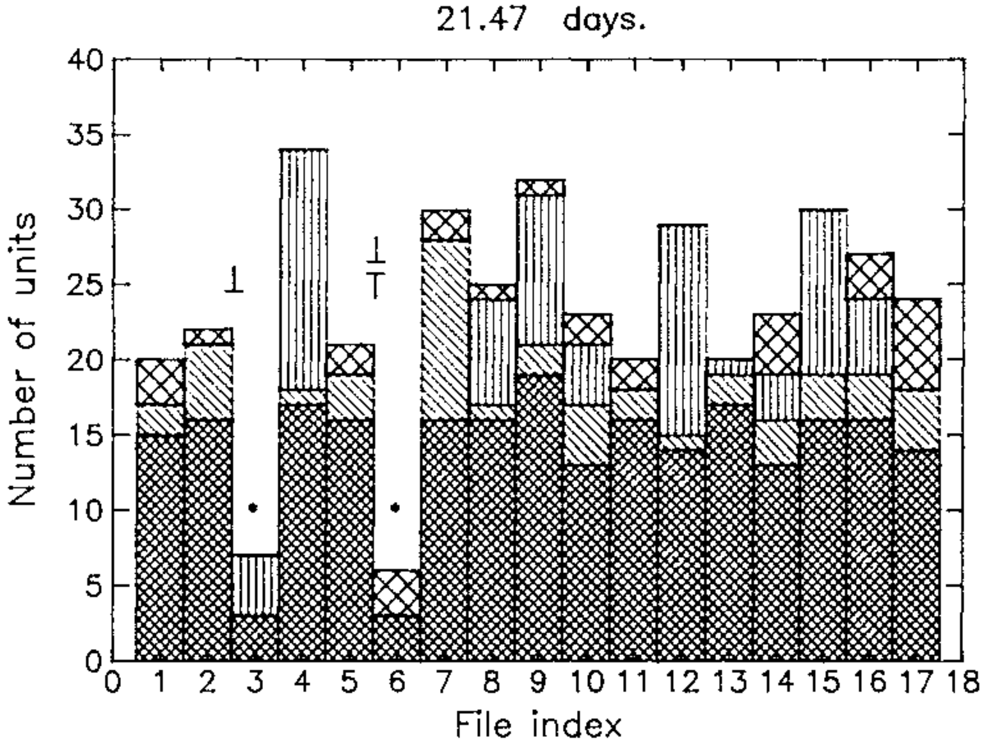


Proportion of unit type:

simple,	.94	.60	1.0	.50	.86	.83	.88	.50	1.0	1.0	1.0	.50	1.0	1.0	.58	.67	.
stomatal,	.00	.00	.00	.28	.05	.06	.00	.46	.00	.00	.00	.50	.00	.00	.25	.33	.
other,	.06	.40	.00	.22	.09	.11	.12	.04	.00	.00	.00	.00	.00	.00	.17	.00	.

▩ 1, simple
▨ 3, ellipse
▤ 4, circle
⊠ 5, g.m.c. +subs.
⊞ 6, g.m.c. +2subs

FIG. 5.5 (c)

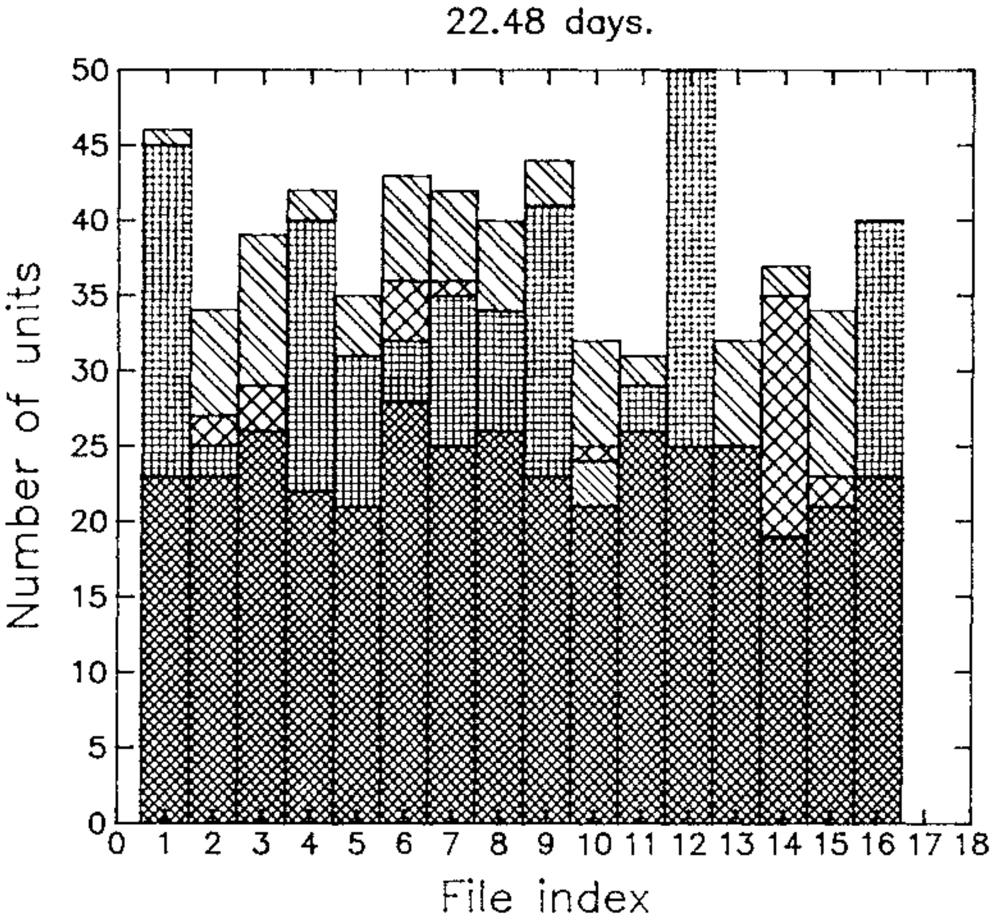


Proportion of unit type:

simple,	.75	.67	.43	.50	.76	.50	.52	.64	.59	.56	.76	.48	.85	.57	.53	.57	.58
stomatal,	.00	.04	.57	.47	.00	.00	.00	.28	.31	.17	.00	.48	.05	.13	.37	.18	.0
elliptic,	.10	.22	.00	.03	.14	.00	.38	.04	.06	.17	.10	.03	.10	.13	.10	.11	.17
other,	.15	.08	.00	.00	.10	.50	.10	.04	.03	.09	.14	.00	.00	.17	.00	.14	.25

▩ 1, simple	☒ 10, hair
▧ 3, elliptic	▧ 11, undefined
▥ 5–8, stomatal	

FIG. 5.5 (d)

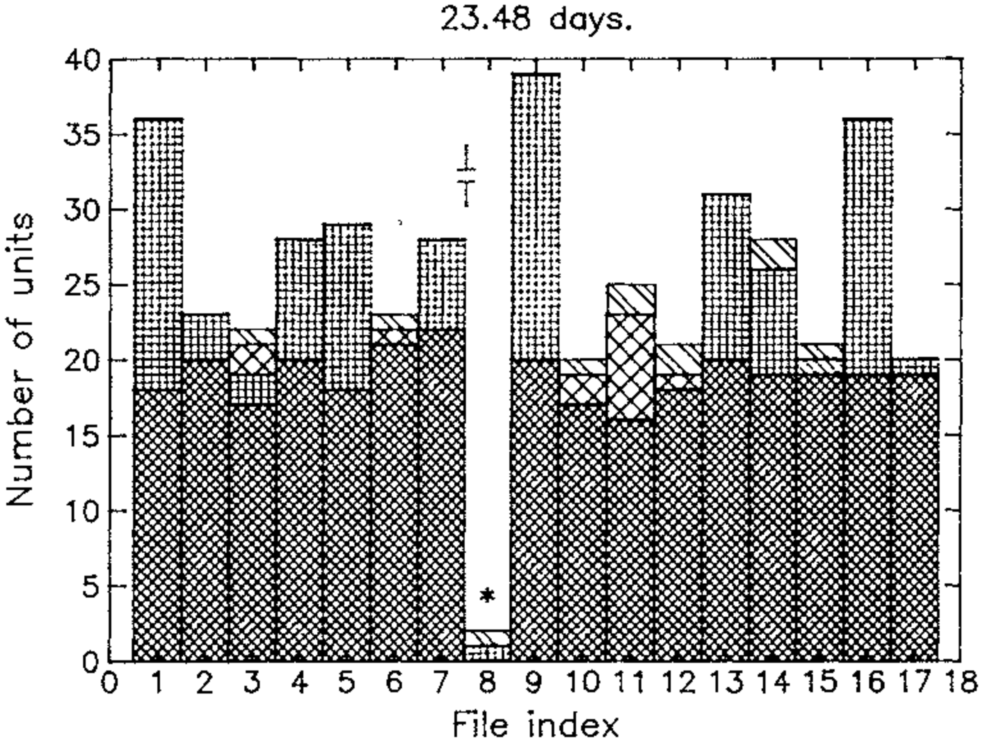


Proportion of unit type:

simple,	.50	.65	.65	.52	.60	.65	.59	.65	.52	.72	.84	.50	.78	.51	.62	.58
stomatal,	.48	.06	.00	.45	.29	.10	.24	.20	.41	.00	.10	.50	.00	.00	.00	.42
other,	.20	.28	.27	.05	.11	.25	.17	.15	.07	.15	.60	.00	.22	.49	.38	.00

▩ 1, simple
▧ 2, short
▦ 9, stomatal
☒ 10, hair
▧ 11, undefined

FIG. 5.5 (e)



Proportion of unit type:

simple,	.50	.87	.77	.71	.62	1.0	.90	.65	.51	.85	.64	.86	.65	.68	.90	.53	.95
stomatal,	.50	.13	.09	.29	.38	.00	.10	.30	.49	.00	.00	.00	.35	.25	.00	.47	.00
other,	.00	.00	.14	.00	.00	.00	.00	.05	.00	.15	.36	.15	.00	.07	.10	.00	.05

▩ 1, simple
▧ 2, short
▦ 9, stomatal
☒ 10, hair
▧ 11, undefined

FIG. 5.5 (f)

Fig. 5.6 a. CATALOGUE OF UNIT TYPES AS SEEN IN A REPLICA OF THE LOWER EPIDERMIS OF A MAIZE LEAF.

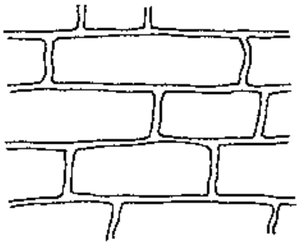
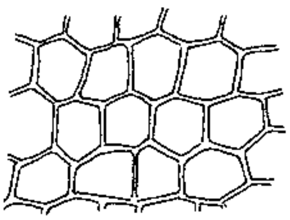
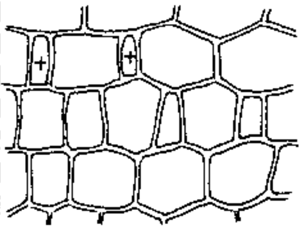
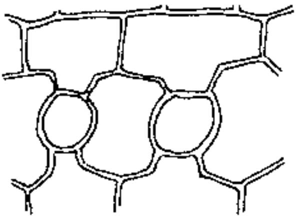
Code	Diagram	No of cells	Description. cells
	(a) Simple or undifferentiated units.		
1.		1	"SIMPLE" epidermal cells. Shape rectangular, with the long axis parallel to the midrib. At maturity the cell walls have deep convolutions
2.		1	"SHORT" cells. Approximately rectangular, but length of side parallel to midrib equal to or shorter than length perpendicular to it. Cell walls not convoluted.
3.		1	"ELLIPTIC" cells (+). Size smaller than mean. Shape lenticular. Dimension parallel to midrib shorter than that perpendicular to it.
4.		1	"CIRCULAR" cells. Size smaller than mean, circular in outline. Walls of cells in adjacent rows may be extended to form a bay at point of contact.

Fig. 5.6 b.

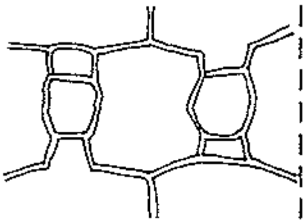
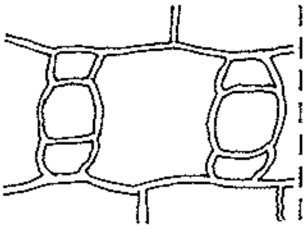
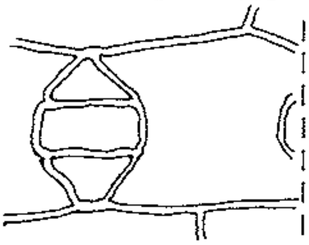
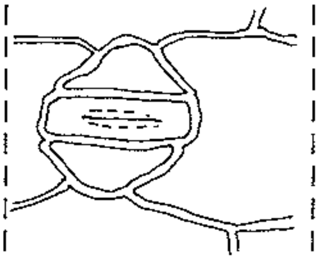
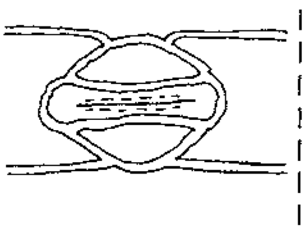
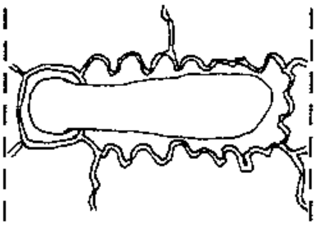
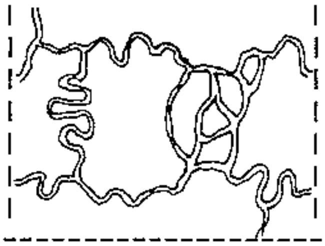
Code	Diagram	No of cells	Description.
(b) Units recognised as stomatal:			
5.		2	"G.M.C+sub". The bay-shaped extension of one lateral epidermal cell cut off by a wall to form a subsidiary cell. This identifies the circular cell as a guard mother cell (GMC).
6.		3	"G.M.C.+2subs". Two subsidiary cells present, one either side of the guard mother cell.
7.		3	"Elongated G.M.C.". The guard mothercell is elongated parallel to the leaf axis but not divided. The subsidiary cells approximate to equilateral triangles.
8.		3	"GUARD CELLS". A stoma can be seen between the two guard cells. Each susidiary cell takes the form of an isosceles triangle with its apex an obtuse angle and its base parallel to the leaf axis.
9.		3	"mature stomata". The stomatal unit resembles a lozenge, elongated parallel to the leaf axis. The guard cells are bell shaped. Subsidiary cells are obtuse angled isosceles triangles

Fig. 5.6 c.

Code	Diagram	No of cells	Description.
	(c) Units recognised as hairs:		
10.		1	"Hair". Basal part is circular. Oblong protrusion often bent over neighbouring tissue, or may be broken off.
	(d) Units with status undefined:		
11.		1	"Undefined". Short portions of cell file cut off by cell walls. These walls deeply convoluted or duplicated to form additional lacunae within the unit. Usually alternate with simple epidermal cells.

The term 'simple' unit was used in preference to 'undifferentiated' since the latter suggests an immature structure and the simple category included fully mature epidermal cells in the older samples. The mature simple epidermal cells have a narrow rectangular shape and convoluted walls. They are easily distinguished from the more or less square and straight walled, very immature, simple cells. No attempt was made to separate the group into more than one category because the change in shape between the two extremes is gradual and not amenable to visual recognition. Short cells were distinguished from other simple cells by shape, their length being never much greater than their width and was usually half that of the surrounding simple cells.

The mother cells of the hairs and stomatal guard cells are the product of asymmetric division (Stebbins and Shah; 1960). They were identified by their rounded shape and their position, alternating with longer cells. They could not be distinguished from one another until either a protrusion or a subsidiary cell had formed indicating respectively a hair or a stomatal complex.

Undefined units were recognised only in the older samples. Although it was impossible to identify them in the early samples it is probable that these units have three sources of origin. Some of them may not have been cells but simply were the result of two convolutions of the opposite long walls of a mature simple cell which coincided across, and apparently cut off, a small portion at the end of the cell. The position of some of the undefined units suggested that they were the products of asymmetrical divisions. Most of these seemed to be abortive hairs or hairs in which the extension had been broken away; but a few may have been stomatal units whose development had been arrested.

DAILY MEAN

The characteristics of the individual samples were recorded in terms of numbers. These varied as a function of age. They also showed natural variation associated with the individual plants on which they were located. The ages at which the sample maize plants were collected were grouped, each group being more or less evenly distributed about midday, age n.5 on a given day. By assuming that there was a linear relationship between age and the changes that occurred as a function of age during the 0.4 of a day over which sampling was spread, an average value at age n.5 was calculated. This average is named the daily mean and is referred to as occurring during the n.5 day.

RESULTS AND DISCUSSION

EXPANSION OF THE SEGMENT AS IT RECEDES FROM THE LEAF BASE.

The calculation of the pathlines of the segment boundaries, $x=x(t;x_0)$, (Chap. 4) gives values at 24 hour intervals. The length of the segment is given by the difference between the positions of these two boundaries, $x^2-x^1=x^2(t;x_0^2)-x^1(t;x_0^1)$. The segment length was calculated at the same 24 hour intervals and a set of values representing segment length at midday each day was obtained (Fig. 5.2 b).

At age 17.5days the segment lay between 3.63791 and 3.61229mm and its length was 0.02562mm. This was slightly less than the length of the average cell in that position and at that age. The upward movement of the segment (fig. 5.2 a) up to day 17.5 was gradual; the rate was increasing, however, and continued to do so until, during day 21, there was a relatively rapid change and the segment swept upwards through the outer part of the growth zone during a single day. This pattern of outward movement is the result of expansion in the parts of the leaf below the segment (Chap. 4). Expansion within the segment itself rose similarly from gradual prior to day 19.5 to very rapid during day 21.5 0 (Fig. 5.2 b). In the 12 hours between day 21.5 and day 22.0 however, this very rapid rate of extension was exchanged for a state of zero extension and the segment left the growth zone. The mature segment was 2mm long. This length must have been reached when the lower boundary left the growth zone. This occurred at or just before age 22 days and the value 2mm at 22 days is included in the set used to plot segment length as a function of age.

THE SAMPLE

At each sampling time the sample included approximately four bands of the stomatal pattern (Stebbins and Shah, 1960). There was apparently no increase in the number of rows across the width of the leaf between days 18 and 23. This was shown by carrying out a comparison between mean file and leaf width, at the segment positions, on each of the sample plants.

Thus the sample represented the same portion of the leaf fabric at each of the sampling ages. It provided values for quantitative characters which were independent of the variability between individual rows and could be used to represent the overall status of that piece of the leaf surface.

INCOMPLETE ROWS IN THE SAMPLE.

Cell rows do not appear to extend the full length of the leaf. Locations at which two rows butted onto a single one within the segment were observed on 32 occasions during the sampling. Of these a pair of distal rows butted onto a single proximal row on 16 occasions and the reverse arrangement was found also on 16 occasions. Thus the discontinuities led to no change in the overall row number. The segment lies in midlamina where the leaf margins are parallel and changes in the number of cell rows required to make up the width would not have been expected.

Although the samples on some plants showed more row discontinuities than those on others at a given age, in general the frequency of such discontinuities within the segment increased with age. This was therefore associated with the increase in transverse subdivisions in the rows. The mean length of the duplicated section within the rows also increased with age but the character was very variable. In the day 21.47 sample two rows occupied the line of one for the length of six units only. Short additional rows were observed frequently in the samples prepared during days 18 and 19. At this age such rows included a small number of comparatively long cells and their transverse walls were frequently coincident across both rows. This structure suggested that construction of a longitudinal cell wall had replaced that of a transverse one in one cell of a multiplying row and subsequent transverse divisions had created the additional cell row.

THE USE OF THE UNIT.

In this study length has been used to describe the distances between positions on a line between the leaf base and tip. In mature grasses most epidermal cells are rectangular with their long axes parallel to this line. In order to be consistent therefore I will continue to use the term length in this work as referring only to distances measured parallel to the leaf axis. I will do this inspite of the fact that in a few cases I will be describing a structure in which the dimension perpendicular to, is greater than that parallel to, the leaf axis.

During the development of the leaf, the segment increases in length. At the same time new cell walls are inserted and subdivision within the segment is increased. The walls involved in the subdivision of length are only those which have a component perpendicular to the length dimension. This excludes walls parallel to the axis such as those formed within the stomatal complex. Use of the unit as defined (Materials and Methods, chap 5.), therefore allows a count to be made of the subdivision of the segment's length and this count is directly related to the mean cell length.

THE NUMBER OF UNITS IN A SAMPLE AS A FUNCTION OF AGE

The total number of units found in each of the samples was examined as a function of age (Fig. 5.7). A line was drawn through the scatter of individual sample counts. It was drawn manually to provide an estimate of the population means. Through days 17, 18, 19 and 20 where the actual values are relatively tightly grouped these lines can be placed with confidence. The total number of units in the sample increases from day 18.5 to day 21.5 (Fig. 5.7). The increase in total number of units between days 20.5 and 21.5 was, however, relatively small. From day 21.5 the samples showed very considerable variation and the position of a mean line is uncertain. It was assumed that no new transverse walls were formed after the segment had ceased to expand on day 22.0, and the mean line that was drawn took this into consideration. This line suggests 488 units in an average sample at maturity. Inspite of the uncertainty about the exact number of units as the sample approaches maturity, there does appear to be a real increase between day 21 and 22, and this means that the construction of transverse cell walls or cell division, of simple epidermal cells, occurs throughout the growth zone and there is no zone of cell extension without division in a maize leaf.

The grass leaf meristem is supposed to occupy a small region at the base of the growth zone. The evidence for this arrangement takes two forms. First, there is the observation of very much larger cells in the distal part of the growth zone. Unless

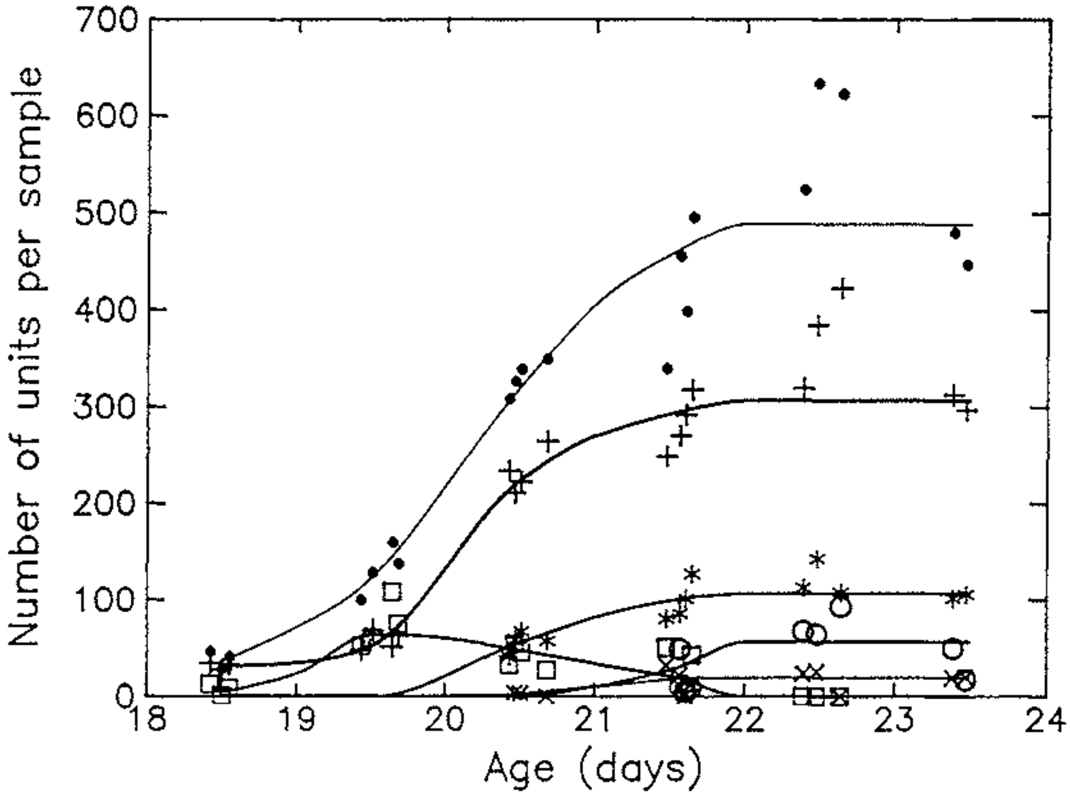


FIG. 5.7. Numbers by types of units in a sample (see text) as a function of age. Zero values are not shown. Symbols indicate the type of unit: total (c); simple, including short(+); elliptic (c); stomatal (*); undefined (o); and hair (x). 'Eyeball' lines have been inserted between the data points.

however,the organ extension in the region can be measured and shown not to be greater than cell extension the fact that the latter is occurring does not preclude cell division. Second the distribution of cell division in growing organs such as a leaf has been studied by searching for mitotic figures in the cells. Grass leaves have not been used in this type of study but other monocotyledonous leaves with a similar shape and growth pattern have been. Denne (1960) worked on *Narcissus pseudonarcissus*. She did not find division figures in any of the simple epidermal cells more than 10 mm from the base of the leaf. This may approximate to the length of the growth zone in these leaves but Denne's values for the mean epidermal cell size at approximately 200mm down from the leaf tip diminished while the leaf was growing from a length between 50mm and 250mm to one of 500mm. If the diminution was real, and cell lengths in leaf epidermes have been shown to exhibit considerable variation (Miranda, Baker and Long 1981), it could only have occurred as a result of cell division in tissue more than 10mm from the leaf base. Denne points out that the number of cell divisions counted in a particular preparation would depend on the duration of the visible part of the cell cycle. I have shown (Chap. 4) that extension is very much more rapid in the outer growth zone. It is possible that the cells in this region are involved not only in a greater rate of increase in size but also in a commensurately more rapid division; and that this has contributed to the reduction in numbers of mitotic figures recorded. In his work on a group of monocotyledonous species including *Narcissus pseudonarcissus* Shanks (1964) observed a small number of divisions among the simple epidermal cells in the outer growth zone. He describes these cells as having de-differentiated in order to divide. Denne observed cell divisions in stomatal mother cells which were more than 10mm from the base of the leaf. These cells were dividing after the other epidermal cells had apparently ceased to do so. If cell division occured between sampling times in ordinary epidermal cells the mitoses would not have been recorded but the appearance of two guard cells in the place of a single guard mother cell would have been visible evidence of divisions having taken place in stomatal initials. Cell extension and cell division are concurrent through the greater part of the leaf expansion in dicotyledons (Dale, 1976). This study of a maize leaf suggests that similarly division takes place through virtually the whole of the growth zone in maize leaves. If maize is representative of other Monocotyledons with regard to leaf growth, then the major difference between the Monocotyledons and the Dicotyledons seems to lie not so much in the distribution of cell division within the growth zone but more in the proportion of the leaf involved in extension during the greater part of the growth period.

THE PROPORTIONS OF UNIT TYPES IN THE SAMPLE AS A FUNCTION OF AGE

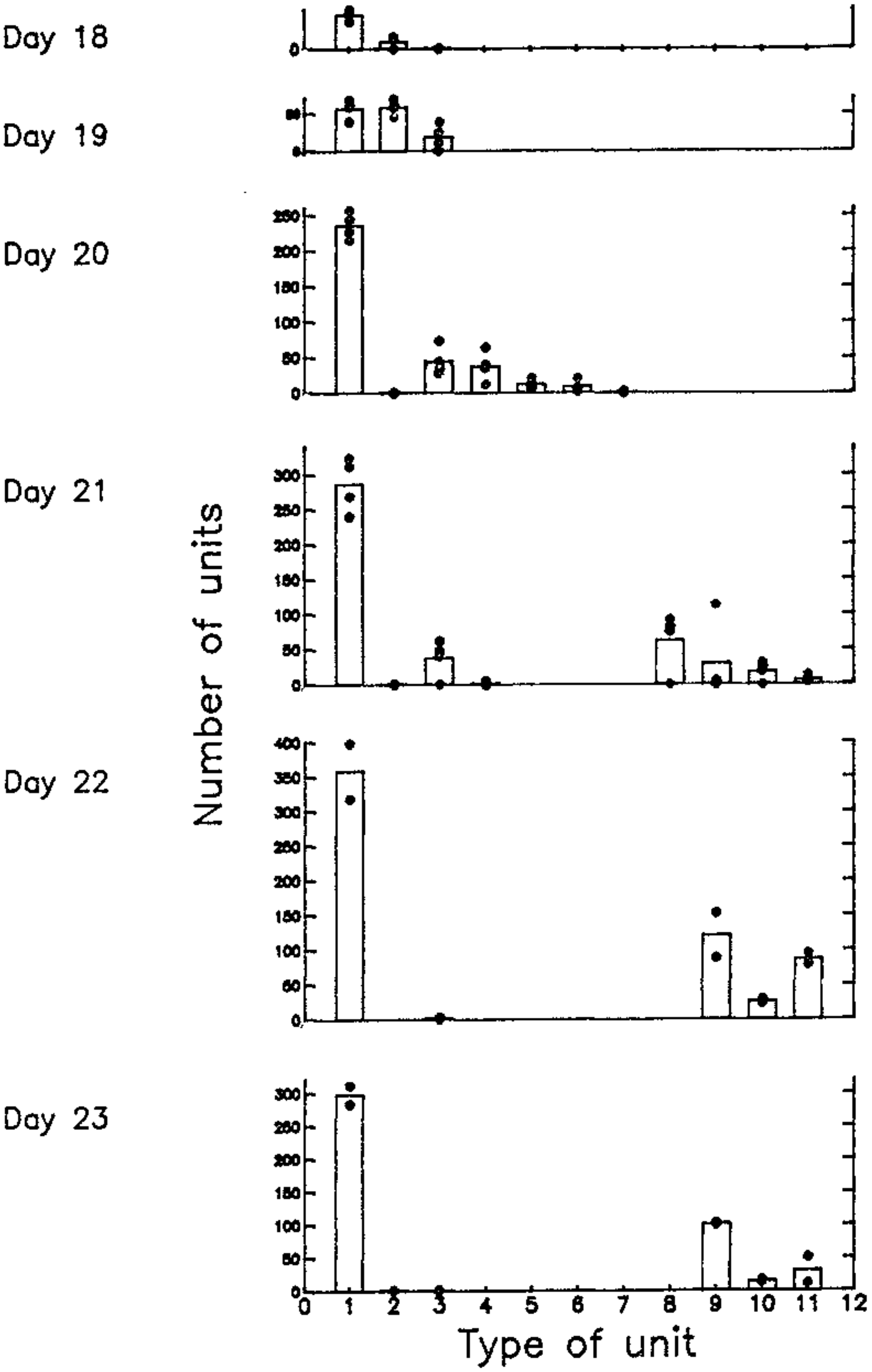
The variety of the unit types, their distribution, and the changes that occur with increasing age are indicated by the samples shown (Fig. 5.5 a,b,c,d,e,f). The number of each type of unit in each sample, and daily mean values for each type in the set of samples collected on a given day were calculated (Fig. 5.8). The relative proportions of the different unit types in terms of the daily means among the samples is shown (Fig. 5.9).

On all days except day 19.5 most of the units were simple. On day 19.5 the samples showed a high proportion, 0.44, of short cells which, together with the 0.14 proportion of mother cells represented more than half of the units. This situation developed during 24 hours from one in which there were half as many units and only 0.2 of them were short units. Between days 19.5 and 20.5 the total number of units more than doubled in number, but the number of short cells was reduced virtually to zero. The average length of this category of unit had increased and the cells were recorded as simple. A large number of asymmetric divisions had occurred during the interval. Nearly 0.25 of the units were elliptic or circular mother cells on day 20.5 and the presence of one or more subsidiary cells identified a 0.06 fraction of these as stomatal guard mother cells.

The small increase in total numbers of units in the segment between day 20.5 and 21.5 included a 0.2 increase in simple units and 0.4 increase in all other types of units. By day 21.5 a considerable number, 0.23 of the total units were recognisable stomatal units. Stomata had formed in virtually all of the stomatal units and 0.3 of them were fully mature. On day 21.5, 0.04 of all the units were recognised as hairs and 0.01 of them as undefined units. Only 0.09 of the units found in the samples on day 21.5 were elliptic.

The samples from days 22.5 and 23.5 showed considerable variability with respect to the comparative numbers of each type of unit found within them but the proportions were similar and in the region of: approximately 0.63 simple; 0.21 stomatal; 0.04 hairs and 0.14 of undefined units.

FIG. 5.8. The number of units of each type found at increasing ages. The count of each sample (see text) is shown (●). The bars represent the mean for the sample counts. The types of unit are representd by numbers on the x axis. Thus: 1, simple; 2, short; 3, elliptic; 4, circular; 5, guard mother cell with one subsidiary cell; 6, guard mother cell with two subsidiary cells; 7, guard mother cell elongated; 8, guard cells formed; 9, mature stomata; 10, hairs; 11, undefined units.


Day 18
0
Day 19
50
0
Day 20
250
200
150
100
50
0
Day 21
300
250
200
150
100
50
0
Number of units
Day 22
400
350
300
250
200
150
100
50
0
Day 23
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Type of unit

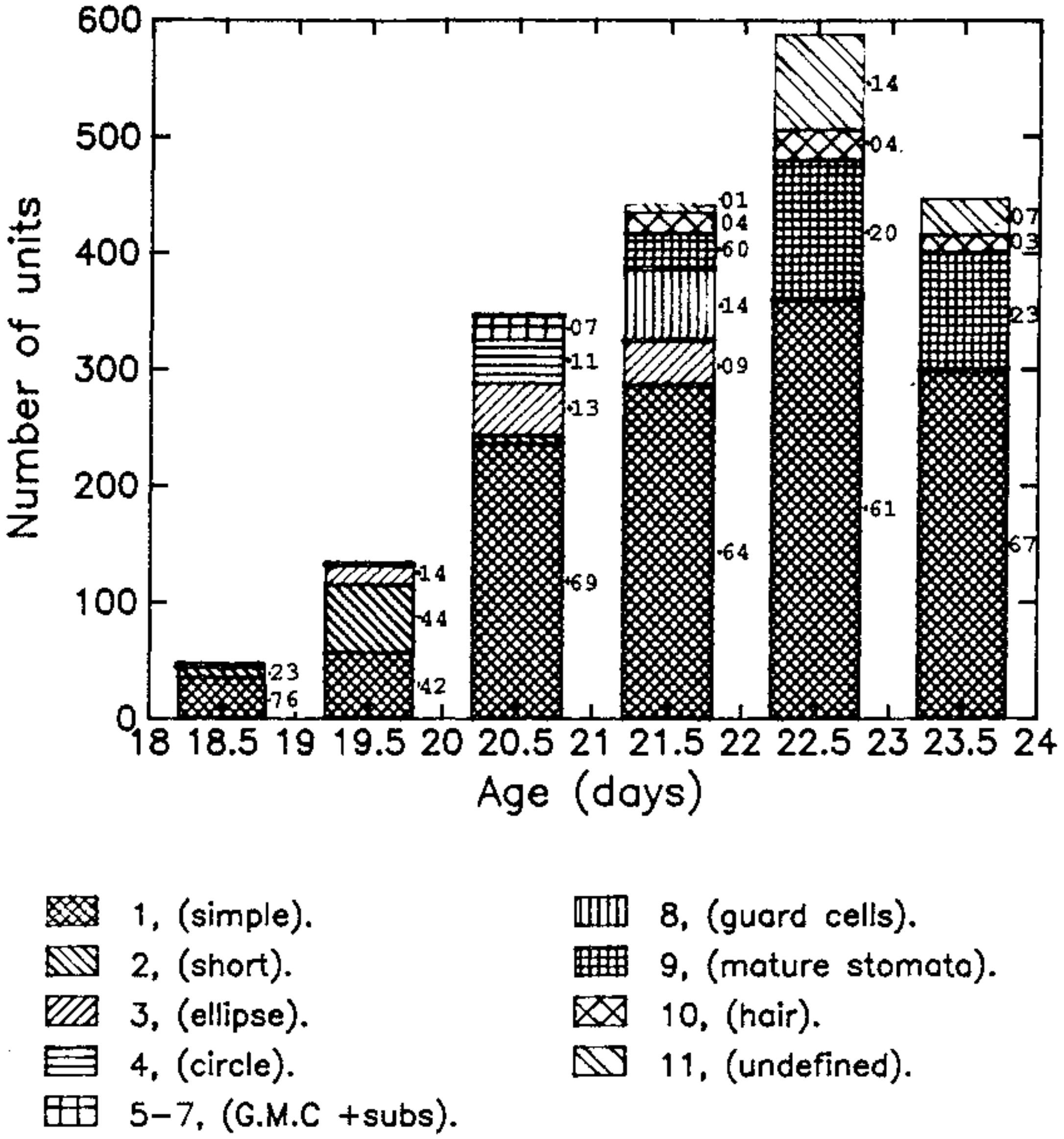


FIG. 5.9 The relative proportions of the different types of unit in a sample as a function of age. The number shown is the mean of the values recorded for the set of samples (see text) obtained during a given day.

MEAN UNIT LENGTH.

The average numbers of all and of stomatal units in a sample, were read from the mean lines representing numbers as a function of age (Fig. 5.7). An average sample consisted of 16 rows. Therefore dividing the average number per sample by 16 yielded an estimate of the number of units in a row and the length of the segment divided by the number of units in a span of it provided values for mean unit length. Values were calculated in this way for midday on each of the sampling days (Table 5.1). Mean unit length decreased from 28µ to 14µ while the lower boundary of the segment moved from 3.6mm to 10.6mm between age 17.5 and 19.5 days. There was a twelvefold increase in length of the tissue between age 17.5 and 20.5 days but it was accompanied by the insertion of an average of 19 new transverse cell walls between the original pair and the mean unit length at the end of the period was half that at the start of it. After day 20.5 while the segment moved from 19mm to the end of the growth zone the mean unit length increased sharply from 15µ to 66µ. During this period the length of both units and segment increased but while the relative increase in length of the unit was 4.4 that of the segment was 6.6. The increase in length had been accompanied by further subdivision and 11.5 new cell walls had been formed.

NUMBERS OF CELLS FOUND IN OF THE SEGMENT AS A FUNCTION OF AGE

The number of cells occurring in all but the stomatal units is one (Fig. 5.6 and 5.3). Therefore the change in cell number as a function of age, recorded in terms of number per row span (Fig. 5.10), follows the change in unit number (Fig. 5.7 and 5.9) until the first subsidiary cells of the stomatal complex are cut off. A few stomatal units were recorded on the age 20.5 samples. These were in two categories with one or two subsidiary cells, and therefore represented 2 or 3 cells each. The separation of the two subsidiary cells of a given stomatal complex from their respective lateral epidermal cells was not necessarily synchronous but normally it appeared to preceed the division of the guard mother cell. No guard cells were seen on the age 20.5 samples. Most of the subsidiary cells and virtually all of the guard cells were formed during the 24 hours between the ages of 20.5 and 21.5. The stomatal units observed on the age 21.5

TABLE 5.1. To show the changes in a segment of epidermis travelling through the growth zone. The unit numbers were read from the "eyeball" fitted mean lines; on the, unit number per sample as a function of age, graphs. Number in the average row span = average number per sample divided by mean number of rows per sample. Cell number = number of other units + number × proportion x number of cells in each type of stomatal unit present.

Age	Segment		Number per row span				Mean
(days)	$x'(t;x_0')$ (mm)	Length (mm)	All Units	Stomatal units	Other units	Cell number	Unit Lengthμ
17.5	3.61289	.02565	.9	0	.9	.9	28
18.5	6.20875	.04402	2.2	0	2.2	2.2	20
19.5	10.60566	.09985	7.3	0	7.3	7.3	14
20.5	18.93119	.30124	20.0	3.4	16.6	23.7	15
21.5	40.23517	.96820	28.6	6.3	22.3	45.3	34
22.5	106.79	2.0	30.5	6.6	23.9	49.0	66
23.5							

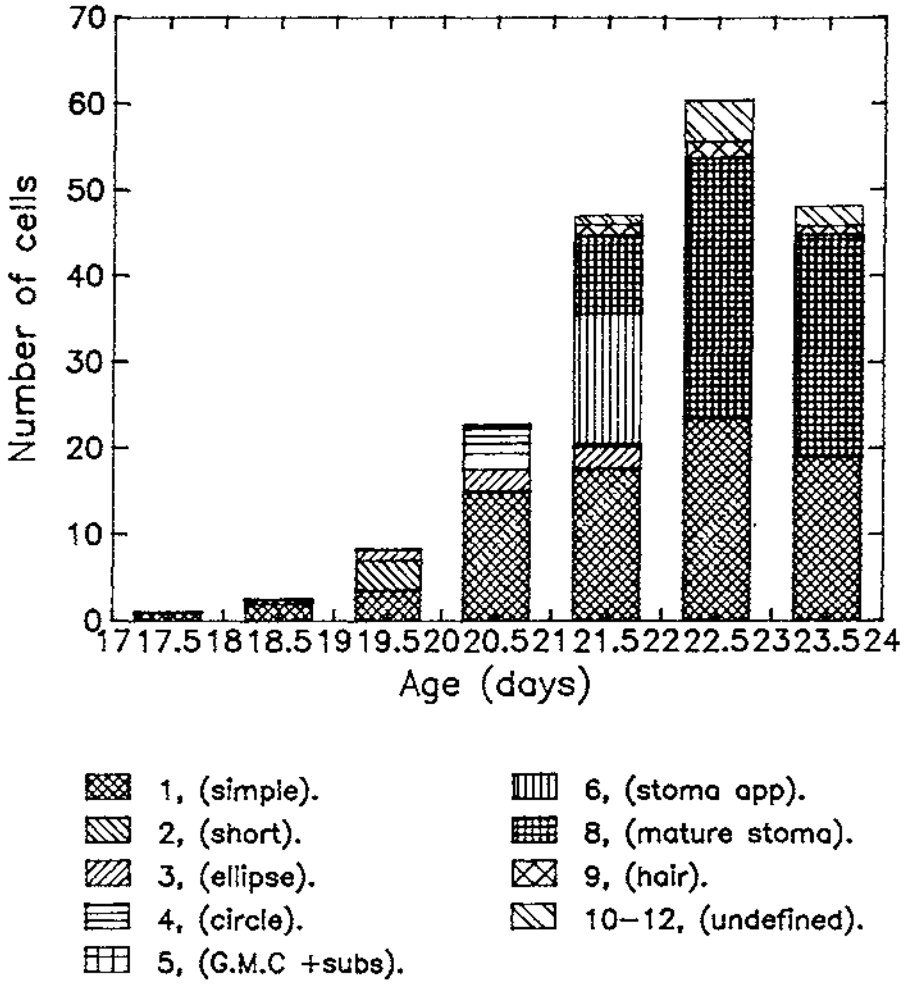


FIG. 5.10 Number and type of cell as a function of age. The numbers are the mean of the counts made for individual complete row spans of the segment through all of the rows collected on a given day.

samples consisted therefore of four cells and this led to a sharp increase in cell number. During this period the number of divisions occurring in the developing stomatal complexes was 0.9 of the total number taking place in the epidermis. The predominance of the stomatal divisions at this age may help to explain why workers in the past hve overlooked the multiplication of other cells and have come to the conclusion that cell division continues in the stomatal complexes after it has ceased elsewhere in grass leaf epidermis.

The variabilty that made it impossible to be certain of the exact increase in unit numbers in samples subsequent to age 21.5days is also relevant to the increase in cell numbers. It was therefore necessary to work with the estimates derived from the eyeball lines for unit numbers per sample as a function of age (Fig. 5.7). The values were calculated in terms of unit number per row span (Table 5.1). The proportions of the stomatal categories found during a particular day gave values for cell numbers in the stomatal units in an average row span and this number plus that of single celled units yielded a value for cell number per row span (Table 1). The estimated increase in the number of cells in a row span between age 21.5 and maturity was 3.7. Apparently 0.3 new stomatal and 1.6 other units were formed in the average row during this period. All the stomatal units included four cells at maturity on day 22.5. One third of all the new cell walls were those required in stomatal subdivision The period between age 21.5 and 22.5 is that during which the segment left the growth zone.

When the maize plant age was 17.5 days, the length of the segment was 0.0257mm and it was spanned by 0.9 of a cell. Four and a half days later the length was 2.0mm and an average cell row which spanned it was divided into approximately 30 units containing 50 cells (Table 1).

THE DISTRIBUTION OF UNIT TYPES WITHIN AND BETWEEN CELL ROWS

The proportions of unit types found in the sample on five of the sample plants, one representing each of the days, is shown (Fig. 5.5 a,b,c,d,e). These diagrams show the considerable variability between rows in the epidermis.

Of the four types of unit found in the mature lower epidermis of the maize leaf (Fig. 5.5 e, f; 5.8 and 5.9) the greatest number were simple units and there was approximately the same number of these in each row at a given age (5.5 e, f). Where other types of unit occurred in a row the total number of units in the row was greater and the simple units were shorter. Other types of unit were almost invariably separated in the row by the presence of at least one simple unit. Stebbins and Shah (1960) name the simple unit that occurs between stomata in a row an intervening cell. The greatest number of units were found in sample rows where stomatal and simple units alternated throughout the length. In these rows the lengths of stomatal and simple units were approximately equal.

Stomatal, hair and undefined units occurred in the mature epidermis in a pattern (Fig. 5.5 c,d,e). Stebbins and Shah (1960) found stomata in up to three adjacent rows in maize. A similar pattern was found in this experiment. The stomata were concentrated in lines. A line would consist of a group of adjacent rows bearing stomata. In the centre row up to 0.5 of the units were stomatal. The proportion fell as the row became more remote from the centre of the stomatal line and there were usually one or more rows between the lines with no stomata .

Each stomatal line runs along a considerable length of the maize leaf. The position of stomatal lines is related to that of the vascular bundles (Stebbins and Shah; 1960). These diverge from the midrib at an acute angle and eventually extend to the margin of the lamina (Russell and Evert, 1985). The coincidence of the stomatal lines and the cell rows is not, however, absolute: they may merge or separate and slant across the rows. Thus in terms of the types of units found in a given row the proportion of stomatal units present in a particular length of it can range from .5 to 0. The change in stomatal representation may occur within the length of a few units but more frequently there is an intervening section of the row with intermediate proportions of stomatal units in it.

Hairs were not invariably present in a sample. Where they did occur they were found in rows or parts of rows adjacent to but, not mingled with, the stomata. Undefined units occurred in the mature samples, and were more common in some samples than in others. They were rarely found in rows with a high proportion of stomatal units but were common in the adjacent rows where they were often associated with the presence of hairs.

My observations tend to support the opinion that the distribution of stomata and hairs depends on the position of the cell on the leaf rather than an inherent character of a particular cell, or set of sibling cells in a row.

ASTOMATAL ROWS

Astomatal rows were recognised by a total absence of stomata. There was an increase in the proportion of rows which included guard mother cells from days 17 to 20 inclusive. Astomatal rows could not be positively distinguished before day 20. On days 20 and 21 the mean numbers of simple units in an astomatal row were 15.25 and 18; and the average unit lengths were 20µ and 58µ respectively. By comparison the combined length of a stomatal unit plus adjacent intervening cell was approximately equal to the length of a single cell in an "astomatal" row. The sample reached its mature length on day 22. Means were therefore calculated for days 22 and 23 combined. As a result of the high variability in the samples for days 22 and 23, together with the fact that there were relatively few astomatal rows in some of them, these means were open to doubt. The values were 26 units per row span and an average cell length of 77µ. While these mean values can not be taken as exact, the increase in the number of units in an astomatal span of the sample between day 20 and maturity was considerable, it was almost certainly real, and it represented continued cell division in the simple cells of the epidermis during this period.

OCCURRENCE OF "MAJOR STOMATAL" ROWS.

"Major stomatal" rows are identified by the abundance of short, elliptic, circular and stomatal units found in them. If the proportion of such units is greater than 0.45 the row is named a "major stomatal" row.

The first visual indication that different types of cell row were to develop in the lamina occurred in the segment on day 18 when short units were found in a number of rows (Figs. 5.3a and 5.5a). Shah (1960) describes such short cells in the basal part of the stomatal rows in grass leaves. It seems very probable that the rows which contained short cells on day 18 developed into major stomatal rows. When I compared the distribution across the segment of the rows of short cells with those showing a high proportion of elliptic, circular or stomatal units in the older samples, the pattern was similar. Counting across the sample the numbers of rows intervening between "major stomatal" rows varied but the most common numbers at all ages were two and three. Moreover in the day 19 samples there were not only short units but also elliptic ones present and the latter were invariably found in the same rows as the short units. Elliptic units could develop into hairs and undefined units instead of into stomata but in mature samples, rows with hairs occur twice as frequently as, and tend to lie on either flank of, the "major stomatal" rows. It is unlikely that on day 18 one of the flanking rows was more advanced than the other, or more advanced than the central row. I have therefore considered the rows of short cells found on day 18 and 19 as "major stomatal" rows.

During day 18 approximately 0.25 of the rows consisted of short cells and were considered "major stomatal". Scattered stomata developed in additional rows at a later age and rows with no stomata at all became less common with increasing age.

The number of rows containing more than 0.45 stomatal units was higher on day 19.5 than any of the other days. In this day's samples these rows were identified by the presence of large numbers of short cells plus in most cases some elliptic cells. The rows were frequently in pairs. The paired arrangement is not apparent in the older epidermal samples. This means that either new rows were formed between members of the pair subsequent to day 19.5, or that one of them ceased to qualify as a major stomatal row. The former is unlikely since I have shown that there was no appreciable increase in the number of rows across the width of the leaf during the experiment and it would be virtually impossible for partially differentiated stomatal units to be involved in duplication. The latter probably happened. Further Dehnel (1960) showed that divisions of guard cells cannot be induced experimentally. A row would however cease to qualify as a "major stomatal" row if, of the new units formed in it, the proportion of new simple units as compared with elliptic ones was high. The following figures support the suggestion that this occurred. The average number of simple cells in a span of the segment increased fourfold from 3.5 on day 19.5 to 14.3 on day 20.5. During the same period the average number of stomatal plus short units in a span rose from 4.62 to 6.04, a much smaller, 1.3 fold, increase. Where pairs of rows with a greater than 0.45 proportion of stomatal units occurred, only that with the higher proportion was nominated a "major stomatal" row.

The temporary rise in the number of rows with a high proportion of stomatal units during day 19 suggests that a visually similar activity had commenced 24 hours later in rows adjacent to the original "major stomatal" rows of day 18. These additional rows formed short cells but apparently a lower proportion of the latter proceeded into asymmetric division to yeild the units which identified the "major stomatal" rows on day 20.5. Further, of the elliptic cells that were formed in these flanking rows, a greater proportion gave rise to hairs or undefined units than to stomata . Bunning (1956) suggested that developing stomata inhibit the formation of other stomata in adjacent cells. His argument would explain the lower numbers recorded in the flanking rows in this experiment. However, the pattern could also be explained in terms of a gradient of a stimulant or suppressant, emanating from the tissues below the epidermis. A mechanism is required which positions the stomatal lines in the wider pattern of structures which are arranged parallel to one another in the grass leaf. With regard to the possible transmission of control from underlying cells, Hake and Freeling (1968) have shown that mutant genotypes present in the mesophyll cells in maize leaves can cause excessive cell division in overlying non mutant epidermal cells.

There was a further fall off in the average number of "major stomatal" rows found in the sample after day 21 but it was impossible to say whether this was the result of an imbalance in the numbers of simple as compared with stomatal units formed during the interval or whether it was an aspect of the variability existing between the individual sample plants.

NUMBERS OF UNIT TYPES WITHIN "MAJOR STOMATAL" ROWS AS A FUNCTION OF AGE.

The numbers of short, simple and incipient or evidently stomatal units in the major stomatal rows in the samples are shown (Table 5.2 and Fig. 5.11). On day 18 the "major stomatal" rows consisted wholly of short units, and there was an average of 3.7 units in each of them as opposed to an average of 2.5 in non stomatal rows. One of the samples on day 18 showed no short units and none were found in the day 17 material. Thus the first visible indication that an epidermal row in this segment of the lamina epidermis was to become a "major stomatal" row occurred during day 18 when the lower boundary of

TABLE 5.2. Unit numbers in the major stomatal rowspans in the samples.

AGE	SHORT
18.42	4
	4
	4
	4
18.51	2
	3
	3
18.56	4
	4
	4
	5
19.43	8
	8
	9
	9
MEAN (DAILY)	3.7

	SHORT	ELPSE	TOTAL
19.51	9	3	12
	11	1	12
	9	4	13
19.64	9	2	11
	9	8	17
	10	8	18
	10	5	15
19.68	8	7	15
	9	7	16
	10	11	21
mean (p.m)	9.4	5.6	15.0

	SMPL	STOM	TOTAL
20.43	12	12	24
	13	13	26
	16	16	32
20.47	10	10	20
	18	15	33
	18	17	35
	17	17	34
20.51	14	12	26
	14	15	29
	16	15	31
20.68	15	13	28
	14	14	28
	16	16	32
	19	16	35
MEAN (DAILY)	15.2	14.3	29.5

AGE	SMPL	STOM	TOTAL
21.60	16	17	32
	17	17	34
	18	19	37
21.64	17	17	34
	18	18	36
	19	17	36
	21	21	42
MEAN (DAILY)	17.4	16.6	34
22.39	19	20	39
	20	21	41
	23	21	44
22.48	23	22	45
	25	25	50
23.39	21	18	31
	16	16	32
	19	18	37
	21	18	39
23.48	18	18	36
	19	17	36
	20	19	39
MEAN (2 DYS)	20.3	19.4	39.7

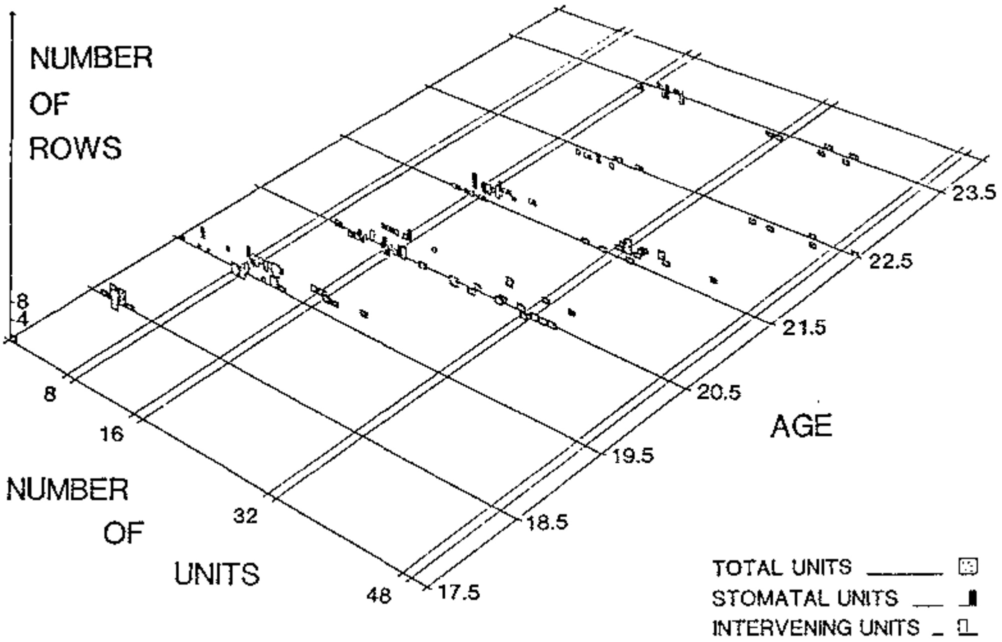


FIG. 5.11. Numbers of units in the major stomatal rows which span the sample. The distribution of values for: intervening short or simple; incipient or established stomatal, and total units as a function of time.

the segment was 6.206mm from the leaf base.

The mean number of units in the "major stomatal" rows had increased to 13.1 by day 19.5 and some asymmetric divisions had taken place. The products of these asymmetric divisions, the elliptic units, usually alternated in the rows with the short cells. The number of elliptic units found in the rows within the sample increased with the progressively later time during day 19 at which the sample had been collected. Whereas there were nine short but no elliptic units in each of the four "major stomatal" rows in the day 19.43 samples, there was an average of 9.0 short, with 8.3 elliptic units distributed among three "major stomatal" rows in the 19.68 sample. Short cells were not observed on day 20 but there was a substantial number of simple units present. The average length of a unit had apparently increased but although they were longer the units now classed as simple were still involved in both symetric and asymmetric division. By age 20.5 there was an average of 15.2 simple units together with 4.3 guard mother cells with either one or two subsidiary cells; and 10.0 elliptic or circular units in each of the "major stomatal" rows. The numbers of subsidiary cells present increased through the sequence of four samples which had been collected at approximately two-hourly intervals during the day. With regard to such comparisons within a day however, the numbers found at a given time are those from a single sample plant, and it is therefore not possible to know how representative they are.

The averages showed further increases between day 20 and day 21: the number of simple units reached 17.4, and the total number of incipient or identifiable stomatal units reached 16.6 per "major stomatal" row span. The additional guard mother cells which were found must have been cut off from simple units during the interval. On day 21 all except the earliest sample shows stomatal units in which the guard cells had formed, and all the stomatal units found on the day 21.64 sample were regarded as mature. Whereas virtually no circular, or two- or three-celled, stomatal units were recorded in the "major stomatal" rows of the day 21 samples, there were some elliptic units present. Elliptic cells were not found on the day 22 or day 23 samples. The difference of 2.5 units between the average number of stomata found in the "major stomatal" rows in the mature epidermis on days 22 and 23 and the total number of immature stomatal units found in them during day 21 suggests that in spite of the fact that the early stages were not seen, a few additional stomata may still have been developing as the segment approached the end of the growth zone. The lower boundary of the segment was at 40mm at age 21.5 and left the growth zone 69mm from the base at age 22days.

STOMATAL DEVELOPMENT AS A FUNCTION OF AGE.

GUARD CELLS

In this experiment development of all the stomatal complexes took place within a four day period but the time of initiation and the rate of differentiation varied. Later initiated stomata appear to have developed more rapidly than the earlier ones.

The first elliptic cells were found on day 19. The first four-celled stomatal complex with visible stomata between the guard cells were recorded on day 21. It appeared that a stomatal initial at this age and in this part of the growth zone required two days to become a fully subdivided stomatal complex. There are very few undefined units in the major stomatal rows at maturity and no partially developed stomatal complexes. The later formed elliptic guard mother cells must therefore have completed their expansion and subdivision into guard cells in times reducing to 12 hours for those present in the samples collected early on day 21, and possibly less for the few which occurred in the afternoon samples. There was an average of 1.3 elliptic units per major stomatal row span on day 21. This increase in the rate of guard cell development is associated with an increase in the rate of tissue expansion (Fig. 5.2). This suggests an extracellular rather than an intracellular constraint over the rate of stomatal construction and that this constraint is related to the position in the growth zone.

SUBSIDIARY CELLS

Before its division to form the stoma, the guard mother cell becomes first circular and then elongated. Its width does not increase as rapidly as that of the surrounding simple cells, however, and as a result bays form as extensions of the lateral epidermal cells where they have a common wall with the guard mother cell. On day 20, 24 hours after the first appearance of the elliptic units, there were an average of 6.9 circular guard mother cells in the "major stomatal" rows, each with a pair of bays associated with it. In an average of 2.3 cases one and in 1.9 cases two, such bays had already been separated from their parental lateral epidermal cells by walls. 24 hours later, on day 21, a few elliptic units were recorded but all of the other developing stomatal complexes seen in the "major stomatal" rows had two completely enclosed subsidiary cells. In the three samples collected later in the day the subsidiary cells had acquired the obtuse isosceles triangle appearance associated with them in the mature epidermis. During the final 12 hours before the tissue left the growth zone the longitudinal extension of these triangles was proportionately greater than the increase in their width

CELL DIVISION IN THE "MAJOR STOMATAL" ROWS.

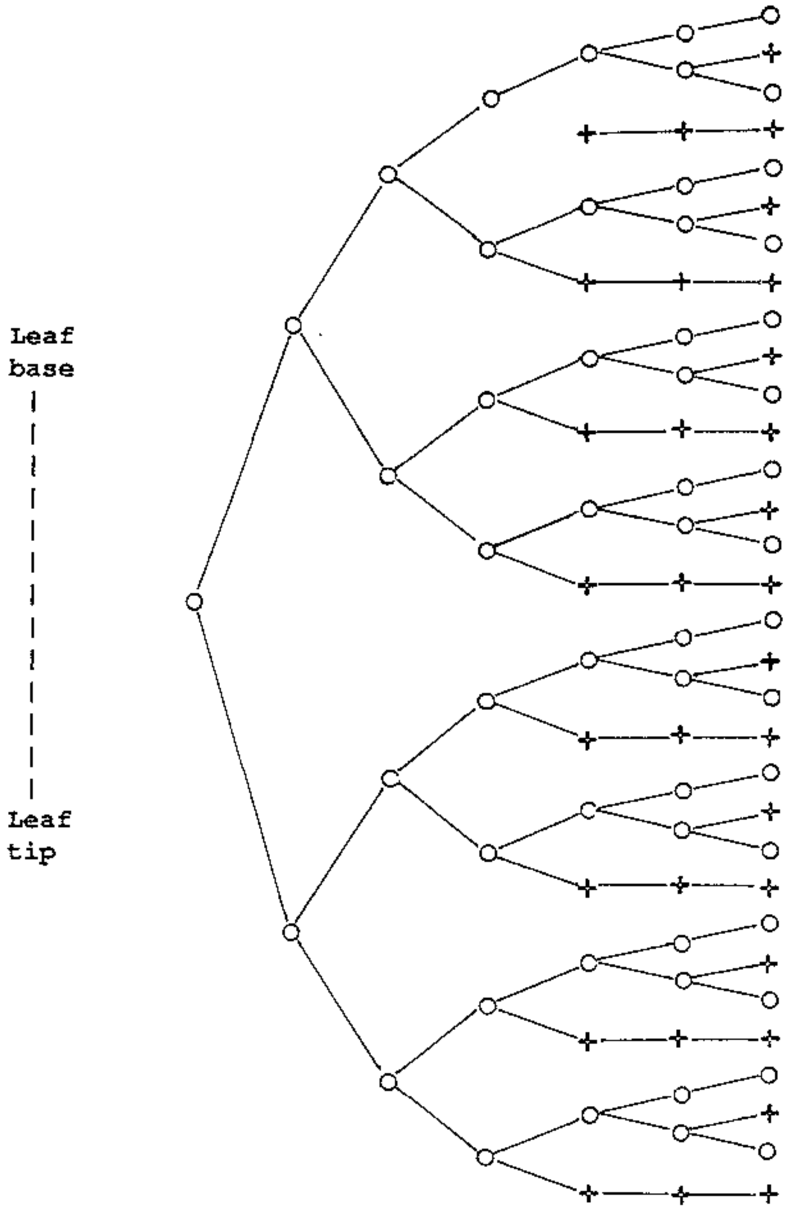
On day 17 there was little difference between the rows with regard to cell length and the average row span included 0.9 cells. During the 24 hours between day 17.5 and day 18.5 the tissue within the segment was divided by a number of transverse walls. There were twice as many of these new end walls in the "major stomatal" rows as compared with the other rows. The mean numbers of units in the rows were four and two respectively. Although the exact values varied, (Fig. 5.11, Table. 2) the situation where the mean numbers for "major stomatal" rows in a sample were approximately twice those for the other rows persisted in the days 19.5 and 20.5 samples. I suggest that it arose as the result of a temporary increase of the rate of cell division in "the major stomatal" rows in the later part of day 17 but that this lasted for the duration of one or possibly two cell cycles only. This enhanced rate of cell division could have affected either or both of the two cell divisions which were involved in the production of the approximately four cells found in the "major stomatal" rows on day 18.

The first asymmetric divisions which gave rise to the stomatal guard mother cells occurred during day 19. In the sample collected at 19.43 there were no elliptic units. There was an average of 8.5 short units in the "major stomatal" rows and of 4.6 units in the other rows in this sample. Elliptic units were observed in the samples obtained at ages 19.51, 19.64, and 19.68. The mean numbers of units present in the "major stomatal" and "astomatal" rows of these three samples were 14.6 and 6.5 respectively. Of the 14.6 units in the "major stomatal" rows an average of 5.2 were recognisable elliptic units. The other 9.4 were short (simple) units.

Comparison of the sample at 19.43 with those collected later in day 19 suggests that the cell wall formation and expansion of the new stomatal guard mother cell to an elliptic appearance is accomplished within six hours. The figures provide an indication of the rate of elliptic cell formation but may not necessarily be accurate because the samples are from separate plants which would be expected to show individual variability.

At age 20.5 days the mean number of simple units in the major stomatal rows was 15.2 and the mean number of stomatal units was 14.3. The number of both simple and stomatal units apparently continues to rise. On day 21.5 the means were 17.4 and 16.6 respectively; and at maturity the mean figures were in the region of 20.3 simple and 19.4 stomatal units in a "major stomatal" row span.

I have set up a comparison between an idealized cell division model and the set of daily means in the "major stomatal" rows of the actual samples (Fig. 5.12). In the model all cells follow a similar pattern of cell division as a function of age. Early divisions in the segment were symetrical and appeared to be more or less synchronised. Asymmetric divisions commenced at a given position and age. The distal of the daughters of an asymmetric division is limited to one further cytokinesis, that which forms the guard cells; the proximal daughter would appear to be available for repeated division. In the experiment 0.9 of an average cell spanned the segment on day 17. Three cell divisions of this 0.9 cell would have yielded approximately eight cells in a row span of the segment. Approximately this number was seen in the day 19.43 sample. Asymmetric divisions in eight cells. would have formed eight guard cells. Approximately 5.5 cells had undergone asymmetric division in the sample collected later during day 19. Ideally if all the cells were behaving in a similar manner and asymmetric divisions occurred in the remaining three short or simple cells the row span would have included 14.4 cells, 7.2 short or simple and 7.2 stomatal ones, and these would have alternated within it. It would appear that some of the short or simple cells infact proceed to a further symetrical division before becoming involved in stomatal formation. Many major stomatal rows in the late day 19 samples include more than eight simple units the mean figure was 9.5. On day 20 the majority of the "major stomatal" rows consisted of alternating simple and stomatal units. The numbers of each increased more or less in parallel during the next two days and the average numbers at maturity were: 18 simple, and 17 stomatal units. If cell division had followed the pattern described in the model, six cell cycles would have yielded 14.4 simple and 14.4 stomatal units. It is apparent that cell division in the segment does not follow the model precisely although it does yield numbers similar to those which were in fact found.



MODEL								
Number of units	o	1	2	4	8	8	16	16
	+	-	-	-	-	8	8	16
EXPERIMENTAL								
Age (days)		17.5		18.5	19.43	19.61	20.5	21.5
Number of units	o	.9	-	3.7	8.6	9.4	15.2	17.4
	+	-	-	-	-	5.2	14.3	16.6

FIG. 5.12. A sequence of divisions which yields and reiterates an alternating pattern of stomatal and intervening cells. The numbers generated by the model are compared with those found experimentally at various ages. Units are shown: simple, o; short, +.

From day 20.5 onwards the "major stomatal" rows were recognised by the fact that the stomatal complexes alternated with ordinary epidermal or intervening cells. Stebbins and Shah (1960) described the alternation of stomatal and intervening units extending through the growth zone. They considered that it was the result of a very early differentiation of stomatal mother cells. In this experiment while the alternating pattern was recognised in the day 19.5 samples the increase in the number of stomatal initials continued after this day. The majority of the simple cells in the "major stomatal" rows in the segment underwent an asymmetric division on day 19.5 to form a stomatal initial at their distal ends. There were ten "major stomatal" rows in the three samples collected during the afternoon of that day. The following table shows the total numbers of short or simple cells in these rows and the number of them that lacked an elliptic cell as its distal neighbour.

sampling time	total units	simple units	Units with nonstomatal distal neighbour
19.51	12	11	10
19.64	11	9	6
19.51	12	9	5
19.51	13	9	5
19.64	15	10	5
19.64	17	8	2
19.68	16	7	2
19.68	15	7	2
19.64	18	10	1
19.68	21	10	0

The total number of short plus simple units in these samples was substantially lower than the numbers found subsequently in "major stomatal" rows. If division in the stomatal units can produce only guard cells (O'Brien, 1982)then this increase in number must be the result of division among the short or simple cells. The smallest number of simple units observed in a "major stomatal" row in the mature samples was 16 and the highest would almost certainly have included more cells by the time it reached maturity, when the lowest number of simple cells in a "major stomatal" row was 16, but each of the intervening units in this row already had a stomatal unit at its distal end. An increase in number in the row would therefore have had to involve the insertion of new initials between those already in place. Similar insertions would probably also have taken place in rows where only a low proportion of cells remained without stomatal initials at their distal ends. It would seem unlikely for example that two out of a total of eight cells in the row span would be the parents of an additional nine or more cells.

The cell division model shown (Fig. 5.12) suggests that if the intervening cells divide after they have cut off a first stomatal initial there should be times when the single simple cells in the alternating pattern are replaced by pairs. Although the intervening cells were sometimes paired the arrangement was not continued through a row span. This suggests that the divisions were not synchronised. With regard to this, the frequency of cell division cannot differ to any extent through a short length of epidermal row such as the length of the segment. The cells in a row are locked (O'Brien, 1982) onto cells in the neighbouring rows in the tissue and whereas there would be a difference in the expansion through the length of the segment it is small and is part of a continuum. In this situation if some cells divide more frequently than others their daughters will be smaller than those of their neighbours and sequences of small cells should be observed. The lengths of simple cells in the "major stomatal" rows did not differ in this way.

Simple cells which had already cut off a stomatal initial must have been involved in further subdivision in order to achieve the increase in number of the simple units in the "major stomatal" rows between day 19.5 and maturity but the additional divisions must have been relatively evenly distributed through the length of the segment. Figure 5.13 shows how such further subdivisions could have occured. The figure shows the growth and subdivision of an hypothetical set of eight simple and seven stomatal units which are supposed to have occupied the length of the segment at age 19.5. The end walls of these units are shown as lines which diverge relative to one another from their positions at age 19.5. The length of the segment increases from 0.1mm to 0.3mm during the day between age 19.5 and 20.5 days. The rate of expansion is taken as constant through the length of the segment and during the 1.4 days shown in the diagram. The lengths of the initials vary through a 4μ range with the median value 13μ. The lengths and variation are similar to those found in the samples. At age 19.5 all except one of the eight simple

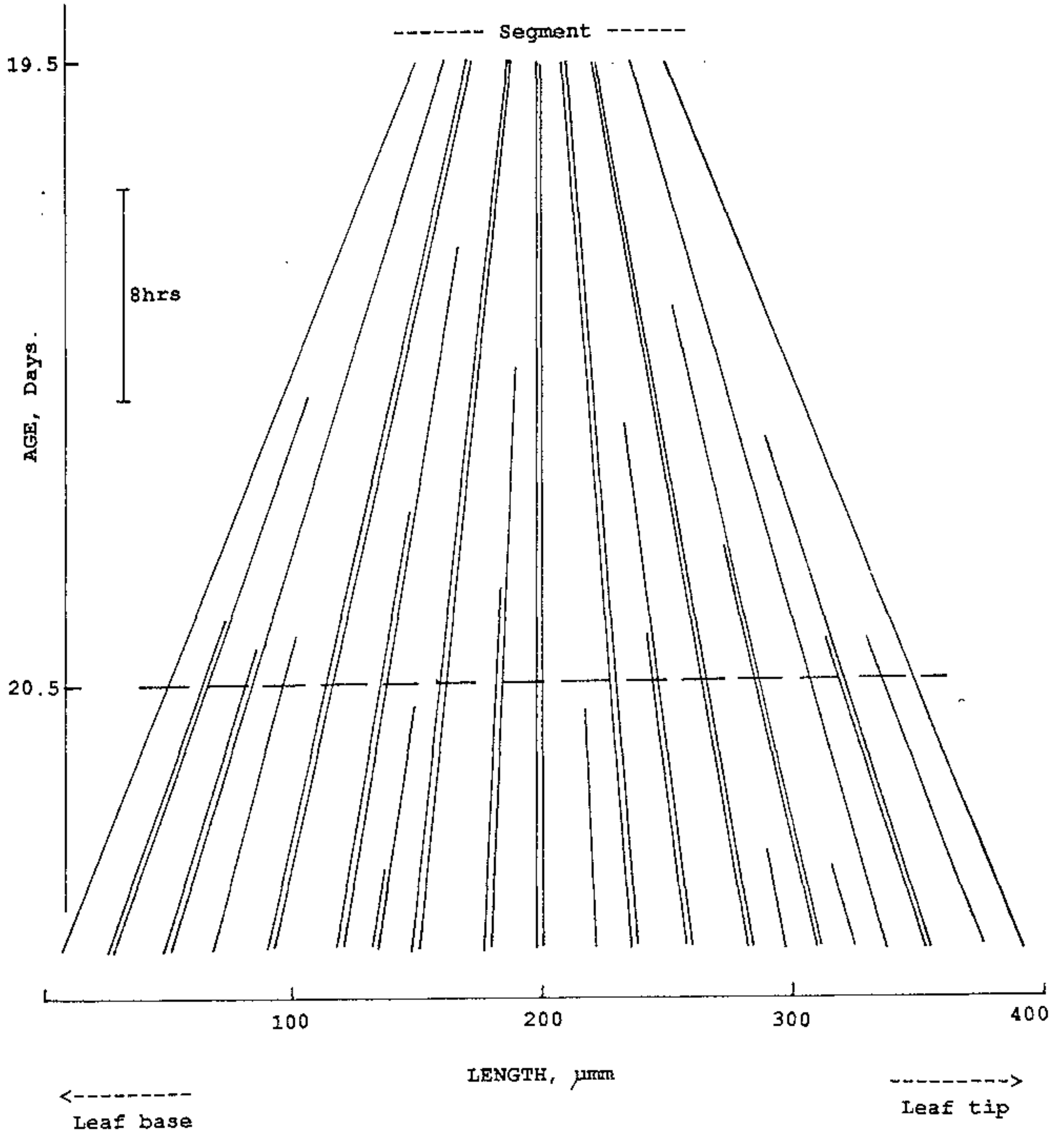


FIG. 5.13. A model of the growth and subdivision of the units in a hypothetical row spanning the segment. Initial cell lengths in basifugal sequence are: 11μ, 11.5μ, 15μ, 12.5μ, 10μ, 12.5μ, 13.8μ, 14μ. Shortest cell is .73 of the length of the longest. The positions relative to the initial central position are shown: cell walls, ——; GMCs, ═══.
Assumption: expansion is uniform through the length of the segment and does not vary with age.
Restraints: a;no simple cell length <9μ or >16μ
b; minimum cell cycle time is 8hrs.

units has a guard mother cell at its distal end. As the cells increase in length and age it becomes likely that they will divide but I have imposed constraints on cell division. These are: that divisions which yield two simple cells can only take place when the parent cell is more than 18μ long; that such a division must intervene before cells become more than 32μ long, and that the least interval between the division of a parent cell and that of either of its daughters is eight hours. Within these constraints cell divisions are supposed to occur at random. Following a cell division a new line, the path of the new cell wall, appears in the figure. The diagram shows how the initial set of units grew and divided under these constraints. If a sample had been collected at age 20.5 days it would have included 16 simple and 14 stomatal units, with two of the simple units lacking a stomatal unit at their distal ends. This arrangement is similar to that found in the real samples collected during day 20. Thus the illustration shows how over a length of time small offsets from a central position of the new cell wall at cytokinesis coupled with limited variation in the cell cycle times of the individual cells could lead to the insertion of additional cells and in particular stomatal units without an obvious disruption of the pattern of alternating intervening and stomatal cells. I suggest that cell cycle time and cell length are linked in the natural system and that both can vary but they do so between limits. I further suggest that the limits are peculiar to a segment of leaf and change as it recedes from the base of the growth zone.

The insertion of new stomatal initials is likely to lead to differences in the degree of differentiation in adjacent stomata in a rows. Stebbins and Shah (1960) reported this type of irregularity along the row in the epidermis of barley and it was evident in the samples examined in this experiment.

The insertion of a new stomatal unit in the space occupied by a single intervening cell has to be preceded by the formation of two simple cells between neighbouring stomatas. This situation was not seen frquently in the samples but the numbers that were observed would have been sufficient to yield the approximately two stomatal units which were added to the mean numbers per row span between day 20.5 and day 21.5. 228 simple cells were found in 15 "major stomatal" rows in the four samples on day 20.5. Seven of these were at the distal boundary of the segment and the nature of their distal neighbour was therefore not recorded, 21 or approximately 0.1 of the remaining 221 simple cells were proximal to another short cell. With regard to the number observed if the second cytokinesis, that which cuts off the guard mother cell, followed the first, that which formed the additional prestomatal cell, within 24 hours, then a proportion of the occasions when a single intervening cell were replaced by two daughter cells will not have been recorded in the samples.

When an intervening cell divides, only one of the daughters can undergo asymmetric division. Stebbins and Shah (1960) showed that the guard mother is always the distal product of division. Two guard mother cells are virtually never found adjacent to one another. Thus the proximal of the two daughters must be the stomatal mother cell. In the absence of erratic changes in cell extension rate along the row this cell would be expected to be longer than its sister by a length proportional to that of the section of it that is to become the new guard mother cell. This difference was not obvious but no measurements were made which would allow it to be critically examined. The fact that the distal daughter cell does not undergo an asymmetric division may be the result of the presence of a differentiating stomatal unit at its distal end which has an inhibitory effect (Bunning, 1956).

Thus there is evidence for the continuing insertion of additional stomatal initials by asymmmetric division in a second and third generation of simple cells. Associated with this the stomata which were found in more remote parts of the growth zone were not in sequence with respect to their age from initiation. The fact that there was little variation in the appearance of these stomata in the outer growth zone suggests that the rate of differentiation was not uniform and was more rapid in later formed initials. The maize leaf studied in this experiment was growing under very favourable conditions. If conditions had not been as favourable, leaf extension would not have been as great. In that event the early formed stomates might have been sufficient to service the length of leaf that was formed. The distribution of the stomates both with regard to their being in stomatal lines parallel to the leaf axis and in their longitudinal placement within these lines during development suggests a regulatory system which involves distance from one another and from other structures. The production of an inhibitory substance has been suggested as a regulator but this has not been tested. Grass leaves have considerable plasticity with regard to growth in size and a mechanism which both allows additional stomatal initials to be inserted, and correlates their formation in the growth zone to the length separating earlier initials, could contribute to this leaf plasticity.

CONCLUSION

In this study I have investigated the growth of the segment of the lower epidermis which occupies the position halfway along the length of a mature maize leaf. The method which was developed in the experiment allowed a number of characters of the growing tissue to be compared with time and position on the leaf and as a result a number of facts about growth in the segment have been revealed.

The experiment gave strong grounds to think that cell division in the epidermis continued through the entire length of the growth zone. Also it suggested that additional new stomatal initials were interpolated between those which are already in place in a section of the growing leaf.

The extension of the segment which was gradual while it was at the base of the growth zone built up to a very rapid rate before it arrived at a sudden cessation. There was a change in the rate of increase in cell number in the segment during the experiment. The increase was gradual while the segment was near the base of the growth zone; it became greater for a period and subdivision outstripped extension in the lower to mid growth zone; but it fell back again as the segment moved further from the base of the leaf and was apparently reduced to zero at approximately the same time as extension. The mean length of units within the segment increased as it passed through the outer part of the growth zone which is the classical region of cell extension. The massive extension in tissue length in this part of the growth zone however was such that while there was an increase in mean unit length subdivision was also continuing

The study as it was carried out in this experiment had severe limitations. The variability that occurred in the population of maize plants used was considerably greater than was anticipated when the work was planned. The number of samples examined was therefore not adequate and it became impossible to place a great deal of confidence in any but the most substantial trends in the results. There was a second inadequacy which was also a result of the fact that this was a novel technique. The rate of development was more rapid than had been anticipated and the intervals between sampling times were too long to record sufficient detail. While the paucity of replication in the records must be borne in mind the results of the experiment are still worth considering.

The location and study of a particular segment in a leaf through the period of its growth has proved to be a valuable approach. It is not only experimentally valid and possible, it is also conceptually simple to handle and therefore allows a relatively straightforward mental appraisal of growth.

CHAPTER VI

DISCUSSION AND CONCLUSION

Much is known about leaf growth in members of the family Gramineae but while the subject has attracted some skilled and meticulous work each piece of research has concentrated on a particular area of interest and the results have not been drawn into a satisfactory overall description. As a consequence the system has not been fully understood. Working with maize as an example of the Gramineae, I have described phytomer growth as being determinate and consisting of the expansion of a disc of tissue originating in the stem apex. I consider that the shape of the phytomer is determined by changes in the pattern of expansion during development. While this expansion proceeds in all directions, I have concentrated my attention on the major component, the extension on a line between the base of the phytomer and the point in the primordium which becomes the leaf tip. My work has shown that on this line extension varies with both position on the phytomer and with time.

I used pin holes to mark the phytomer in order to locate the extension, which I measured in terms of displacement from the base. The treatment and harvest procedure was repeated to establish displacement as a function of age as well as of position. My figures for displacement extend the descriptions of expansion already in the literature. Initially the whole length of the phytomer is expanding but expansion is more rapid near the leaf tip. The region undergoing the most rapid expansion moves basipetally through the phytomer and is followed by the distal end of the growth zone. The length of the growth zone increases and then decreases during the period of phytomer growth. Since phytomer growth varies with respect to time the results of experiments on it will be affected by the part of the growth pattern which is encountered at the sampling time and it is important that scientists are aware of this.

I concur with the argument that it is not satisfactory to discuss developmental changes in terms of position within a growing structure. In order to obtain an age function it is necessary to know the change in position of tissue between sampling times. I have shown that the referential pathline of the position of any particular point on a growing leaf as a function of time can be obtained if the displacement field has been established. I have used two referential pathlines as the boundaries of a growing segment of leaf lamina and this has enabled me to study the extension and differentiation of the epidermis as a function of age. It would be possible to follow the development of a section of the epidermis of the leaf by keeping it under observation during a period of time but whereas this is a suitable technique for roots, the early growth of most internodes and leaves takes place inside a bud. The enclosing structures would need to be dissected away and the dissection would probably cause damage which might affect subsequent growth. There was an effect on growth when I punched holes through the leaf spindle (Figs. 3.3, 3.4) but it should not have been as severe since only a small proportion of the plant was destroyed. The expanded photosynthetic surfaces remained in place and the supply of the photosynthates should have continued. In my pinhole technique the most important effect was the immediate response to wounding which retarded leaf extension for about eight hours.

A much greater distortion of the growth would be expected if internal tissues were exposed for measurement. In practice neither histological studies of internal tissues nor biochemical analyses can be conducted without removing the sample from the growing system. In order to study these characters it is therefore necessary to have a method such as that described here to relocate the equivalent section of tissue in other plants after suitable time intervals. My study of development in the epidermis was intended to test the value and validity of the method for locating a section of a growing organ over a period of time. The epidermis was chosen as being a relatively easily sampled and geometrically simple structure to work with. The method however is equally suitable for studies of other tissues and characters of the organ such as the development of leaf mesophyll and vascular tissue and the change in levels of biochemical factors.

The referential pathlines not only yield the position of a piece of tissue as a function of time, they also allow the expansion of the material to be calculated. When quantitative characters are studied the inflow of material is of interest and it can be measured only when the dilution which results from the concurrent increase in volume of the tissue is known. Thus for example, cell division rates could be obtained from values for cell density and for tissue expansion as a function of time.

With regard to cell division rates I have suggested that since there are upper and lower limits to the sizes of members of a particular type of cell in any given part of the growth zone, factors which influence tissue expansion combine with cell size determinants to have an effect on the frequency of cell division. Thus I suggest that where there is a high capacity for expansion in a piece of growing leaf tissue because it has a good supply of the raw materials for growth, there will also be an increase in the number of cell divisions which take place within it during development. This hypothesis could be investigated in future work using the technique described in this thesis.

This experiment was carried out under a chosen set of conditions. Altered conditions would be expected to change the overall rate of increase in length of the leaf but it would be useful to know more precisely what aspect of tissue expansion was being affected and at what stage in development the tissue was most responsive to environmental change. The chlorotic bands studied by Faris for example occupy short lengths of the leaf lamina. The tissue in a Faris band had presumably reached a critical stage in its development at the time of the sharp change in temperature. Using the technique for evaluating referential pathlines, experiments could be carried out to investigate the effect during growth of changes in environmental factors such as light, temperature and water level. This includes the effect of short term changes where the results might depend on the section of the phytomer involved in a particular phase of development at the time of the change.

The technique that I have described is then a valuable tool. Its drawback is the size of the population of plants that is required to provide values for the displacement field. The samples for analysis are taken from the plants used to measure displacement, but the validity of the results of the technique depends on there being either a very uniform population or a large number of replicate plants. It should be possible to eliminate a substantial part of the variability reported in these investigations by obtaining a more uniform set of seed, eliminating disease and avoiding the 'onion tip' problem (Chap. 3). In the absence of these factors which altered the growth of some individuals, the number of plants used in the experiment would have provided an acceptable level of variation about the mean. The second inadequacy in the experiment lay in the length of the intervals between sampling times. When the experiment was planned the very high rate of displacement of tissue in the upper part of the growth zone was not anticipated, otherwise the intervals would have been shorter. Additional sampling times should be inserted. If they were, there would not necessarily need to be a greater number of plants grown for the experiment. Attention could be concentrated on a particular part of leaf growth over a shorter period of time and the harvest times of the available plants could be planned to give more intensive sampling during that period.

This has been a preliminary investigation of a method of evaluating the distribution of growth through the length of a maize leaf and the pathlines of chosen points in the leaf fabric during growth. It has shown that the method is practicable and potentially useful. It has also led to a description of maize leaf growth which allows the significance of the previous work on specialized aspects of it to be properly appreciated. Finally it has indicated that cell division may be encountered throughout the growth zone of the maize leaf.

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