LEAF GROWTH AND DEVELOPMENT IN THE YOUNG TOBACCO PLANT

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Summary

The growth of the vegetative shoot of tobacco, *Nicotiana tabacum* L., and the associated changes in dry weight of the whole plant and its major parts are described. By means of serial reconstruction, the volume changes of successive young leaf primordia are followed, and this information is integrated with the dry weight data for older leaves.

Initially the relative growth rates of the primordia are very high (about 1·6 per day), but fall rapidly for 2 or 3 days to about 1·1 per day. At this level growth is exponential for a few days. The relative growth rate starts to fall again before leaf emergence, and then falls asymptotically to zero during leaf expansion.

Correlations are noted between major changes in relative growth rate and vascular differentiation in the primordium.

The eighth leaf is described in detail in terms of increase in cell number in both lamina and vein tissue. At first the primordium consists only of vein-like tissue; the lamina is initiated after 2 or 3 days, and its relative growth rate is higher than that of the veins for a considerable time. The proportion of cells in the vein tissue at leaf emergence is greater at higher leaf positions.

The pattern of leaf growth is contrasted with that for the wheat leaf, and it is suggested that the two patterns may be dependent on different modes of vascular differentiation in the shoots.

I. Introduction

Descriptions of shoot development which include the early phases of leaf growth have been made for various plants. Some refer to the "typical" leaf whose growth characteristics are derived from those of successive leaves on the shoot, or to single leaves at specified nodes. Such studies have been made of the tobacco leaf (Avery 1933), the strawberry leaf (Arney 1954), the leaflets of *Trifolium repens* (Denne 1966), the fifth leaf of *Lupinus albus* and the second pair and the tenth leaf of *Helianthus annuus* (Sunderland 1960), the first two leaves of *Phaseolus vulgaris* (Dale 1964), and the third leaf of cucumber (Wilson 1966).

Other workers have investigated the growth of the whole embryonic region comprising the vegetative shoot apex and its attached leaf primordia. Sunderland and Brown (1956) described the distribution of growth in the stem apex and the young leaf primordia of *Lupinus albus*, and later estimated respiration rates and protein production in the same system (Sunderland, Heyes, and Brown 1957). Williams (1960) described seedling growth in wheat, including a detailed study of apical growth, and Williams and Rijven (1965) supplemented this description by

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measuring changes in DNA, RNA, protein, and cell wall materials in the fourth leaf. The effects of temperature and light intensity on the growth of the shoot apex and leaf primordia in the tomato were reported by Hussey (1963*a*, 1963*b*, 1965).

Of these growth studies, few have dealt with leaf primordia smaller than those which could be dissected with ease from the shoot axis. Hussey succeeded in weighing dissected leaf primordia only one plastochron after initiation, that is, after the attainment of a recognizable form. Williams (1960) used a serial reconstruction technique to measure volume changes in intact leaf-bearing apices, and described the growth of the leaves in terms of changes in their relative growth rates. This method of description is a useful means of comparing the growth of successive leaves on the shoot and also of shoots of different plant types, and the technique allows consideration of very early stages.

The growth patterns disclosed in the wheat shoot led Williams to suggest firstly that wheat, a monocotyledon, might exemplify a universal pattern of leaf primordial growth, and secondly, that changes in the relative growth rate of the leaf might be related to morphogenetic development, for example, the onset of cell expansion, or the phases of vascular differentiation.

When the opportunity to study growth in tobacco arose, it was decided to study the vegetative shoot in the same way as Williams had done with wheat. Besides providing information of specific relevance to the growth of tobacco, this would enable comparisons to be made between the primordial growth patterns of a herbaceous dicotyledon and a monocotyledon.

II. Materials and Methods

(*a*) *Plant Culture*

Seeds of a blue-mould-resistant line of *Nicotiana tabacum* L. bred at the Tobacco Research Institute, Mareeba, were sown in soil in 6-in. plastic pots in a controlled-environment cabinet. The length of the light period was 14 hr; the radiation, from 28 125-W high-output fluorescent tubes and four 100-W incandescent bulbs, was about 10 cal/cm^2/hr, measured by a Kipp solarimeter. Temperatures were 32°C (day) and 26°C (night). The soil was watered with a nutrient solution [0·00325M KH_2PO_4, 0·0021M $MgSO_4$, and 0·0043M $Ca(NO_3)_2$].

Plants were harvested daily until the seventh day from sowing and thereafter at intervals of 2 days. They were thinned to one plant per pot on day 20.

(*b*) *Sampling and Measurement*

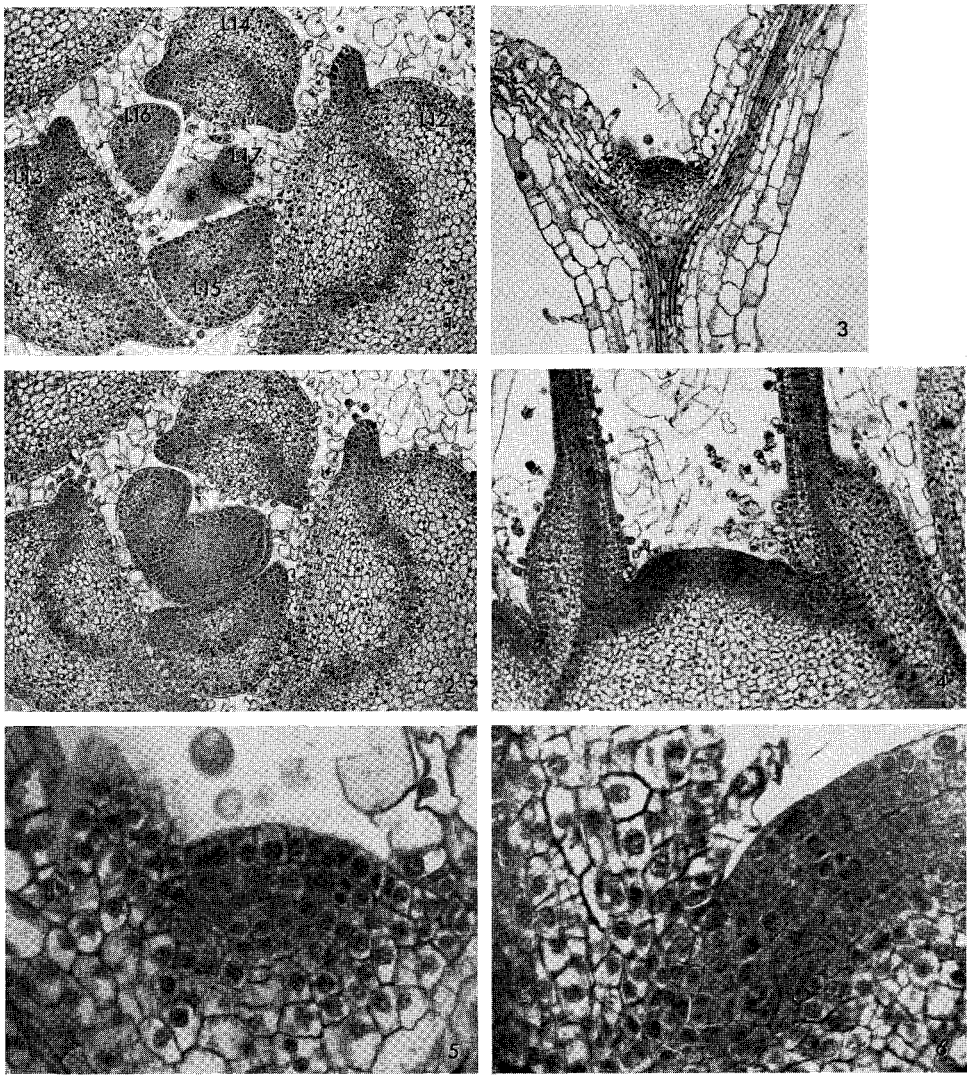
At harvest, five replicates were used for volume measurement and five for dry weight measurement. Leaf area measurements were made for all ten replicates on all leaves which had emerged from the bud (that is, which were unfolded and had reached an area of 1 cm^2).

Material for volume measurement was killed and fixed in formalin–acetic acid–alcohol (F.A.A.) under reduced pressure and stored in fresh F.A.A. Up to day 19 all plant parts were preserved; after that the shoot apex with all leaf primordia less than about 3 cm long was dissected out under a dissecting microscope and fixed in F.A.A.; one-half of each remaining leaf was also preserved for cell number estimation.

The plants used for dry weight determinations were dissected into all their major parts, particular care being taken with the smaller leaves so that their separation from the stem was as close as possible to the morphological limits used in the volume determinations. The stem part included the youngest three or four primordia which could not be separated. The total number of leaves and primordia was counted under the dissecting microscope. The parts were dried in an oven at 80°C, the smaller parts for 24 hr and the larger for 48 hr or more.

(c) *Volume Method*

The fixed, whole-stem apices with attached primordia were dehydrated by Johansen's butanol schedule (Johansen 1940), embedded in paraffin wax, and sectioned on a Reichert rotary microtome at 10 μ. The sections were stained with a safranin–orange G–tannic acid combination (Sharman 1943), and mounted in Canada balsam. For each harvest, transverse serial sections of four apices and longitudinal sections of one apex were made. The method of volume determina-



Figs. 1–6.—Sections through shoots of tobacco plants. **1**, Transverse section through day 23 shoot at tip of apex. ×70. **2**, Transverse section through the same shoot as in Figure 1, 20 μ below the apex. ×70. **3**, Median longitudinal section through day 7 shoot. ×70. **4**, Median longitudinal section through day 23 shoot. ×70. **5**, Median longitudinal section through day 7 shoot (as in Fig. 3). ×270. Periclinal division in the second tunica layer. **6**, A section adjacent to that in Figure 4. ×270. Periclinal division in the second and third tunica layers.

tion was essentially that of Williams (1960), in which the volume of a wheat leaf primordium was estimated from several cross-sectional areas equally spaced along the long axis of the primordium.

Area measurements of sections (Figs. 1 and 2) were made by placing a grid over the screen of a Reichert Visopan projection microscope and counting squares and tenths of squares covering the image of the section. Tracings of larger areas were measured with a planimeter; grid and planimeter measurements agreed within one percent. Figure 7 is a volume distribution diagram for an apex at day 15.

The lower limit of the blade, or free part of the primordium, was defined as the section in which the leaf base was half united with the stem (leaf 5 in Fig. 7). The buttress or attached part of the primordium was measured with an upper limit at the section showing half-junction with the axis and a lower limit at the section in which the perimeter of the leaf base was indistinguishable from that of the stem. The outer limit of the stem and the inner limit of the buttress was defined as the circle enclosing the procambial or vascular cylinder with the minimum radial extent of the cortex.

On the grounds that the primordium was derived from the outer layers or tunica of the apical meristem, and that these layers should be included as part of the leaf (Williams 1960), a volume calculated to represent the volume of the tunica was added to the direct volume measurement of each primordium. A definite limit to the extent of the tunica, which was available for wheat (Barnard 1955), was not so readily found in tobacco. Crockett (1957) and Bonnand (1959) report a two-layered tunica in *N. tabacum* and a corpus rather regularly arranged in layers, but do not comment on the extent to which these layers contribute to the primordia. In the present work, the epidermal layer did not show any periclinal divisions in any longitudinal section examined, and the hypodermal layer showed periclinal divisions only at the site of a newly initiating primordium. Apices with four leaves initiated showed no periclinal divisions in deeper layers, but these appeared in the third layer at the sites of leaves 5–17 approximately, and in the fourth layer at the sites of still later leaves (Figs. 5 and 6).

The mean depths of these cell layers contributing to primordia were 28 μ (two layers, in leaves 1–4), 36 μ (three layers, in leaves 5–17), and 50 μ (four layers, in leaves 18–24). The inconsistency of the increments is due to changes in the dimensions of the tunica cells, those in the seedling apex being about 14 μ deep while those in the older apices were about 12 μ deep (Figs. 5 and 6).

The angle α at which each leaf buttress was inserted on the stem apex was estimated graphically for one apex of each harvest, from a plot of the height of the apex against its radius in successive sections. This angle varied within one apex, being flatter at the tip of the apical cone, and also became generally flatter with increasing age. The area of that part of the tunica (A_T) which could be associated with each primordium was taken to be the vertical projection of the leaf base at half-junction (A_1 in Fig. 7) on the surface of the apical cone, i.e.

$$A_T = A_1 \sec \alpha.$$

The tunica volume (V_T) for each primordium was the product of the tunica area and the appropriate depth of the tunica (D) for the leaf position:

$$V_T = A_1 \sec \alpha \,.\, D.$$

For the smallest primordia every section was measured, and for slightly larger ones (up to 100 sections or 1 mm in length) usually about 10 equally spaced areas were measured. For the largest primordia, up to 1000 sections or 10 mm in length, the following regression equation derived from direct measurements was used:

$$\log_{10} Y = 1{\cdot}0076X - 0{\cdot}4250,$$

where Y is the volume and X is $\log_{10} L(A_1 + A_2)$, L being the length of the free part of the primordium, A_1 the area at half-junction with the stem, and A_2 the area half-way up the blade (Fig. 7).

Shrinkage during fixation and dehydration of the material must be expected, but little distortion of cell walls was seen. Intercellular spaces were noticeable in the oldest stages of leaves 1 and 2, so dry weight measurements are more meaningful than volumes in the data from these harvests (day 11). Most of the visible distortion was caused by imperfect expansion of the sections from time to time, so care was taken to avoid making area measurements on such sections.

(*d*) *Estimation of Cell Number*

Estimations of cell numbers were made for leaves 1, 2, 8, and 14, using sections for unemerged leaves and disks cut from unfolded leaves.

In sections, the method of Williams (1960) was used; the mean length of the nuclei in longitudinal section was 7 μ, so that there were 10 whole nuclei for every 17 nuclei counted in each section. The numbers of lamina cells, vascular cells, and the total numbers of cells were calculated in this way. The term "vascular" was applied to all tissue forming the midrib and principal veins, that is, those which protruded from the "lamina" tissues (see outline diagrams in Fig. 7). In leaves 8 and 14 the volumes of lamina and vascular tissues in the primordia were also estimated separately.

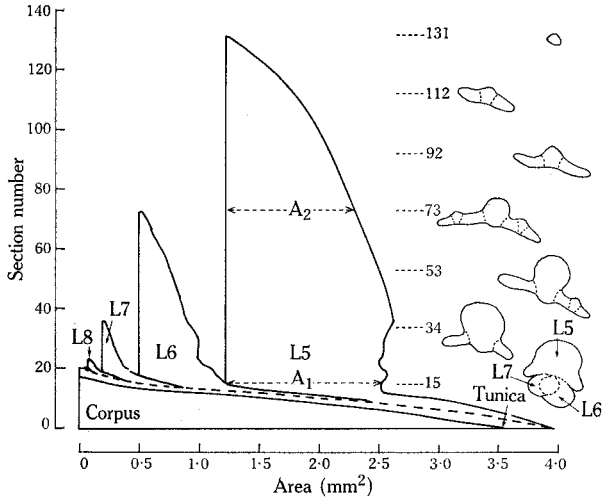


Fig. 7.—Diagram of volume distribution in a tobacco shoot at day 15, showing the method of reconstruction from area measurements. A_1, area at half-junction with axis; A_2, area at mid-section; L5, L6, etc., successive leaf primordia; – – – outer limit of tunica; ——— inner limit of tunica. Outline drawings at right are of sections of leaf 5 at the positions indicated.

Palisade cell numbers were counted at the same leaf positions in unfolded, preserved leaves. Three disks, about 8 mm in diameter, were cut from each of three positions (near the base, the middle, and the tip), decolourized in warm 95% alcohol, and simultaneously stained and cleared in 0·25% aniline blue in lactophenol for 5 min at 60°C (Arney 1954). The tobacco leaf has a palisade layer only one cell deep; good paradermal views were obtained in cleared disks by focusing through the upper epidermis. Cells were counted under a Reichert Biozet microscope with a known field area, and total palisade cell numbers were estimated from the leaf area. No corrections were made for the area of the vascular tissue.

Leaf areas were calculated from a formula established at Mareeba for the same breeding line:

$$A = 0{\cdot}66 L_M \,.\, B,$$

where A is the leaf area, L_M the length of the midrib, and B the maximum leaf breadth.

(e) *Phloem Differentiation*

The rate of differentiation of the vascular system in the primordia was also investigated. In sections near the leaf bases the presence of phloem elements, identifiable by their thick walls and lack of nuclei (Esau 1938) was recorded. Three stages were recognized:

(1) several immature sieve tubes present;

(2) a few mature sieve tubes present;

(3) many (more than 10) mature elements present.

III. Results

(a) *Whole Plant*

The young seedling possessed a well-developed radicle and cotyledons by the fourth day after sowing. No leaf primordia were present in the seed. The first primordium was visible on day 5 and the second on day 6. Up to about day 19 leaves were initiated at intervals of about 1·2 days; the rate of initiation then increased slightly to one primordium in about 0·9 days [Fig. 8(*a*)]. The plants remained in a rosette state through most of the experiment; the stems began to elongate at about day 28, when 21 leaves had been initiated.

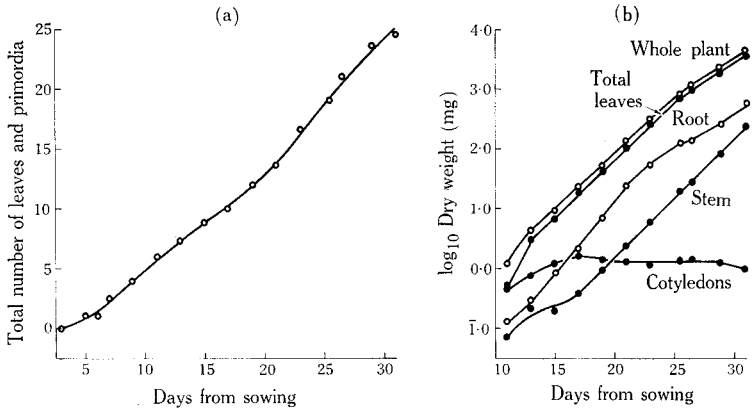


Fig. 8.—(*a*) Total number of leaves and primordia plotted against time in days from sowing. (*b*) Dry weights on logarithmic scale of plant parts and whole plant of tobacco.

The dry weights of the whole plant and of the major plant parts are plotted on a log scale in Figure 8(*b*). Dry weights for the youngest primordia still attached to the stem were estimated from the volume data, and the stem and leaf dry weights were corrected on this basis. The cotyledons increased in weight until about day 15 and then remained constant. The weight of the leaves had already surpassed that of the cotyledons at the time of the first harvest, and quickly dominated the growth of the whole plant. This growth is seen to be exponential between days 13 and 25,

with a fall in the rate thereafter. The root system was remarkably small at first, but grew exponentially at a somewhat greater rate than the leaves until day 21, after which the rate was less than that for the leaves. The data for the stem fraction was erratic at first, but its rate of growth thereafter was rather constant.

The leaves, including the cotyledons, account for at least 80% of the plant, and the differential between root and leaf growth gains expression in a maximal root weight ratio of 0·19 on day 21. The stem accounted for only 2% of the dry weight of the plant for the greater part of the experiment, but rose to 5% by day 31. This rise is correlated with the onset of stem elongation mentioned above.

(*b*) *Leaf Growth*

Changes in the shapes and arrangement on the apex of primordia at different times during the plant's life are reflected in volume distribution diagrams for days 7, 15, and 31 in Figure 9, and in median sections through day 7 and day 23 apices in Figures 3 and 4.

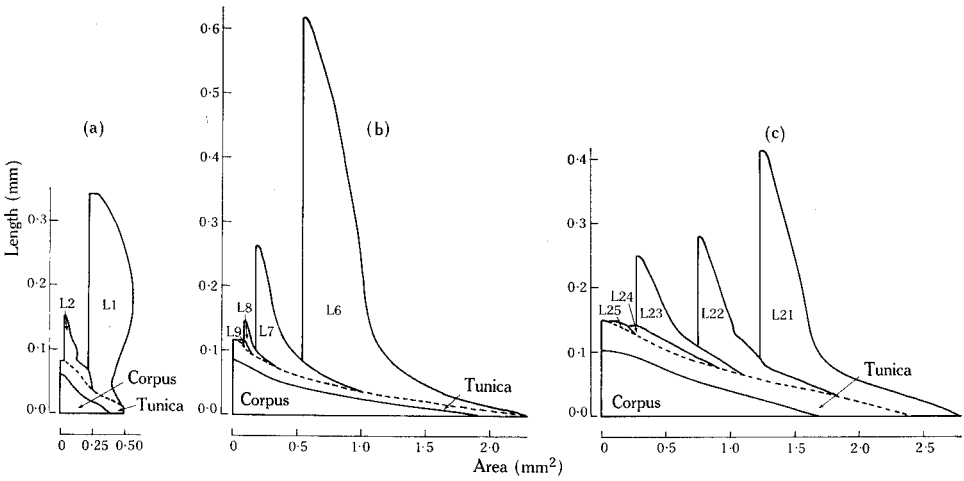


Fig. 9.—Diagrams illustrating volume distribution in tobacco shoots at day 7 (*a*), day 15 (*b*), and day 31 (*c*) after sowing.

Leaves 1 and 2 [Fig. 9(*a*)] were rather different from the later leaves, being almost opposite on the axis, almost circular in shape, and attached to the narrow apical cone by broad shallow buttresses. With higher leaf position, the volume distribution down the long axis of the primordium altered from the blunt-tipped shape of leaf 1 to the broad-based, elongated shape of leaf 6 [Fig. 9(*b*)] with the buttress also extending further down the axis. However, between day 23 and day 31 this tendency was partly reversed in newly formed primordia, and lateral growth was more pronounced than vertical growth, so that the diagrams were more squat in shape [Fig. 9(*c*)]; the bases were broader, with relatively shallower buttresses enclosing a broadening apex (Fig. 4).

In Figure 10 the volume changes for the individual primordia and the subsequent dry weight changes for the unfolding leaves are plotted on logarithmic scales. The dissection of the plants and the recording of data in later harvests took a considerable time, so that the time of the dry weight harvest differed by up to 12 hr from the time of the corresponding volume harvest. Appropriate allowance was made for this in plotting the experimental data. Both dry weight and volume were available on at least one occasion for every leaf position, providing a basis for linking the two sets of data. The mean density over the whole range of leaf positions at the linkage points (for example, on day 13 for leaf 3 and day 23 for leaf 8), was fairly constant at about 0·25 mg/mm^3; there was no consistent drift in density with rising leaf position. The curves of Figure 10 were fitted visually, the fact that they constitute a family of curves being kept in mind.

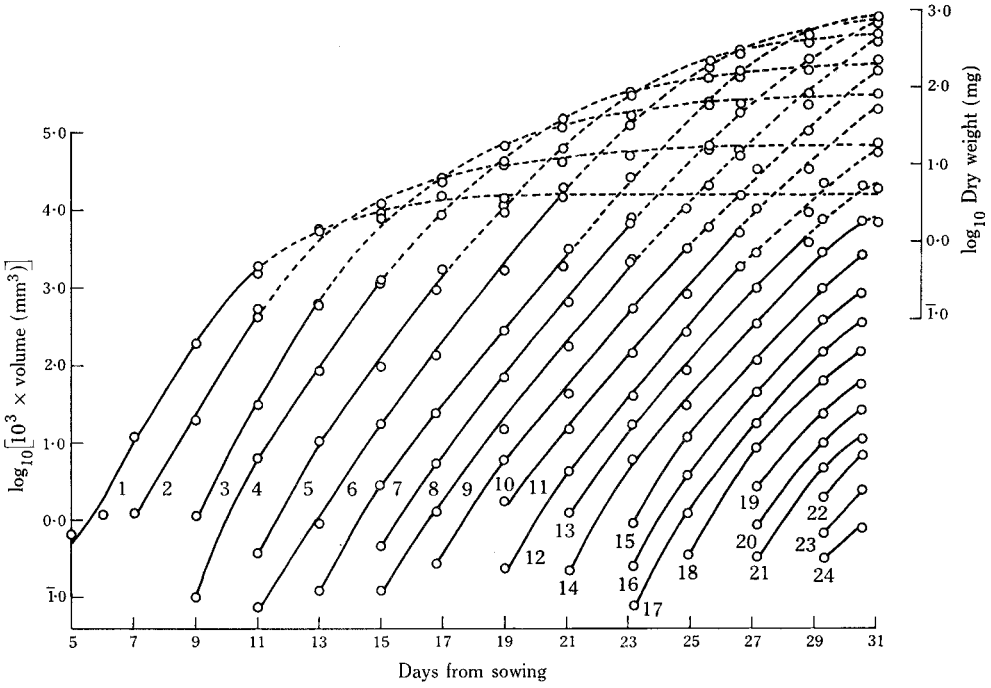


Fig. 10.—Dry weight and volume for individual leaves (1–24) plotted on logarithmic scales. Leaves numbered in order of appearance.

Though the growth of leaf 1 had ceased by about day 21, and of leaf 2 by about day 29, the other leaves were still increasing in dry weight at the end of the experiment. The maximum leaf size clearly increased with higher leaf position, though evidence from a later experiment shows that in the uppermost leaves the maximum size eventually decreases.

The volume values for each primordium were got by adding the calculated tunica volume to the blade volume estimated from area measurements. At first, the tunica fraction may make up as much as 90% of the total primordium volume; this proportion decreases rapidly with increasing primordium volume to about 1·5%

at leaf emergence. Figure 11 illustrates for two representative leaves (6 and 14) the volume changes with time in the blade plus buttress, the tunica alone, and the total primordium. It also shows the effect on total volume change of arbitrarily increasing the tunica depth from three layers (36 μ) to five layers (about 60 μ). It will be seen that, while an underestimation of the contribution of the tunica, or its omission altogether, would grossly underestimate the early volumes, the general pattern of relative volume change is maintained, that is, after a steep initial portion the curve straightens to give an exponential phase of growth.

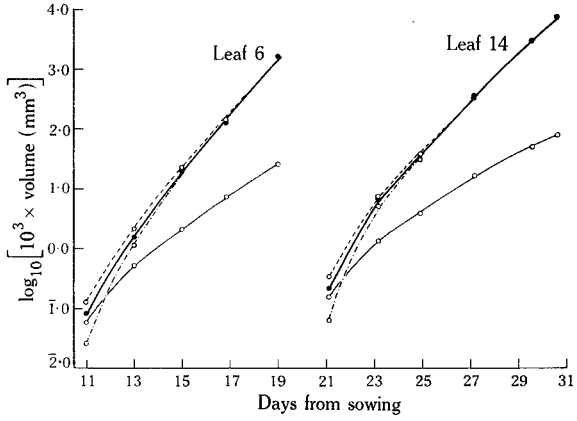


Fig. 11.—Effect on $\log_{10}$ primordium volume of changing depth of the tunica layer in leaves 6 and 14. O–·–·–O Blade plus buttress; O———O three-layered tunica; ●———● blade, buttress, and three-layered tunica; O– – –O blade, buttress, and five-layered tunica.

The implications of Figures 10 and 11 may be read more easily from Figure 12(*a*) which presents the changes with time in the relative growth rate R of the successive leaves, R having been derived by graphical analysis from the slopes of the curves in Figure 10. To avoid confusion, curves for alternate leaves only are presented.

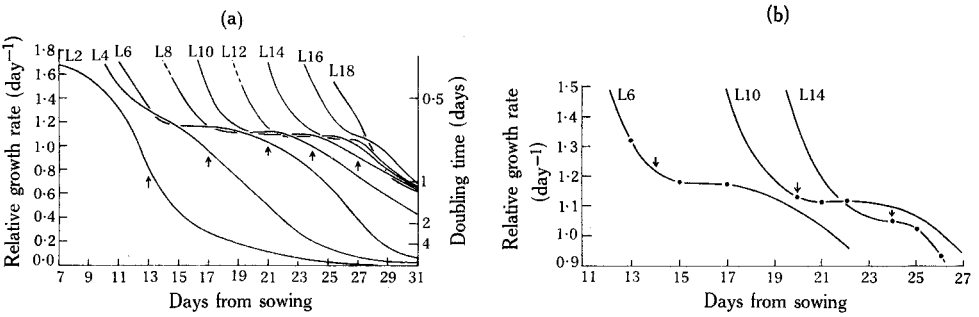


Fig. 12.—(*a*) Change in relative growth rate of individual leaves as a function of time. Alternate leaves only are shown. Arrows mark time of leaf emergence. (*b*) Relationship between changes in relative growth rates and appearance of lamina and phloem in leaves 6, 10, and 14. Arrows indicate time of first appearance of lamina; closed circles indicate three stages in sieve-tube development (see text).

The R values for all leaves fall from high initial values of about 1·6 units, which correspond to doubling times of only two-fifths of a day. The pattern of change thereafter varies with leaf position. Thus, with leaves 1 and 2 there is virtually

no check in the asymptotic fall to zero, but this fall is progressively checked at values ranging from 1·3 to less than 1·1 units to give periods of up to 4 days of exponential growth in leaves 4–15. In later leaves there is little suggestion of any exponential phase of growth, though the experiment did not continue long enough to confirm the expected asymptotic fall to zero. Where an exponential phase of growth was present, it tended to end shortly before leaf emergence.

(c) *Phloem Maturation*

Three stages of phloem development are indicated on the relative growth rate curves for three of the leaves in Figure 12(*b*). The time at which the lamina was first seen developing is also shown in Figure 12(*b*). Since the lamina when first seen was already relatively well-developed (see Fig. 1, leaves 14 and 15), it may be assumed that the initiation of the lamina had begun earlier, probably as long as 24 hr previously in some cases, and phloem differentiation could be expected to have begun at least a few hours before detection.

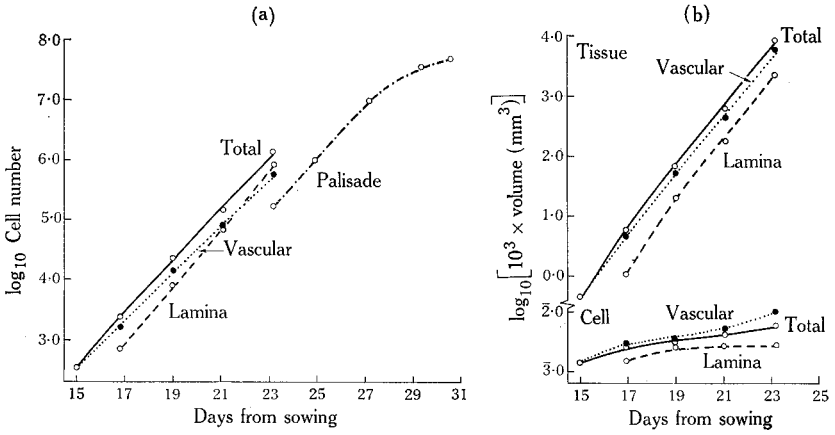


Fig. 13.—(*a*) Vascular, lamina, palisade, and total cell numbers for cell 8. (*b*) Vascular, lamina, and total primordium tissue volumes, and mean cell volumes for cells in these tissues in leaf 8.

Three main points may be noted in Figure 12(*b*). Firstly, the time interval between the appearance of immature phloem and the differentiation of mature sieve tubes decreases with higher leaf position. Secondly, the appearance of the lamina is fairly closely correlated with the appearance of immature sieve tubes. Thirdly, the start of sieve tube differentiation tends to precede the start of the exponential phase of primordium growth.

Esau (1938) has shown that the phloem in tobacco differentiates acropetally from the axis into the leaf base, so the recognition of sieve tubes in the base indicates a connection between the primordium and the vascular system. Such a connection should provide a more efficient supply of substrate for growth than that available through undifferentiated meristematic tissue, and it is possible that such an improvement in the substrate supply could cause the check in the fall in R which occurs at about the same time, and allow the simultaneous development of the lamina.

(*d*) *Cell Numbers*

Figures 13(*a*) and 13(*b*) summarize on a log scale the data for cell numbers and cell sizes for leaf 8, and also the volume changes in the vascular and lamina parts of the primordium. From the absolute values of Tables 1 and 2, it will be seen that the

TABLE 1

SUMMARY OF LENGTH, TISSUE VOLUME, CELL NUMBER, AND CELL VOLUME FOR PRIMORDIA BEFORE EMERGENCE OF LEAF 8

	Time (days from sowing)				
	15	17	19	21	23
Length (mm)	0·02	0·27	0·90	2·50	15·22
$10^3 \times$ tissue volume (mm^3)					
Total	0·62	6·17	69·32	634·39	8570·7
Vascular	0·62	5·02	52·94	451·87	6251·8
Lamina		1·15	16·38	182·52	2318·9
$10^{-3} \times$ cell number					
Total	0·43	2·46	22·78	151·84	1496·21
Vascular	0·43	1·72	14·60	83·12	570·32
Lamina		0·74	8·18	68·72	925·89
Palisade					172·41
$10^6 \times$ cell volume (mm^3)					
Total	1·35	2·52	3·04	4·18	5·73
Vascular	1·35	2·90	3·63	5·44	10·96
Lamina		1·55	2·00	2·66	2·50

lamina, which was initiated on the flanks of the elongating cone-shaped primordium 2–3 days after leaf initiation, contained shortly before leaf emergence the greater proportion (about 62%) of the total cells, while making up only about 28% of the

TABLE 2

SUMMARY OF LENGTH, DRY WEIGHT, LEAF AREA, AND PALISADE CELL NUMBER FOR UNFOLDING OF LEAF 8

	Time (days from sowing)				
	23	25	27	29	31
Length (mm)	15·22	30·00	80·00	160·00	200·00
Dry weight (mg)	1·94	25·65	50·37	257·39	629·71
Area (cm^2)	0·29	1·74	23·36	110·88	164·82
Palisade cell number (million)	0·17	1·02	9·96	38·85	50·63

total primordium volume. The mean cell volume over the same period increased only fourfold; this increase was due mainly to cell expansion in the vascular tissue as the mean volume of the lamina cells barely doubled.

At the end of the experiment (day 31) when the leaf had reached an area of about one-third of its final area (estimated from unpublished data from a later experiment), the leaf size was still largely determined by the number of cells, as indicated by the number of palisade cells. The rate of increase in cell number showed a decline at about this time, however, so it may be assumed that the subsequent increase in leaf area was due chiefly to cell expansion.

If it is assumed that all cells had been dividing in all parts of the apex, and that cell number $= 2^n$ where n is the number of cell generations, there were 8·4 generations in leaf 8 before it was recognizable as a primordium, and a further 12 generations before leaf emergence. The mean cell generation time calculated for this last interval (day 15–23) is 16 hr. This is considerably less than the 27 hr calculated by the same method by Williams (1960) for wheat grown at 20°C, though the number of generations before the primordium is seen is close to that in wheat (8·6). The high initial values for R, and the intensity of staining of the cytoplasm in cells in the vicinity of the first periclinal divisions in the apex (see Figs. 2–5) point to a high rate of cell division just before the appearance of a primordium.

The growth of leaves 1, 2, and 14 will not be described in detail but some comparisons may be made with that of leaf 8. At comparable volumes, all these leaves had about the same total cell numbers, though leaves 1 and 2 unfolded at much smaller sizes than did the others and cell division in them stopped earlier. Leaf 8 emerged about 8 days, and leaf 14 10 days after initiation. Just before emergence, the two leaves had similar volumes and total cell numbers, the cell number being about 1·5 million. However, there was a marked change with leaf position in the distribution of cells between the lamina and the vascular tissue.

The proportion of vascular cells was progressively higher in leaves further up the stem. In leaves 1 and 2 the proportion of vascular cells at unfolding was about 20%, while in leaf 8 it was about 38% and in leaf 14 about 52%.

Thus these leaves at three positions (counting leaves 1 and 2 together since they are very similar) emerged with an increasingly better-endowed vascular system. However, these results do not give any indication of the numbers of cells engaged in active transport, since the vascular cell numbers included all tissues of the veins.

From Figure 13 and the trends mentioned above, it follows that for a considerable time R for the lamina must have been higher and that for the vascular tissues lower than R for the leaf as a whole.

IV. Discussion

Clowes (1961) has pointed out that "leaves cannot be regarded as distinct from stems in their early stages of development"; the young primordium closely resembles a stem during the first two plastochrons, and the midrib retains many stem-like features, distinguishing it from the lamina which arises from it. Williams (1956) has commented on this physiological distinction between veins and mesophyll, noting that their integration within one organ must lead to some modification of each by the other.

However, there can be no doubt that the initiation of a leaf primordium is the result of an exceptional burst of growth in a restricted area of the otherwise slowly

growing stem apex (Clowes 1961; Denne 1966). Estimations of the intensity of this growth will have a more precise meaning if based on all the tissues involved than if based only on tissues protruding from the apical dome.

In this study of growth in the tobacco shoot, the internal limit of each primordium is based on specified layers of the tunica (two, three, or four according to age) as these appear at the earliest recognizable stage. As development proceeds, such a limit becomes artificial because of the obvious continuity of structure and function across it. Nevertheless, estimations of volume change based on such limits are believed to be fairly objective if only because it seems unlikely that there can be an actual migration of cells into the developing primordium. It has been shown (Fig. 11) that the form of the curve for volume increase is little affected by assuming the tunica to consist of three or five layers of cells. However, the initial slopes (R values) of the curves are more affected and would be very different from those for blade plus buttress alone (usually buttress alone at this point), so there is need for a careful assessment of the number of tunica layers likely to contribute to primordium growth.

The leaf primordia produced on the apex seem to differ very little during early stages with respect to volume, cell number, and their high relative rates of growth, R. These rates fall rapidly for 2 or 3 days before entering a period of constancy implying fairly strict exponential growth. In leaf 8 this period lasts for 3 or 4 days, after which R starts to fall prior to leaf emergence. During emergence and the later expansion of the lamina R falls asymptotically to zero over quite a long period.

This generalized account of leaf growth in tobacco does not hold for the early seedling leaves, and the exponential phase seems to disappear for leaves above position 15 or 16.

The increase in cell number, as far as can be shown from only two leaf positions, closely parallels the increase in total size [Figs. 13(a) and 13(b); Tables 1 and 2], but there is also a steady increase in mean cell volume. Information on increase in cell numbers after unfolding is available for leaf 8 only, and then only for palisade cells, but there is no indication that any large changes in the rate of cell division take place when the leaf emerges into the light, nor is there any marked increase in mean cell volume. These results are similar to those obtained by Sunderland (1960) for *Lupinus albus* and *Helianthus annuus* leaves, but in marked contrast to the results of Denne (1966) who found large increases, at least in cell area, shortly after emergence of leaflets of *Trifolium repens*.

The relative importance in the tobacco leaf of the two periods of development (before and after emergence) may be compared with the results of Sunderland and Denne. In the fifth lupin leaf, 14 million of the 15 million cells, and in the tenth sunflower leaf, 23 million of the 25 million cells (about 93 and 92% respectively), were formed after leaf emergence. The eighth tobacco leaf at emergence had produced 1·5 million cells, of which only about 172,000 were palisade cells. At the end of the experiment, when cell division in the palisade had practically ceased, the total number of palisade cells was about 50 million, about one-fifth of the total number of cells in the lamina. Thus more than 99% of the palisade cells were produced after

emergence, and though cell division probably persists longer in the palisade than in other tissues (Avery 1933), a very high proportion of the other lamina cells would have been produced after leaf emergence.

Denne (1966) does not give direct data for total cell numbers in the *T. repens* leaflet, but it seems that about 77% of the palisade cells and about 63% of the total number of cells were formed after emergence. The emerging leaflets contained about 1·5 million cells, including about 500,000 palisade cells.

Once the tobacco leaf had attained a substantial number of cells, the prolonging of cell division through even one more cycle would have had a large effect on the final cell number, and on the final leaf area. The present results support the view that the events occurring after leaf emergence are of more importance in controlling the final size of the leaf than are those before emergence, interesting though these may be.

The major changes in R for a single leaf are associated to some extent with morphological developments within the apex and the leaf. The cause of the initial high level of R is not known, but the subsequent fall in R may be due to increasing inefficiency in the supply of substrate to the growing primordium. Priestley (1929) points out that, in a meristematic tissue, substrate molecules will tend not to be passed to a cell through one which has similar requirements for them. By the time a primordium is visible, the apex itself is expanding in preparation for the initiation of the next primordium. Three developmental events are correlated fairly closely with the change in R from a falling to a steady rate. Thus the next consecutive primordium appears on the far side of the apex, within the leaf itself the lamina appears on the sides of the midrib, and a few nearly mature sieve tubes are detectable in the basal region [Fig. 12(*b*)], having developed acropetally from the stem into the leaf (Esau 1938).

Though it is difficult to see how the first of these events could affect the growth potential of the previously formed primordium, the presence of functional phloem suggests that the halt in the fall in R, expressed morphologically in the outgrowth of the lamina, is made possible by the establishment of a more efficient supply of substrate than that available by diffusion through meristematic cells. The establishment of such connections has been put forward as a likely prerequisite for the increase in R in the wheat leaf (Williams 1960). This interpretation does not exclude the operation of a hormonal control mechanism, since gradients in hormone concentration, though not yet established, are no doubt present in the shoot apical system.

The end of the phase of exponential growth when R again begins to fall, is correlated for a leaf at position N with the initiation of leaf $N+5$, that is, the leaf immediately above it on the axis. This may be seen in Figures 1 (L12 and L17) and 12(*a*).

The growth pattern of the leaf primordia in the tobacco plant was different from that in wheat in several ways. The initial values of R for tobacco leaves (about 1·60 per day) were much higher than those for wheat (approximately 0·5 per day) and there was a single phase of exponential growth with no increase in R at any time. The wheat leaf primordium "tends to show an early phase of exponential growth

followed by a substantial *increase* in the exponent to what may well prove to be a second, though briefer, phase of exponential growth" (Williams 1960). The high relative growth rates in young tobacco primordia may well be supported by high levels of substrate in the apex. Phloem differentiation is acropetal, and primordia high on the apex develop vascular connections at early stages. In wheat, on the other hand, where phloem differentiation is basipetal, the apex and the first four leaf primordia are supplied by diffusion and have no direct vascular connection with the rest of the plant (Sharman and Hitch 1967).

Thus one general pattern of leaf growth seems unlikely, but in view of the correlations discussed above between vascular differentiation and changes in the relative growth rates, the different modes of vascular differentiation — acropetal in tobacco (Esau 1938) and basipetal in wheat (Sharman and Hitch 1967) — may well result in different potentials for growth in and around the two shoot apices.

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VI. References

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