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THE DEVELOPMENT OF FOLIAGE LEAVES

by

Beryl Taylor Mounts

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INTRODUCTION

Beryl Taylor Mounts

This paper presents the results of a study of leaf development in two mesophytic plants, *Vitis vulpina* L. and *Catalpa bignonoides* Walt. The investigation was undertaken with the hope of gaining additional information on the development of the islet tissue which lies between the veinlets and constitutes the main body of the leaf. *Vitis* was chosen as one type because new leaves are produced throughout the summer months. *Catalpa* on the other hand has limited leaf development, normally producing but one set of leaves each year. Mesophytic plants were selected since they are related to intermediate habitats and their leaves may be sectioned readily. Facts concerning mesophytic leaf development establish a basis for comparisons with hydrophytic, xerophytic, or other types.

The leaf, which is perhaps the most important vegetative organ of the plant, is a highly specialized structure. Its work involves a large superficial area, mutitudinous openings for gaseous exchange, an extensive series of internal air spaces, and a ramifying system of veins and veinlets for conduction and support. The whole structure is denied adequate protection since it cannot function with other than a transparent covering. Such an organ encounters numerous problems in the performance of its major functions as it must expose extensive areas to external conditions while denied the protection of other plant parts.

Detailed studies of leaf development are necessary to round out our knowledge of the higher plants. Information on the normal course of leaf development affords a basis for interpretation of abnormal growths, for the study of changes due to environmental conditions, and may possibly strengthen conclusions regarding phylogeny. For practical workers also it is important to know at what stage the stomata become functional and the structure of the leaf at varying ages. Especially helpful is such information to all botanical workers if it enable one to correlate internal structure and developmental stages with obvious external changes or characters of the leaf.

Papers concerned with leaf development while numerous have dealt chiefly with their external aspects. Such of the developmental investigations as involved internal structure have been concerned mainly with the venation or with the vascular system in its relation to leaf form. Deinega (2) expressed the view that the mid-vein of the leaf is laid down first, then the lateral veins, and that the mature shape and venation of the leaf depends on whether elongation of the cells is greater in the longitudinal or in the transverse direction. Freundlich (4) stated that the mid-vein appears first; that there are lateral veins of the first, second and third order, and that the kind of vascular development is dependent upon the position of the meristem. Many papers have dealt with the structure of the mature leaf and especially with the morphology of the leaf in relation to its habitat but have not discussed the development of these organs. Goebel (5) who reviewed the history of the study of leaf development and discussed the growth of the leaf as a whole states "We may say in general that parts which have earlier functions to perform appear earlier." Development of the islet tissues has received relatively little attention with modern methods of study and interpretation.[1]

[1] Foster's paper (Foster, Adriance S. Investigations on the morphology and comparative history of development of foliar organs. I. The foliage leaves and cataphyllary structures in horsechestnut (*Aesculus Hippocastanum* L.) Am. Jour. Bot. 16:441-501. 1929), published since this study was begun, discusses certain phases of foliar development. The bud scales of *Vitis* are found in the winter bud only, are without chlorophyll and not persistent, so are not comparable to the cataphylls of *Aesculus*. His discussion of the development of the blade is chiefly on the order in which the leaflets appear. *Vitis* and *Catalpa* with their simple blades do not have separate leaflets but it is interesting that in both the apical tip of the leaf develops first, paralleling the early growth of the median leaflet in *Aesculus*. Foster does not follow the development of the islet tissue.

MATERIAL AND METHODS

The investigation of *Vitis* was begun in June and series of buds and leaves in various stages were collected at intervals during the summer. During the winter buds were collected periodically and from twigs kept in the laboratory buds were removed and preserved as they began to swell or open. Collections were continued through the following spring and summer. Buds of *Catalpa* were taken in February and collections of buds and leaves in various stages were continued until June.

For microscopic study material, killed in 1% chromo-acetic and imbedded in paraffin, was cut 5-20 μ in thickness. In addition to the usual transverse sections others were cut parallel with the surface of the leaf. The latter proved especially helpful in studying the palisade tissue and the relations of interior cells to epidermal layers. Longitudinal sections of buds of various ages were necessary for the earlier stages of leaf development. Several stains were used but Safranin-Haematoxylin proved the most useful in this study. All drawings from sections were made by means of a Spencer drawing apparatus; these preliminary drawings were completed in detail, cell by cell, with the compound microscope under oil immersion lens.

In determining rate of growth certain tagged leaves were measured daily. The first series of measurements, on very small leaves, involved length only, but later series included both length and breadth. In determining distribution of growth for leaves over 3 cm. in length a special rubber stamp was devised for marking the leaf surface. This stamp had a ruled surface 8 x 8 cm. divided into 5 mm. squares, and was mounted on a wooden block having a thick rubber pad between the block and the stamping surface. A soft support, slit at one side to receive the petiole, was placed under a leaf while the upper surface was being marked by the stamp. After considerable experiment a mixture of three parts India ink and one part glycerine was found satisfactory for marking leaves. This medium did not harm the leaves nor was it washed off by the heaviest rains.

Permanent records of the further growth of leaves thus marked were made in the following manner. Unexposed and undeveloped photographic dry plates were fixed, washed and dried. A plate was held firmly over a leaf, supported by a pad below, and the ink lines of the stamped area were traced with a sharp steel point in the transparent film on the plate. In this way a series of records could be made during the expansion of a leaf blade, showing the relative growth of all its parts. The lines on the plate were then intensified with India ink and the plates numbered and filed for reference.

LEAF DEVELOPMENT

I. *Vitis vulpina* L. (Wild grape)

General description of the mature leaf

The mature leaf of *Vitis* may be 20 x 22 cm. but the average of those studied was about 11 x 12 cm. This leaf is usually three-lobed, with broad rounded or truncate sinuses and large acute or acuminate teeth (fig. 6). Rarely there are five instead of three lobes, and in some the margin is not lobed at all. The mature leaf of *Vitis* averages 144 μ in thickness, the upper epidermis making up about 10% of this total, the palisade 35%, the spongy mesophyll 46% and the lower epidermis 9%. The upper epidermal cells are relatively large, averaging 32 μ in surface width. The cells of the lower epidermis are somewhat smaller, about 26 μ, their outer walls being less convex and less heavily cutinized than those of the upper epidermis. Stomata are confined strictly to the lower epidermis, (fig. 8).

The palisade cells, consisting of one row, are slender, usually slightly bell-topped, average about 9.6 μ in diameter near the middle and 51 μ in length. They do not form a compact tissue as there are intercellular spaces about each cell. Sections cut parallel to the surface disclose certain relations not evident in the transverse view. The palisade cells, seen thus in cross section are approximately circular in outline except in the junction plane with the upper epidermis. They are always at least partly free from lateral contacts (compare figs. 8 and 10).

The spongy mesophyll tissue consists originally of three cell layers, or rarely four, but at maturity these cells are loosely arranged and irregularly elongated in shape, usually with the longer axes parallel to the epidermis (fig. 8). In sections cut parallel to the surface the cells of the spongy mesophyll appear as a course network with lobes drawn out about the air spaces. These connected intercellular spaces occupy over 50% of the total mesophyll volume (fig. 8).

Early Development of the Leaf

The winter bud of *Vitis* is covered with three or four hard brown scales and five or six densely hairy bracts. The most advanced foliage leaves in the winter bud are mere rudiments about 1 mm. long. The leaf starts as a lateral protuberance near the vegetative point (fig. 1). The stipules appear on its sides as two small papillae which grow more rapidly and are longer than the leaf itself until the blade is 2 mm. long, after which their growth is relatively slower, reaching their maturity by the time the leaf blade is about 8 mm. long. The stipules when mature are about 5 mm. long, 4-5 cells thick and contain no chlorophyll. They protect the young leaf in the bud and after the leaf emerges dry up and drop off.

The petiole is differentiated from the leaf base when the stipules are about 0.5 mm. long. The petiole remains short during the early development of the blade, beginning to elongate slowly when the lamina is about 3 mm. long. About the time the blade reaches 10 mm. in length the rate of elongation becomes rapid, forming a petiole one-half to two-thirds the length of the blade.

The apex of the blade early pushes out into a long rounded tip which lengthens rapidly and makes up about half the length of the blade when it is 2 mm. long. Small papillae appear along the lateral margin of the blade shortly after the tip begins to push out, the larger of these papillae forming the lateral lobes, the smaller ones the teeth (fig. 2). The meristematic tissue is differentiated into six (rarely seven) distinct layers before any specialization is evident. The cells of these layers at an early stage look alike and are distinguishable only by their position in cross section.

The mid-vein begins to differentiate in the second layer below the upper epidermis when the blade is about 1 mm. long. Its appearance is early indicated by a modified staining reaction and soon also by differences in the shape and arrangement of the cells. Annular markings on the tracheal tissue of the mid-vein are distinct by the time the blade reaches 2 mm. in length. The major lateral veins running to the margins appear next. While the development of the veins was not followed in detail in this study it should be noted here that new veinlets form from the uppermost mesophyll layer.

Differentiation of the Tissues of the Leaf Blade

This study has been concerned mainly with the development of the leaf tissue within the islet which is bordered laterally and supported by veinlets. These areas have most of the chlorophyll bearing cells, stomata, and internal air spaces. In brief, the islet is the chief working portion of the leaf, all other parts being auxiliary to it.

The Epidermal Cells

The cells of the several layers begin to differentiate when the blade is 5-7 mm. long (fig. 5). The cuticle begins at this stage as a slight thickening on the outer wall. Mitoses in the upper epidermal cells continue, as evidenced by figures, until the blade is approximately 20 mm. long. All cells of this layer remain about the same size until division ceases but the lower epidermal cells, which have for a time a deeper staining reaction, continue dividing somewhat longer. By the time the blade is about 30 mm. long this deeper staining reaction is localized in scattered cells which are probably to form stomata.

Cell enlargement begins as mitoses cease. The epidermal cells through this phase increase little in thickness but their lateral expansion is marked. The cells of the upper epidermis at the time enlargement begins average 7 μ in width; in the mature leaf they average 32 μ, an increase of 357% in diameter, making 21 times the original area. In the mature leaf the lower epidermal cells average 26 μ across, an increase of 270% in diameter or about 14 times in area. As stated above mitoses continue longer in the lower epidermal layer, thus there are more cells in a given area of this tissue than in the upper epidermis.

The earlier stomata make their appearance when the blade is some 14 mm. long. Some stomata begin to open when the blade is 35-40 mm. long but all do not become functional at one time. A leaf 50-60 mm. has functional stomata separated by only a few intervening cells from guard cells in the initial stages of development (fig. 7).

The Palisade Layer

The cells of the palisade as differentiation begins, appear, in transverse section, tightly packed together and slightly longer than in other zones of the leaf, averaging approximately 15 μ in length. Cell division, however, is here continued longer than in the contiguous upper epidermis, for while at this stage the palisade and upper epidermal cells are about equal in number in a given area, at maturity there are approximately five palisade cells which increase 240% in length but show only moderate change in diameter. Their average width at the conclusion of cell division in the epidermis is 7 μ and at maturity 9.6 μ in the middle plane and 11 μ at the upper end where they meet the epidermis. It should be noted here that these two adjacent layers with cells of equal size, enlarge in different degrees. While the upper epidermal cells enlarged to approximately 21 times their original area the adjacent palisade cells in the same leaf area have enlarged, after allowing for increase in number to a total cell-area of less than ten times that of early differentiation stage. The shape of the palisade cells, seen in cross section changes, from the initial polygonal outline to approximately circular form except at the upper junction plane where they keep much of their angular shape (compare figs. 4, 9, and 10). This change of shape is correlated with the development of the intercellular spaces among the palisade cells, which in turn is related to the unequal expansion of epidermis and palisade.

The Mesophyll Tissue

The three layers of cells which give rise to the spongy mesophyll are somewhat less uniform in size and stain more deeply than the palisade (fig. 5). The uppermost layer of the mesophyll tissue is slightly different from the other layers in having smaller cells and staining more deeply. This is doubtless related to the development of the vascular system of the leaf since the veinlets originate from this layer and mitoses continue here after cell division has ceased in all other layers of the islet.

During the differentiation of the mesophyll its cells not only enlarge but change their relations. At the time cell division ceases their longer dimension is perpendicular to the surface of the leaf. As the blade enlarges the cells are partly separated from one another, and from the epidermis in part, leaving intercellular spaces, the larger of these appearing above the differentiating stomata (fig. 12). The mesophyll cells at maturity are lobed and irregularly stretched out with their longer axes in most cases parallel to the epidermis, thus forming a coarse net-work (fig. 11). In the transverse section these relations and connections are not apparent (fig. 8).

Intercellular spaces occur throughout the interior of the leaf. They were first clearly noted as tiny spaces between rounded corners of adjacent cells above the developing stomata when the blade

was about 20 mm. long. At about the same time gaps appear between other mesophyll cells and then among palisade cells. As the leaf expands these spaces are enlarged, uniting with other spaces, and forming a system which ramifies throughout the islet area. At maturity probably more than half the internal volume of the *Vitis* leaf is air space. Every chlorophyll bearing cell of the leaf borders an intercellular space and is often laterally surrounded by these air chambers. The development of these spaces is undoubtedly related to the unequal expansion of the different tissues of the islet.

Rate of growth of the leaves

Spring leaves grew more slowly than those formed during the summer. The rate of expansion differed little for organs of approximately equal size under like conditions. The developing leaf passes over from the cell division stage to the cell enlargement phase when about 20 mm. long. Leaves 7-11 mm. long, measured for ten days during July, showed an average increase of 1.7 mm. per day. During April considerably larger leaves, entered upon the enlargement phase, showed an average daily gain in length of

TABLE 1. Rate of growth of spring leaves of *Vitis*.

No. of leaf	Date tagged	Initial length	Length at end of ten days	Average daily increase in length
1	April 24	25 mm.	30.6 mm.	0.56 mm.
2	” ”	25 mm.	30.6 mm.	0.56 mm.
3	” ”	27 mm.	32.2 mm.	0.52 mm.
4	” ”	20 mm.	24.3 mm.	0.43 mm.
5	” ”	39 mm.	44.7 mm.	0.57 mm.
Average for 5 leaves		27.2 mm.	32.48 mm.	0.52 mm.

TABLE 2. Rate of growth of summer leaves of *Vitis*.

No. of leaf	Date tagged	Initial length	Length at end of ten days	Average daily increase in length
101	June 26	33 mm.	70 mm.	3.7 mm.
102	” ”	45 mm.	100 mm.	5.5 mm.
103	” ”	53 mm.	143 mm.	9.0 mm.
104	” ”	33 mm.	76 mm.	4.2 mm.
105	” ”	35 mm.	65 mm.	3.0 mm.
106	” ”	64 mm.	119 mm.	5.5 mm.
107	” ”	72 mm.	109 mm.	3.7 mm.
108	” ”	77 mm.	128 mm.	5.1 mm.
109	” ”	63 mm.	96 mm.	3.3 mm.
110	” ”	47 mm.	113 mm.	6.6 mm.
Average for ten leaves		52.2 mm.	101.9 mm.	4.97 mm.

only 0.52 mm. (Table 1). Slightly larger leaves made an average daily increase of 4.97 mm. through a ten day period beginning in late June (Table 2).

The distribution of growth in leaves of 30 mm. or more in length was studied from the series of records drawn on fixed photographic dry plates by the method described above. These records revealed a uniform distribution of growth and an absence of any special growing regions (fig. 6). The apical point, which earlier had grown so rapidly, enlarges but slightly during this phase. Near the midvein expansion in a transverse direction is frequently a little less than elsewhere but this difference is not marked. There are other minor variations here and there but these are slight and are not constant, so may be attributed to differences in surroundings. The expansion of the leaf in general is uniformly distributed throughout the blade.

II. *Catalpa bignonoides* Walt.

General description

Catalpa presents a very different habit since it is a tree instead of a vine. Only one set of leaves is regularly produced each year and these average several times larger than the *Vitis* leaf. *Catalpa* leaves are arranged alternately or in whorls of three, varying in length from 5-35 cm. The leaf has a cordate base and acuminate apex with smooth, unlobed margin. There are no stipules.

The foliage leaf of *Catalpa* varies considerably in structure. The thickness ranges from about 100 μ to 200 μ, depending chiefly on the width of the palisade tissue. Some leaves show two layers of regular palisade and a third layer of elongated cells which appears intermediate between palisade and spongy mesophyll. Other *Catalpa* leaves have only one complete palisade layer with an additional modified layer (fig. 18).

TABLE 3. A comparison of the mature *Catalpa* leaf with that of *Vitis*. These figures except where maximum and minimum are stated are averages of several measurements.

	Vitis	*Catalpa*
Thickness of leaf	144 μ	100-200 μ
Thickness of upper epidermis	15 μ	17 μ
Thickness of palisade layer	51 μ	40-100 μ
Thickness of mesophyll	65 μ	26-66 μ
Thickness of lower epidermis	13 μ	17 μ
Diameter of upper epidermal cells	32 μ	25.8 μ
Diameter of palisade cells at junction plane	11 μ	11.4 μ
Diameter of palisade cells at middle plane	9.6 μ	10 μ

Development of the Blade

The *Catalpa* leaf follows the same general course of development as *Vitis*. The primorida push out as lateral protuberances from the vegetative point and differentiate into leaf base, petiole and blade when the leaf is approximately 0.5 mm. long. The meristematic layers are clearly differentiated by the time the blade is 1.5 mm. long. The number varies; some leaves show six layers of cells, others only five (figs. 14, 15). Veins make their appearance when the blade is 2-3 mm. in length, developing from the uppermost mesophyll layer as in *Vitis*. The size of the leaf at the time cell division ceases varies considerably in different leaves on the same shoot and is related to the dimensions of the leaves at maturity. In some there is no division after a length of 15 mm. is reached as evidenced by changes in the cells of the various layers; in others division continues until the blade is 60 mm. long; the latter resulting of course in a much larger leaf. The epidermal cells when they cease division, average 7.9 μ in surface width; at maturity they average 25.8 μ, an increase of 226% in width or ten times in area; that is, at maturity each is about 11 times its original area.

The mature palisade differs somewhat from *Vitis*. Originally there is but one layer of palisade tissue, but periclinical divisions frequently occur, giving rise to a double or even triple palisade layer. There appear to be two anticlinal divisions of the palisade cells after the upper epidermal cells cease to divide. When the latter begin their expansion, the number of palisade and epidermal cells in a given area is equal; at maturity the ratio is 4 to 1 (fig. 21). The palisade cells average 7.1 μ in diameter when they cease dividing, at maturity they average 11.4 μ at the junction plane with the upper epidermis and 10 μ at the middle plane, an increase of 184.5% in diameter giving approximately eight times the total cross sectional area at the middle plane (fig. 19). Meanwhile the contiguous epidermis had expanded to eleven times its original area.

The mesophyll tissue in some leaves is differentiated as two layers, in others as three layers (figs. 14-15). Mitoses cease at about the same time as in the epidermal layers. In maturity the mesophyll cells are less changed than in *Vitis* and about half of them have their long axis parallel to the epidermis (figs. 18, 20).

Measurements on the rate and distribution of growth showed an average increase in length of 9 mm. per day through a ten day period, for leaves that were approximately 40 mm. long. Growth during this period of expansion is uniformly distributed throughout the area of the blade.

Discussion

The results of this investigation are difficult of correlation or comparison with the work of earlier botanists for those workers were concerned mainly with the external aspects of leaf development. Such as made structural study of development were interested in veins rather than in islet tissue. Yet many of their general findings harmonize with the detailed development of *Vitis* and *Catalpa* as worked out in this paper.

The appearance of the mid-vein followed by the major lateral veins while the leaf is still in the bud supports the view of Deinega (2), Freundlich (4) and others who state that these veins arise very early.

Goebel's (5) statement that parts which function first mature earlier finds support by several points in the development of these leaves: (a) in *Vitis* the early appearance and growth of the stipules which function as a protection to the young leaf in the bud, (b) the delayed growth of the petiole which keeps the young blade in the bud and later by rapid elongation brings it out into more favorable light relations when the leaf is ready for photosynthesis, (c) the appearance of the intercellular spaces first above the differentiating stomata.

The intercellular spaces of higher plants are analogous to the air chambers of lower plants. The origin of the air chambers in the liverworts has been studied by a number of botanists. Hill (6), Evans (3) and Pietsch (8) stated that the air spaces are schizogenous in origin. Hirsh (7) argues that in the Ricciaceae there are two methods of origin for the air spaces:—by internal cleavage resulting in the formation of broad, irregular chambers separated by plates of green tissue one layer of cells thick; by the upward growth of filaments at right angles to the surface of the thallus, resulting in the formation of narrow elongated air chambers. This latter process has of course no possible equivalent in the foliage leaf of higher plants.

Barnes and Land (1) in their paper on the origin of air chambers in the Marchantiales state, "In as much as new cells are produced by division, and the partitioning wall is a joint product of the two severed protoplasts, *a priori* reasoning leads to the hypothesis that the intercellular spaces arise by secondary splitting of the membrane, on account of unequal growth and turgor." They cite no figure of air spaces originating in this way in vascular plants, nor do they refer to any paper discussing such origin. Their own paper is limited to the Marchantiales. Smith (9) found that in Isoetes the air spaces formed by the disorganization of certain groups of cells. No disorganizing cells are visible at any stage in the leaf development of *Vitis* and *Catalpa*.

This study leads to the suggestion that these air spaces result in part from the unequal expansion of the epidermal layers and the interior tissues of the leaf. This strain must be marked especially in the lateral plane. Cells of the upper epidermis of *Vitis* when they cease division average 7 μ in diameter. The palisade cells at this stage are closely fitted together and have also an average diameter of about 7μ. From this stage forward the cells of the two layers do not enlarge at the same rate. At maturity the epidermal cells average 32 μ, an increase of nearly twenty times in surface area. Cell division is continued somewhat longer in the palisade layer and at the end of the expansion phase there are about five palisade cells, averaging 9.6 μ in diameter at their mid-plane, to one epidermal cell. This results in an eight-fold increase of the total cross sectional area of palisade cells (fig. 13, A, B, C). Since the epidermal cells expand much more than the attached palisade, a strain obviously results which tends to separate the palisade cells. While the epidermis of the islet has increased about twenty times in area, the adjacent palisade cells in their mid-plane have enlarged meanwhile only eight fold in total cell area. The upper ends of the elongating palisade cells usually expand with the contiguous epidermal cells but small spaces occur even in the junction plane (fig. 9). Lower down these palisade cells separate quite fully and the columnar cells are usually nearly surrounded laterally by air space. It seems clear that the disparity in expansion of epidermis and palisade must set up stresses favoring such separation of interior cells. In *Catalpa* also there is an unequal expansion of superficial and interior tissues. The epidermal cells increase from 7.9 μ to 25.8 μ in diameter, or over nine times in area. At the end of the expansion phase there are four palisade cells to one epidermal cell, each averaging 10 μ at their mid-plane, a seven fold increase in total cross sectional area of palisade cells in this plane. The fact that the *Catalpa* leaf is more compact is probably correlated with the lesser difference in total expansion of these contiguous layers of the blade.

This greater epidermal expansion seems also to pull apart the spongy mesophyll cells. Their mature form is such as to make accurate measurement difficult but they appear to increase comparatively little in diameter after mitosis ceases which is earlier than in the palisade. The lateral strain placed upon the cells is evidenced by their elongated, lobed outline. This also suggests an explanation of the fact that their longer axes in these species are parallel to the epidermal layers.

Insufficient stress has heretofore been placed upon the mechanico-dynamic function of the epidermis. These layers as noted above appear to be a major factor in leaf expansion, for the veins, while they keep pace with the enlargement of the leaf, are probably not a force in its expansion. Mechanically the epidermis constitutes the only continuous compact tissue between veins and in considerable measure carries and supports the interior mesophyll of the islet.

Time necessary for the development of a leaf

The time required for the development of a foliage leaf is of considerable interest and importance. This study enables one to approximate the length of each epoch in the growth of a *Vitis* leaf. The period from primordium to about 1 cm. in length varies considerably because of seasonal conditions but in midsummer it would probably be passed through in ten days or even less time. The duration of this period of development, however, is relatively unimportant since the leaf is protected meanwhile by overlapping leaves and stipules. By the time the leaf is 1 cm. long it is partly out of the bud and directly affected by external conditions. The time required for a leaf of *Vitis vulpina* to grow from 1 cm. to 12 cm., the length of an average mature leaf, may be estimated with definiteness. Accepting 0.17 cm., given above, as the average rate of growth in length for a leaf under 3 cm. long it will require 11-12

days for the leaf to attain such size. The average daily increase in length obtained for summer leaves over 3 cm. long was 0.5 cm. per day. At this rate it will require an additional 18 days for the leaf to grow from 3 cm. to maturity, or probably 40 days from primordium to adult leaf. No doubt the growth period would be longer under less favorable conditions of earlier spring.

The developmental period is long enough to avoid adjustment to any temporary factors. If this were much reduced accidental shading of part of the leaf for a short time might result in unequal growth of the parts of the leaf. With too short a growth period we might expect the leaf as a whole to be unduly influenced by a succession of sunny or cloudy days while it was expanding. The time, however, is long enough to allow for considerable variations in light and temperature as well as moisture conditions with adjustment to the average environment.

Possibly of great significance is the fact that the growth period of foliage leaves seems ample to permit safe adjustment to the increasing shade within and between branch systems. When leaves emerge from the bud the light is many times brighter for all interior leaves than at the conclusion of their development. There must therefore be a gradual adjustment throughout their plastic phase to average conditions that are to surround the adult leaf during its functional period.

Summary

1. The leaf of *Vitis* or *Catalpa* arises as a lateral protuberance on the stem.

2. The stipules of *Vitis* appear as small protuberances on the central papilla when it is very small (60-100 μ long). *Catalpa* has no stipules and shows no rudiments of them at any stage.

3. Distinct cell layers are evident in the developing leaf. *Vitis* has six of these layers, three of them giving rise to the spongy mesophyll. *Catalpa* may have either five or six, the sixth layer when present becoming part of the mesophyll tissue.

4. *Vitis* shows a more marked apical tip in the embryonic stage and has lateral papillae which develop the lobes and teeth. *Catalpa* which has a smooth margin shows no suggestion of lobing in the embryonic stage.

5. The cell layers begin to differentiate into epidermis, palisade and spongy mesophyll when the blade is 5-8 mm. long.

6. In *Vitis* cell division in epidermal and mesophyll (except cells concerned with vascular development) ceases when the leaf is approximately 2 cm. long. Mitoses continue somewhat longer in the palisade.

7. In *Catalpa* cell division continues until the blade is 15-60 mm. in length. Division continues longer in the palisade cells than in the epidermal. It may also continue longer in mesophyll cells concerned with vascular development.

8. Stomata in both leaves do not all become functional at the same time. Some guard cells are barely differentiated when other stomata of the same leaf region are mature.

9. Growth in both species is uniformly distributed over the leaf blade during the major phases of expansion except that the apical point develops early and soon ceases growth.

10. The development of a *Vitis* leaf under summer conditions from primordium to maturity probably covers a period of 40 days.

11. The upper epidermis of *Vitis* increases in area to more than twice that of the total cross sectional area of the adjacent palisade-cells at their mid-plane. The upper epidermis of *Catalpa* also increases in area much more than the total cross sectional area of the contained palisade cells at their mid-plane.

12. The intercellular spaces are schizogenous in origin. The greater expansion of the epidermal layers doubtless tends to separate cells of both palisade and mesophyll tissue and is an important factor in the development of the intercellular spaces.

13. The epidermis not only aids in leaf expansion but provides the major mechanical support of the islet area.

The writer takes pleasure in expressing her thanks to Dr. Robert B. Wylie, at whose suggestion and under whose direction the investigation was carried on, for his helpful advice and criticism.

Ballard Normal School
Macon, Georgia

Plate I *Vitis vulpina*

Figure 1. Longitudinal section through a bud.

Figure 2. Outline of a leaf 2 mm. long.

Figure 3. Transverse section of a leaf 3 mm. long, showing the six meristematic layers.

Figure 4. Horizontal section of palisade of a leaf about 6mm. long.

Figure 5. Transverse section of a leaf 10 mm. long.

Figure 6. Successive stages in the expansion of a single leaf. First three outlines beginning with smallest taken at 24 hour intervals; next three at 48 hour intervals; and last outline after an interval of 72 hours.

Figure 7. Lower epidermis, surface view, of an immature leaf.

PLATE I

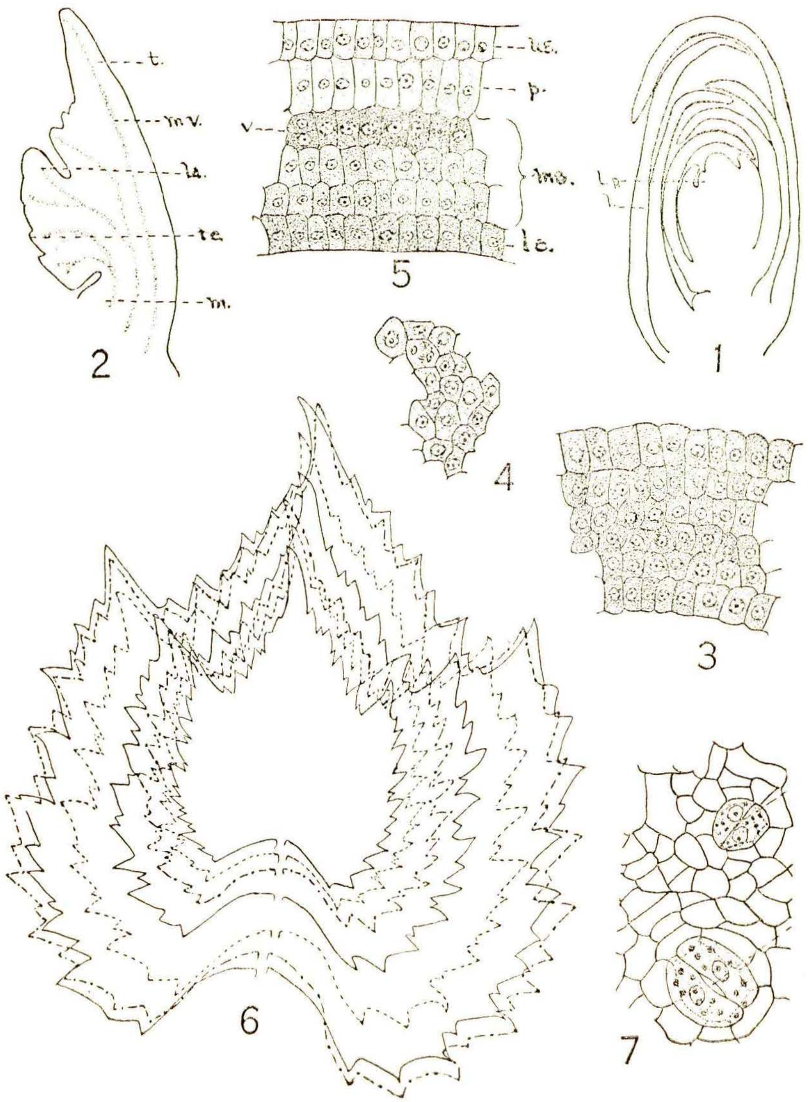


Plate II *Vitis vulpina*

Figure 8. Transverse section of a mature leaf.

Figure 9. Upper epidermis, surface view, of a mature leaf. Ends of subjacent palisade cells dotted in outline.

Figure 10. Horizontal section of palisade of a mature leaf, middle plane.

Figure 11. Horizontal section of spongy mesophyll of same leaf as fig. 10.

Figure 12. Transverse section of an immature leaf.

Figure 13. Diagrams drawn to common scale, illustrating the relative enlargement of upper epidermis and palisade cells of *Vitis*, in the horizontal plane.

A. Represents a given area of leaf, involving epidermis and contiguous solid palisade, at the time mitoses cease in the upper epidermal cells.

B. Represents total area in horizontal section of palisade cells developed from the original area (A). Cells measured midway between upper and lower ends.

C. Represents the area of the epidermal cells, included in area A, at the time cell enlargement ceases in the upper epidermis. The disparity in area of the squares B and C represents the unequal expansion of palisade and epidermis of the original area A, as these contiguous layers mature.

PLATE II

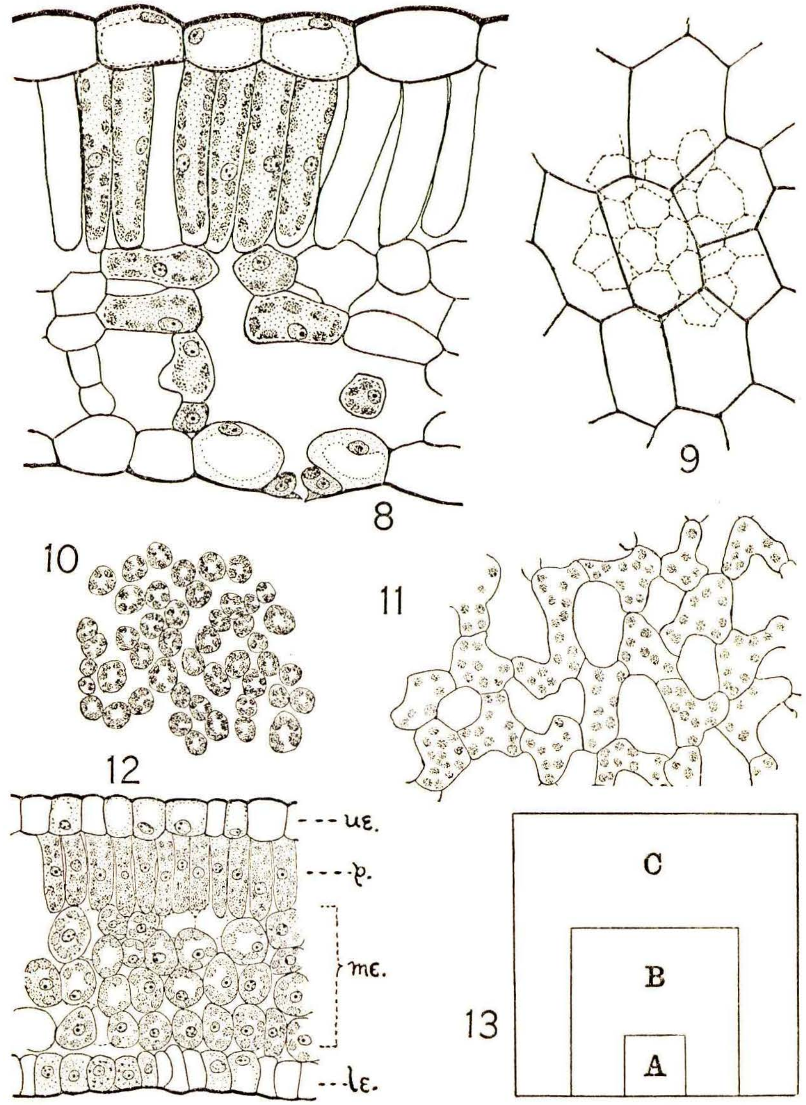


Plate III *Catalpa bignonoides*

Figure 14. Transverse section of a leaf showing germinal layers.

Figure 15. Transverse section of a leaf 8 mm. long.

Figure 16. Transverse section of a leaf 14 mm. long.

Figure 17. Transverse section of an immature leaf.

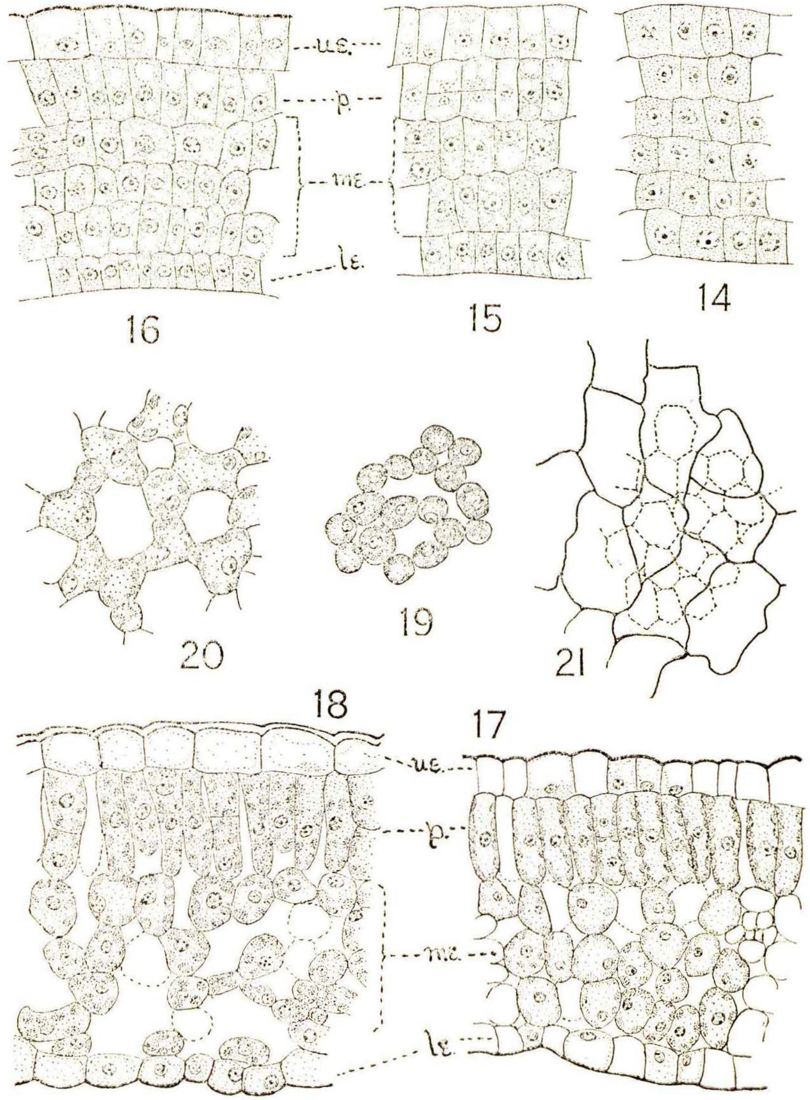
Figure 18. Transverse section of a mature leaf.

Figure 19. Horizontal section of the palisade of a mature leaf.

Figure 20. Horizontal section of the spongy mesophyll, same leaf as fig. 19.

Figure 21. Upper epidermis, surface view, of a mature leaf. Ends of subjacent palisade cells dotted in outline.

PLATE III



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