

Epidermal Characters in Fraxinus

JONATHAN W. WRIGHT, Purdue University

Difficulty is often encountered in the identification of sterile specimens of some of the ashes of eastern America. Particularly hard to separate from each other are leaves of the Biltmore white ash (*F. biltmoreana* Beadle) and the pumpkin ash (*F. tomentosa* Michx.), as well as entire or nearly entire specimens of white ash (*F. americana* L.) and the green ash (*F. pennsylvanica* Marsh. var. *lanceolata* (Borkh.) Sarg.). These species may be easily separated by certain microscopic characters of the lower epidermis. As these characters have not heretofore been used, they are here described in detail for the ashes found in eastern America.

Methods

The epidermal characters were studied from colodion peels made from the lower leaf surface of herbarium specimens. The peels were made by applying a solution of colodion in butyl acetate plus 5 per cent butyl alcohol to the under surface of the leaf (Sax and Sax, 1937). Rather thin solutions were found satisfactory with all but the white ash and the Biltmore white ash. With these, a much more viscous solution was needed, in order to provide a film tough enough to be removed from the leaf. It was found unnecessary to remove the pubescence prior to the application of the collodion, as the unicellular hairs did not interfere with the observation of the peels. The collodion film was dried in the open air. Upon drying, the peels were affixed to microscope slides by a drop of the collodion solution at each corner of the peel. No cover glasses were added to the mounts. Stomata measurements were made from camera lucida drawings using a 10x ocular and a 44x objective.

According to Solereder (1908) "the characteristic form of hair in this order (family) is that of peltate trichomes of variable size, having a unicellular stalk and a shield which is usually divided by vertical walls only. These peltate hairs are usually grandular, and in *Olea* alone having a clothing function; . . ." These peltate hairs (Figs. 6, 7) were of general occurrence over the entire lower surface of the leaf in all the species studied as well as in several of our western ashes and appeared of little diagnostic value. They are umbrella-shaped structures of slightly less than twice the diameter of the stomata. The stalk is usually very short, and the shield is composed of from 4 to 8 cells. Unicellular hairs, often reaching 0.5 mm. in length are also found, most commonly occurring along the midrib. It is these unicellular hairs which give the velvety appearance to the leaves and twigs of *F. biltmoreana* and *F. pennsylvanica*. The cells of the lower epidermis of ash generally have an irregular outline.

SPECIES KEY BASED ON EPIDERMAL CHARACTERS

- a. Stomata oval-acute, variable in size; epidermal ridges numerous, usually obscuring the impression of the cell outlines on a leaf peel..... subsection *Melioides*
- b. Stomata 15-30 microns long, not grouped; cells with coronulate papillae (except those of veins); peels removed with difficulty
 -1. *F. americana*
 -2. *F. biltmoreana*
- bb. Stomata in groups of 10-12, separated by small veinlets with rectangular cells; epidermal ridges numerous within groups; no coronulate papillae; peels removed easily.
- c. Stomata 15-24 microns long.....3. *F. pennsylvanica*
 -4. *F. caroliniana*
- cc. Stomata 22-34 microns long.....5. *F. tomentosa*
- aa. Stomata oblong, of uniform size, not grouped; epidermal ridges usually emanating only from stomata, not obscuring impression of cell wall on leaf peel; peels removed easily... subsection *Bumelioides*
- d. Epidermal ridges usually present in vicinity of all stomata; stomata 14-20 microns long.....6. *F. nigra*
- dd. Epidermal ridges often absent; stomata 11-16 microns long
 -7. *F. quadrangulata*

The two subsections of the genus present in eastern America are as easily distinguishable by the appearance of their leaf peels (Figs. 4, 7) as by their gross morphology. In subsection *Melioides*, containing white ash and red ash (*F. pennsylvanica* Marsh.) the stomata¹ are oval acute, under high power (x 440) looking much like eyes. They are relatively large, and quite variable in size, even on the same leaf. In the red ash especially it is usual to find a variation of 50 per cent in guard cell length within the same microscope field. This subsection is also characterized by abundant fine epidermal ridges which obscure the cell outlines. These ridges give all the species a whitish cast to the under leaf surface when viewed by the unaided eye.

Leaf peels of white ash (Figs. 1, 2) and Biltmore white ash are easily distinguishable from those of our other eastern species by their "coronulate papillae united by a network of ridges" (Solereder, 1908). Fine epidermal ridges, visible only under high magnification, cover the entire lower leaf surface with the exception of the veins. These ridges end in the centers of the cells, curving upward and outward, giving the papillae their coronulate appearance. This outward curving of the ridges gives the leaf an exceptionally firm grip on the leaf peel, making the removal of the latter difficult. The roughness caused by these papillae causes the under surface of the leaf to be much whiter than that of any of our other species when viewed in the field. The papillae somewhat obscure the stomata, making their observation and measurement difficult. When examined under low power (x 100) the peels have the appearance

¹ The term stomata is here used to denote not only the openings in the leaf surface but the guard cells as well.

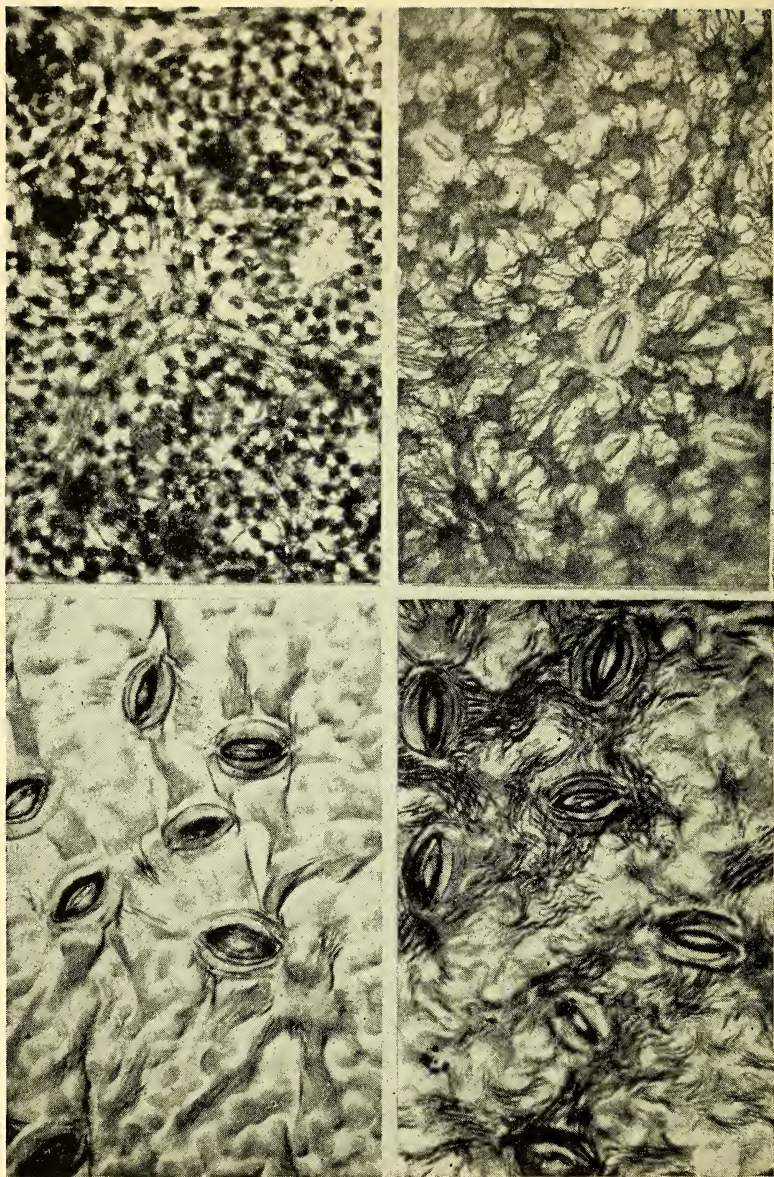


Plate I

Figures 1-4. Photographs of collodion peels of the lower epidermis of species of *Fraxinus*. Fig. 1. Diploid *F. americana* (mature tree). At low magnifications the coronulate papillae and anastomosing ridges give to the peel the appearance of a reticulum. (x 250). Fig. 2. Diploid *F. americana* (mature tree). At higher magnifications, the tips of the coronulate papillae are out of focus, but the ridges running between cells can be clearly seen. Note the considerable variation in size of the individual stomata which is typical of all species of the subsection *Melioides*. (x 490). Fig. 3. Diploid *F. americana* (two-year tree). In young trees the papillae are lacking and the ridges are not well developed (x 490). Fig. 4. *F. caroliniana*. Under high magnification, both red and Carolina ash show numerous epidermal ridges radiating out from the stomata, giving the appearance of eyes with long curly eyelashes (x 490).

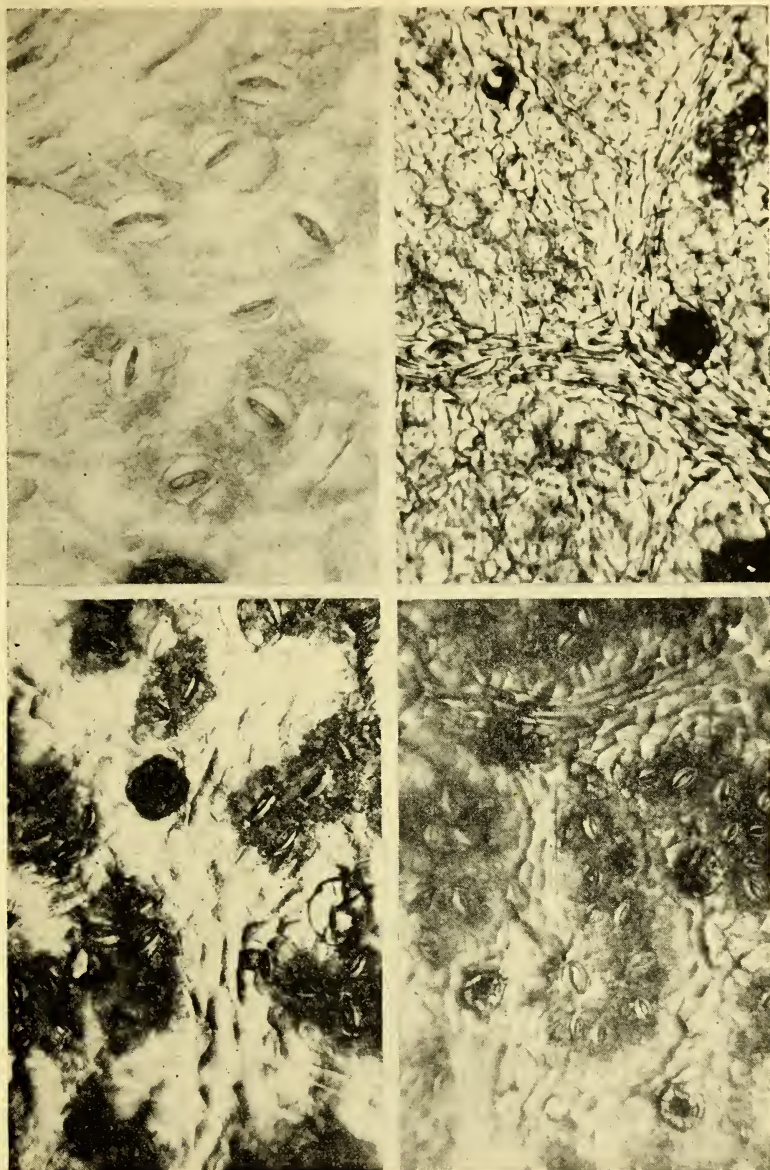


Plate II

Figures 5-8. Photographs of collodion peels of the lower epidermis of species of *Fraxinus*. Fig. 5. *F. pennsylvanica*. The stomata are characteristically grouped. There are no coronulate papillae, and the epidermal ridges are nearly confined to the portions of the leaf within stomata groups. In the left center is a peltate hair, characteristic of the entire family Oleaceae. Peels of *F. caroliniana* are indistinguishable from peels of *F. pennsylvanica*. (x 250). Fig. 6. *F. tomentosa*. Leaf peels of the pumpkin ash are distinguishable from those of red ash by the larger stomata size. (x 250). Fig. 7. *F. nigra*. The stomata are of uniform, small size in both black and blue ash, and are evenly distributed over the surface of the leaf. They are more numerous than in the other species studied. In the center is a peltate hair. (x 250). Fig. 8. *F. nigra*. Under high magnification, black ash stomata are oblong. Epidermal ridges are lacking, or emanate only a short distance from the stomata. (x 490).

of a reticulum, and the stomata are hardly visible. The cell outlines are usually not visible under either high power or low power. Unfortunately, this character cannot be used in the identification of young trees, since white ash seedlings up to two years of age lack the papillae, although they do have some fine ridges emanating from the stomata (Fig. 3).

Leaf peels of the Mexican *F. papillosa* Lingelsh. are similar to those of white ash.

The stomata of *F. americana* and *F. biltmoreana* are variable in density from 30 to 200 per square millimeter on different individuals, although the density is remarkably constant on peels made from any one tree. An attempt was made to correlate number of stomata per unit of area with chromosome number within the species, as Sax (1938) had done for different species of ash. This was impossible, as there was no constant relation between the two variables. However, stomata size did prove a reliable criterion of polyploidy within *F. americana*. The base number in the genus is $n = 23$ (Sax and Abbe, 1933). Wright (1944) found a polyploid series in the southern portion of the species' range. Stomata measurements made on peels of trees with known chromosome number revealed significant differences between the stomata of the plants with different chromosome number (Table 1). Stomata size is a most

Table I.
Length of stomata guard cells in diploid and polyploid
Fraxinus americana

Chromosome number	Number of collections included in mean	Mean length, guard cells Microns	Limits within which a collection mean may vary ($P = \pm .05$) * Microns
PROGENY†			
2n	14	$18.8 \pm 0.2\ddagger$	17.5-20.0
4n	2	21.6 ± 0.3	20.1-23.0
6n	4	24.2 ± 0.6	22.5-25.8
PARENTS§			
2n	11	18.2 ± 0.3	16.3-20.1
4n	2	22.5 ± 1.1	20.2-24.8
6n	4	23.7 ± 0.4	21.2-26.2

* Calculated by assuming a coefficient of variability equal to that found in the diploids.

† Mean lengths and limits of variability of mean determined from samples of 100 stomata (20 stomata on a single peel from 1 leaf on each of 5 seedlings).

‡ Standard error.

§ Mean lengths determined from samples of 40 stomata (two peels from each tree), and limits of variability of mean from samples of 20 stomata (one peel from each tree).

reliable criterion of polyploidy for young trees grown under constant conditions in the nursery, for which it is possible to separate with certainty diploids, tetraploids and hexaploids. It is less reliable in ordinary field-grown material, in which it is only safe to distinguish between diploids and polyploids.

The lower surface of *F. pennsylvanica* (Figs. 4, 5) and *F. caroliniana* are similar in appearance, lacking entirely the papillae so characteristic of *F. americana*. The stomata are aggregated into groups of ten or twelve. These groups are separated by small veinlets composed of rectangular cells without epidermal ridges. In the areas within stomata groups the cells are irregular in outline and usually have abundant epidermal ridges although in a few specimens these are lacking. The ridges seems to emanate from the stomata, which have the appearance of eyes with long wavy eyelashes.

The lower epidermis of *F. tomentosa* (Fig. 6) is similar to that of the two preceding species in all quantitative characters. However, with a little practice, peels of this tree may be distinguished by cell size, the stomata being about $1\frac{1}{2}$ times as large as those of either red or Carolina ash. They are less abundant, having a density of 120 to 300 per square millimeter.

The stomata of *F. nigra* (Figs. 7, 8) and *F. quadrangulata*—our two species of the subsection *Bumelioides*—are oblong, and of a smaller and less variable size than are those of the subsection *Melioides*. The guard cells are relatively broader and less pointed. The stomata are very abundant (up to 1000 per square millimeter), and are uniformly distributed over the entire lower leaf surface with the exception of the larger veins, in contrast with those of the subsection *Melioides*. The minute epidermal ridges when present emanate only short distances from the stomata, rarely obscuring the impression of the cell walls on the peel. In *F. quadrangulata*, which has the smallest and most abundant stomata of any of our species, the ridges are almost entirely absent. The differences between epidermal peels of blue and black ash are too slight to make positive separation possible.

It should be made clear that these epidermal characters are of little use in field identification, for it is impossible for a collector to carry with him the equipment necessary for making and examining the peels. (In any case, the peels are best made from dried material.) Nevertheless, they should be of great help to the taxonomist classifying specimens in the herbarium. By their use difficult sterile specimens may be referred definitely to one species with more certainty than is possible by an examination of gross morphology alone. Too, further study of the leaf peels of living material should help to clarify the relation between living and fossil species of *Fraxinus*.

The epidermal characters are most useful in the separation of sterile or male specimens of *F. americana* and *F. pennsylvanica* var. *lanceolata*, normally distinguished by the fruits. Leaves of these two species are often quite indistinguishable from gross morphology; a peel of the white ash leaf can only be removed with great difficulty, and will reveal the coronulate papillae, absent in the latter. Likewise, non-fruiting specimens of *F. biltmoreana* and *F. tomentosa* are similar in gross morphology, but

are easily distinguishable upon microscopic analysis by the presence or absence of the coronulate papillae. Unfortunately, the peels are of no help in distinguishing leaves of the green ash and the Carolina ash, which are also hard to separate on gross morphology, nor can they be used to separate black from blue ash.

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