

THE STATUS OF *FRAXINUS PAPILLOSA* (OLEACEAE): SEM STUDY OF EPIDERMAL FEATURES

Justin K. Williams¹ and Guy L. Nesom²

¹Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341-2116

²2925 Hartwood Drive, Fort Worth, TX 76109, USA

www.guynesom.com

Abstract: The abaxial leaf surfaces of *Fraxinus* in the southwestern USA and northwestern Mexico are sometimes white-glaucous and have been described as “papillose.” Individuals exhibiting this morphology have been identified as *F. papillosa*, a species reported from Arizona, New Mexico, Texas, and Mexico. In the original description of *F. papillosa*, its leaf epidermal structure was compared to *F. americana* L., but the abaxial surface morphology of *F. papillosa* is distinctly different from that of *F. americana*. In the latter, a cuticular reticulum overlays the epidermis and partially obscures it, while the abaxial surface of *F. papillosa* is similar to that of *F. velutina* in its lack of a cuticular overlay. In *F. papillosa* each epidermal cell is abruptly convex or folded upward into a papilla-like structure, compared to the relatively flat-surfaced cells of *F. velutina*—intergradation occurs but whether the two are appropriately considered conspecific remains to be determined. SEM photographs of the abaxial leaf surface morphology of *F. papillosa*, *F. velutina*, and other species that are similar to *F. papillosa* are provided.

Keywords: *Fraxinus papillosa*, *F. velutina*, *F. americana*, Scanning Electron Microscopy, leaf epidermis.

Fraxinus papillosa Lingelsh. has been widely recognized as a species of the southwestern United States and northwestern Mexico (e.g., Lingelsheim, 1920; Standley, 1924; Sargent, 1933; Little, 1952, 1976; Miller, 1955; Kearney and Peebles, 1960; Martin and Hutchins 1980; Elias 1980; Powell 1988; USDA, NRCS 2009). The original description of the species (Lingelsheim, 1907) was based on only the type collection from northwestern Chihuahua. Its attribution to the USA was first reported by Rehder (1917), who cited *Mearns* 2533 from the San Luis Mountains in Grant Co., New Mexico; Sargent (1933) repeated the same citation. Little’s account of the species (1952) was the first to describe a wider geographic range and was accompanied by voucher citations (from MICH and US) from Arizona, New Mexico, Chihuahua, and Sonora.

The citations and map of *Fraxinus papillosa* by Little (1952, 1976) apparently have been the basis for most subsequent accounts and summaries of its range. In fact, it appears that the recognition of the species in the USA stems mostly from Little’s

accounts. It is among the names on the Flora of Arizona checklist posted on the Southwest Environmental Information Network (SEINET, 2009), but there is no voucher specimen identified as *F. papillosa* for any state in any of the herbaria associated with the SEINET database.

Correll and Johnston (1970) included *Fraxinus papillosa* in the Texas flora on the basis of identifications by Little: “Specimens from Presidio Co. have been tentatively identified as this species (fide E.L. Little, Jr.). Additional material is needed for complete verification.” Little’s Atlas map (1976) unequivocally (though without documentation) added trans-Pecos Texas to the overall range of *F. papillosa*. Simpson (1988) mapped *F. papillosa* in Presidio County, noting (p. 156) that it occurs “in the Chinati Mountains ... and is also thought to occur in the Bofecillos Mountain range to the east and the Sierra Vieja range just north of Chinati Peak.” The Texas distribution of *F. papillosa* later was seemingly corroborated in the Atlas by Turner et al. (2003) through a

dot in Presidio County, based on a TEX voucher.

The feature generally emphasized in distinguishing *Fraxinus papillosa* is the “papillose” nature of the abaxial leaf surface. A papillose abaxial surface also has been noted as a constant feature of the *F. americana* L. complex (including *F. albicans* Buckley [see Nesom 2010a], the diploid, tetraploid, and hexaploid entities of *F. americana*, and *F. pauciflora* Nutt. [see Nesom 2010b]). Photos of this feature by Wright (1944) and line drawings by Miller (1955) are remarkably similar to SEM photos by Hardin and Beckmann (1982). At high resolution, the minute areolae formed by the cuticular reticulum that overlays the epidermis are equally as conspicuous as the papillae in leaves with the “papillose” morphology—the whole reticulum appears minutely, shallowly, and evenly “reticulate-perforate,” or “micro-foveolate.” The term “coronulate” was used by Solereder (1908, “coronulate papillae united by a network of ridges”) and preferred by Wilkinson (1979), Hardin and Beckmann (1982), and Kremer et al. (2008) to describe this aspect of the abaxial epidermis. Once one becomes familiar with this morphology, the nature of the abaxial epidermis of *F. americana* and *F. texensis* can be readily identified, making it easy to differentiate the *F. americana* group from the other North American ash species by a quick inspection.

In the original description of *Fraxinus papillosa*, Lingelsheim (1907) described the leaflets as having a network of papilla beneath and noted that the new species was related to *F. americana*. Rehder (1917, p. 211) noted that *F. papillosa* differs from *F. velutina* Torr. “chiefly in the glaucous papillose under surface of the leaflets and from *F. americana* in its sessile smaller leaflets.” Little (1952, p. 377) observed the abaxial epidermal features of *F. papillosa* to be similar to those of *F. americana*. “As its specific epithet indicates, *F. papillosa* is characterized by the papillose, glaucous or whitish under surface of the leaflets. This

feature, which has been mentioned under *F. americana*, is found also in *F. texensis* [= *F. albicans*] and suggests a relationship with those species in addition to the similarity with *F. velutina* (or *F. standleyi*). These minute papillae, though not shown by a hand lens, are revealed by a high power (40x) dissecting microscope or by low power (50x to 100x) of a compound microscope through direct examination of the leaves or collodion leaf peels.”

Wright (1944, p. 88) noted that “Leaf peels of the Mexican *Fraxinus papillosa* Lingelsh. are similar to those of white ash,” but he did not show a photo of a *F. papillosa* sample or cite a voucher. Wallander (2008) also observed that “Like the eastern and much larger *F. americana* [*F. papillosa*] has a distinctly papillose lower leaf epidermis.”

The observations of Miller (1955, p. 54), however, were different—she noted that *Fraxinus papillosa* “is perhaps more easily confused with *F. velutina* than with any other species. The elliptical leaflets and the fruits are often very ‘velutina-like;’ however, it is distinguished by the papillose condition of the leaflets. It may be closely related to *F. americana* ssp. *texensis*; but the papillose pattern in *F. papillosa* is a very close one, quite unlike the typical network pattern of the typical white ash complex.”

Regarding *Fraxinus papillosa*, Felger et al. (2001, p. 262) noted that its “[geographic] distribution is wholly within the range of *F. velutina*.” Although most authors have treated *F. velutina* and *F. papillosa* as distinct, Standley (1924: 1136) expressed the opinion that they ‘would have to be united ultimately’ and Powell (1988) likewise cast doubt on their distinctiveness. We also have found that the distinctions lack clarity.

The present study investigates the abaxial foliar features of *Fraxinus papillosa* and those of potentially and putatively closely related species. The null hypothesis, as its epithet suggests, is that *F. papillosa* is most closely similar in morphology, due to its papillose epidermis, to the *F. americana* complex.

MATERIALS AND METHODS

Seven putative species are included in the study: *F. papillosa*, *F. velutina*, *F. berlandieriana* A. DC., *F. pennsylvanica* Marsh., *F. profunda* Bush, *F. albicans*, and *F. americana*—including diploid, tetraploid (*F. smallii* Britton), and hexaploid (*F. biltmoreana* Beadle) variants, as identified from chromosome counts and morphological features (Nesom in prep.). Leaf samples from the seven taxa were collected from dried herbarium specimens housed at BRIT, LL, MO, and TEX. Vouchers with brief localities are given in Table 1. In order to provide unambiguous identifications, samples were taken from collections with mature fruits, except for *Powell 3148* and *Sanders 2316*. Vestiture on the abaxial surface is variable; for this study, we selected leaflets with reduced vestiture in order to simplify views of the epidermal features. All samples were taken from the distal one-fourth of the leaflets. The samples were not rehydrated, acetolyzed, or critical-point dried. The specimens were mounted onto an SEM stud with conductive graphite tape and coated with gold using a LADD sputter coater. Observations of the abaxial leaf surface were made with a Vega Tescan 5130 (Department of Biological Field Studies, Sam Houston State University) and photographed digitally. Each specimen was photographed at 400x and 20kV using both scanning and backscatter electron microscopy.

RESULTS

The abaxial leaf surfaces of *Fraxinus berlandieriana*, *F. pennsylvanica*, *F. profunda*, and *F. velutina* are similar (Figs. 1 and 2a–e). The abaxial surfaces of *F. papillosa* (Figs. 1a, b, and c) lack the specialized cuticular overlay characteristic of *F. americana* and thus are more similar to those of *F. velutina* and its relatives. The abaxial surfaces of *F. velutina* are particularly similar to those of *F. berlandieriana* (Fig. 2a).

Each abaxial epidermal cell of a *Fraxinus papillosa* leaf is abruptly convex (Fig. 1a, the type of *F. papillosa*, from Chihuahua), or perhaps better described as folded upward, presenting the papillose aspect, and the whole surface is prominently white. These leaves have often been described as “glaucous,” but the white color apparently results from increased reflectivity of the raised cells, as there is no obvious evidence of deposition of any kind of material. Trees with such leaves occur in northwestern Mexico and into New Mexico and Arizona. They intergrade to some degree with trees of *F. velutina*, which produce leaves with green abaxial surfaces, the individual epidermal cells without abruptly convex cell surfaces (Figs. 1c, e, f, g, h) but the details of the biological interaction between the two taxa remain to be delineated.

A Texas collection, as the basis for the inclusion of *Fraxinus papillosa* in the Texas Atlas (Turner et al. 2003), has proved to be a specimen of *F. velutina*: Presidio Co: Arroyo Primero, a tributary of Fresno Creek on the Big Bend Ranch, common tree, 14 Jun 1975, *Butterwick & Strong 954* (TEX). This plant appears to be within the variability of other TEX collections of *F. velutina* made from the Fresno Creek drainage in Presidio County—in fruit and leaf morphology, vestiture, and foliar epidermal features. As in other *F. velutina*, the epidermal cells may be slightly raised but not to the exaggerated extent of typical *F. papillosa*. Presumably the Presidio County plants identified by Little and by Simpson as *F. papillosa* also are similar.

In *Fraxinus pennsylvanica*, 8–12 closely arranged stomata are produced within each ultimate areole, and the surfaces of the intervening epidermal cells are delicately but conspicuously striate (Fig. 2b–d). Within each ultimate areole, 1 or 2 peltate multicellular scales (Hardin and Beckmann, 1982) usually occur. In *F. berlandieriana*, *F. velutina*, and *F. papillosa*, the stomata are less densely arranged than in *F. pennsylvanica* and often highly variable in size; the intervening epidermal cells are more nu-

TABLE 1. Species and voucher citation for the specimens examined. SE = Image using scanning electron microscopy. BS= Image using back-scatter electron microscopy.

Species	Specimen	Figure Identification and Image Type
<i>Fraxinus papillosa</i>	MEXICO. Chihuahua. <i>Townsend & Barber 354</i> (MO, isotype)	1a (SE); type of <i>F. papillosa</i>
<i>Fraxinus papillosa</i>	MEXICO. Sonora. <i>White 4414</i> (TEX)	1b (SE); 1d (BS)
<i>Fraxinus velutina</i>	MEXICO. Coahuila. <i>Johnston et al. 11804</i> (LL)	1c (SE)
<i>Fraxinus velutina</i>	MEXICO. Chihuahua. <i>Spencer 704</i> (TEX)	1e (BS)
<i>Fraxinus velutina</i>	USA. Arizona. Graham Co. <i>Thornber 8769</i> (TEX)	1f (BS)
<i>Fraxinus velutina</i>	USA. Texas. Presidio Co. <i>Studhalter s.n.</i> (TEX)	1g (SE)
<i>Fraxinus velutina</i>	USA. Texas. Jeff Davis Co. <i>Powell 3148</i> (TEX)	1h (SE); Originally identified as <i>F. aff. texensis</i>
<i>Fraxinus berlandieriana</i>	USA. Texas. Val Verde Co. <i>Correll 12908</i> (LL)	2a (SE)
<i>Fraxinus pennsylvanica</i>	USA. Texas. Lamar Co. <i>Holmes 10534</i> (TEX)	2b (SE)
<i>Fraxinus pennsylvanica</i>	USA. Alabama. Lawrence Co. <i>Kral 61941</i> (TEX)	2c (SE)
<i>Fraxinus pennsylvanica</i>	USA. Texas. Red River Co. <i>Sanders 2316</i> (BRIT)	2d (SE)
<i>Fraxinus profunda</i>	USA. Louisiana. St. John Baptist Par. <i>Ewan 21196</i> (TEX)	2e (SE)
<i>Fraxinus americana</i>	USA. Texas. Freestone Co. <i>Do 815</i> (TEX)	2f (BS)
<i>Fraxinus americana</i>	USA. Illinois. Fayette Co. <i>Hill 34207</i> (TEX)	2g (SE)
<i>Fraxinus smallii</i>	USA. Texas. Newton Co. <i>Tharp & Gimbrede 52-627</i> (TEX)	2h (SE); Originally identified as <i>F. americana</i> and <i>F. biltmoreana</i> tetraploid
<i>Fraxinus biltmoreana</i>	USA. North Carolina. Mecklenberg Co. <i>Taylor et al. s.n.</i> (TEX)	3a (SE)
<i>Fraxinus albicans</i>	USA. Texas. Cooke Co. <i>Correll 29496</i> (LL)	3b (BS)
<i>Fraxinus albicans</i>	MEXICO. Coahuila. <i>Riskind 2389h</i> (TEX)	3c (SE); Originally identified as <i>F. velutina</i>

Index to scientific names:

Fraxinus albicans Buckley.; Fig. 3b,c

Fraxinus americana L.; Fig. 2f, g

Fraxinus berlandieriana A. DC.; Fig. 2a

Fraxinus biltmoreana Beadle; Fig. 3a

Fraxinus papillosa Lingelsh.; Fig. 1a

Fraxinus pennsylvanica Marshall; Fig. 2b–d

Fraxinus profunda Bush; Fig. 2e

Fraxinus smallii Britton; Fig. 2h

Fraxinus velutina Torr.; Fig. 1b–h

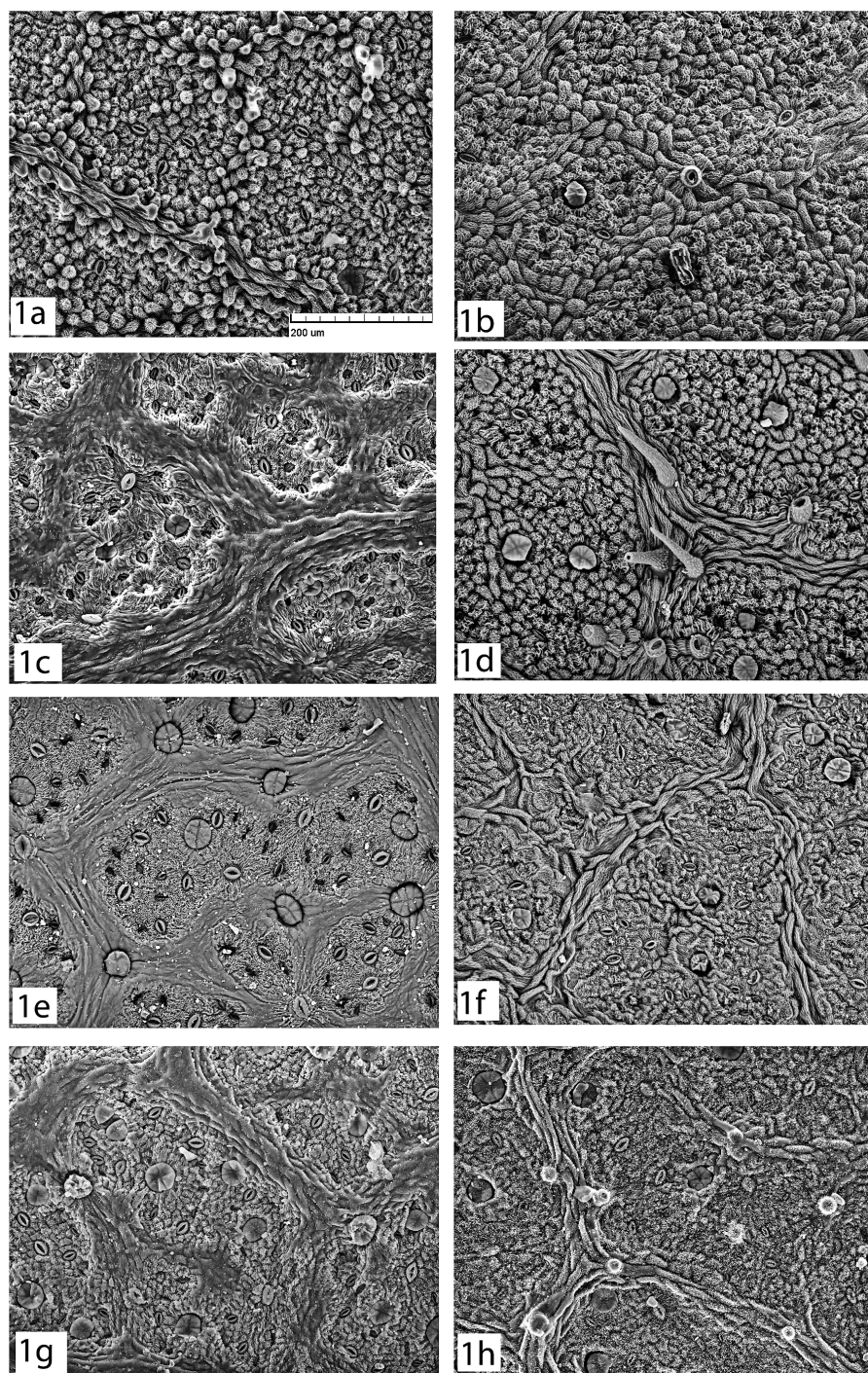


FIG. 1. Scanning electron micrographs of adaxial leaf surfaces of: 1a) *Fraxinus papillosa* and 1b-h) *F. velutina*. Each specimen was photographed at 400x and 20kV; scale bar [1a] shows actual magnification. Voucher specimen, herbarium and collecting locations found in Table 1.

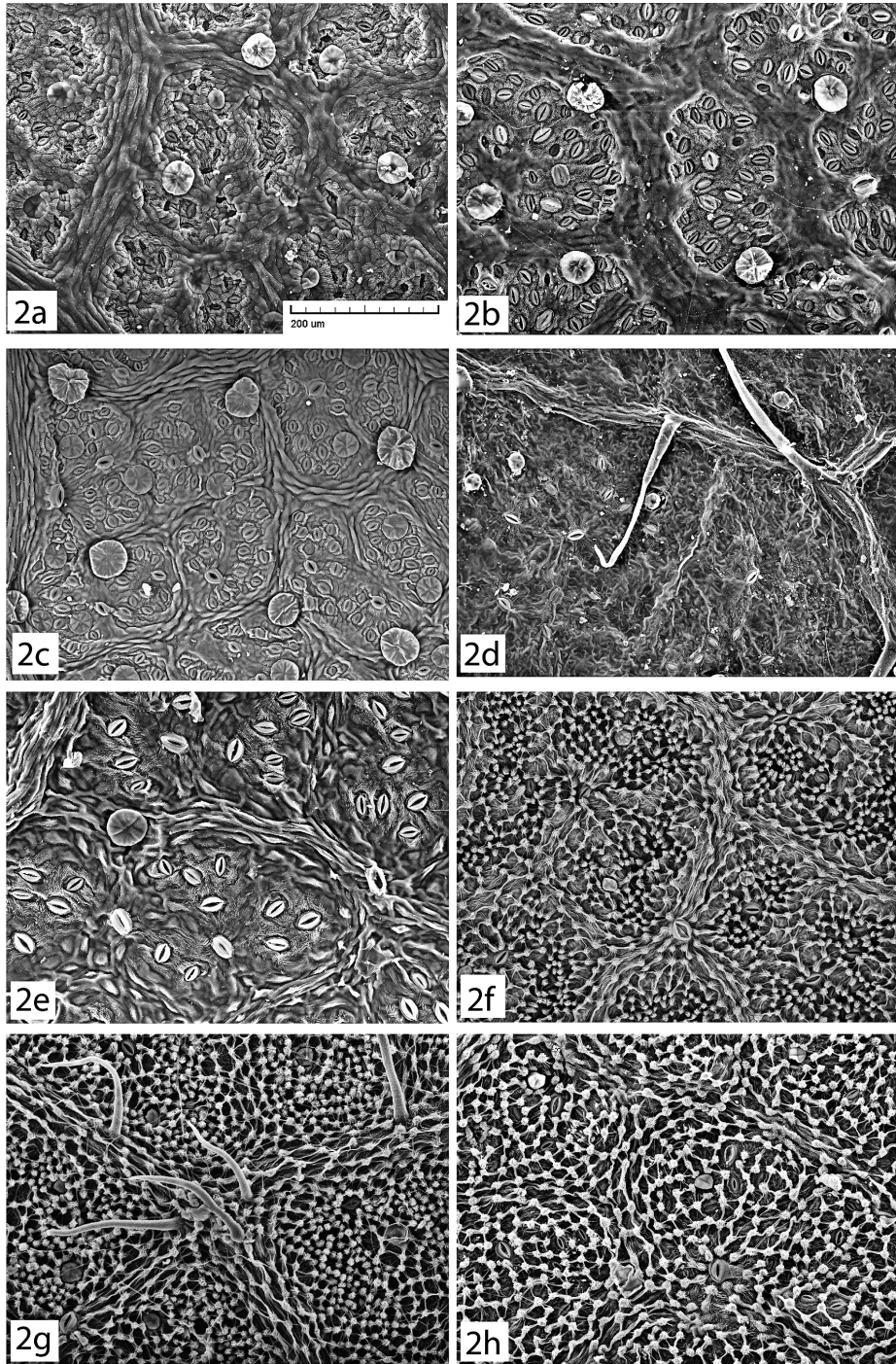


FIG. 2. Scanning electron micrographs of adaxial leaf surfaces of: 2a) *Fraxinus berlandieriana*; 2b–d) *F. pennsylvanica*; 2e) *F. profunda*; 2f, g) *F. americana* and 2h) *F. smallii*. Each specimen was photographed at 400x and 20kV; scale bar [2a] shows actual magnification. Voucher specimen, herbarium and collecting locations found in Table 1.

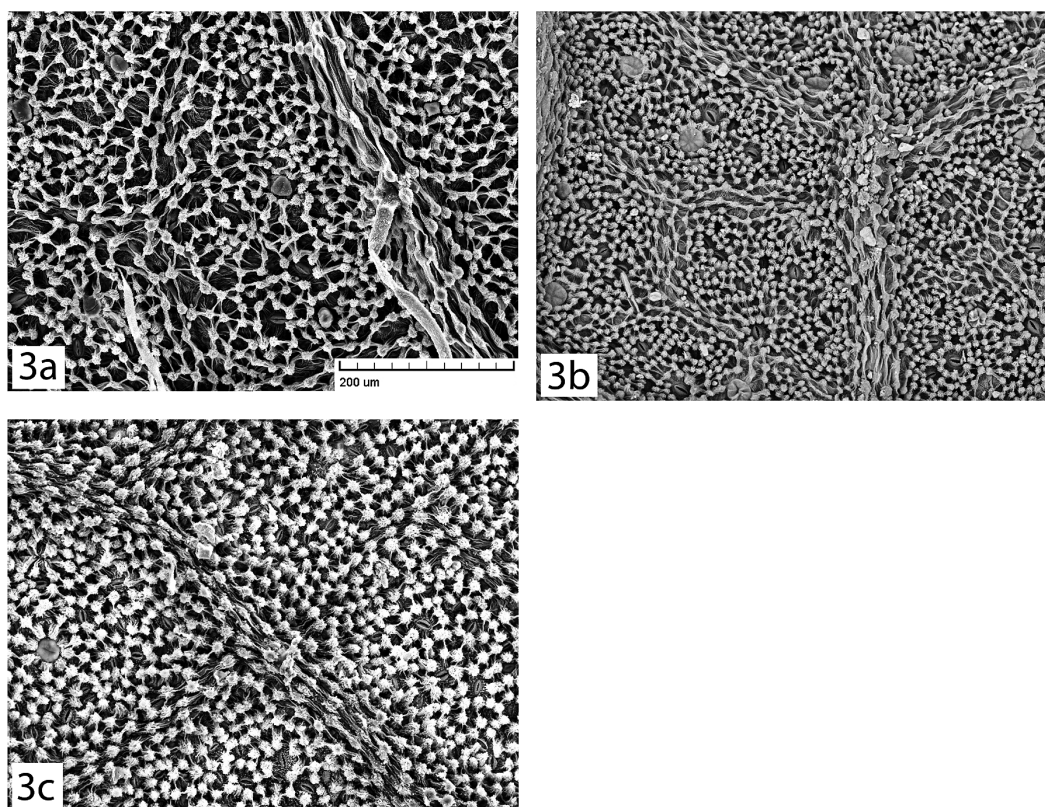


FIG. 3. Scanning electron micrographs of adaxial leaf surfaces of: 3a) *Fraxinus biltmoreana*; and 3b, c) *F. albicans*. Each specimen was photographed at 400x and 20kV; scale bar [3a] shows actual magnification. Voucher specimen, herbarium and collecting locations found in Table 1.

merous and their surfaces are convex with pronounced striations. In *F. profunda* (Fig. 2e), the stomata are conspicuously larger and only the epidermal cells immediately surrounding each stomate have striate surfaces—the others are smooth-surfaced; the same morphology was observed by Hardin and Beckmann (1982, SEM photo, Fig. 24) and by Wright (1944, photo of a collodion peel, Fig. 6).

All three ploidal entities of *Fraxinus americana* [*F. americana* (2x), *F. smallii* (4x), and *F. biltmoreana* (6x)] are nearly identical among themselves and to *F. albicans* in the “papillose” structure of their abaxial leaf surfaces (Figs. 2f–h and 3). *Fraxinus pauciflora*, of northern Florida and southern Georgia, has not yet been studied with SEM technology but also appears to be

similar. Through a survey at MO of the whole genus, including Asian species, it appears that these are the only species where this morphology occurs.

In the *Fraxinus americana* group, the abaxial epidermal leaf surface is mostly obscured by an overlay (presumably cuticular) composed of a reticulum of thin ridges with thickenings at the ridge intersections. The thickenings are the papillae of the “papillose” feature commonly described as characteristic and diagnostic of the species group. The papillae, each of which occurs near the center of an epidermal cell (fide Hardin & Beckmann 1982), vary in size relative to the rest of the reticulum of ridges. The system of ridges and papillae also usually covers the veinlets. Within the areoles defined by papillae and ridges,

thinner lines or incomplete ridges of the same material are formed at a lower level. The stomata, as well as parts of the immediately surrounding epidermal cells, are partly obscured but still visible through the reticulum.

DISCUSSION

Abruptly convex surfaces of abaxial epidermal cells produce a papillose appearance that presumably has been the basis for the recognition of *Fraxinus papillosa* and for the association of these plants with *F. americana*. As implied by Miller (1955), however, the papillose pattern of *F. papillosa* is not homologous with that of *F. americana*. The present report shows that *F. papillosa* is more similar to *F. velutina* in the lack of a cuticular overlay on its abaxial leaf surfaces, this presumably a primitive character state compared to the specialized one in *F. americana*.

The epidermal morphology of *Fraxinus papillosa* has proved to be surprisingly and unexpectedly distinct. Initially, we thought that *F. papillosa* and *F. velutina* probably were completely intergrading, but such may not be the case. A decision regarding the taxonomic status of *F. papillosa* awaits a detailed study of the geographic distribution and assessment potential genetic interactions between the two (Nesom and Williams in prep.).

The phylogenetic study of *Fraxinus* by Jeandroz et al. (1997) did not include *F. papillosa*, but in the study by Wallander (2008), the two samples of *F. papillosa* are positioned cladistically basal to the *F. pennsylvanica* group (including samples of *F. berlandieriana*, *F. caroliniana*, *F. latifolia*, *F. pennsylvanica*, *F. profunda*, and *F. velutina*; also including one sample of *F. albicans*).

In Wallander's study (2008), the two samples of *Fraxinus albicans* were placed in different cladistic positions, each among taxa of the *F. pennsylvanica* group, each far-removed from *F. americana*. Wallander concluded that "presence or absence of epidermal papillae appears not to be phylo-

genetically informative in this section [sect. *Melioides*]." From our perspective, *F. albicans* is a relatively uniform species and the two samples should be expected to appear together on a cladogram. Further, it is difficult to believe that the papillose morphology in *F. americana* and *F. albicans* has evolved in parallel—reexamination of the sample identifications in Wallander's study appears to be in order.

ACKNOWLEDGEMENTS

We are grateful to the staff at herbaria BRIT, MO, and TEX for their hospitality and help with this study and to Jim Henrickson for discussions of epidermal features in ashes. Samples for the SEM study were taken with permission from TEX-LL, MO, and BRIT. We thank the Department of Biological Sciences at Sam Houston State University for providing support and access to the SEM.

LITERATURE CITED

- Correll, D. S. and M. C. Johnston. 1970. *Manual of the Vascular Plants of Texas*. Renner: Texas Research Foundation.
- Elias, T. S. 1980. *The Complete Trees of North America*. New York: Van Nostrand Reinhold Co.
- Felger, R. S., M. B. Johnson, and M. F. Wilson. 2001. *The Trees of Sonora, Mexico*. New York: Oxford University Press.
- Hardin, J. W. and R. L. Beckmann. 1982. Atlas of foliar surface features in woody plants: 5. *Fraxinus* (Oleaceae) of eastern North America. *Brittonia* 34: 129–140.
- Jeandroz, S., A. Roy, and J. Bousquet. 1997. Phylogeny and phylogeography of the circumpolar genus *Fraxinus* (Oleaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. *Molec. Phylogenet. Evol.* 7: 241–251.
- Kearney, T. and R. H. Peebles. 1960. *Arizona Flora with Supplement*. Los Angeles: University of California Press.
- Kremer, D., E. Stabentheiner, Ž. Borzan, and R. J. Grubešić. 2008. Micromorphological differences between some European and American *Fraxinus* (Oleaceae) species. *Biologia* 63: 1111–1117.
- Lingelsheim, A. 1907. Vorarbeiten zu einer Monographie der Gattung *Fraxinus*. *Bot. Jahrb.* 40: 185–223.

- . 1920. Oleaceae-Oleoideae-Fraxineae. Pflanzenreich. IV 243: 1–61.
- Little, E. L., Jr.** 1952. Notes on *Fraxinus* (ash) in the United States. *J. Wash. Acad. Sci.* 42: 369–380.
- . 1976. *Atlas of United States Trees*: Vol. 3, minor western hardwoods. U.S.D.A. Misc. Publ. 1314.
- Martin, W. C. and C. R. Hutchins.** 1980. *A Flora of New Mexico*. Vols. 1 and 2. Vaduz, Germany: J. Cramer.
- Miller, G. N.** 1955. The genus *Fraxinus*, the ashes, in North America, north of Mexico. Cornell. Expt. Sta. Memoir 335, Cornell Univ., Ithaca, New York.
- Munz, P. A. and J. D. Laudermilk.** 1949. A neglected character in western ashes (*Fraxinus*). *Aliso* 2: 49–62.
- Nesom, G. L.** 2010a. Observations on *Fraxinus albicans* Buckley (Oleaceae), the correct botanical name for *Fraxinus texensis*. *Phytoneuron* 2010-33: 1–12.
- . 2010b. Taxonomy of the water ashes: *Fraxinus caroliniana*, *F. cubensis*, and *F. pauciflora* (Oleaceae). *Phytoneuron* 2010-39: 1–13.
- Powell, A. M.** 1988. *Trees and Shrubs of the Trans-Pecos and Adjacent Areas*. Austin: University of Texas Press.
- Rehder, A.** 1917. The genus *Fraxinus* in New Mexico and Arizona. *Proc. Amer. Acad. Arts* 53: 199–212.
- Sargent, C. S.** 1933. *Manual of the Trees of North America (exclusive of Mexico)* (ed. 2). Boston and New York: Houghton Mifflin Co.
- SEINET.** 2009. Southwest Environmental Information Network. Southwest Biodiversity Consortium. <<http://swbiodiversity.org/seinet/index.php>>
- Simpson, B. J.** 1988. *A Field Guide to Texas Trees*. Austin: Texas Monthly Press.
- Solereder, H.** 1908. *Systematic Anatomy of the Dicotyledons*. Translated from German by L.A. Boodle and F.R. Fritsch. Rev. by D.H. Scott. Oxford: Clarendon Press.
- Standley, P. S.** 1924. Oleaceae. Trees and Shrubs of Mexico. *Contr. U.S. Natl. Herb.* 23: 1132–1141.
- Turner, B. L., H. Nichols, G. Denny, and O. Doron.** 2003. Atlas of the Vascular Plants of Texas. *Sida, Bot. Misc.* 24: 1–888.
- USDA, NRCS.** 2009. The PLANTS Database. National Plant Data Center, Baton Rouge, Louisiana. <<http://plants.usda.gov>>
- Wallander, E.** 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Pl. Syst. Evol.* 273: 25–49.
- Wilkinson, H. P.** 1979. The plant surface. In: C. R. Metcalfe and L. Chalk, (eds.). *Anatomy of the Dicotyledons* (ed. 2). Vol. 1, *Systematic Anatomy*. Oxford: Clarendon Press.
- Wright, J. W.** 1944 [1945]. Epidermal characters in *Fraxinus*. *Proc. Indiana Acad. Sci.* 54: 87–90.