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A Study of the Leaf Epidermis of Selected Species of Veerbena

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A STUDY OF THE LEAF EPIDERMIS

OF SELECTED SPECIES

OF VERBENA
(TITLE)

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A STUDY OF THE LEAF EPIDERMIS OF SELECTED SPECIES OF VERBENA

INTRODUCTION

The investigation was of the epidermal layers of leaves of the genus Verbena. Attention was given to such characteristics as epidermal cell size, epidermal cell form, distribution of stomates, and epidermal trichomes. In addition, a limited attempt was made to correlate the epidermal characteristics with the reported somatic chromosome numbers for these species.

Verbena is one of the larger genera of the family Verbenaceae. There are 98 genera and more than 2600 species in the family, and most of these are native to subtropical and tropical regions. The family consists of herbs, shrubs, or trees having tetragonal, often annulate, stems and branches. The leaves are mostly opposite, but in a few species they are alternate, whorled, or scattered. The leaves are simple, more rarely palmately or pinnately compound, and without stipules. The family Verbenaceae is closely related to the Labiatae or Mint family. The Verbenaceae can be distinguished from the Labiatae by the undivided ovary, the terminal style, and

the usually nonverticillate inflorescence (Lawrence, 1951).

There are approximately 270 species in the genus Verbena, of which many are hybrids. Most species of Verbena grow in tropical or subtropical regions; some species are native to the temperate regions of the New World and a smaller number to the cooler parts of the Old World. The genus includes both herbs and woody plants. The members of the genus may be either erect or creeping. The leaves are opposite or whorled and may be obtuse, ovate, lanceolate, or acuminate in form with margins being deeply incised or regularly toothed (Fernald, 1950). The ovary is superior with two carpels, four cells, and with a single ovule in each cell. The fruit consists of four hard indehiscent nutlets at maturity. The stigma usually is two-lobed and the style arises from the terminal part of the ovary. The flowers are sessile in dense or loose spikes at the ends of the stem and the branches. Those species growing in the United States usually flower during the summer. Each flower is subtended by bracts. The calyx is tubular and 5-lobed. The tubular and sympetalous corolla usually has 5 lobes and is frequently bilabiate. Four stamens, each with two-celled anthers, are present (Gleason and Cronquist, 1963). The most recent materials toward a monograph of the genus Verbena is by Harold N. Moldenke, (Moldenke, 1963[a], 1963[b], 1963[c], 1963[d], 1964[a], 1964[b], 1964[c], 1964[d], 1964[e], 1964[f], 1964[g], 1964[h], 1964[i], 1965[a], 1965[b], and 1965[c]).

Materials and Methods

A dried leaf from each of 12 different species of the genus Verbena was obtained from the herbarium of the Missouri Botanical Garden. The following 12 species of Verbena were studied:

1. V. brasiliensis Vell. was collected by W. H. Lewis (#5301) in Harris County, Texas, August 1, 1959. The plant is an erect herb with stout stems. It has short flower spikes and is found in dry, sandy soil and waste places from Virginia to Florida, Arkansas, and Texas. A haploid chromosome number of 14 has been reported by W. H. Lewis and R. L. Oliver.
2. V. cameronensis L. I. Davis was collected by Alfred Traverse (#1107) in Cameron County, Texas, April 26, 1959. The plant is an herb with stems lying on the ground and ascending. It has sparsely flowered heads. V. cameronensis is a new species found in Texas. A haploid chromosome number of 15 has been reported by W. H. Lewis and R. L. Oliver.
3. V. carolina L. was collected by J. C. Blumer in Tallahassee, Florida, April, 1843. The plant is an herb with erect stems that branch at the base. Terminal flower spikes are present. It is found in the southeastern United States. A haploid chromosome number of 7 has been

reported by W. H. Lewis and R. L. Oliver.

4. V. cloveri Moldenke was collected by F. A. Barkley in Frio County, Texas, February 26, 1944. The herbaceous plant branches near the base and has terminal flower spikes. The plant is found in most of the United States. A haploid chromosome number of 7 has been reported by W. H. Lewis and R. L. Oliver.

5. V. delticola Small was collected by C. L. Lundell (#10788) in Cameron County, Texas, March 16, 1942. The stems of this herbaceous plant are branching and creeping on the ground. Flowers occur in spikes. The plant is found in the southwestern United States. A haploid chromosome number of 15 has been reported by W. H. Lewis and R. L. Oliver.

6. V. halei Small was collected by B. Shimek in Natchez, Mississippi, June 18, 1898. The plant is an herb with erect branching stems and slender flower spikes. It is found in sandy soil of the southwestern United States. A haploid chromosome number of 7 has been reported by W. H. Lewis and R. L. Oliver.

7. V. perennis Wooten was collected by W. H. Lewis in Mescalero, New Mexico, September 2, 1960. The plant is shrubby with numerous erect stems

and is found growing in crevices of rocks. The plant is found in the southwestern United States. A haploid chromosome number of 7 has been reported by W. H. Lewis and R. L. Oliver.

8. V. pumila Rydb. was collected by W. H. Lewis and R. L. Oliver (#5435) at Richland Springs, Texas, April 14, 1960. The plant is a creeping herb with several stems originating at the base. Short flower spikes are present. It is common in southeastern United States. A haploid chromosome number of 10 has been reported by W. H. Lewis and R. L. Oliver.

9. V. quadrangulata Heller was collected by W. Lewis and R. L. Oliver at Raymondville, Texas, March 21, 1960. The plant is a creeping herb with several stems branching from the base. Short terminal flower spikes are present. The plant is common in southwestern United States. A haploid chromosome number of 10 has been reported by W. Lewis and R. L. Oliver.

10. V. rigida Spreng. was collected by C. D. Smith at San Augustine, Texas, May 7, 1941. The plant is shrubby with terminal cymes. It is common in the southwestern United States. A 2n somatic chromosome number of 42 has been reported by W. H. Lewis and R. L. Oliver.

11. V. tenuisecta Briq. was collected by W. H. Lewis

and R. L. Oliver in Angelina County, Texas, March 5, 1960. The plant is a creeping herb with many branches. Terminal flower spikes are present. It is found in sandy woods and fields from South Carolina to Florida and west to Missouri, Arkansas and Texas. A 2n somatic chromosome number of 10 has been reported by W. H. Lewis and R. L. Oliver.

12. V. urticifolia L. was collected by J. C. Blumer in the Chiricahua Mountains of Arizona, October 12, 1907. The plant is an erect herb with stems branching near the base. Slender flower spikes are present. It is found in moist fields and meadows from Quebec and Ontario south to Florida and west to Nebraska, Oklahoma and Texas. A 2n somatic chromosome number of 14 has been reported by W. H. Lewis and R. L. Oliver.

The chlorophyll was extracted from the dried leaves by boiling in alcohol. The leaves were then transferred to vials containing a 5% aqueous solution of sodium hydroxide. Frequent changes of approximately 15 minutes duration were necessary for the removal of cell contents. If the leaves were not devoid of discoloration after several changes of sodium hydroxide, the specimens were washed in 3 changes of distilled water and then placed in a vial of 5% sodium hypochlorite for a period of 1 to 3 minutes for bleaching. When most of the color had been removed from the leaf, the

bleach was poured off and the specimens were washed in tap water for a few minutes and then placed in 50% ethanol to reharden the leaf tissue. Then the specimens were washed in running tap water for a period of twelve hours. The leaves were placed in 50% ethanol for 15 minutes and then stored in 70% ethanol.

Staining of the cleared leaves in 1% safranin in 50% ethanol for 24 hours was tried, but the epidermal layers were much more visible in unstained specimens. Permanent mounts in Canada Balsam were attempted, but the results were inferior to those obtained by making temporary microscopic mounts of the material.

The material was viewed with a Zeiss microscope and drawings were made with the aid of an attached camera lucida.

Results

Stomates were observed on the lower epidermis of the leaves of all the species studied. Stomates were also present on the upper epidermis of eight of the species, but no stomates were observed on the upper epidermis of four species. The upper epidermis of *Verbena brasiliensis* Vell., *V. cloveri* Moldenke, *V. halei* Small, *V. pumila* Rydb., and *V. quadrangulata* Heller had sparsely distributed stomates. The upper epidermis of *Verbena rigida* Spreng. and *V. tenuisecta* Briq. had densely distributed stomates and *V. perennis* Wooten had very densely distributed stomates. No stomates were observed on the upper epidermis of *Verbena cameronensis*, *V.*

carolina L., V. delticola Small, and V. urticifolia L.

All of the species studied had unicellular trichomes on both the upper and lower epidermal layers. Verbena cameronensis Davis, V. carolina L., V. cloveri Moldenke, V. delticola Small, V. pumila Rydb., V. quadrangulata Heller, and V. urticifolia L. had longer trichomes on the upper epidermis than on the lower epidermis. There seemed to be no definitive difference in length for Verbena brasiliensis Vell., V. halei Small, V. perennis Wooten, V. rigida Spreng., and V. Tenuisecta Briq.

The average length (based on 10 cells per species) of the upper epidermis cells ranged from 49.06μ in Verbena carolina L. to 96.53μ in V. delticola Small. The average length (based on 10 cells per species) of the lower epidermal cells ranged from 41.16μ in Verbena cloveri Moldenke to 102.13μ in V. cameronensis Davis.

Discussion and Conclusions

It appears there is not a systematic manner in determining, prior to examination, the presence or absence of stomates on the upper epidermis of leaves of species of the genus Verbena.

From this study it appears that all species will have unicellular trichomes on both the upper and the lower epidermis. Since 7 of the 12 species studied had longer trichomes on the upper epidermis than on the lower epidermis, further research of the comparative length of these struc-

tures could be justified. This study does not show any correlation between the average epidermal cell length and chromosome number.

In order to make a more definitive conclusion, one would need to examine many more specimens of the same species and many more species of the genus Verbena.

Explanation of Figs. 1-16

Fig. 1.--Upper epidermis of *Verbena perennis* Wooten x375

Fig. 2.--Lower epidermis of *V. perennis* Wooten x375

Fig. 3.--Lower epidermis hair of *V. perennis* Wooten x375

Fig. 4.--Upper epidermis hair of *V. perennis* Wooten x375

Fig. 5.--Lower epidermis hair of *V. carolina* L. x375

Fig. 6.--Upper epidermis hair of *V. carolina* L. x375

Fig. 7.--Lower epidermis of *V. carolina* L. x375

Fig. 8.--Upper epidermis of *V. carolina* L. x375

Fig. 9.--Upper epidermis of *V. halei* Small x375

Fig. 10.--Lower epidermis of *V. halei* Small x375

Fig. 11.--Lower epidermis hair of *V. halei* Small x375

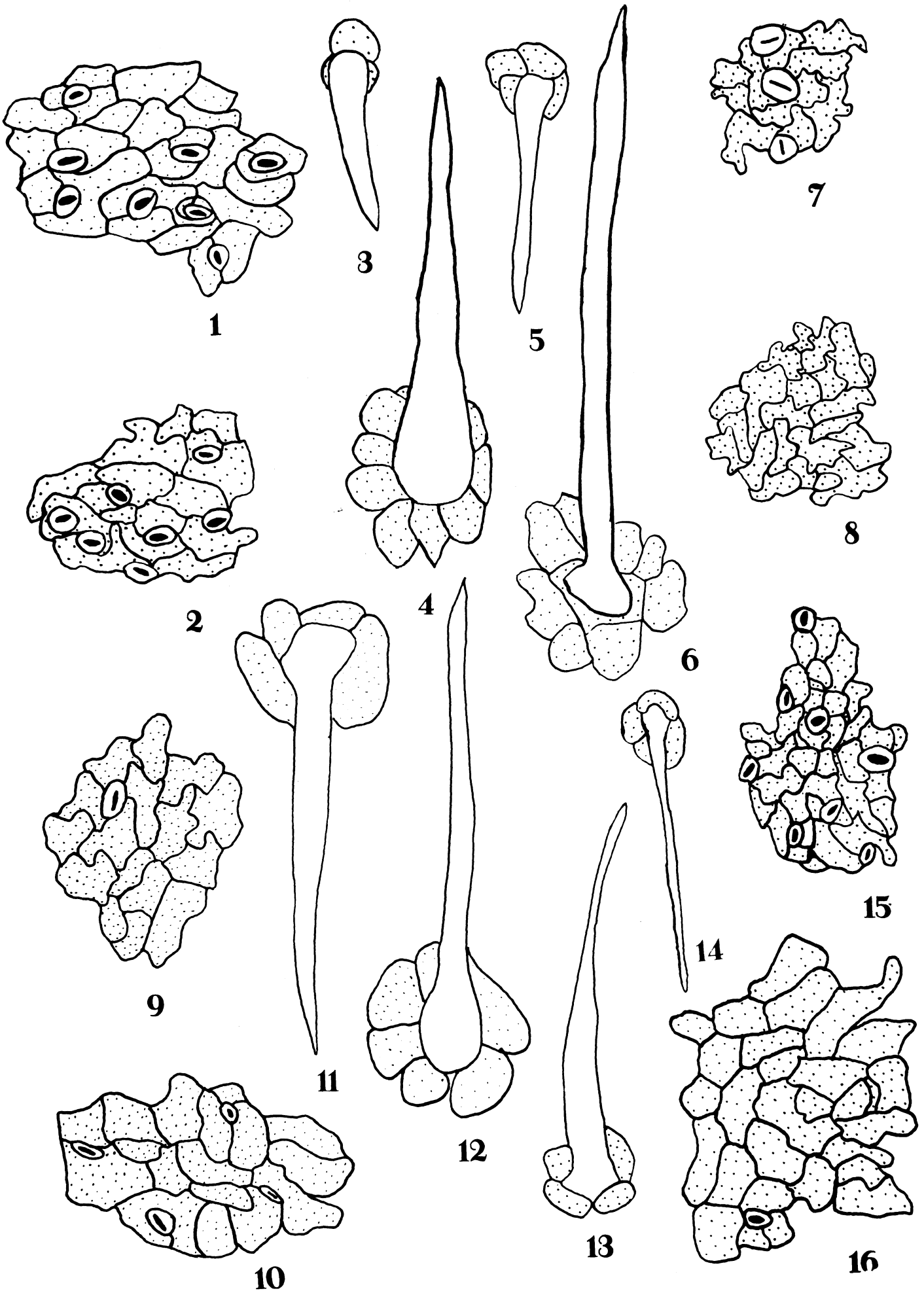
Fig. 12.--Upper epidermis hair of *V. halei* Small x375

Fig. 13.--Upper epidermis hair of *V. cloveri* Moldenke x375

Fig. 14.--Lower epidermis hair of *V. cloveri* Moldenke x375

Fig. 15.--Lower epidermis of *V. cloveri* Moldenke x375

Fig. 16.--Upper epidermis of *V. cloveri* Moldenke x375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Explanation of Figs. 17-28

Fig. 17.--Lower epidermis of Verbena urticifolia L. x375

Fig. 18.--Upper epidermis hair of V. rigida Spreng. x375

Fig. 19.--Upper epidermis of V. urticifolia L. x375

Fig. 20.--Lower epidermis hair of V. rigida Spreng. x375

Fig. 21.--Upper epidermis hair of V. urticifolia L. x375

Fig. 22.--Lower epidermis hair of V. urticifolia L. x375

Fig. 23.--Upper epidermis of V. rigida Spreng. x375

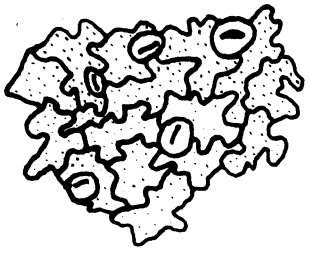
Fig. 24.--Lower epidermis of V. rigida Spreng. x375

Fig. 25.--Upper epidermis hair of V. tenuisecta Briq. x375

Fig. 26.--Lower epidermis hair of V. tenuisecta Briq. x375

Fig. 27.--Lower epidermis of V. tenuisecta Briq. x375

Fig. 28.--Lower epidermis of V. tenuisecta Briq. x375



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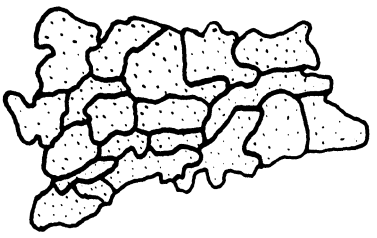

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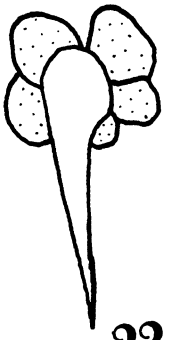

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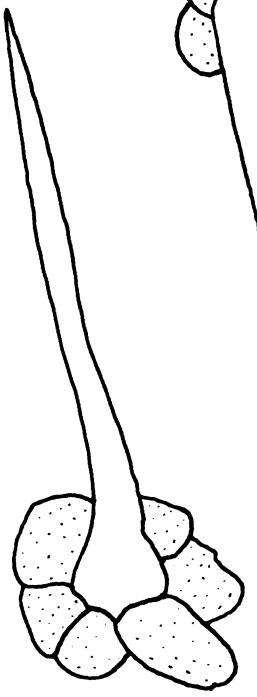

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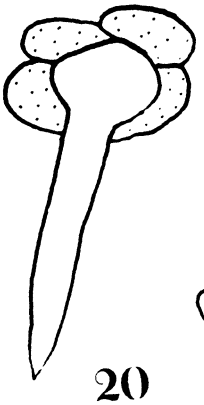

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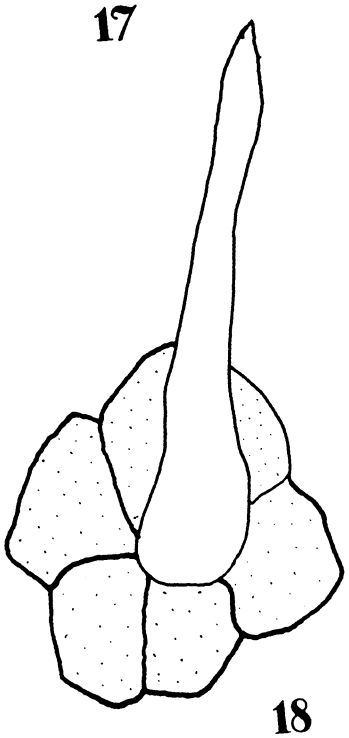

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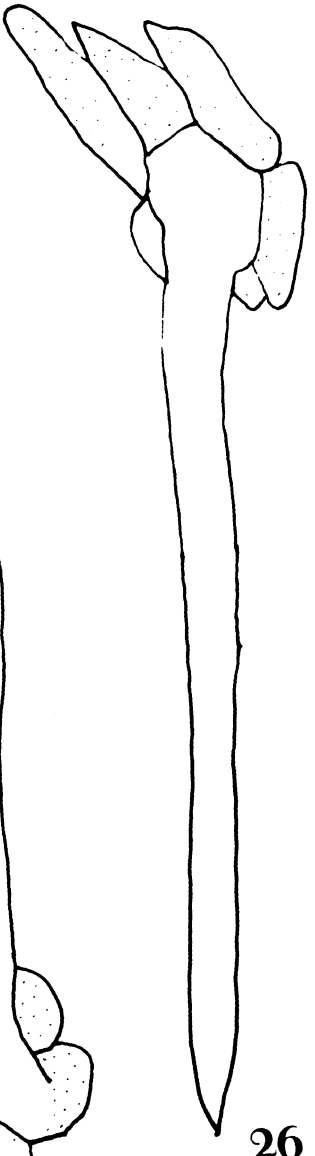

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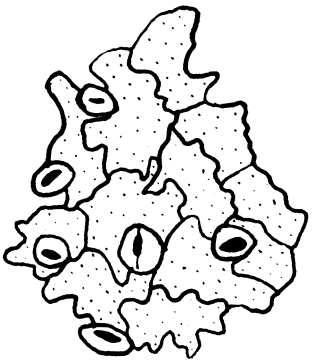

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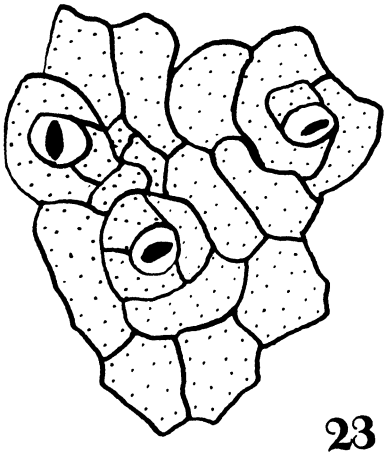

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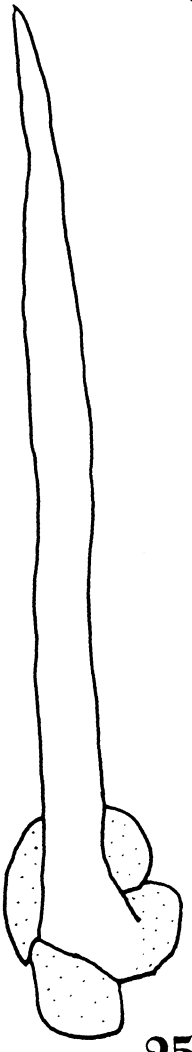

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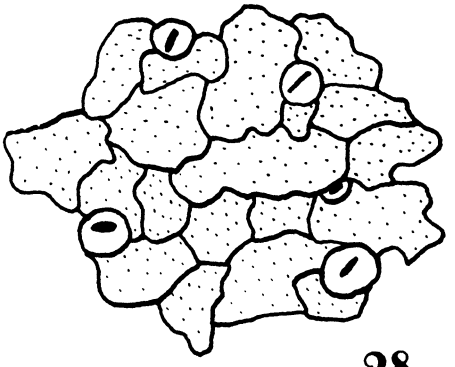

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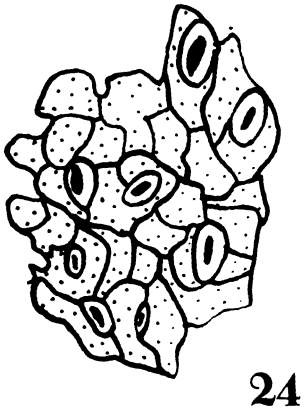

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Explanation of Figs. 29-40

Fig. 29.--Upper epidermis of Verbena quadrangulata Heller x375

Fig. 30.--Lower epidermis of V. quadrangulata Heller x375

Fig. 31.--Lower epidermis hair of V. brasiliensis Vell. x375

Fig. 32.--Upper epidermis hair of V. brasiliensis Vell. x375

Fig. 33.--Upper epidermis hair of V. quadrangulata Heller x375

Fig. 34.--Lower epidermis hair of V. quadrangulata Heller x375

Fig. 35.--Lower epidermis of V. pumila Rydb. x375

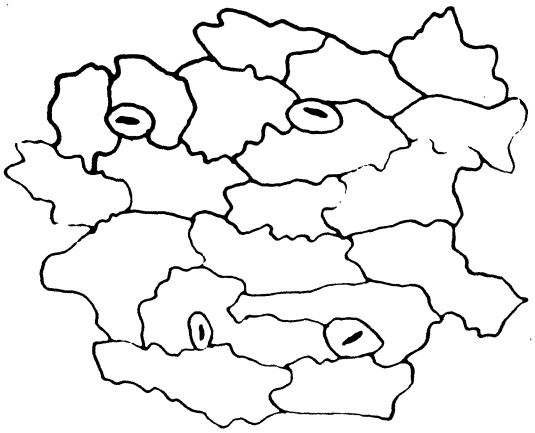
Fig. 36.--Lower epidermis of V. pumila Rydb. x375

Fig. 37.--Upper epidermis hair of V. pumila Rydb. x375

Fig. 38.--Lower epidermis hair of V. pumila Rydb. x375

Fig. 39.--Upper epidermis of V. pumila Rydb. x375

Fig. 40.--Lower epidermis of V. brasiliensis Vell x375



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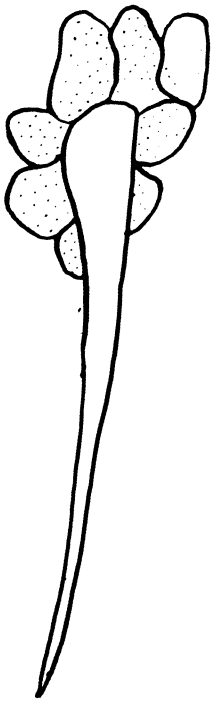

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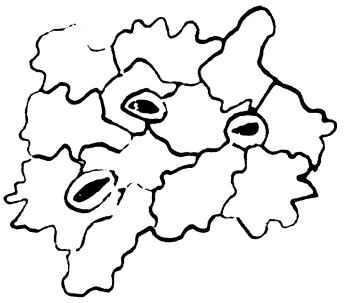

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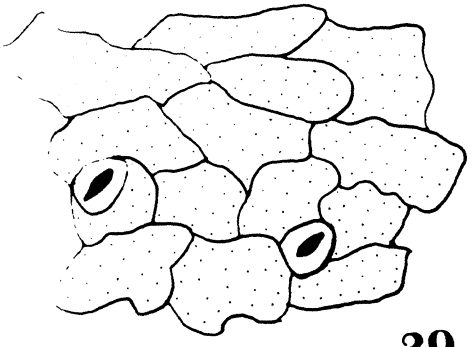

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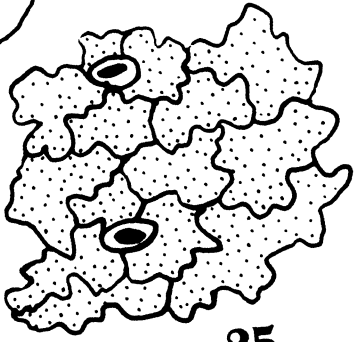

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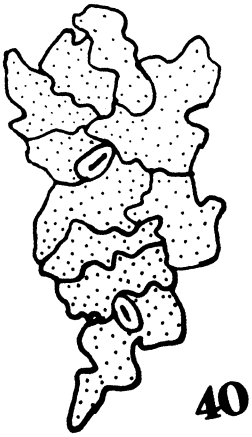

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Explanation of Figs. 41-48

Fig. 41.--Lower epidermis of *Verbena cameronensis* Davis x375

Fig. 42.--Upper epidermis of *V. cameronensis* Davis x375

Fig. 43.--Lower epidermis hair of *V. delticola* Small x375

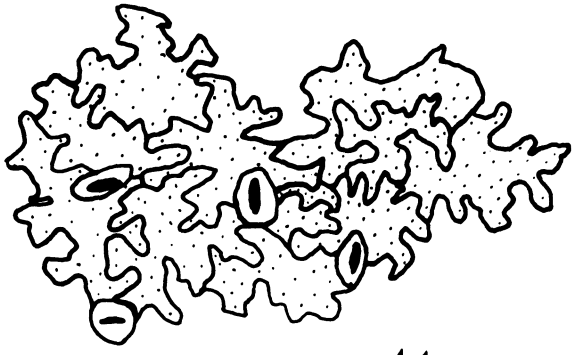
Fig. 44.--Upper epidermis hair of *V. delticola* Small x375

Fig. 45.--Upper epidermis hair of *V. cameronensis* Davis x375

Fig. 46.--Lower epidermis hair of *V. cameronensis* Davis x375

Fig. 47.--Lower epidermis of *V. delticola* Small x375

Fig. 48.--Upper epidermis of *V. delticola* Small x375



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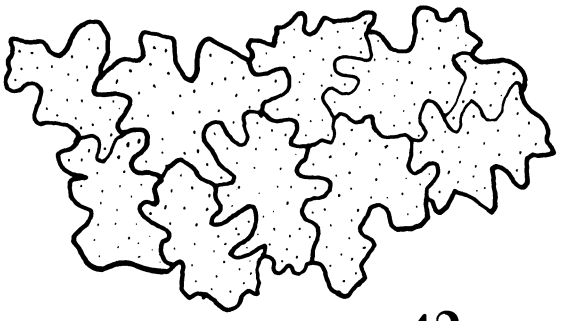

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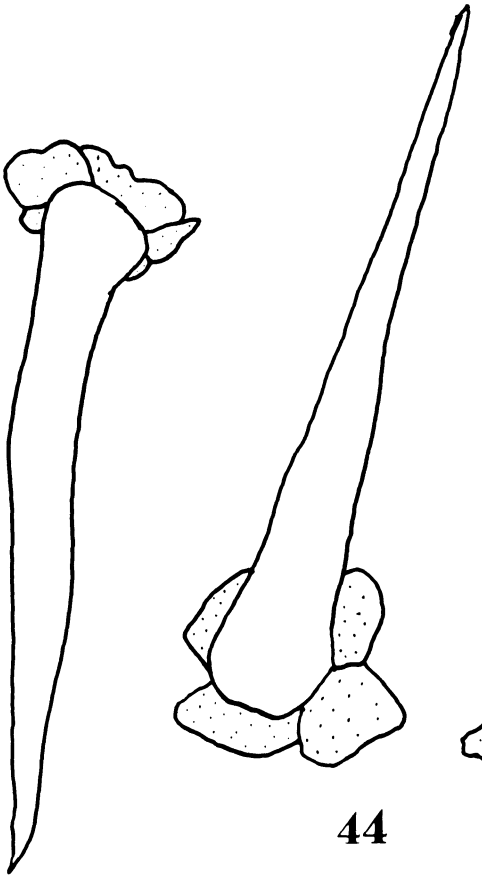

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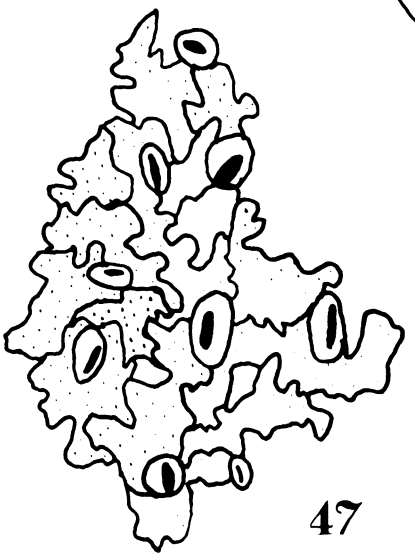

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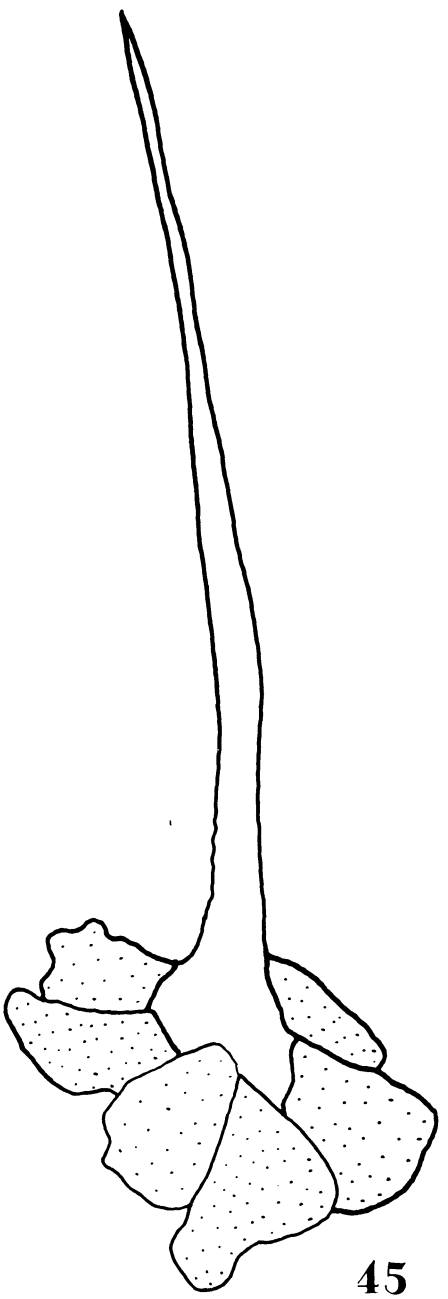

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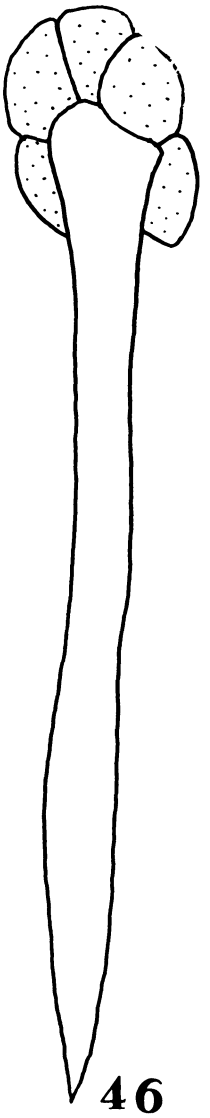

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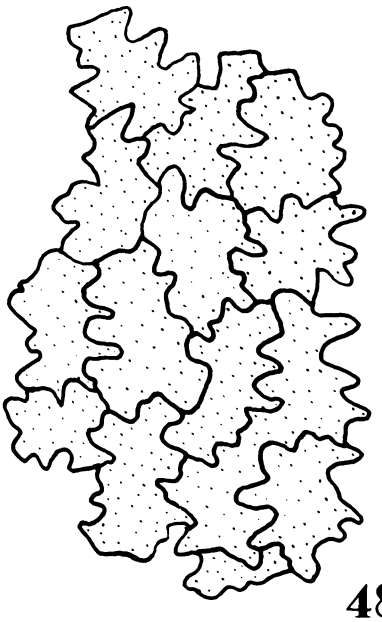

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TABLE 1.-Leaf Characteristics of species of Verbena

Species	Hairs Present or Absent		Hairs Uni- or Multi-Cellular	Hairs Longer on Upper or Lower Epidermis
	Upper	Lower		
Verbena brasiliensis Vell.	+	+	Uni	E
V. cameronensis	+	+	Uni	U
V. carolina L.	+	+	Uni	U
V. cloveri Moldenke	+	+	Uni	U
V. delticola Small	+	+	Uni	U
V. halei Small	+	+	Uni	E
V. perennis Wooten	+	+	Uni	E
V. pumila Rydb.	+	+	Uni	U
V. quadrangulata Heller	+	+	Uni	U
V. rigida Spreng.	+	+	Uni	E
V. tenuisecta Briq.	+	+	Uni	U
V. urticifolia L.	+	+	Uni	U

+ denotes presence of hairs.

Uni denotes hairs are unicellular.

E denotes no definitive difference in length of upper and lower epidermis hairs.

U denotes longer hairs on upper epidermis.

Distribution of Stomates		Average Epidermal Size in μ (based on 10 cells)		Chromosome Number	
Upper	Lower	Upper	Lower	n	2n
+	++	75.73	74.93	14	28
-	++	92.26	102.13	15	
-	++	49.06	58.13	7	
+	++	71.73	41.06	7	
-	+++	96.53	85.33	15	
+	++	60.53	72.00	7	
+++	+++	69.06	72.26	7	
+	+++	90.13	65.86	10	
+	+++	88.55	74.13	10	
++	+++	76.80	54.66		42
++	+++	67.73	63.46		10
-	+++	52.80	48.53		14

+ denotes sparse distribution of stomates.

++ denotes dense distribution of stomates.

+++ denotes very dense distribution of stomates.

- denotes absence of stomates.

Note: Chromosome numbers as reported by Lewis and Oliver (1961) are listed.

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