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Anatomical data for the classification of Muhlenbergia pungens.

C. Martin Warwick

ANATOMICAL DATA FOR THE CLASSIFICATION OF *MUHLENBERGIA PUNGENS*

A Thesis

Presented to the

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and the

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

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THESIS ACCEPTANCE

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INTRODUCTION

The genus *Muhlenbergia* Schreb. is a member of the Gramineae. Of the 110 species described to date, 55 species are American (Gleason, 1963). The species are most numerous in the southwestern states. *Muhlenbergia* includes members of considerable diversity and several efforts have been made to subdivide the genus. Hitchcock (1913) states that members of *Muhlenbergia* have been classified in other genera such as *Vilfa*, *Sporobolus*, *Calamagrostis*, *Clomena*, *Bealia*, *Chaboissaea*, and *Trichloa*. However, no classification has been totally satisfactory.

Muhlenbergia pungens was first described by Thurber (1863). Bush (1921) reassigned the species to the genus *Podosaemum*, but he has not been followed by later workers. Sutherland (1975) placed the species in a group with *Redfieldia flexuosa* using vegetative characteristics.

Gross morphological characteristics have been the primary basis for classification of the grasses, but it is evident that additional characteristics are needed to properly classify this species. The purpose of this paper is to present anatomical characteristics that will supplement the gross morphological characteristics in classifying *Muhlenbergia pungens*.

MATERIALS AND PROCEDURES

Specimens of Muhlenbergia pungens were collected from the Monroe Sandhills, an outcropping of sand about four miles south of Monroe, Nebraska. Three collections were made and dried for storage. Some were air dried, some were pressed in a plant press, and the rest were rolled in newspapers.

Herbarium specimens of Muhlenbergia pungens were obtained from the University of Nebraska at Omaha and from the University of Nebraska at Lincoln so that material from other locations could also be examined. As a basis for comparison, herbarium specimens of Muhlenbergia asperifolia (Nees and Meyen) and Sporobolus airoides (Torr.) were examined.

Center sections of the leaves were placed in boiling water, to which had been added a small amount of detergent as a wetting agent. After a few minutes the leaf sections were dehydrated by using a modification of the Tertiary Butyl Alcohol Dehydration Method suggested by Johansen (1940). The entire dehydration series is shown on Table I.

The leaf sections were placed on top of the partially solidified wax and placed in the embedding oven. The molten Carowax was changed twice at two-hour intervals. The sections were then embedded in fresh Carowax and set aside for sectioning.

Hand sections of the leaves were taken at a point midway between the tip of the blade and the ligule. The sections were affixed to clean glass slides with Haupt's or Mayer's Albumin adhesives for staining. Staining was accomplished by using the Sass (1958) Safranin and

Fast Green Combination staining procedure shown on Table II. The stained sections were made permanent by mounting in Histoclad and were covered with clean glass cover slips for examination.

Ligules of the three grasses were examined. Ligules of Muhlenbergia pungens were preserved by mounting in Hoyer's Mounting Medium after being softened in Aerosol-O-T as suggested by Pohl (1965).

Culms of Muhlenbergia pungens were softened as above and sectioned and mounted unstained.

The epidermis of softened leaves of Muhlenbergia pungens was stripped and mounted in Hoyer's Medium for examination.

Table I

Tertiary-Butyl Alcohol Dehydration Series

Step 1 5% ethyl alcohol 95% distilled water	Step 2 11% ethyl alcohol 89% distilled water
Step 3 18% ethyl alcohol 82% distilled water	Step 4 30% ethyl alcohol 70% distilled water
Step 5 40% ethyl alcohol 10% tertiary butyl alcohol 50% distilled water	Step 6 50% ethyl alcohol 20% tertiary butyl alcohol 30% distilled water
Step 7 50% ethyl alcohol 35% tertiary butyl alcohol 15% distilled water	Step 8 45% ethyl alcohol 55% tertiary butyl alcohol
Step 9 25% absolute ethyl alcohol 75% tertiary butyl alcohol	Step 10 100% tertiary butyl alcohol
Step 11 100% tertiary butyl alcohol	Step 12 50% tertiary butyl alcohol 50% paraffin oil
Step 13 100% paraffin oil	

The leaf pieces were left in each solution for two hours. The series was run non-stop from Step 1 through Step 12, as the alcohols act as hardening agents. Step 13, 100% paraffin oil, is a non-reactive storage or holding step. The leaf pieces were accumulated in paraffin oil and several groups were embedded at the same time for convenience.

Table II

Safranin-Fast Green Combination Stain

Step 1	Step 2	Step 3
xylene 3 minutes	absolute ethyl alcohol 3 minutes	95% ethyl alcohol 3 minutes
Step 4	Step 5	Step 6
70% ethyl alcohol 3 minutes	50% ethyl alcohol 3 minutes	30% ethyl alcohol 3 minutes
Step 7	Step 8	Step 9
distilled water 3 minutes	Safranin O 3 minutes	distilled water 3 minutes
Step 10	Step 11	Step 12
30% ethyl alcohol 5 minutes	50% ethyl alcohol 5 minutes	70% ethyl alcohol 5 minutes
Step 13	Step 14	Step 15
95% ethyl alcohol 5 minutes	Fast Green 10 seconds	xylene 5 minutes
	Step 16	
	Mounted in Histoclad	

RESULTS

Morphological and Anatomical Data

Muhlenbergia pungens (Fig. 1) has a scabrous, involute, stiff leaf, 1-2 mm wide, tapering to a sharp point, and measuring 23-38 mm from the collar to the tip of the blade. The leaf lacks auricles and has a hairy ligule measuring 0.5-1.0 mm. The culms are long and slender, 20-40 cm tall, decumbent and much branched at the base. The panicle is scabrous, persistent, purplish, open and 10-18 cm long; the primary branches 20-45 mm long and the secondary branches 15-13 mm long. The spikelets are single flowered, with the florets articulated above the glumes. The two glumes are nearly equal, being 1-2 mm long and ending in a short awn, 0.5-1.0 mm long. The lemma is 4-5 mm long (of this, 2 mm is awn) and 3-nerved, the two lateral nerves faint. The caryopsis apparently falls readily from the floret as none were found in the spikelets.

In the leaf epidermis, short cells (1/3-1/2 the average length) are located over the veins singly, in twos, in threes, and sometimes in short rows of 4-6 cells, but never in long rows. Square or dumbbell-shaped silica bodies are present over the veins (Fig. 9). The micro-hairs (Fig. 2) found over the veins are long and tapered to a point, a few having two cells with the apical cell slightly pointed.

In cross section (Fig. 2) it is apparent that the blade of the leaf is divided into four general types of tissue: vascular bundles, sclerenchyma, parenchyma, and chlorenchyma. The arrangement of

Figure 1. Muhlenbergia pungens

Figure 2. Muhlenbergia pungens leaf cross section

s- sclerenchyma

x- xylem

p- phloem

m- micro-hair

p. sh- parenchyma sheath

c- chlorenchyma

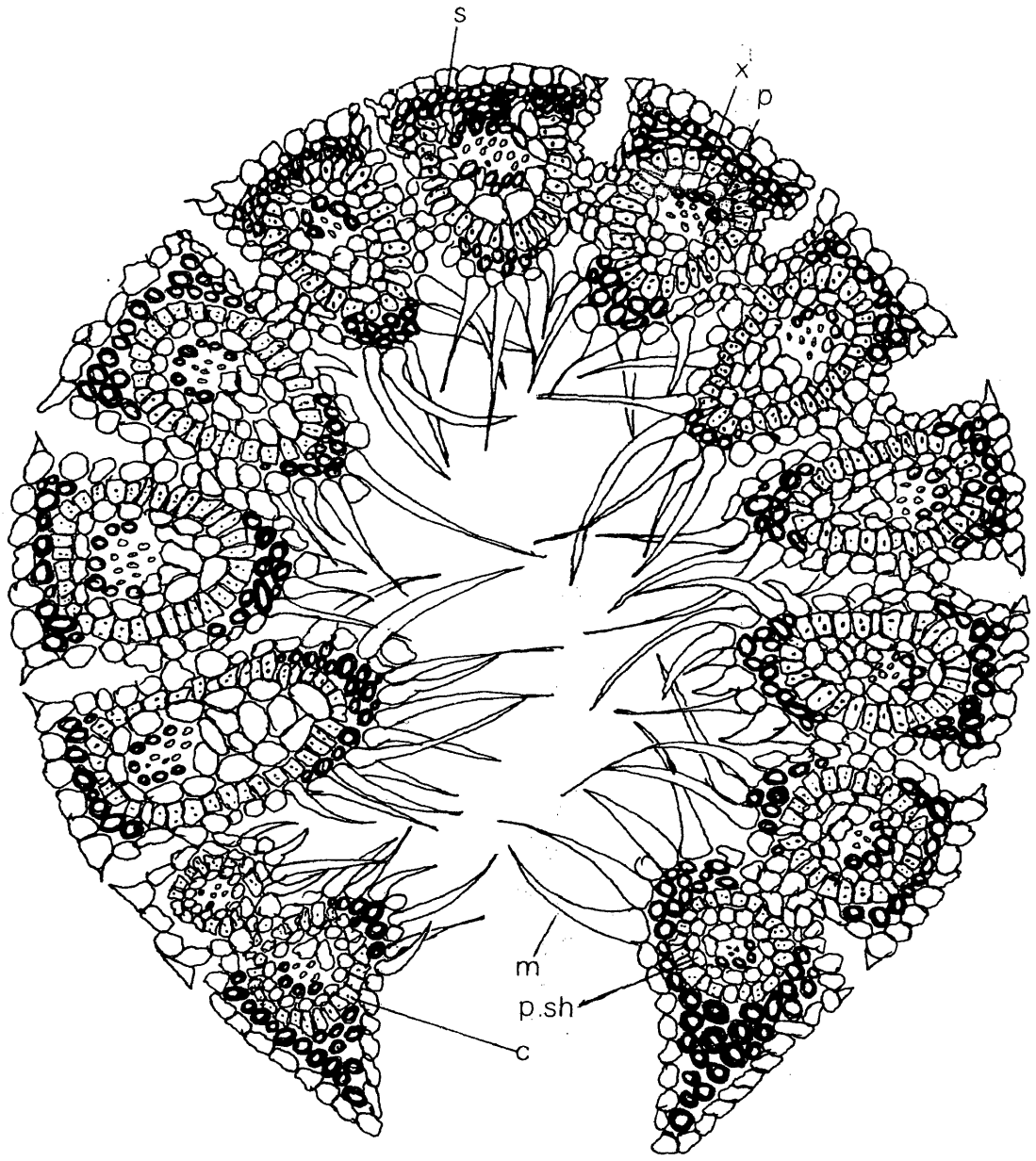

s
x
p
m
p.sh
c

Figure 3 Muhlenbergia pungens leaf cross section

s- sclerenchyma

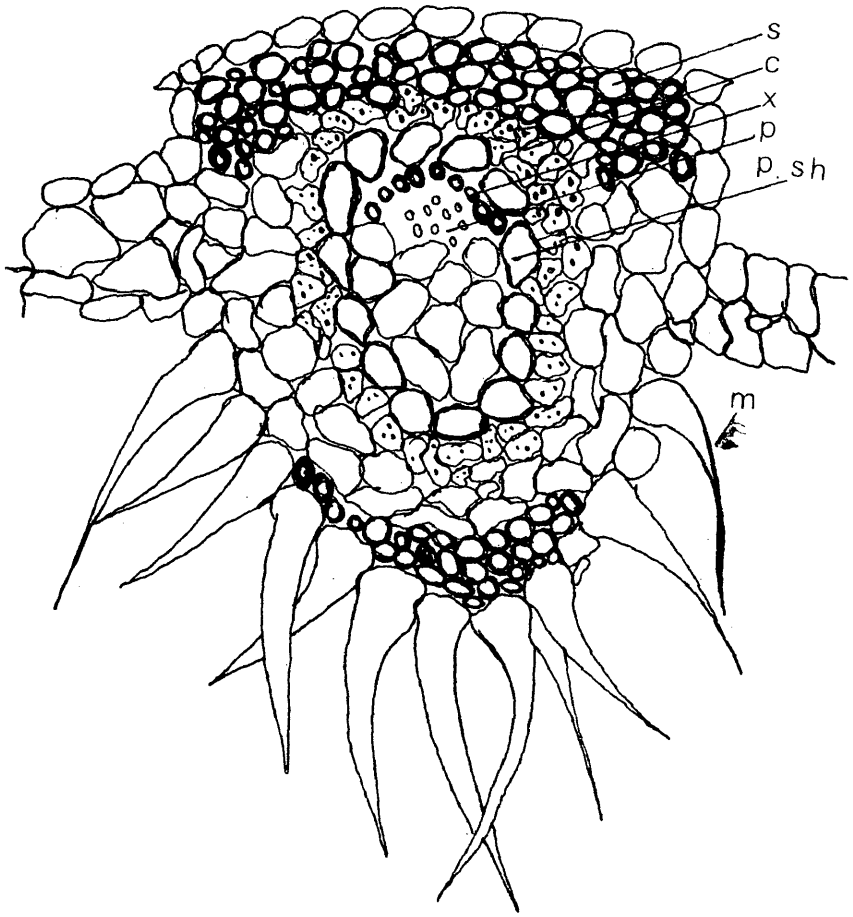
c- chlorenchyma

x- xylem

p- phloem

p. sh- parenchyma sheath

m- micro-hair


s
c
x
p
p. sh
m

sclerenchyma is distinctive, with about twice as much being found dorsal to the bundles as ventral to them. In most cases a parenchyma sheath surrounds the vascular bundles; sometimes this sheath contains sclerenchymatous cells continuous with those of the sclerenchyma cap. Parenchyma cells are also found inside the sheath. Chlorenchyma is usually found as a layer of cells around the vascular bundles, appearing to radiate outward from the bundle.

In cross section the leaf appears to be divided into sections, each section (Fig. 3) containing a vascular bundle surrounded by a parenchyma sheath which is, in turn, surrounded by the layer of chlorenchyma. A large sclerenchyma cap is dorsal to the bundle and a smaller schlerenchyma zone is ventral to it. These sections are connected by parenchyma cells. Micro-hairs are prominent on the ventral surface of the leaf.

The stem cross section (Fig. 4) is as distinctive as the leaf. The culm is best described as being in layers or rings of tissue. The center is large parenchyma cells, filled with a substance which appears almost black when dehydrated and mounted. This "filled" center is surrounded by a ring of "clear" parenchyma cells with small vascular bundles rather evenly spaced in it. Some of these bundles have a differtiated sheath of parenchyma cells. Surrounding this is a rather solid layer of sclerenchymatous cells 3-5 cells thick. The stem "core" is surrounded by a ring of vascular bundles. The vascular bundles are similar in size and appear to "sit" on the ring of sclerenchyma. A typical vascular bundle is as follows: a xylem-phloem "core" partially surrounded by a parenchyma sheath, with a layer of radiate chlorenchyma

Figure 4 Muhlenbergia pungens stem cross section

v.b.- vascular bundle

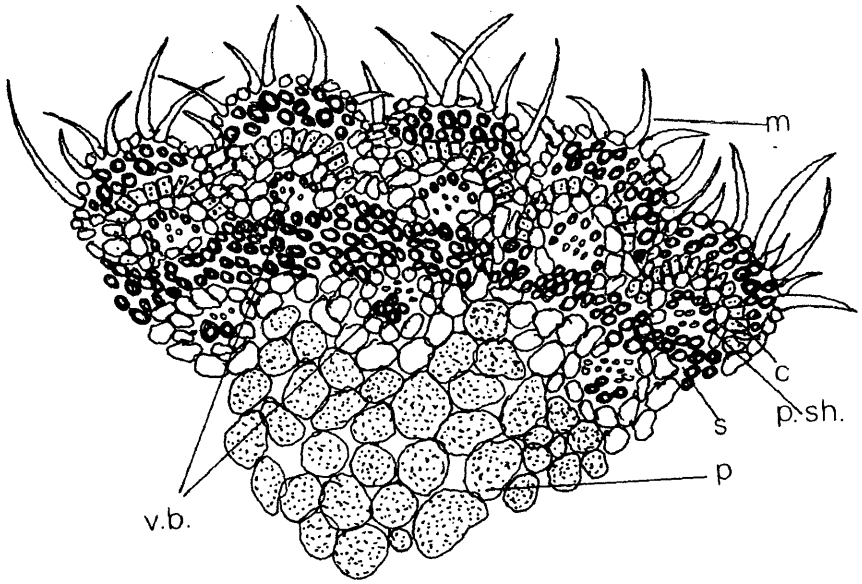
m- micro-hair

c- chlorenchyma

p. sh- parenchyma sheath

s- sclerenchyma

p- parenchyma "core"


m
c
p.sh.
s
p
v.b.

adjacent to and outside the sheath. The vascular bundle section is topped with a sclerenchyma cap. The epidermis has many micro-hairs, all tapering to a point.

Comparative Features

Ligule

Of the grasses placed in the genus Podosaemum (Bush, 1921), six species were examined. Five of the grasses had a membraneous ligule and one, Muhlenbergia pungens, had a hairy ligule (Fig. 5).

The ligules of Muhlenbergia asperifolia are (like those of most muhlenbergias) of the membraneous type, while those of Sporobolus airoides (and other Sporobolus) are fringes of hairs.

Caryopsis

The grain of Muhlenbergia pungens is a true caryopsis, with the ovary wall adhering tightly to the seed (Fig. 6). In Muhlenbergia asperifolia, the wall also adheres tightly, but in Sporobolus airoides, the wall separates readily from the seed. Muhlenbergia asperifolia, on the other hand, tends to hold its grain within the floret, while in Muhlenbergia pungens, it drops out, as it does in Sporobolus airoides.

Lemma

The lemma of Muhlenbergia asperifolia has the three veins characteristic of Muhlenbergia, while Sporobolus airoides has one vein as in most Sporobolus. Muhlenbergia pungens has three veins, but the lateral veins are very faint.

Figure 5 Muhlenbergia pungens ligule

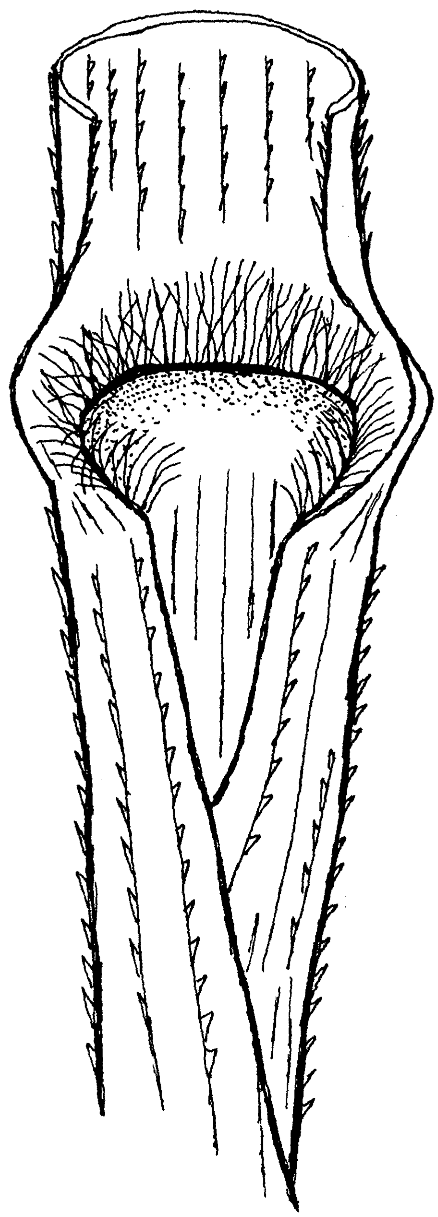
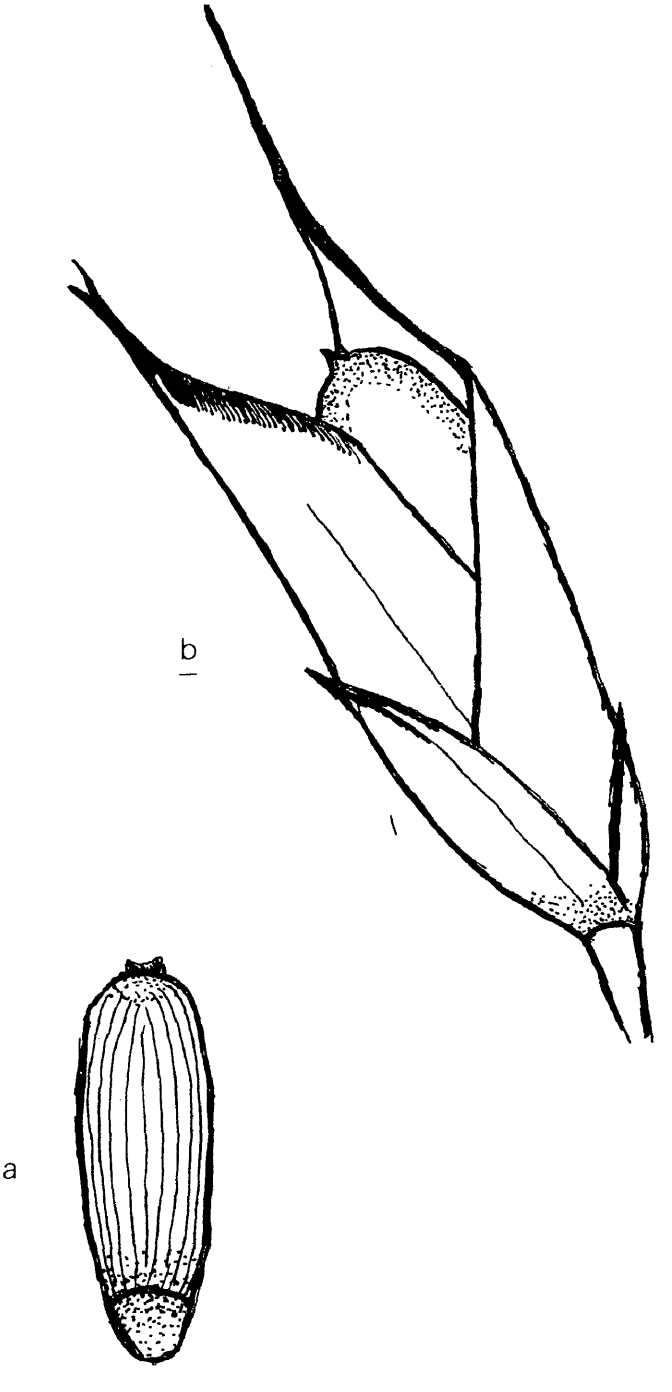


Figure 6 Muhlenbergia pungens

a- caryopsis

b- floret


b
a

Vascular Bundles

The vascular bundles of Muhlenbergia asperifolia (Fig. 7) and Muhlenbergia pungens (Fig. 2) are relatively uniform in size in the leaf blade. Sporobolus airoides (Fig. 8), in contrast, has bundles of varying sizes.

Sclerenchyma

The quantity and placement of sclerenchyma relative to the vascular bundle is distinctive in the leaf blade. Muhlenbergia asperifolia (Fig. 7) has sclerenchyma dorsal and ventral to the vascular bundle in cross section in fairly equal quantities. In Sporobolus airoides (Fig. 8) the sclerenchyma is found primarily dorsal to the bundle and rarely ventral to it. When sclerenchyma is found ventral to the bundle, only small amounts are present. Approximately twice as much sclerenchyma is found dorsal to the Muhlenbergia pungens vascular bundles as is found ventral to them (Fig. 2).

Micro-hairs

Short, tapered micro-hairs are found dorsal to the vascular bundle in Muhlenbergia asperifolia's leaf cross section (Fig. 7). Sporobolus airoides (Fig. 8) has the micro-hairs primarily ventral to the bundle. These are short and tapered. The micro-hairs on Muhlenbergia pungens are found primarily ventral to the vascular bundle and are long and tapered (Fig. 2).

Chlorenchyma

In Muhlenbergia asperifolia (Fig. 7) the chlorenchyma associated

Figure 7 Muhlenbergia asperifolia leaf cross section

c- chlorenchyma

s- sclerenchyma

x- xylem

p- phloem

p.sh- parenchyma sheath

m- micro-hair

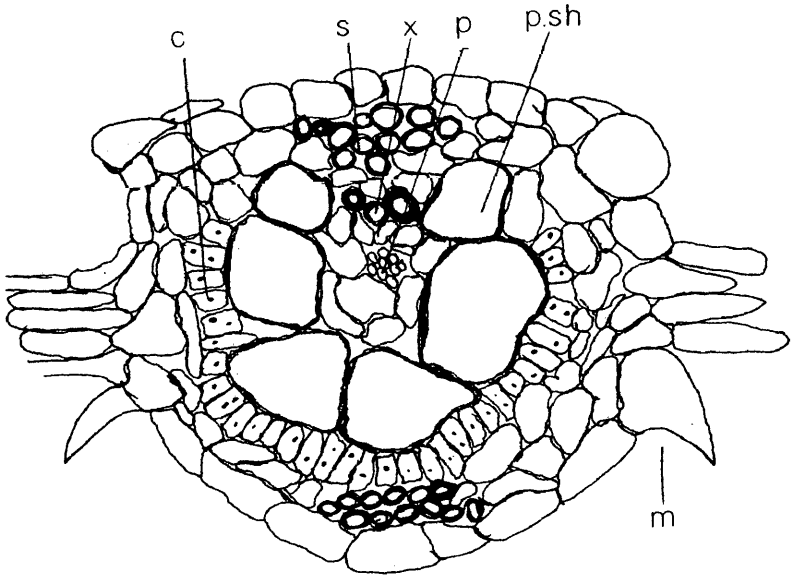

c
s
x
p
p.sh
m

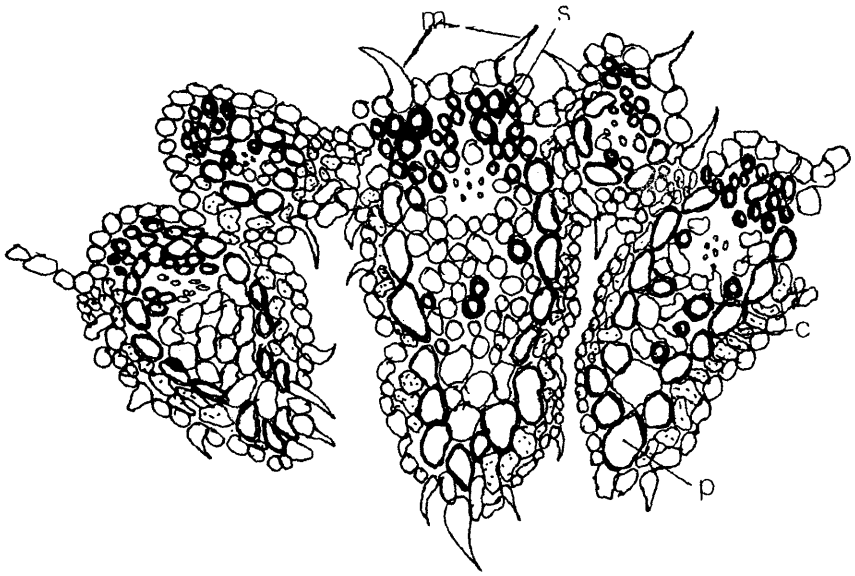
Figure 8 Sporobolus airoides leaf cross section

m- micro-hair

s- sclerenchyma

c- chlorenchyma

p- parenchyma sheath


m
s
c
p

with the vascular bundles radiates outward from the center of the bundle, while the chlorenchyma in Sporobolus airoides (Fig. 8) is dispersed, with some of the cells appearing radiate. Muhlenbergia pungens (Fig. 2) has both radiate and dispersed chlorenchyma.

Parenchyma Sheath

The parenchyma sheath in leaf cross section was found to be open (not completely enclosing the vascular bundle) in Muhlenbergia asperifolia (Fig. 7). In Sporobólus airoides (Fig. 8) the sheath around the primary vascular bundles is open and closed around the secondary bundles. Closed or complete parenchyma sheaths are found in Muhlenbergia pungens (Fig. 2).

Silica-bodies

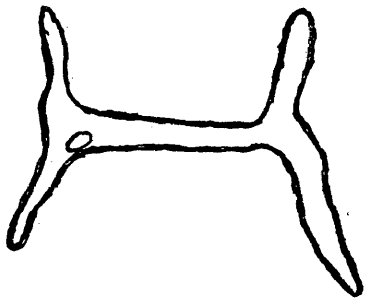
Silica-bodies found in the leaf epidermis of Muhlenbergia asperifolia (Fig. 10) are dumbbell shaped, with a few saddle shaped. The silica-bodies in Sporobolus airoides (Fig. 11) are somewhat saddle shaped. Muhlenbergia pungens (Fig. 9) has cross shaped silica-bodies with some dumbbell shaped and some saddle shaped.

Figure 9 Muhlenbergia pungens silica-bodies

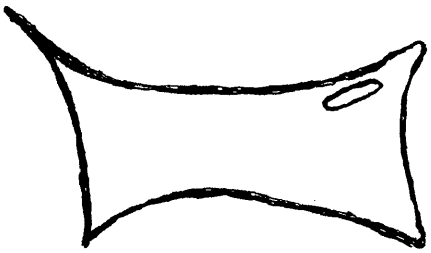
a- cross shaped

b- saddled shaped

c- dumbbell shaped



a



b

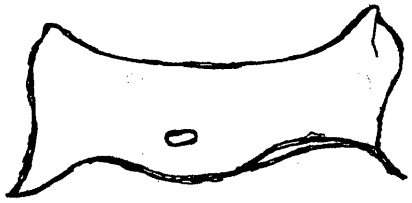
c

Figure 10 Muhlenbergia asperifolia silica-bodies

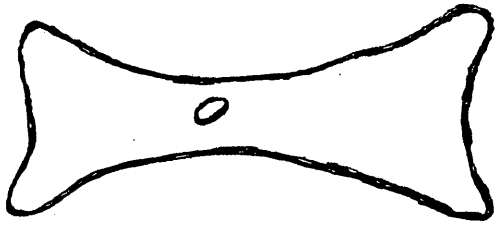
a- saddle shaped

b- dumbbell shaped

c- dumbbell shaped



a



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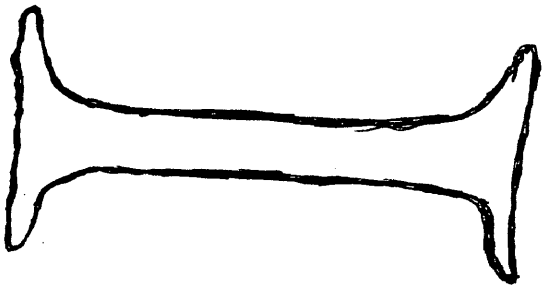


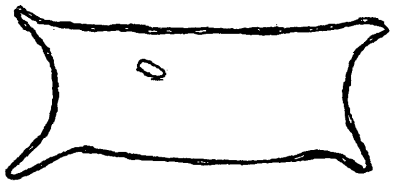
c

Figure 11 Sporobolus airoides silica-bodies

a- saddle shaped

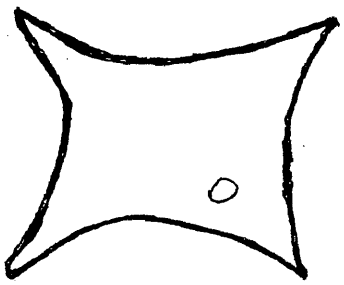
b- saddle shaped

c- saddle shaped



a

b



c

DISCUSSION

The gross morphological characteristics of Muhlenbergia pungens, M.asperifolia, and Sporobolus airoides are shown in Table 3. This table emphasizes the difficulty of using such characters to classify Muhlenbergia pungens.

Metcalfe (1960) considered certain anatomical features to be diagnostic of the genera Sporobolus and Muhlenbergia. These are summarized in Table 4.

Muhlenbergia pungens resembles other members of the genus in some respects, while in others it appears to be sporobolus-like or intermediate (Table 5). Muhlenbergia pungens is like M. asperifolia with respect to the vascular bundles, since both species have only primary bundles in the leaf blade. Muhlenbergia pungens is rhizomatous as are most of the muhlenbergias. Muhlenbergia pungens is sporobolus-like with respect to three characteristics studied. The caryopsis drops out of the floret; the ligule is a fringe of hairs and the chlorenchyma is both dispersed and radiate. Three of the characteristics studied suggest that Muhlenbergia pungens is intermediate between Muhlenbergia and Sporobolus. The Muhlenbergia lemma is typically 3-nerved and the lemma in Sporobolus is 1-nerved. The lemma in Muhlenbergia pungens is 3-nerved, however the lateral nerves are much reduced. The sclerenchyma in Muhlenbergia asperifolia is found dorsal and ventral to the vascular bundles in fairly equal amounts. Sporobolus airoides has mostly dorsal sclerenchyma, rarely ventral. Muhlenbergia pungens has dorsal and ventral sclerenchyma; however, twice the amount

Table III

MORPHOLOGICAL DATA

Muhlenbergia pungens	Muhlenbergia asperifolia	Sporobolus airoides
perennial	perennial	perennial
strong creeping rhizome	slender rhizome	non-rhizomatous
culm erect from a decumbent base	culm branching at base, spreading	culm erect to spreading not decumbent
20-40 cm, sometimes taller	10-50 cm tall	50-100 cm tall
panicle open, 5-15 cm long	panicle open, 5-15 cm long	panicle open, nearly half the height of the plant
spikelets 4-5 mm long	spikelets 1.5-2 mm long	spikelets 2-2.5 mm long
glumes about 1/3 as long as spikelet	glumes half or longer as spikelet	first glume about half as long as spikelet
blades short, involute, sharp-pointed	blades flat, crowded, scabrous mostly 2-5 cm long	blades elongate, flat soon becoming involute
lemma terete, tapering into and awn, faintly 3-nerved	lemma thin, broad, minutely mucronate from an obtuse apex, 3-nerved	second glume, lemma and palea about equal, lemma 1-nerved
caryopsis drops out of floret ovary wall adheres tightly to seed	caryopsis held within floret ovary wall adheres tightly to seed	caryopsis drops out of floret ovary wall readily separates from seed

Table IV

DIAGNOSTIC GENERIC CHARACTERISTICS

Muhlenbergia:

1. short cells, over the veins, in long rows
2. silica-bodies over the veins, mostly saddle shaped; but sometimes cross to dumbbell shaped
3. mesophyll chlorenchyma distinctively radiate

Sporobolus:

1. short cells, over the veins, mostly paired but sometimes solitary
2. silica-bodies mostly elliptical or slightly cresent shaped more rarely somewhat saddle shaped or tending to be cross shaped
3. mesophyll chlorenchyma inconspicuously to conspicuously radiate

Table V

COMPARATIVE DATA

Feature	Muhlenbergia asperifolia	Muhlenbergia pungens	Sporobolus airoides
Lemma	3-nerved	3-nerved, lateral reduced	1-nerved
Caryopsis	retained	drops out	drops out
Ligule	membraneous	fringe of hairs	fringe of hairs
Vascular Bundle	all primary	all primary	primary and secondary
Sclerenchyma with v.b.*	dorsal and ventral (equal)	dorsal and ventral (twice as much dorsally)	mostly dorsal rarely ventral
Micro-hairs	dorsal to v.b.* some short	mostly ventral, long, tapered	mostly ventral, not as many as pungens, short, tapered
Chlorenchyma	radiate	dispersed, some radiate	dispersed radiate
Parenchyma	open	closed	open, primary v.b. closed, secondary v.b.

* vascular bundle

is dorsal to the bundle as ventral to it. Muhlenbergia asperifolia has an open parenchyma sheath around the vascular bundle. The primary vascular bundles of Sporobolus airoides have an open parenchyma sheath, with a closed sheath around the secondary bundles. The closed parenchyma sheath of Muhlenbergia pungens does not strictly resemble Sporobolus, inasmuch as Sporobolus shows an open sheath with the primary bundles and a closed sheath around the secondary bundles. But it is important since a closed sheath is not a Muhlenbergia characteristic.

Based on these findings, I would conclude that Muhlenbergia pungens does not fit well in either genus. It may be a transition species between Muhlenbergia and Sporobolus or may well deserve to be in a genus by itself.

Further research may help clarify the problem of classification of Muhlenbergia pungens. Protein analysis, leaf phenolics, and chromosome studies could be undertaken to provide additional data.

SUMMARY

Anatomical data were collected to aid the classification of Muhlenbergia pungens. Data were collected on Muhlenbergia asperifolia and Sporobolus airoides as a basis for comparison.

The venation of the lemma, the caryopsis retention, the ligule type, vascular bundle, sclerenchyma quantity and placement relative to the vascular bundle, chlorenchyma, micro-hair placement relative to the vascular bundle, and the parenchyma sheath were studied, in addition to the usual morphological characteristics.

Muhlenbergia pungens resembled the other muhlenbergias in that it contained all primary vascular bundles and was a rhizomatous grass. In the other characteristics studied, Muhlenbergia pungens is either sporobolus-like or appears to be intermediate. With respect to the caryopsis retention, ligule, and mesophyll chlorenchyma Muhlenbergia pungens showed a resemblance to Sporobolus airoides. The lemma venation, sclerenchyma, and parenchyma sheath characteristics lead one to believe that Muhlenbergia pungens is an intermediate species between Muhlenbergia and Sporobolus.

Further research may clarify the problem of classification of Muhlenbergia pungens.

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