

A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section

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ABSTRACT

Descriptive "keys", including definitions and diagrams, for standardizing and simplifying the description of grass leaf structure as seen in transverse section are given. Over 500 characters are included with the possibility for expansion to 999. Notes on variation and taxonomic importance of the characters are also included.

Résumé

UN PROCEDE POUR STANDARDISER L'ANATOMIE COMPAREE DE LA FEUILLE DE POACEAE. I. LE LIMBE VU EN SECTION TRANSVERSALE

Des "clefs" descriptives incluant des définitions et des diagrammes pour standardiser et simplifier la description de la structure des feuilles de graminées vues en section transversale sont données. Plus de 500 caractères sont inclus avec la possibilité d'expansion jusqu'à 999. Des notes sur la variation et l'importance taxonomique des caractères sont également fournies.

INTRODUCTION

Anatomical investigations of the grass leaf-blade have long provided valuable taxonomic information. In fact, nowadays, it is generally accepted that anatomical details, especially of the leaf-blade and embryo, when used in conjunction with a wide spectrum of other diagnostic characters, are an essential ingredient of any satisfactory treatment of grass taxonomy. Furthermore, in the Poaceae (=Gramineae), with their highly specialized and reduced flowers, very fine morphological distinctions are often necessary to define differences between taxa. Anatomical data is, therefore, regarded as being of undoubted importance in the jigsaw of complete systematic evidence in this numerically large and important family.

The importance of anatomy in agrostological studies has resulted in the rapid accumulation of an extensive body of literature with attendant problems of lack of uniformity with definitions and descriptions. Valuable data is, therefore, commonly not applicable to the family as a whole and comparisons cannot be drawn with any degree of assurance. This problem was greatly ameliorated by the publication in 1960 of *Anatomy of the Monocotyledons. 1. Gramineae* by C. R. Metcalfe and the present paper is a further attempt to stabilize this terminology, and, at the same time to present a system whereby description and comparison of grass leaf anatomy will be simplified and standardized.

DESCRIPTIVE KEYS

In an attempt to achieve the necessary uniformity, descriptive "keys" have been compiled for use as a framework for anatomical descriptions of the grass leaf-blade as viewed in transverse section. The "keys", which incorporate both definitions and diagrams, have been designed to enable the user to standardize descriptions and the hierarchical layout has been chosen to facilitate the speed and ease at which

complete, comparative descriptions can be compiled. Anatomical characters, and all other information considered to be of diagnostic or taxonomic importance, and gathered from an extensive survey of the relevant literature, have been included. The keys should, therefore, prove adequate for all tribes of the Poaceae except for the Bambuseae where additional data on the fusoid cells must be included to satisfactorily distinguish between certain genera.

The hierarchical tabulation of the characters has been used to expedite their use but they do not in any other way conform to any acknowledged key format or design. However, if a statement, at any level of the hierarchy, does not apply to the specimen being examined, no subsequent statements or characters of a lower rank are relevant. The user, therefore, merely proceeds to the next character of equal rank or indentation, and, if applicable, works inwards noting all the relevant numbered end points before working back outwards until the same level or rank as that of the originally chosen statement is reached. Thus, the "keys" are not dichotomous or true indented keys, but by using this type of format all the possible characters are recorded in a constant descriptive sequence. Each character is assigned a constant number and the recording of these numbers effects a saving in time and space. Furthermore, this system enables easy conversion to edge punched and feature cards as well as for electronic data processing by computer. In addition, it is ensured that all possible structures are rapidly and routinely noted in a rational sequence which simplifies compilation of descriptions of the various taxa. By employing a standard sequence significant differences become more readily evident.

In order for the standardization of terminology and descriptions to be effective it is essential that comparative material be examined. Therefore, in this study, all descriptions refer to transverse sections taken at a point about halfway between the blade apex and the ligule of mature basal leaves. Flag leaves on flowering culms were avoided where possible. By standardizing on the material studied in this way intraspecific differences may be assessed.

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1. OUTLINE OF THE LAMINA IN TRANSVERSE SECTION

Outline usually determined by the shape of the abaxial surface. Includes the entire transverse section of the leaf blade at a point about halfway between the leaf apex and the base of the lamina. Subdivided into leaves that can be or are relatively flattened and permanently infolded, acicular leaves.

OUTLINE OF THE LAMINA OF OPEN LEAVES:

Includes all leaves which are not permanently infolded to such a degree that the internal structure is altered. These leaves are normally open or expanded under favourable environmental conditions but exhibit various degrees of infolding or inrolling under conditions of water stress. Projecting and grooved midribs and keels must be considered in relation to the overall shape.

DESCRIPTION:

Expanded; commonly flattened; a line connecting both margins and the median bundle straight:

Nature of blade; ribs and furrows not considered:

Flat, even or straight 101*

Undulating gently 102*

Distinctly wavy or undulating:

Corrugated leaf i.e. waves rounded 103*

Pleated leaf i.e. waves pointed or angled 104*

V-shaped; two halves of the lamina folded toward each other on either side of the midrib i.e. a line connecting both margins with the median bundle angled at the median bundle; carinate:

Angle formed by the two arms of the lamina at the midrib:

Narrow; closed V i.e. less than 45° to each other 105*

Standard V i.e. between 45° and 90° to each other 106*

Broad, open V i.e. more than 90° to each other 107*

Wide, very open V i.e. almost 180° ; presence of projecting keel gives appearance of V-shape 108*

Shape of arms of the lamina:

Straight 109*

Outwardly bowed; concave 110*

Outwardly curving; convex 111*

Outline heart-shaped 112*

Distinctly wavy or undulating:

Corrugated leaf i.e. waves rounded 113*

Pleated leaf i.e. waves pointed or angled; plicate 114*

Irregularly wavy or bent 115*

Symmetry of two halves of the lamina:

Symmetrical on either side of the median vascular bundle 116

Two halves asymmetrical on either side of the median vascular bundle 117*

U-shaped; two halves of the lamina curved upwards on either side of the midrib or with a prominent rounded keel i.e. no definite angle formed with the midrib; canaliculate:

Shape of the U formed:

Broad, wide i.e. horizontally elongated 118*

Tall, narrow i.e. vertically elongated 119*

Base and sides same length i.e. equidimensional 120*

Inrolled; lamina rolled inwards towards the adaxial surface:

Nature of inrolling:

Convolute; inrolled from ONE margin only; margins wrapped around each other 121*

Involute; inrolled from BOTH margins 122*

Degree of inrolling:

Tightly inrolled 123

Loosely inrolled 124

DESCRIPTION CONTINUED. Measurements taken by estimation utilizing the known diameter of the field of view of the objective where the thickness or width of the leaf almost fill this field of view. This standardisation is for the Zeiss Standard RA microscope fitted with X 10 Kpl W eyepieces.

Width of one half of the lamina i.e. from blade margin to midrib or median vascular bundle if midrib indistinguishable:

X16 Objective:

Less than half the field of view at x 160 i.e. less than 0,56 mm wide 125

Between half and the full field of view at x160 i.e. from 0,56 mm – 1,125 mm wide 126

X2,5 Objective:

Less than half the field of view at x25 i.e. 1,125 mm – 3,6 mm wide 127

Between half and the full field of view at x25 i.e. from 3,6 mm – 7,2 mm wide 128

Up to twice the field of view at x25 i.e. from 7,2 mm – 14,4 mm wide 129

More than twice the field of view at x25 i.e. more than 14,4 mm wide 130

Thickness of lamina taken at a point about halfway between the median bundle and the margin. Depth of largest rib in central area of lamina measured:

X40 Objective:

Less than one quarter of the field of view at x400 i.e. less than 0,112 mm thick 131

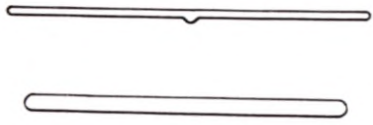
Between one quarter and half the field of view at x400 i.e. from 0,112 mm – 0,225 mm thick 132

Between half and three quarters of the field of view at x400 i.e. from 0,225 mm – 0,336 mm thick 133

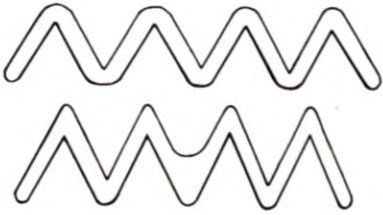
Between three quarters and the full field of view at x400 i.e. from 0,336 mm – 0,450 mm thick 134

More than the full field of view at x400 i.e. more than 0,450 mm thick 135

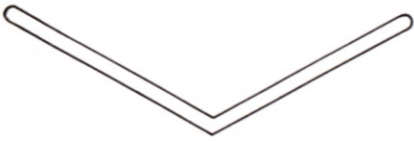
OUTLINE OF OPEN LEAVES



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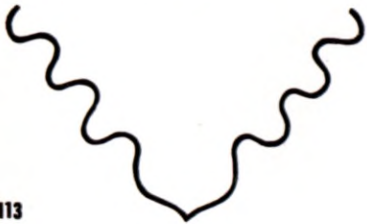
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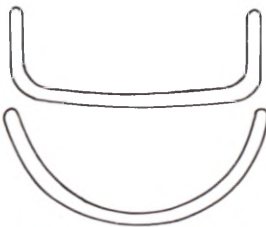
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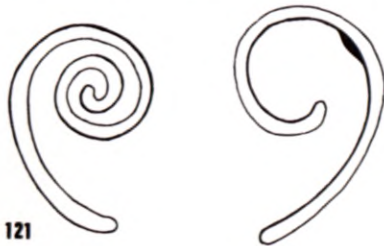
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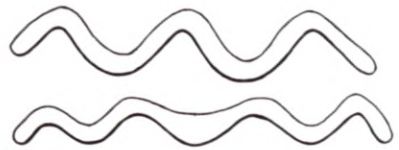
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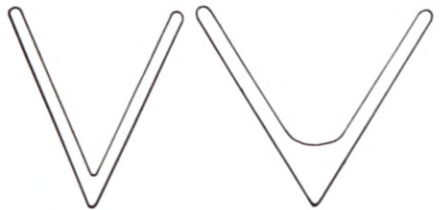
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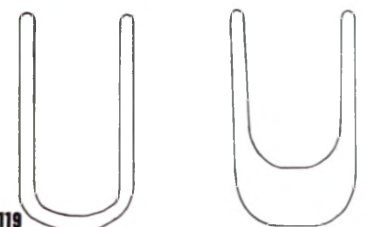
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112



115



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122

1. OUTLINE OF THE LAMINA IN TRANSVERSE SECTION CONTINUED

OUTLINE OF THE LAMINA OF PERMANENTLY INFOLDED LEAVES:

Includes only leaves that are permanently infolded to the extent that the internal structure is altered. Often includes acicular, setaceous, filiform and junciform leaves. The entire section is considered under low magnification with the abaxial surface determining the outline.

DESCRIPTION:

Shape, outline; as formed by the abaxial surface:

Reduced V-shaped; two halves of the lamina folded toward each other on either side of the median bundle i.e. a definite angle formed:

- Arms straight 150*
- Arms angled; often hexagonal 151*

Reduced U-shaped; two halves of the lamina curved upwards on either side of the median bundle i.e. no definite angle formed:

- Arms vertical or parallel to one another 152*
- Arms rounded or outwardly bowed:
 - Elliptical; forming an incomplete oval or ellipse 153*
 - Rounded; forming an incomplete circle 154*

Triangular; base broad on either side of the median bundle 155*

Circular or Round in transverse section:

- Hollow cylinder; margins not fused; adaxial surface not reduced; horseshoe-shaped 156*
- Solid cylinder; margins fused or nearly so:
 - Adaxial surface reduced to a minute groove 157*
 - Adaxial surface not distinguishable; radially symmetrical; terete 158*

Number of Vascular Bundles present in section; includes median bundle or midrib if present:

- 3 vascular bundles in T/S 159
- 5 vascular bundles in T/S 160
- 7 vascular bundles in T/S 161
- 9 vascular bundles in T/S 162
- 11 vascular bundles in T/S 163
- 13 vascular bundles in T/S 164
- 15 vascular bundles in T/S 165
- 17 or more vascular bundles in T/S 166

Adaxial channel bounded by the arms of the lamina; may be open or closed at the leaf margin:

Vertical channel sides; parallel to each other:

- Width of the channel; compared with the blade thickness at a point midway between median bundle and margin:
 - Wider than the leaf thickness 167*
 - Nearly the same as the leaf thickness 168*
 - Narrower than the leaf thickness 169*
 - Narrow cleft; less than half the leaf thickness 170*
- Depth of the channel; compared with the blade thickness at the median vascular bundle:
 - Shallower than the leaf thickness 171*
 - Nearly the same as the leaf thickness 172*
 - Deeper than the leaf thickness 173*
 - Deep; more than twice the leaf thickness 174*

Non-vertical channel sides:

- Rounded channel 175*
- Triangular channel 176*
- Channel reduced to small groove 177*
- No channel present 178*

DESCRIPTION CONTINUED. Measurements taken by estimation utilizing the known diameter of the field of view of the objective where the thickness or width of the leaf almost fill this field of view. This standardisation is for the Zeiss Standard RA microscope fitted with X10 Kpl W eyepieces.

Width of one half of the lamina i.e. from blade margin to midrib or median vascular bundle if midrib indistinguishable:

X16 Objective

- Less than half the field of view at x160 i.e. less than 0,56 mm wide 179
- Between half and the full field of view at x160 i.e. from 0,56 mm – 1,125 mm wide 180

X2,5 Objective:

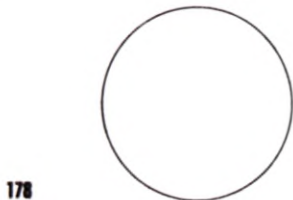
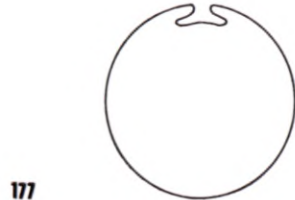
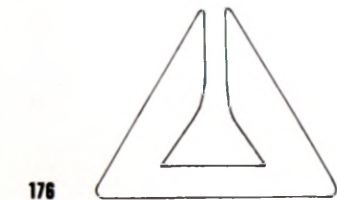
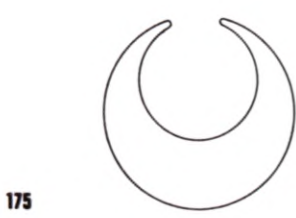
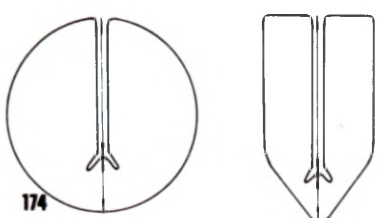
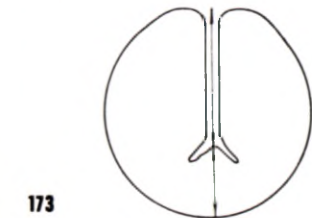
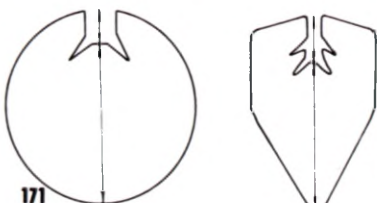
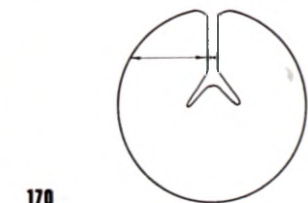
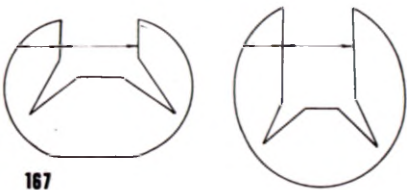
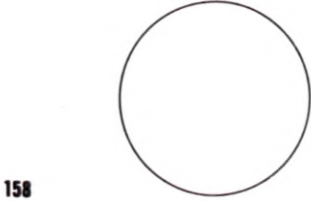
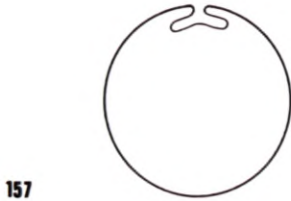
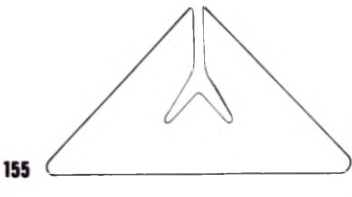
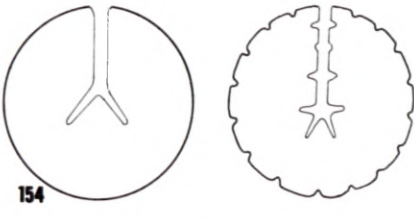
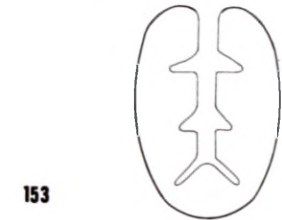
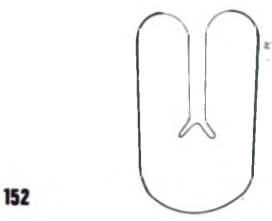
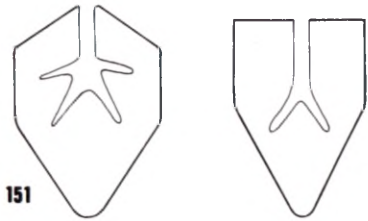
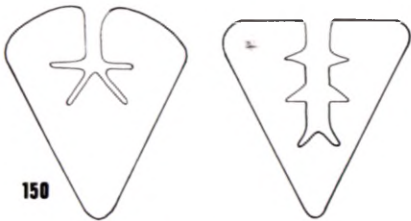
- Less than half the field of view at x25 i.e. 1,125 mm – 3,6 mm wide 181
- Between half and the full field of view at x25 i.e. from 3,6 mm – 7,2 mm wide 182
- More than the full field of view at x25 i.e. more than 7,2 mm wide 183

Thickness of lamina taken at a point about halfway between the median bundle and the margin. Depth of largest rib in central area of lamina measured:

X40 Objective:

- Less than one quarter of the field of view at x400 i.e. less than 0,112 mm thick 184
- Between one quarter and half the field of view at x400 i.e. from 0,112 mm – 0,225 mm thick 185
- Between half and three quarters of the field of view at x400 i.e. from 0,225 mm – 0,336 mm thick 186
- Between three quarters and the full field of view at x400 i.e. from 0,336 mm – 0,450 mm thick 187
- More than the full field of view at x400 i.e. more than 0,450 mm thick 188

OUTLINE OF PERMANENTLY INFOLDED LEAVES



I. THE LEAF-BLADE AS VIEWED IN TRANSVERSE SECTION

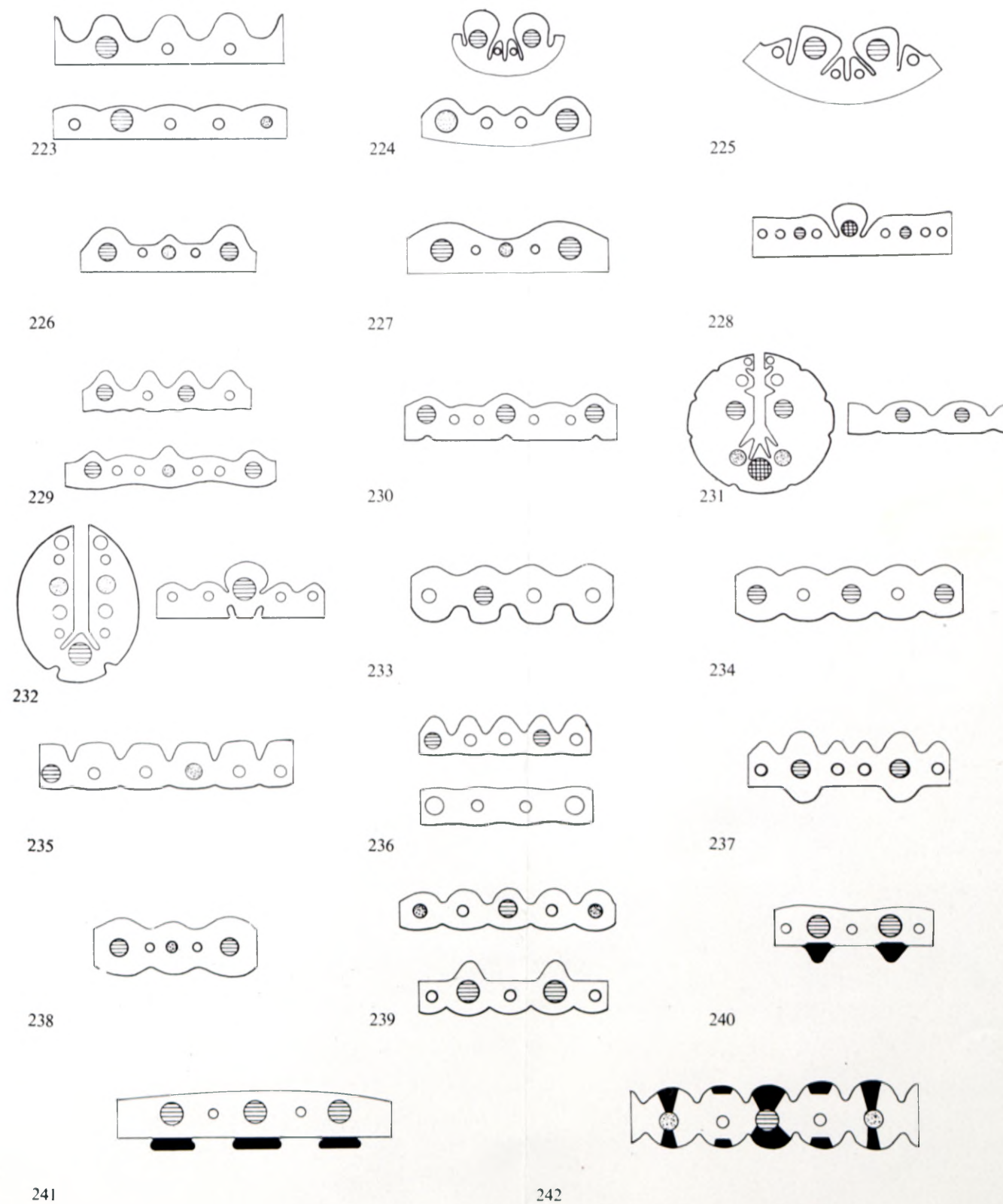
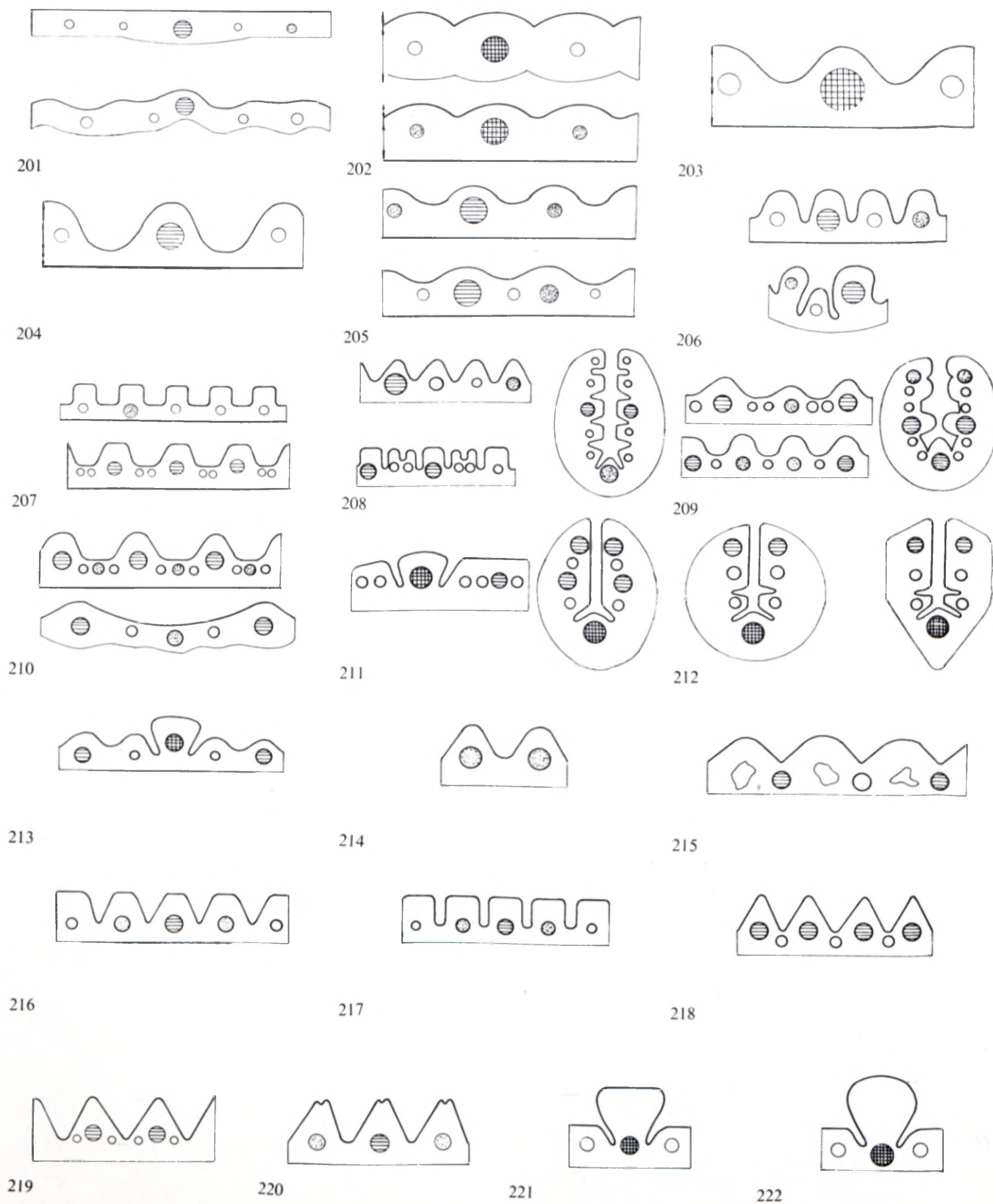
2. ADAXIAL AND ABAXIAL LONGITUDINAL RIBS AND FURROWS

Includes all longitudinal ribs or ridges as well as the associated furrows or grooves commonly found on the adaxial leaf surface but sometimes on the abaxial surface as well. If ribs are present there must be corresponding furrows and vice versa. If furrows are not found between the ribs but in any other situation they are termed grooves, e.g. the grooves present on some ribs. Ribs and the associated vascular bundles form regular associations and patterns. The furrows and the adaxial channel of acicular leaves can be distinguished by the fact that the channel is the area bounded by the permanently infolded lamina and the furrows lie between the ribs which may also be in the channel.

DESCRIPTION:

No ribs or furrows present on either surface i.e. lamina surface straight or only slightly undulating with no regular pattern associated with the vascular bundles	201*
Adaxial ribs and furrows present:	
Depth of adaxial furrows in comparison to the leaf thickness i.e. the depth of the larger ribs in the central region of the lamina between the margin and the median vascular bundle regarded as leaf thickness:	
Slight, shallow furrows i.e. less than a quarter of the leaf thickness	202*
Medium furrows i.e. a quarter to one half the leaf thickness	203*
Deep furrows i.e. more than one half the leaf thickness	204*
Shape of adaxial furrows:	
Wide, open furrow i.e. obtuse angle formed by furrow sides at base	205*
Narrow furrow i.e. sides of furrow almost vertical:	
In form of cleft	206*
Base fairly broad but sides steep	207*
Distribution of adaxial furrows; important for acicular leaves:	
Furrows between all vascular bundles	208*
Furrows between 1st and 2nd order vascular bundles i.e. present over 3rd order vascular bundles	209*
Furrows between 1st order vascular bundles i.e. present over 3rd and 2nd order vascular bundles	210*
Furrow on either side of the median vascular bundle only	211*
Two furrows on either side of median vascular bundle only	212*
Deepest furrows on either side of the median vascular bundle	213*
Shape of adaxial ribs as seen in T/S; all types present must be included:	
Rounded, obtuse ribs i.e. apex rounded:	
Situated over the vascular bundles	214*
Situated between the vascular bundles (usually occupied by air spaces)	215*
Flat-topped, square ribs i.e. apex flattened:	
Sides rounded with flat top	216*
Sides angled with flat top	217*
Triangular ribs i.e. apex pointed:	
One vascular bundle in each rib	218*
Three vascular bundles in each rib	219*
Grooved at apex	220*
Massive ribs i.e. very large usually with bases narrow in relation to upper parts of rib:	
Flattened apices	221*
Rounded apices	222*
Distribution of different ribs in association with the vascular bundles:	
Ribs present over all vascular bundles:	
Similar ribs over all vascular bundles	223*
Ribs over 1st and 2nd order vascular bundles larger than those over the 3rd order vascular bundles	224*
Ribs over 1st order vascular bundles with flattened tops and those over the 3rd order bundles triangular	225*
Ribs not associated with all vascular bundles:	
Ribs present only over 1st and 2nd order vascular bundles	226*
Ribs present only over 1st order vascular bundles	227*
Ribs present over midrib only	228*
Abaxial ribs and furrows present:	
No abaxial rib development:	
Surface smooth or with few undulations not regularly associated with the vascular bundles	229*
Grooves present on abaxial surface:	
Opposite larger vascular bundles	230*
Between vascular bundles; often shallow	231*
Groove on either side of midrib	232*
Ribs present on abaxial surface:	
Size of abaxial ribs:	
Taller than the adaxial ribs	233*
Same size as adaxial ribs i.e. section resembles a string of beads; moniliform	234*
Smaller than adaxial ribs	235*
Slight undulation associated with the vascular bundles	236*
Distribution of abaxial ribs:	
Present opposite 1st order vascular bundles only	237*
Present opposite 1st and 2nd order vascular bundles only	238*
Present opposite all vascular bundles	239*
Composition of abaxial ribs:	
Composed of sclerenchyma:	
In form of rounded caps	240*
In form of flat-topped caps	241*
Composed of girder or strand of sclerenchyma in contact with the epidermis	242*

RIBS AND FURROWS



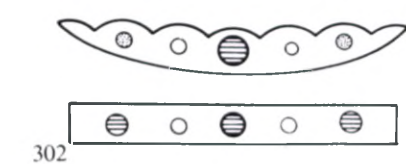
3. MEDIAN VASCULAR BUNDLE, MIDRIB AND KEEL STRUCTURE

Keel and midrib distinguished by the presence or absence of parenchyma associated with the median vascular bundle. It is termed a midrib if the median bundle is solitary, structurally distinct and without associated parenchyma and a keel if parenchyma or bulliform cells are associated with the median bundle or bundles. Thus one or many vascular bundles may be incorporated in a keel. The keel does not necessarily project. In V-shaped leaves, only the median bundle is considered to comprise the keel (if parenchyma is present) unless there is a marked thickening in relation to the rest of the lamina. The number of vascular bundles in a keel is, in a general way, roughly proportional to the size of the keel.

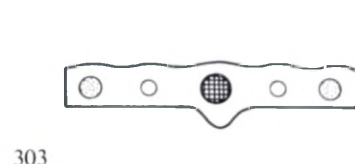
DESCRIPTION:

Not possible to determine from preparation i.e. entire width of blade not sectioned	301
Median bundle only present i.e. entire or more than half the blade width sectioned and no structurally distinct midrib is distinguishable	302*
Midrib only present; distinguishable from other first order vascular bundles; no associated parenchyma developed i.e. keel inconspicuous:	
Projection of midrib:	
Projects abaxially:	
Sclerenchyma causes projection	303*
Projection due to position or size of bundle	304*
Projection inconspicuous or marked; not projecting abaxially only:	
Blade expanded or slightly inrolled	305*
Lamina V-shaped but no parenchyma at angle	306*
Acicular or terete leaves; parenchyma may be developed but no specifically in association with the midrib	307*
Keel developed; colourless parenchyma and/or bulliform cells associated with the median bundle; sclerenchyma development may also occur:	
Number of vascular bundles comprising keel; in V-shaped leaves, if there is no thickening in the keel area the median bundle is considered to constitute the keel:	
One vascular bundle comprising the keel:	
Parenchyma of small, rounded cells surrounding or immediately adaxial to the median bundle	308*
Bulliform cells in adaxial epidermis above median bundle:	
Leaf expanded, flat or slightly inrolled	309*
Leaf V-shaped:	
Bulliform cells penetrate into leaf on either side of median bundle forming an inverted V	310*
Bulliform cells elongated and arranged in U above median bundle	311*
Elongated bulliform cells in U as well as layers of elongated parenchyma cells	312*
Bulliform cells and rounded parenchyma cells present	313*
Two vascular bundles present in keel; one being a superposed adaxial amphivasal 3rd order bundle	314*
Three vascular bundles present in keel:	
Adaxial groove present	315*
No adaxial groove present	316*
Four vascular bundles present in keel; three abaxial and one adaxial amphivasal bundle	317*
Many vascular bundles present in keel:	
All vascular bundles abaxially arranged:	
Median bundle indistinguishable from other 1st order bundles:	
One 1st order bundle and smaller bundles comprise keel	318*
Three 1st order bundles and other smaller bundles comprise keel	319*
Five or more 1st order bundles and other smaller bundles comprise keel	320*
Median bundle structurally distinct (size, bundle sheath or sterome) from other 1st order bundles:	
Large median bundle and other 3rd and 2nd order bundles only in keel	321*
Normal 1st order bundles present in addition to the median bundle	322*
Vascular bundles distributed adaxially, abaxially and in the interior of the keel; usually includes superposed and amphivasal bundles	323*
Shape of keel in transverse section:	
Not really distinct from leaf outline	324*
V-shaped, pointed or inverted triangular keel:	
Leaf V-shaped with V-shaped keel	325*
Leaf V-shaped with inverted triangle-shaped keel	326*
Leaf expanded with inverted triangular or pointed keel	327*
Rounded or semicircular keel; leaf not necessarily U-shaped:	
U-shaped keel; slightly thicker than rest of lamina	328*
U-shaped keel; much thicker than rest of lamina	329*
Rounded or semicircular keel i.e. adaxial side of keel flat	330*
Rounded keel with raised, flattened adaxial side	331*
Rounded keel with single, central adaxial groove	332*
Rounded keel with distinct adaxial rib developed	333*
Rounded keel with numerous adaxial ribs and furrows	334*
Massive U-shaped keel; often with air-spaces	335*
Sclerenchyma associated with the keel:	
Adaxial sclerenchyma:	
Strands in subepidermal layers	336*
Strands fused forming a hypodermal band	337*
Abaxial sclerenchyma:	
Most bundles with abaxial strands	338*
Most bundles with abaxial girders	339*
Central or solitary bundle with anchor-shaped girder	340*
Air spaces or Lacunae incorporated in the keel; definite lacunae present or enlarged parenchyma cells of keel breaking down:	
One air-space in keel	341*
Two air-spaces in keel	342*
Four or more air-spaces in keel	343*

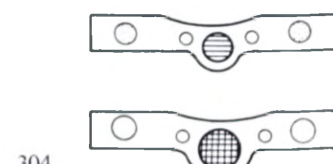
MIDRIBS AND KEELS



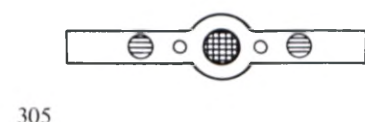
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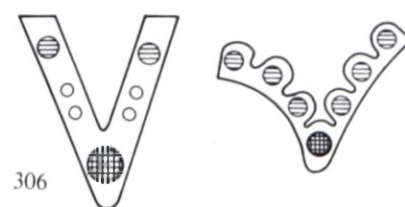
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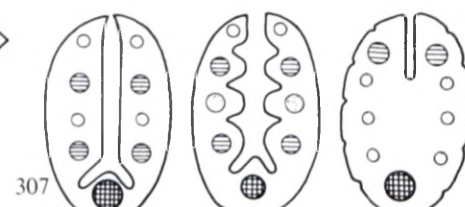
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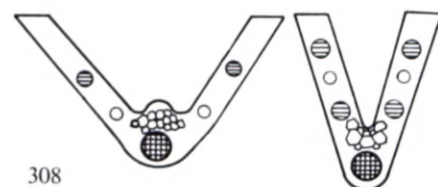
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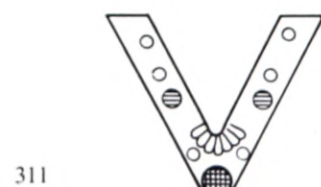
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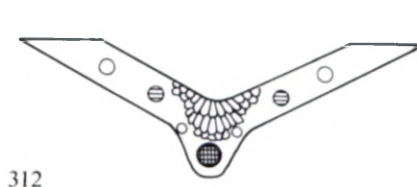
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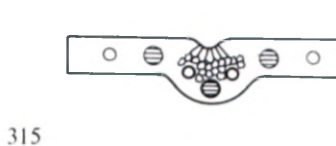
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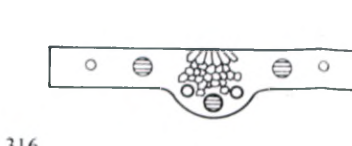
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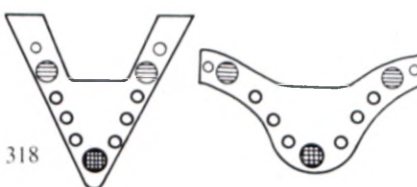
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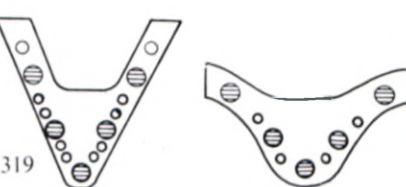
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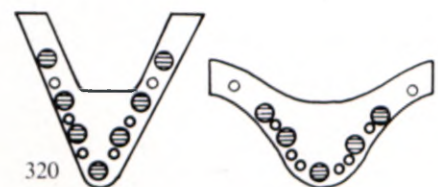
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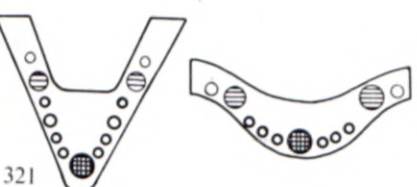
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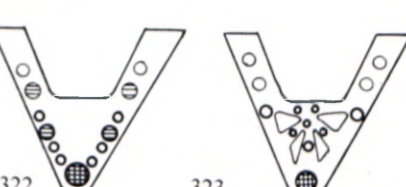
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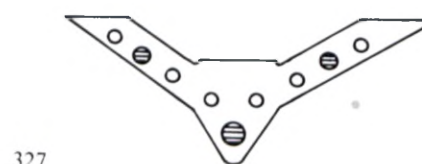


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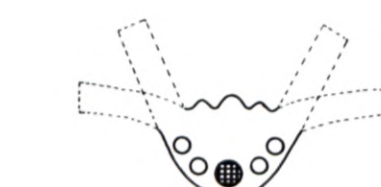
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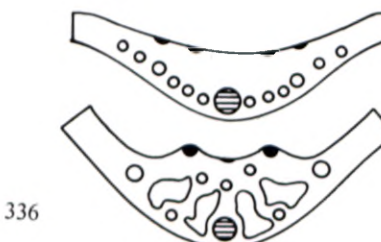
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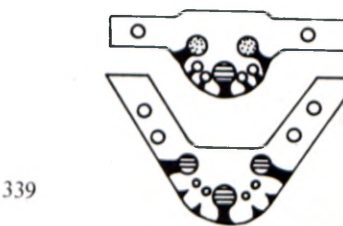
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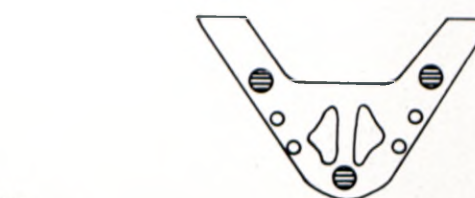
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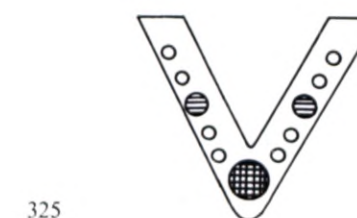
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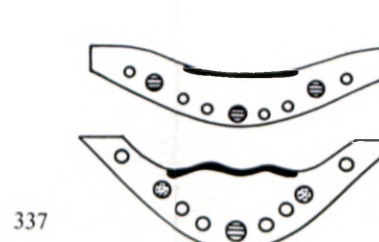
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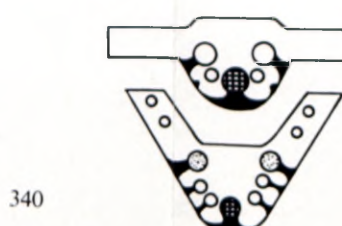
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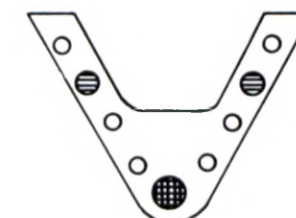
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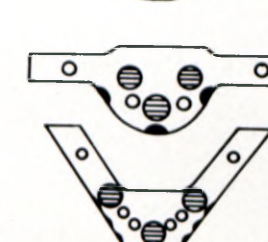
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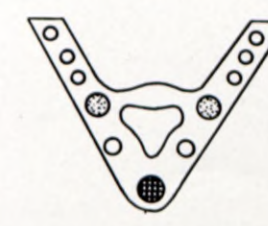
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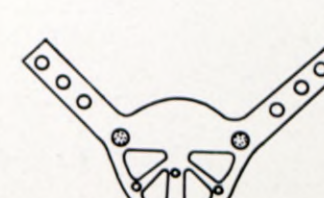
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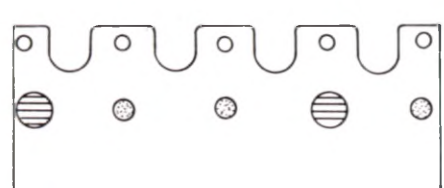
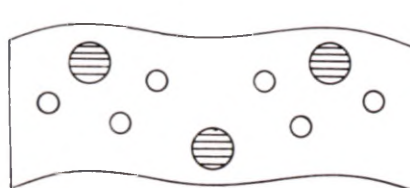
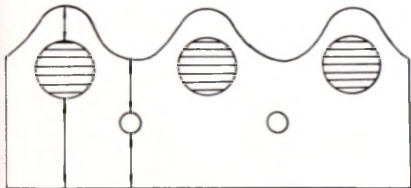
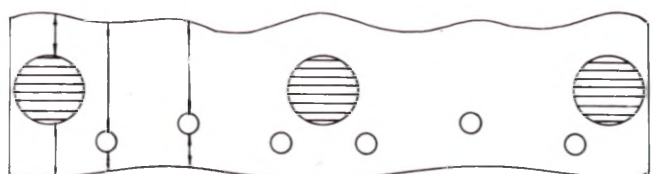
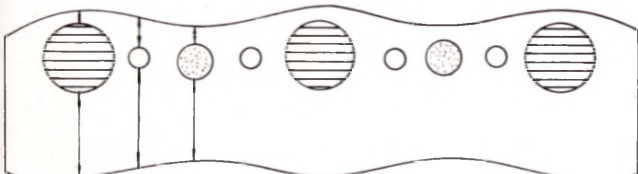
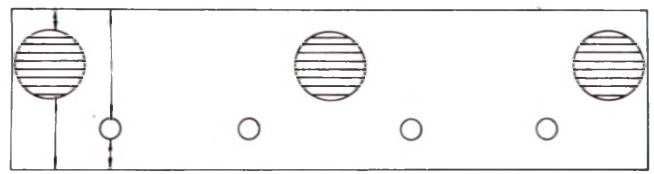
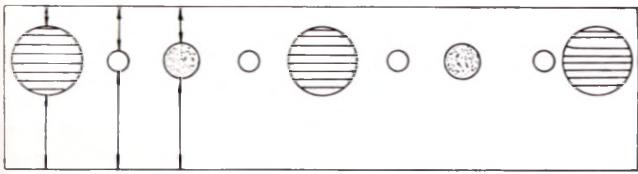
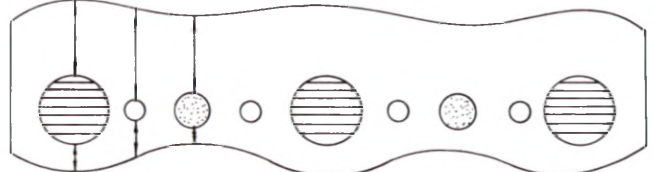
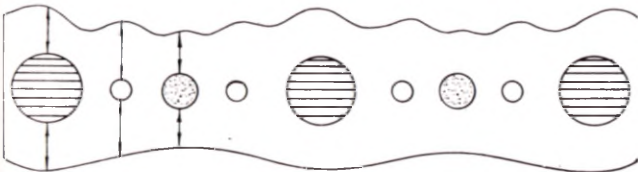
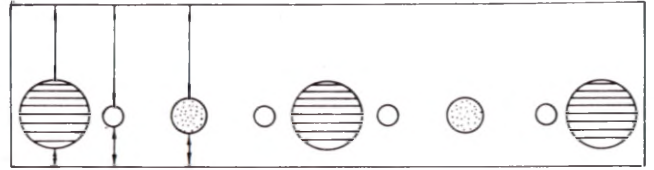
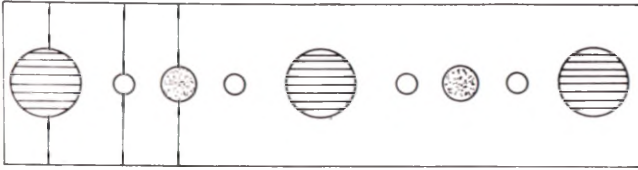
4. VASCULAR BUNDLE ARRANGEMENT

Usually studied under low magnification. Only half the width of the lamina, from the margin to and including the median vascular bundle considered. When the section is incomplete and the median bundle is indistinguishable only those characters that are possible to determine are considered. In certain cases where the 2nd order vascular bundles are not clearly distinguishable from the 1st order bundles they are considered as 1st order bundles for the purposes of arrangement.

ARRANGEMENT:

Total number of 1st ORDER bundles in half the width of the lamina; includes the median vascular bundle:	
Not possible to determine; entire blade not sectioned	401
1 1st order vascular bundle in blade section i.e. median bundle	402
2 1st order vascular bundles in half lamina i.e. 3 in entire blade	403
3 1st order vascular bundles in half lamina i.e. 5 in entire blade	404
4 1st order vascular bundles in half lamina i.e. 7 in entire blade	405
5 1st order vascular bundles in half lamina i.e. 9 in entire blade	406
6 1st order vascular bundles in half lamina i.e. 11 in entire blade	407
7 1st order vascular bundles in half lamina i.e. 13 in entire blade	408
8 1st order vascular bundles in half lamina i.e. 15 in entire blade	409
9 1st order vascular bundles in half lamina i.e. 17 in entire blade	410
10 or more 1st order vascular bundles in half lamina	411
Arrangement and Alternation of different orders of vascular bundle along width of blade; central part of lamina between margin and midrib studied:	
Variable from median bundle to margin; no regular pattern discernible:	
Progressively fewer 1st and more 3rd order bundles towards the margin	412
Progressively more 1st and fewer 2nd and 3rd order bundles toward the margin	413
Arrangement near margin differs from remainder	414
Regular arrangement from median bundle to margin:	
Number of 3rd order bundles between consecutive 2nd or 1st order bundles at a position halfway to margin:	
No 3rd order bundles between consecutive larger bundles	415
1 3rd order bundle between consecutive larger bundles	416
2 3rd order bundles between consecutive larger bundles	417
3 3rd order bundles between consecutive larger bundles	418
4 3rd order bundles between consecutive larger bundles	419
5 3rd order bundles between consecutive larger bundles	420
6 3rd order bundles between consecutive larger bundles	421
7 3rd order bundles between consecutive larger bundles	422
8 3rd order bundles between consecutive larger bundles	423
9 3rd order bundles between consecutive larger bundles	424
10 or more 3rd order bundles between consecutive larger bundles	425
Number of 2nd order bundles between consecutive 1st order bundles:	
No 2nd order bundles between consecutive 1st order bundles	426
1 2nd order bundle between consecutive 1st order bundles	427
2 2nd order bundles between consecutive 1st order bundles	428
3 2nd order bundles between consecutive 1st order bundles	429
4 2nd order bundles between consecutive 1st order bundles	430
5 or more 2nd order bundles between consecutive 1st order bundles	431
Position of vascular bundles in blade; vertical situation in the section about halfway between median bundle and margin:	
Same level of positioning for all bundles of different orders:	
All bundles situated in the centre of the blade	432*
All bundles situated closer to the abaxial surface	433*
All bundles situated closer to the adaxial surface	434*
Different levels of positioning for bundles of different orders:	
1st order bundles central and 3rd order bundles abaxial	435*
3rd order bundles central and 1st order bundles displaced adaxially in ribs	436*
All bundles arranged irregularly and inconsistently	437*
Bundles of all orders abaxial and an adaxial superposed, often amphivasal bundle in each rib	438*

VASCULAR BUNDLE ARRANGEMENT



5. VASCULAR BUNDLE DESCRIPTION

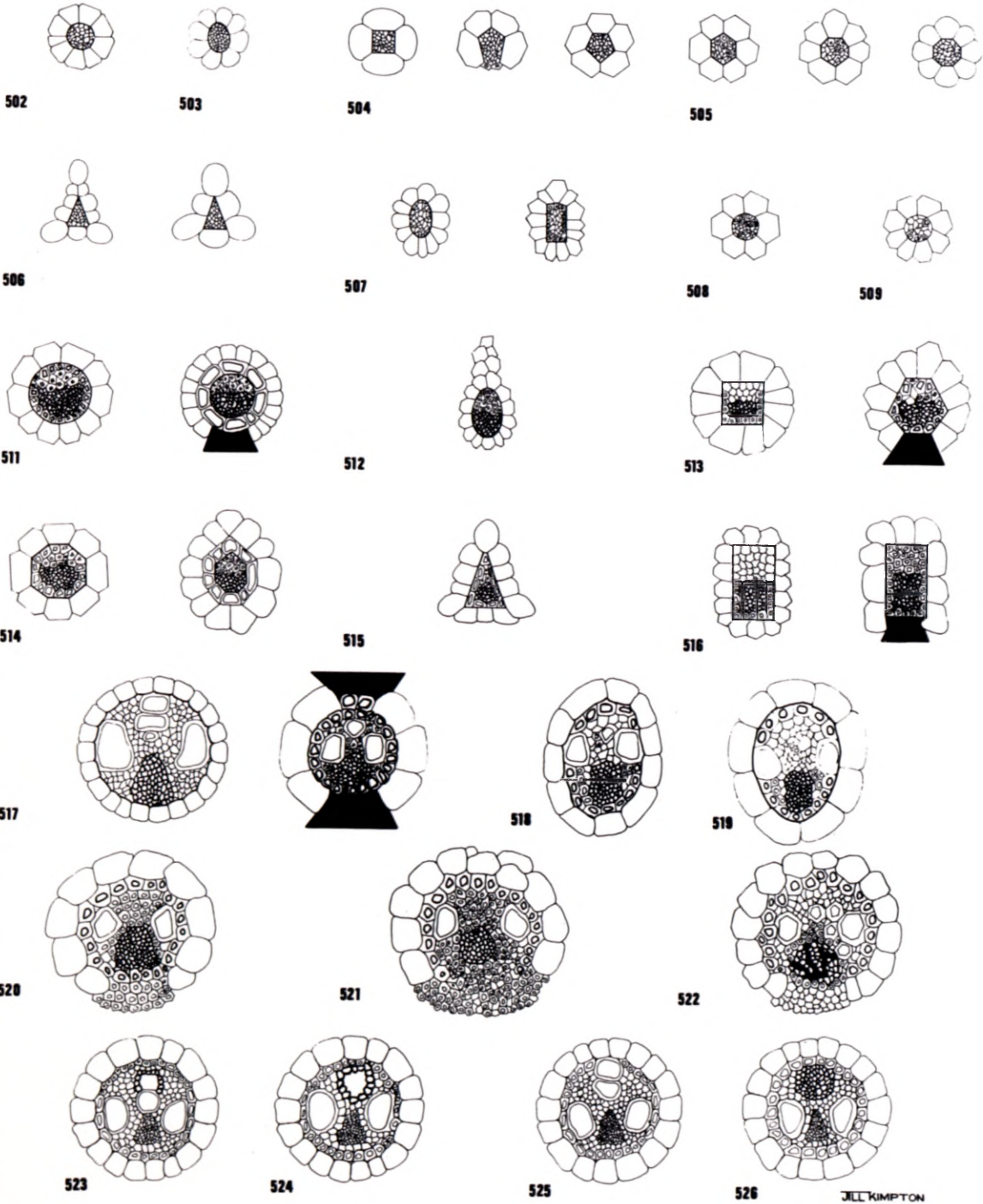
Vascular bundles are considered to consist solely of the xylem and phloem tissue. For the purposes of describing the shape of the vascular bundles the bundle sheaths are not included as being part of the bundles. Thus, when a bundle is described as being circular or angular in outline, these terms refer only to the vascular tissue and exclude the inner or single bundle sheath.

DESCRIPTION:

Third order vascular bundles; usually very small bundles often with xylem and phloem indistinguishable and consisting of only a few lignified cells and a few phloem elements; when not obviously smaller than basic type bundles often distinguishable by the absence of sclerenchyma strands and/or the presence of bulliform cell groups adaxially:

No third order bundles present in section	501
<i>Shape</i> of third order bundles in section:	
Rounded in outline; usually with many small parenchyma sheath cells:	
Circular in outline	502*
Elliptical; vertically elongated	503*
Angular in outline:	
Square-shape or pentagonal i.e. surrounded by a sheath of 4 or 5 large parenchyma cells	504*
Hexagonal or octagonal i.e. surrounded by 6 or more relatively small parenchyma cells	505*
Triangular in outline	506*
Vertically elongated; tall and narrow	507*
<i>Nature</i> of vascular tissue of third order bundles:	
Xylem and phloem groups distinguishable	508*
Vascular tissue consists of only a few vascular strands	509*
Second order vascular bundles; xylem and phloem easily distinguishable; bundles usually fairly large, often of similar size to the first order bundles; no conspicuously large metaxylem vessels or lysigenous cavities present; sclerenchyma arrangement usually the same as for first order bundles:	
No second order bundles present in section	510
<i>Shape</i> of second order bundles in section:	
Rounded in outline; usually with many small parenchyma sheath cells:	
Circular in outline	511*
Elliptical; vertically elongated	512*
Angular in outline; parenchyma sheath of relatively few large cells; inner mestome sheath may also be present:	
Square-shaped or pentagonal	513*
Hexagonal or octagonal	514*
Triangular in shape; often rather large	515*
Tall and narrow in outline; vertically elongated; bundles with straight vertical sides	516*
First order or Basic type vascular bundles; large metaxylem vessel present on either side of protoxylem elements; lysigenous cavity commonly present; associated with sclerenchyma girders or strands:	
<i>Shape</i> of first order bundles in section:	
Circular or round in outline	517*
Elliptical; vertically elongated	518*
Egg-shaped; broadest side adaxial	519*
<i>Relationship</i> of phloem to vascular fibres:	
Phloem adjoins the inner or parenchyma sheath	520*
Phloem completely surrounded by thick-walled fibres	521*
Phloem divided by intrusion of small fibres resulting in sclerosed phloem	522*
<i>Nature</i> of lysigenous cavity and protoxylem:	
Lysigenous cavity and enlarged protoxylem vessel present	523*
Lysigenous cavity but no protoxylem vessel present	524*
Enlarged protoxylem vessel present but no lysigenous cavity	525*
No lysigenous cavity or protoxylem vessel present	526*
<i>Size</i> of metaxylem vessels in relation to parenchyma sheath cells in T/S:	
Narrow vessels i.e. parenchyma sheath cells wider than vessels	527
Wide vessels i.e. vessels with width equal to or slightly greater than parenchyma sheath cells	528
Very wide vessels i.e. width of vessels very much more than that of parenchyma sheath cells	529
<i>Shape</i> of metaxylem vessels as seen in T/S:	
Angular in T/S	530
Circular in T/S	531
<i>Thickening</i> of walls of metaxylem vessels as seen in T/S:	
Unthickened; secondary wall barely visible	532
Slightly thickened; intermediate between unthickened and distinctly thickened state	533
Distinctly thickened; inner secondary wall distinct	534

VASCULAR BUNDLE DESCRIPTION



JILL KIMPTON

6. VASCULAR BUNDLE SHEATHS

Includes the single or double sheath completely or partially surrounding each vascular bundle and adjoining the mesophyll. May also include adaxial or abaxial extensions of parenchyma cells which are considered to be part of the bundle sheath and not part of the system of colourless parenchyma. The different orders of vascular bundles may be surrounded by sheaths of different structure and thus the sheaths associated with the different orders of vascular bundles are considered individually.

ORDERS OF VASCULAR BUNDLE present in section; bundle sheaths of each order of vascular bundle present completely described before continuing with the descriptions of the sheaths of the other orders of vascular bundle present:

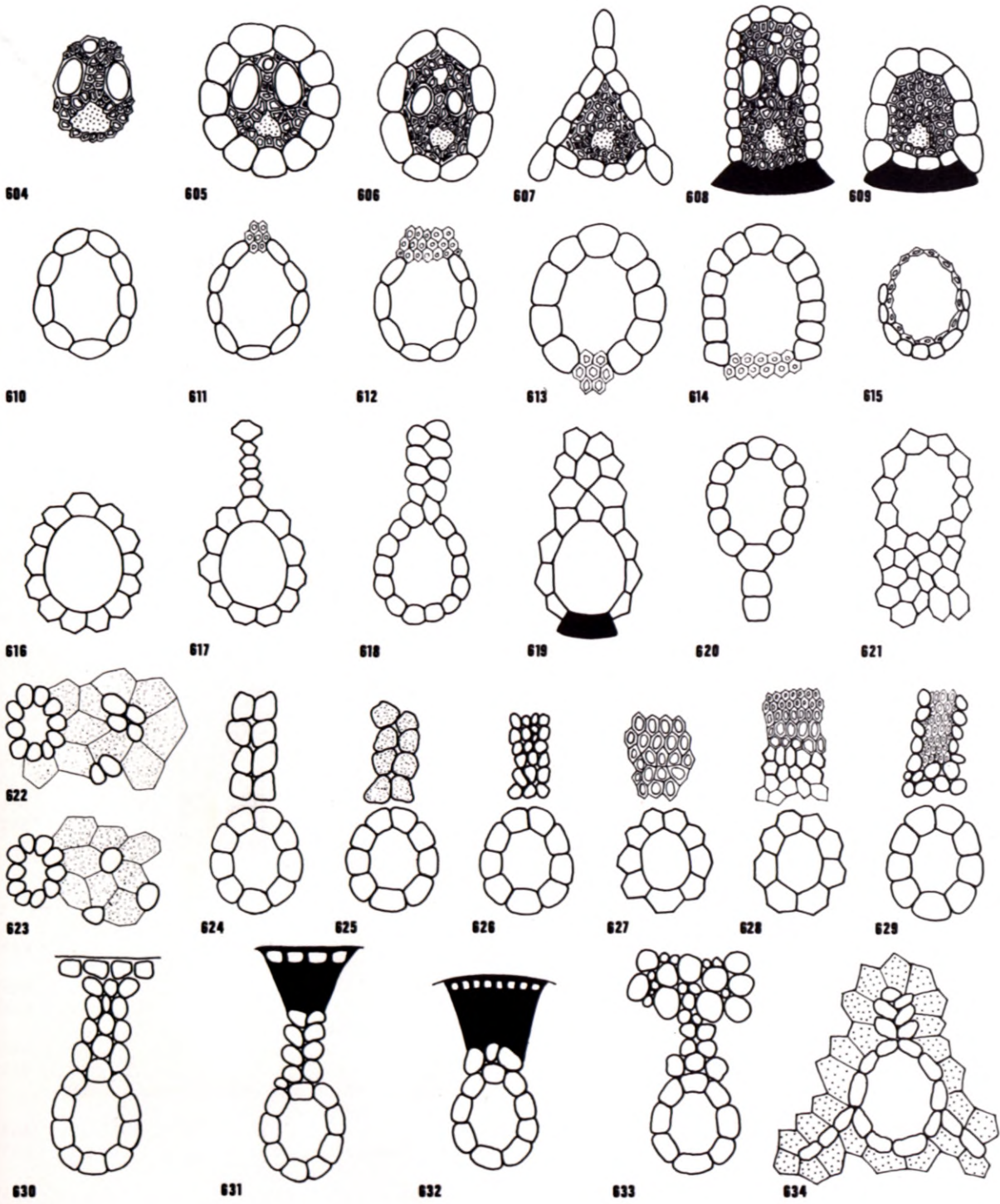
- Third order vascular bundle sheaths; describe fully: 601
- Second order vascular bundle sheaths; describe fully: 602
- First order vascular bundle sheaths; describe fully: 603

DESCRIPTION. A complete description is given separately for each order of vascular bundle present:

- No bundle sheaths surrounding the vascular bundles 604*
- Outer or Single bundle sheath; parenchyma sheath; includes vascular bundles with single and those with double sheaths:
 - Shape of bundle sheath:
 - Sheath round, circular 605*
 - Sheath elliptical, vertically elongated 606*
 - Sheath triangular with adaxial apex 607*
 - Sheath tall and narrow with vertical sides 608*
 - Sheath horse-shoe shaped 609*
 - Extent of bundle sheath:
 - Sheath complete; completely surrounding the bundle 610*
 - Sheath incomplete due to interruption of sclerenchyma girders of various sizes:
 - Adaxial interruption:
 - Slight interruption caused by a narrow girder of 1 – 3 fibres wide 611*
 - Wide interruption caused by broad girder of more than 3 fibres wide or by colourless parenchyma 612*
 - Abaxial interruption:
 - Slight interruption caused by a narrow girder of 1 – 3 fibres wide 613*
 - Wide interruption caused by broad girder of more than 3 fibres wide or by colourless parenchyma 614*
 - Sheath reduced to two lateral strips at the sides of the phloem; adaxial interruption not due to sclerenchyma girders 615*
 - Extensions of the bundle sheath; comprised of parenchyma cells associated with the sheaths and not part of the bulliform groups; extend to adjacent sclerenchyma girders or strands or to the epidermis:
 - No extensions of the bundle sheath present 616*
 - Nature of the extensions:
 - Adaxial extension of the bundle sheath:
 - Narrow extension:
 - Uni-seriate; consisting of one column of cells 617*
 - Bi-seriate; consisting of two columns of cells 618*
 - Broad extension; tri- or multi-seriate 619*
 - Abaxial extension of the bundle sheath:
 - Narrow extension; uni- or bi-seriate 620*
 - Broad extension; tri- or multi-seriate 621*
 - Distinctive cells similar to those of the parenchyma sheath found scattered in the mesophyll:
 - In groups of 2 – 6 with vascular tissue lacking 622*
 - Solitary 623*
 - Composition of the extensions:
 - Consist of large, thin-walled colourless cells; the same size or bigger than sheath cells 624*
 - Consist of large, thin-walled cells with chloroplasts; the same size or bigger than sheath cells 625*
 - Consist of relatively small, thin-walled colourless cells; smaller than the sheath cells 626*
 - Consist of thickened parenchyma cells 627*
 - Consist of colourless cells gradually decreasing in size as walls increase in thickness and eventually merge into the sclerenchyma strand 628*
 - Consist of parenchyma cells on either side of sclerenchyma strands or girders 629*
 - Extent of the extensions of the bundle sheath:
 - Extend to and in contact with the epidermis or an inconspicuous sclerenchyma strand (1 – 4 fibres) 630*
 - Extend to conspicuous sclerenchyma strand 631*
 - Extend to sclerenchyma strand almost extending to the bundle sheath 632*
 - Extend to colourless subepidermal parenchyma 633*
 - Extend into mesophyll tissue 634*
 - Length of extension:
 - 1 – 2 cells long or deep 635
 - 2 – 5 cells long or deep 636
 - More than 5 cells long or deep 637
- Number of cells comprising the bundle sheath:
 - 4 parenchyma cells comprise the sheath 638
 - 5 parenchyma cells comprise the sheath 639
 - 6 parenchyma cells comprise the sheath 640
 - 7 parenchyma cells comprise the sheath 641
 - 8 parenchyma cells comprise the sheath 642
 - 9 parenchyma cells comprise the sheath 643
 - 10 parenchyma cells comprise the sheath 644
 - 11 parenchyma cells comprise the sheath 645
 - 12 parenchyma cells comprise the sheath 646
 - 13 parenchyma cells comprise the sheath 647
 - 14 parenchyma cells comprise the sheath 648
 - 15 or more parenchyma cells comprise the sheath 649

CONTINUE ON FOLLOWING PAGE AND THEN RETURN AND DESCRIBE SHEATHS OF ANY OTHER ORDERS OF VASCULAR BUNDLES THAT ARE PRESENT.

VASCULAR BUNDLE SHEATHS



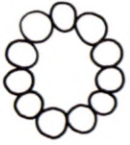
6. VASCULAR BUNDLE SHEATHS CONTINUED

DESCRIPTION CONTINUED. A complete description is given separately for each order of vascular bundle present:

<i>Structure</i> of the parenchyma sheath cells; : cells of each order of vascular bundle considered separately and all cell types included:	
Not well differentiated from the chlorenchyma cells	650
Distinct from chlorenchyma cells:	
Shape of cells comprising sheath; include all shapes relevant for each order of vascular bundle:	
Inflated, round, circular	651*
Radial walls straight; tangential walls inflated	652*
Radial and outer tangential walls straight; inner tangential wall inflated	653*
Radial and inner tangential walls straight; outer tangential wall inflated; fan-shaped	654*
Cells elliptical; elongated; often rather irregular	655*
Arrangement of cells of different shapes comprising the bundle sheath:	
All cells similar in shape	656
Irregular; cells of different shapes comprise sheath	657
Two enlarged elongated cells abaxially situated:	
Adjoining girder of sclerenchyma	658*
Normal parenchyma sheath cells between the elongated cells	659*
Cells at adaxial side of sheath elongated vertically; elliptical	660*
Sheaths of adjacent bundles continuous with each other; <i>Triodia</i> type	661*
Size of cells comprising sheath:	
Cells of sheath similar in size:	
Large and inflated; generally larger than mesophyll cells; conspicuous	662*
Not markedly larger than the mesophyll cells; conspicuous	663*
Cells smaller than the mesophyll cells; inconspicuous	664*
Cells smaller than the inner bundle sheath cells	665*
Cells small; resemble cells of a mestome sheath i.e. thickened walls often especially the inner tangential walls; parenchyma sheath apparently absent	666*
Cells of sheath of various sizes:	
Cells of different sizes comprise sheath; irregular	667*
Gradation in size with largest cells adaxially situated	668*
Gradation in size with largest cells abaxially situated	669*
Gradation with smallest cells in the centre; abaxial and adaxial cells larger	670*
Gradation with largest cells in the centre on each side	671*
Wall structure of bundle sheath cells:	
Walls thin; no secondary thickening	672*
Walls slightly thickened; secondary cell wall not distinct	673*
Walls distinctly thickened; secondary wall distinct	674*
Inner tangential and radial walls thickened	675*
Chloroplast structure of the bundle sheath cells:	
Translucent; sheath cells without chloroplasts	676
With small chloroplasts; not distinct from chloroplasts of the chlorenchyma:	
Numbers of chloroplasts comparable to the mesophyll cells	677*
Numbers of chloroplasts fewer than in mesophyll cells	678*
With large, specialised chloroplasts:	
Chloroplasts fill entire cell lumen	679*
Chloroplasts concentrated near the outer tangential wall	680*
Chloroplasts concentrated near the inner tangential wall	681*
Chloroplasts centrally situated	682*
Inner or Mestome bundle sheath; endodermis; only applicable when two sheaths are present; often difficult to distinguish from fibrous ground tissue of the vascular bundle; in contact with the metaxylem i.e. if metaxylem vessels in contact with parenchyma sheath then there is no inner bundle sheath:	
<i>Extent</i> of sheath:	
No inner sheath present	683*
Sheath complete; completely surrounding the xylem and phloem	684*
Sheath incomplete due to interruptions of sclerenchyma girders:	
Adaxial interruption	685*
Abaxial interruption	686*
Sheath reduced; intermediate type; interruptions not due to sclerenchyma girders:	
Abaxial; opposite phloem only	687*
Adaxial; opposite xylem only	688*
<i>Structure</i> of cells of inner bundle sheath in region adjacent to phloem; cells of each order of vascular bundle considered separately:	
Relatively large with inner tangential and radial walls thickened	689*
Small, with inner tangential and radial walls thickened; U-shaped thickenings	690*
Small, with uniformly thickened walls:	
Walls heavily thickened; lumen small	691*
Walls not conspicuously thickened	692*
Larger than outer bundle sheath; parenchymatous; with chloroplasts	693*
Adaxially situated cells larger than lateral cells of the sheath	694*

N.B. SHEATH OF EACH ORDER OF VASCULAR BUNDLE PRESENT IN SECTION DESCRIBED COMPLETELY BEFORE CONTINUING WITH SCLERENCHYMA OF THE LEAF.

VASCULAR BUNDLE SHEATHS



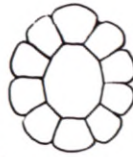
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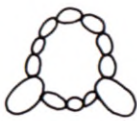
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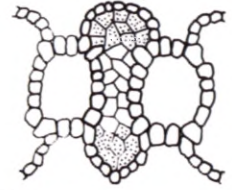
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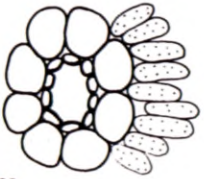
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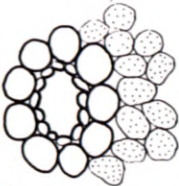
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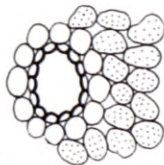
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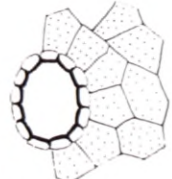
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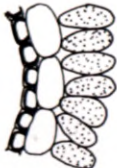
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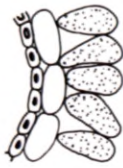
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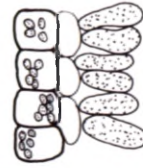
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7. SCLERENCHYMA OF LEAF

Includes all thickened fibrous tissue in the leaf, except that constituting part of the vascular tissue i.e. xylem and phloem fibres and thickened parenchyma. The sclerenchyma can be sub-divided into the circumvascular sclerenchyma surrounding, in contact with, or opposite the vascular bundles in the form of girders or strands, sclerenchyma found in the intercostal areas between successive bundles, sclerenchyma of the leaf margin and sclerenchyma of acicular leaves. Sclerenchyma associated with the midribs or keels included under midribs and keels.

SCLERENCHYMA STRANDS AND GIRDERS

Circumvascular sclerenchyma surrounding, in contact with, or opposite the bundles; refers only to longitudinal subepidermal sclerenchyma following the course above and below the vascular bundles. Complete description is given of the circumvascular sclerenchyma associated with each of the different orders of vascular bundle present in the leaf.

ORDERS OF VASCULAR BUNDLES present in leaf section; sclerenchyma associated with each order of vascular bundle present in section considered separately and each is completely described before commencing with other orders that might be present:

Sclerenchyma associated with third order vascular bundles	701
Sclerenchyma associated with second order vascular bundles	702
Sclerenchyma associated with first order vascular bundles	703

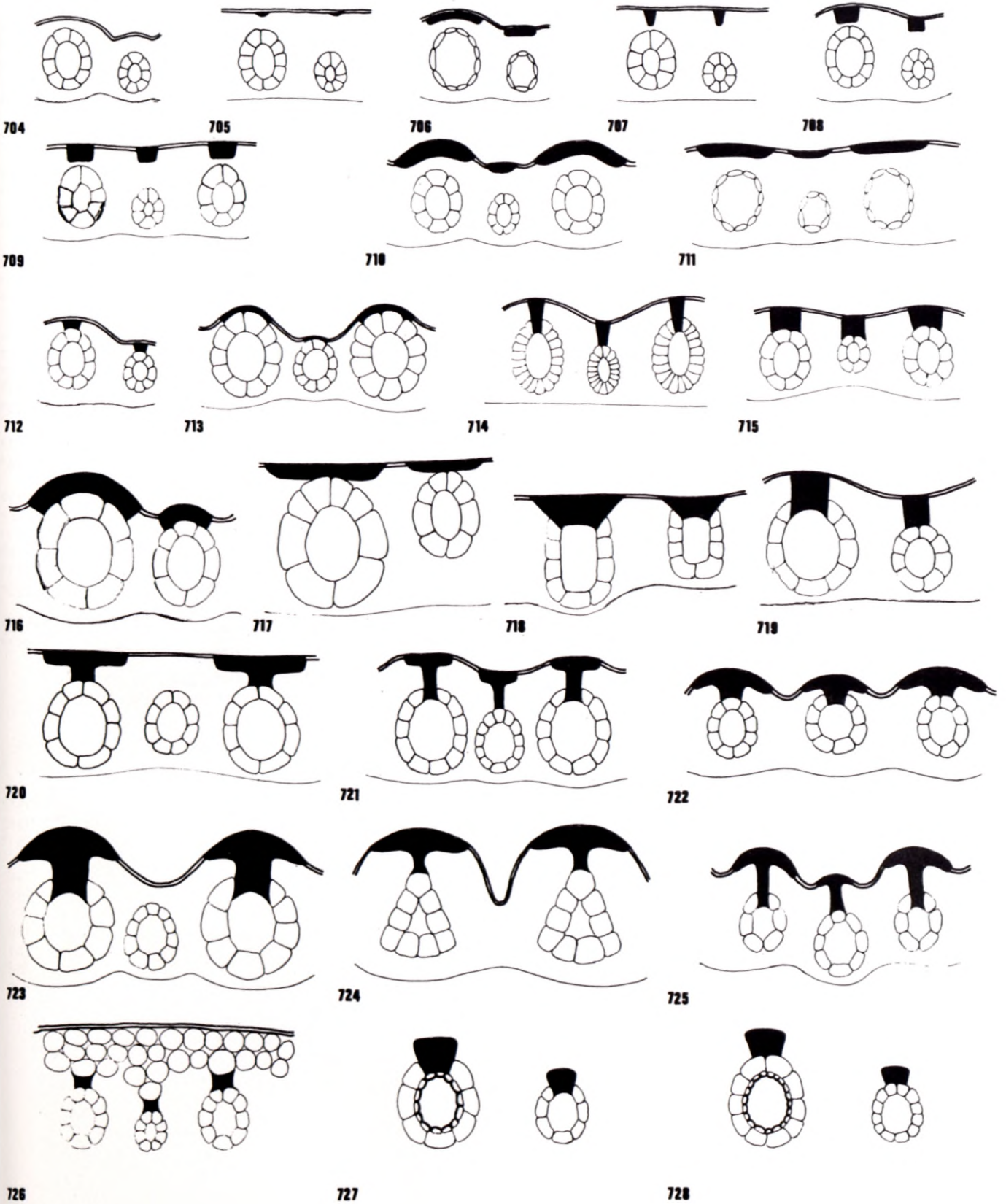
DESCRIPTION

ADAXIAL sclerenchyma; sclerenchyma found above the vascular bundles; associated with the bundles:

No adaxial sclerenchyma present	704*
<i>Strand</i> present; sclerenchymatous; not in contact with the bundle sheath; separated by parenchyma or mesophyll from sheath:	
Minute strand consisting of only a few subepidermal fibres	705*
Small strand; inconspicuous; epidermal cells unaltered over the strand:	
Shallow, forming a strip; only 2 – 4 fibres deep; subepidermal	706*
Narrow; only 2 – 4 fibres wide	707*
As deep as wide as seen in T/S	708*
Well-developed strand; conspicuous; epidermal cells over strand usually small and thick-walled:	
As deep as wide as seen in T/S	709*
Wider than deep as seen in T/S; in form of band:	
Arched; follows the shape of the adaxial rib	710*
Straight, horizontal band	711*
<i>Girder</i> present; sclerenchymatous; in contact with, or interrupts the bundle sheath:	
Very small girder; inconspicuous; epidermal cells usually unaltered over girder	712*
Small girder; epidermal cells over the girder usually small and thick-walled:	
Small thin subepidermal strip	713*
Narrow; deeper than wide	714*
As deep as wide as seen in T/S; equidimensional	715*
Well-developed girder; conspicuous; epidermal cells over girder small and thickened:	
Relatively wide and deep band; as wide or wider than the vascular bundle:	
Arched; follows the shape of the adaxial rib	716*
Straight; horizontal band	717*
Narrowing towards bundle	718*
Relatively narrow and deep girder; narrower than the vascular bundle	719*
T-shaped; horizontal band connected to bundle or bundle sheath by a stem:	
Stem short; shorter than horizontal cross-piece	720*
Stem relatively long; as long or longer than horizontal cross-piece	721*
Inversely anchor-shaped; arched band follows shape of adaxial rib:	
Stem short and sturdy; more than tri-seriate	722*
Stem long or tall and sturdy; more than tri-seriate	723*
Stem short and thin or narrow; 1 – 3 seriate	724*
Stem long or tall and thin or narrow; 1 – 3 seriate	725*
<i>Girder</i> extends from bundle sheath to bulliform or colourless cells; not to epidermis	726*
<i>Girder</i> description continued:	
Fibres interrupt the cells of the single or outer bundle sheath	727*
Fibres in contact with the cells of the single or outer bundle sheath	728*

CONTINUE ON FOLLOWING PAGE AND THEN RETURN AND DESCRIBE STRANDS AND GIRDERS OF ANY OTHER ORDERS OF VASCULAR BUNDLES THAT ARE PRESENT IN THE SECTION.

ADAXIAL SCLERENCHYMA OF LEAF



7. SCLERENCHYMA OF LEAF CONTINUED

Continuation of circumvascular sclerenchyma surrounding, in contact with, or opposite the bundles; refers only to longitudinal subepidermal sclerenchyma following the course above and below the vascular bundles. Complete description is given of the circumvascular sclerenchyma associated with each of the different orders of vascular bundle present in the leaf.

ABAXIAL sclerenchyma; sclerenchyma found below the vascular bundles:

No abaxial sclerenchyma present	729*
<i>Strand</i> present; sclerenchymatous not in contact with the bundle sheath; separated by parenchyma or mesophyll from sheath:	
Minute strand consisting of only a few subepidermal fibres	730*
Small strand; inconspicuous; epidermal cells unaltered under the strand:	
Shallow, forming a strip; only 2 – 4 fibres deep; subepidermal	731*
Narrow but fairly deep; only 2 – 4 fibres wide	732*
As deep as wide as seen in T/S	733*
Well-developed strand; conspicuous; epidermal cells under strand usually small and thick-walled:	
As deep as wide as seen in T/S	734*
Wider than deep as seen in T/S; in form of a band:	
Arched, follows the shape of the abaxial rib	735*
Straight, horizontal band	736*
<i>Girder</i> present; sclerenchymatous in contact with, or interrupts the bundle sheath:	
Very small girder; inconspicuous; epidermal cells usually unaltered under girder	737*
Small girder; epidermal cells under the girder usually small and thick-walled:	
Small, thin subepidermal strip	738*
Narrow, deeper than wide	739*
As deep as wide as seen in T/S; equidimensional	740*
Well-developed girder; conspicuous; epidermal cells under the girder usually small and thickened:	
Relatively wide and deep band; as wide or wider than the vascular bundle:	
Arched, follow the shape of the abaxial rib	741*
Straight, horizontal band	742*
Narrowing towards bundle; triangular or trapezoidal	743*
Relatively narrow and deep girder; narrower than the vascular bundle	744*
Inverted T-shaped; horizontal band connected to bundle or bundle sheath by stem:	
Stem short; shorter than horizontal cross-piece	745*
Stem relatively long; as long or longer than horizontal cross-piece	746*
Anchor-shaped; arched band follows shape of abaxial rib:	
Stem short and sturdy; more than tri-seriate	747*
Stem long or tall and sturdy; more than tri-seriate	748*
Stem short and thin or narrow; 1 – 3 seriate	749*
Stem long or tall and thin or narrow; 1 – 3 seriate	750*
Continuous or almost continuous abaxial hypodermal band	751*
<i>Girder</i> extends from bundle sheath to bulliform or colourless cells; not to the epidermis	752*
<i>Girder</i> description continued:	
Fibres interrupt the cells of the single or outer bundle sheath	753*
Fibres in contact with the cells of the single or outer bundle sheath	754*

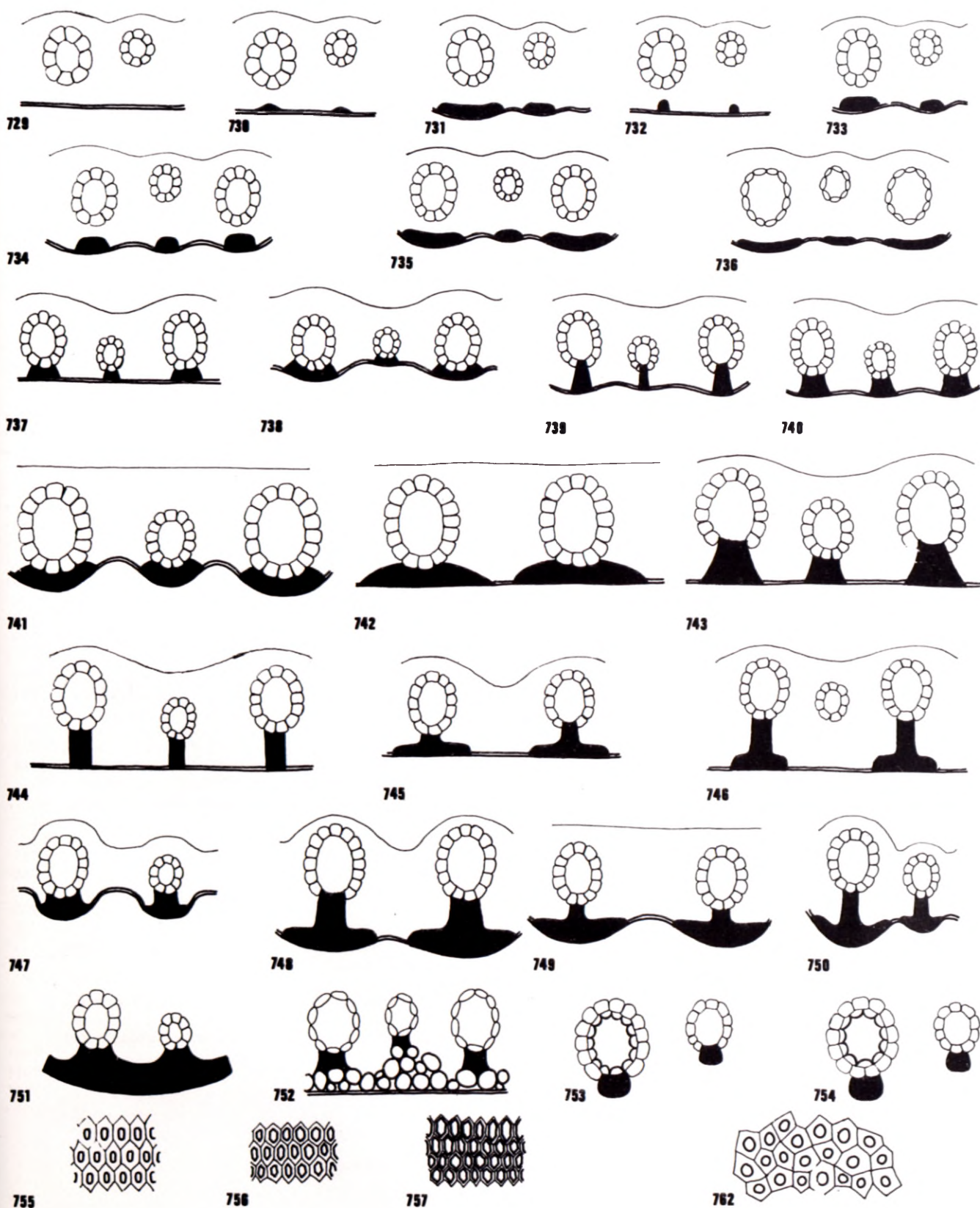
N.B. CIRCUMVASCULAR SCLERENCHYMA OF EACH ORDER OF VASCULAR BUNDLE PRESENT IN SECTION DESCRIBED SEPARATELY BEFORE COMMENCING WITH SCLERENCHYMA CELL STRUCTURE AND OTHER SCLERENCHYMA TISSUE OF THE LEAF.

SCLERENCHYMA CELL STRUCTURE:

Fibres, circumvascular sclerenchyma comprised of thickened; elongated fibres:

Cell wall structure:	
Very thick walled; lumen almost excluded	755*
Cell walls of fibres of medium thickness; secondary wall distinct	756*
Not markedly thickened; secondary wall not thick	757*
Cell wall composition:	
All cell walls lignified i.e. stain red with saffranin	758
All cell walls of cellulose i.e. stain blue-green with fast green	759
Some sclerenchyma cell walls lignified others with cellulose walls:	
In individual girder or strand groups	760
In different parts of the leaf section	761
<i>Collenchyma</i> ; thickened parenchyma cells; wide lumens	762*

ABAXIAL SCLERENCHYMA OF LEAF



7. SCLERENCHYMA OF LEAF CONTINUED

Continued from previous page and in contrast to the circumvascular sclerenchyma the types of sclerenchyma described below are not described in relation to the different orders of vascular bundles.

SCLERENCHYMA BETWEEN BUNDLES:

Supernumary strands not associated with the vascular bundles but situated intermediate between them, usually beneath the abaxial epidermis opposite the bulliform cell groups or furrows. Excludes sclerenchyma at the leaf margin and of the keel or midrib.

DESCRIPTION:

No sclerenchyma present between the vascular bundles	763
<i>Abaxial strands</i> of sclerenchyma fibres present:	
Opposite adaxial furrows with or without bulliform cells	764*
Opposite bulliform cell groups not associated with furrows	765*
<i>Hypodermal band</i> ; sclerenchyma in form of more or less continuous abaxial band:	
Extensions of girder or strand groups	766*
Hypodermal layer continuous	767*

SCLERENCHYMA IN LEAF MARGIN:

Includes all mechanical tissue situated in, or directly associated with the margin of the lamina. May incorporate or fuse with the ultimate, the penultimate or other lateral bundles.

DESCRIPTION:

No sclerenchyma developed in association with the margin	768
<i>Cap</i> of sclerenchyma at the margin; not in contact with the lateral bundle:	
<i>Size</i> of cap:	
Very small; consists of a couple fibres	769*
Relatively small; less than the width of a third order vascular bundle	770*
Well-developed; wider than third order vascular bundles	771*
<i>Shape</i> of cap:	
Rounded cap	772*
Pointed cap	773*
Narrow, very pointed projection	774*
Crescent-shaped cap; sclerenchyma extends shortly along both abaxial and adaxial side of leaf	775*
Curved in shape with sclerenchyma extending along adaxial side of the leaf	776*
Curved in shape with sclerenchyma extending along abaxial side of the leaf	777*
<i>Epidermal cells</i> at margin:	
Not fibrous; thin-walled or outer walls thickened; distinct from fibres	778
Small, fibrous; thickened on all walls; similar to fibres in T/S	779
<i>Angular prickles</i> of margin:	
Enlarged bases present in margin	780
No prickle bases visible in margin	781
<i>Nature</i> of junction with mesophyll and remainder of lamina:	
Adjoins normal mesophyll cells	782
Specialised enlarged parenchyma cells near cap or ultimate bundle	783
Lateral intercellular canal near margin; lysigenous duct not associated with the bundle	784
<i>Hood</i> of sclerenchyma; hood-like structure formed by sclerenchyma extensions above or below ultimate and other lateral bundles; may or may not be in contact with bundles or bundle sheaths but must extend above or below or beyond ultimate lateral vascular bundle:	
<i>Position</i> of fibrous hood:	
Fibres extend along adaxial side of the leaf	785*
Fibres extend along abaxial side of the leaf	786*
<i>Extent</i> of fibrous hood:	
Short hood; extends as far as or over ultimate lateral bundle	787
Well-developed hood; extends as far as or beyond penultimate bundle	788
Penultimate bundle and associated sclerenchyma; may be no sclerenchyma development at the extreme margin but the penultimate lateral bundle may be intimately associated with specialized girders, strands or bands:	
<i>Nature</i> of lateral fibrous groups:	
Bundle sheath of penultimate bundle modified and cells are horizontally elongated and in contact with the normal sheath cells of the ultimate bundle	789*
Bundle sheath of penultimate bundle not modified but sclerenchyma continuous between the ultimate and penultimate bundles from adaxial to abaxial epidermis	790*
Well-developed adaxial girder or band developed above the penultimate bundle	791*
Extensive adaxial band developed above the lateral 3 or 4 bundles	792*

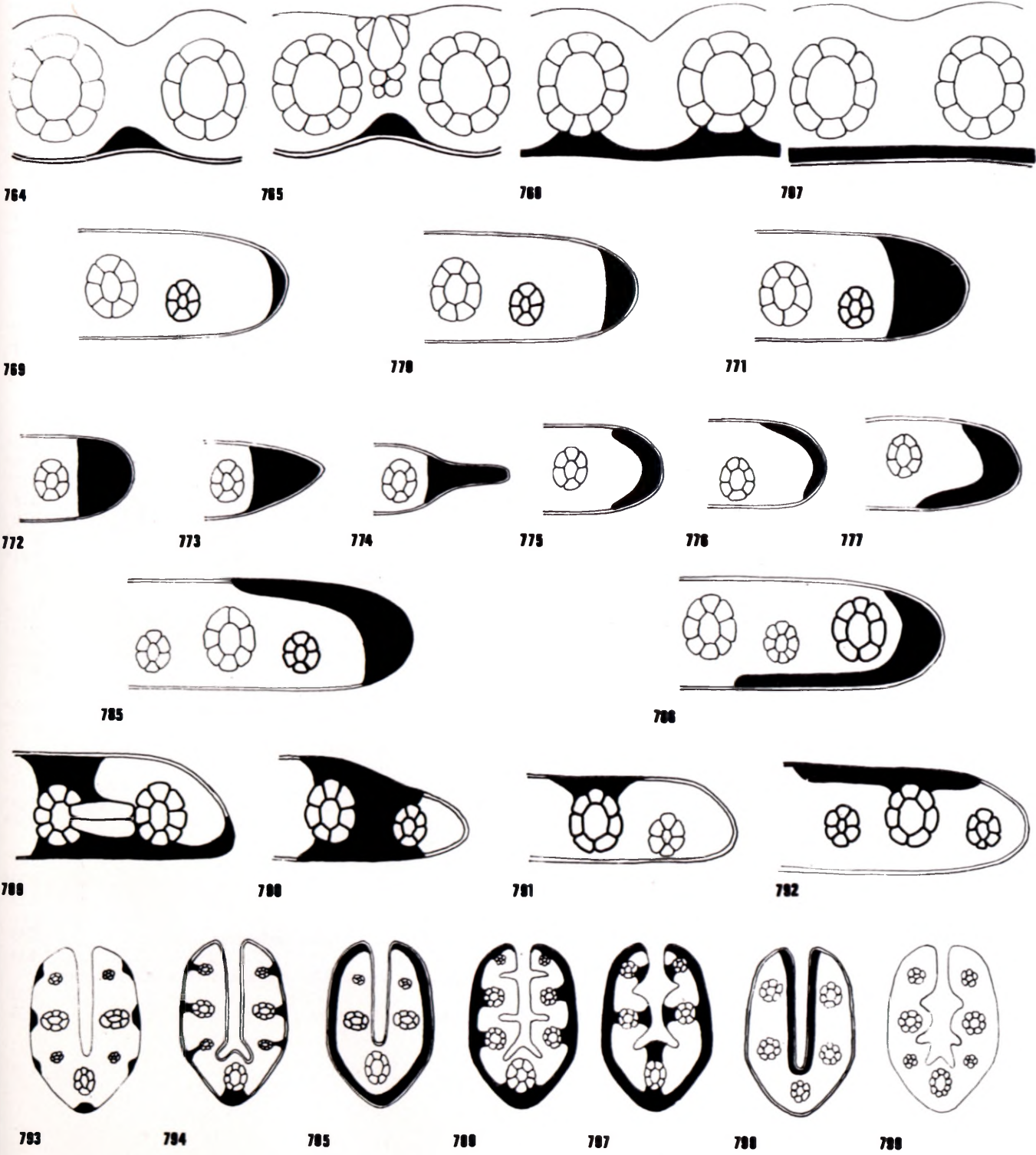
SCLERENCHYMA ARRANGEMENT IN ACICULAR LEAVES:

Refers to the sclerenchyma as seen in transverse section of permanently infolded leaves.

DESCRIPTION:

Abaxial strands present opposite the vascular bundles	793*
Abaxial girders present opposite the vascular bundles	794*
Continuous, abaxial, subepidermal band; not connected to the vascular bundles	795*
Continuous, abaxial, subepidermal band connected to bundles by girders	796*
Continuous, abaxial, subepidermal band and girders extending to adaxial surface	797*
Continuous, adaxial, subepidermal band not connected to the vascular bundles	798*
No, or very poor sclerenchyma development	799*

SCLERENCHYMA BETWEEN BUNDLES, AT MARGIN AND IN ACICULAR LEAVES



8. MESOPHYLL OF LEAF

Applies to all the ground tissue between the adaxial and abaxial epidermides. It usually consists mainly of the assimilatory tissue or chlorenchyma but may be partly composed of translucent colourless cells. It does not include the bulliform cells (which are part of the epidermis) the sclerenchyma or the bundle sheaths or their extensions.

CHLORENCHYMA

All the tissue containing chloroplasts excepting the parenchyma bundle sheath which may or may not contain chloroplasts and is described under bundle sheaths. Does not include all the ground tissue between the adaxial and abaxial epidermides especially if any translucent cells without chloroplasts are present.

DESCRIPTION:

Radiate chlorenchyma; radially arranged around the vascular bundles which are often close together; panicoid; may be interrupted by sclerenchyma girders and thus divided into two groups on either side of the vascular bundle:

Number of layers of chlorenchyma cells arranged around the bundles:

Single layer of cells:

Tabular

801*

Isodiametric

802*

Two layers of radiating cells

803*

Numerous layers of radiating cells; cells long and narrow; *Isachne* type

804*

Extent of radiating cells around bundles:

Radiating cells completely surrounding bundles

805*

Interrupted above bundles by sclerenchyma or colourless parenchyma girders

806*

Interrupted below bundles by sclerenchyma or colourless parenchyma girders

807*

Reduced to two strips of chlorenchyma by large girders or colourless parenchyma

808*

Relationships of successive radiating mesophyll groups to each other:

Separated by bulliform and colourless cell groups:

Completely divided; colourless cells continuous to abaxial epidermis

809*

Adaxially divided; colourless cells in adaxial half of leaf

810*

Separated by irregular chlorenchyma and intercellular air-spaces

811*

Radiating cells of successive bundles adjoin one another

812*

Indistinctly or incompletely radiate chlorenchyma; intermediate between radiate type and that not arranged in a definite pattern:

Continuous between bundles

813*

Chlorenchyma divided by groups of colourless cells; tending to be radiate

814*

Irregular chlorenchyma; not radiate or arranged in a definite pattern; vascular bundles usually widely spaced; festucoid:

Vertical arrangement in the mesophyll between successive vascular bundles:

Occupying the major or entire area between the adaxial and abaxial epidermides:

All cells of the chlorenchyma similar; homogenous:

Regular small cells; isodiametric; tightly packed

815*

Irregular; cells of different size and shape; often with intercellular air-spaces

816*

Cells palisade-like in the adaxial chlorenchyma

817*

Chlorenchyma cells adjoining the bundles larger than those further removed

818*

Occupying the lower abaxial half of the leaf thickness

819

Occupying the lower abaxial third of the leaf thickness

820

Confined to small patches surrounding abaxial and adaxial grooves which contain stomata

821*

Horizontal arrangement in the mesophyll between successive vascular bundles:

Continuous between bundles:

Strap-shaped; horizontally elongated; bundles widely spaced

822*

Tall and narrow groups; bundles close together

823*

U-shaped; occupying the sides and bases of furrows or around bulliform cell groups

824*

Mesophyll groups divided by colourless cell groups

825*

Arm cells present or comprise chlorenchyma; walls invaginated:

Invaginations extend almost to opposite wall; cells divided into compartments

826*

Invaginations relatively short

827*

Fusoid cells present in mesophyll; translucent, elongated narrow collapsed cells; cavity between fusoid cells usually only visible in T/S; bamboo type:

Successive fusoid cells separated by a vertical column of chlorenchyma one cell wide

828*

Successive fusoid cells separated by numerous chlorenchyma cells

829*

Distinctive cells, similar to parenchyma sheath cells but not associated with bundles present in mesophyll

830*

Lacunae or air spaces present in mesophyll:

Extent of lacunae:

Between all or most adjacent vascular bundles

831*

Continuous air spaces above third order bundles

832*

Aerenchyma associated with the lacunae:

Distinct lacunae bounded by compact mesophyll with no aerenchyma traversing the lacunae

833

Lacunae distinct but traversed by colourless aerenchyma cell chains

834

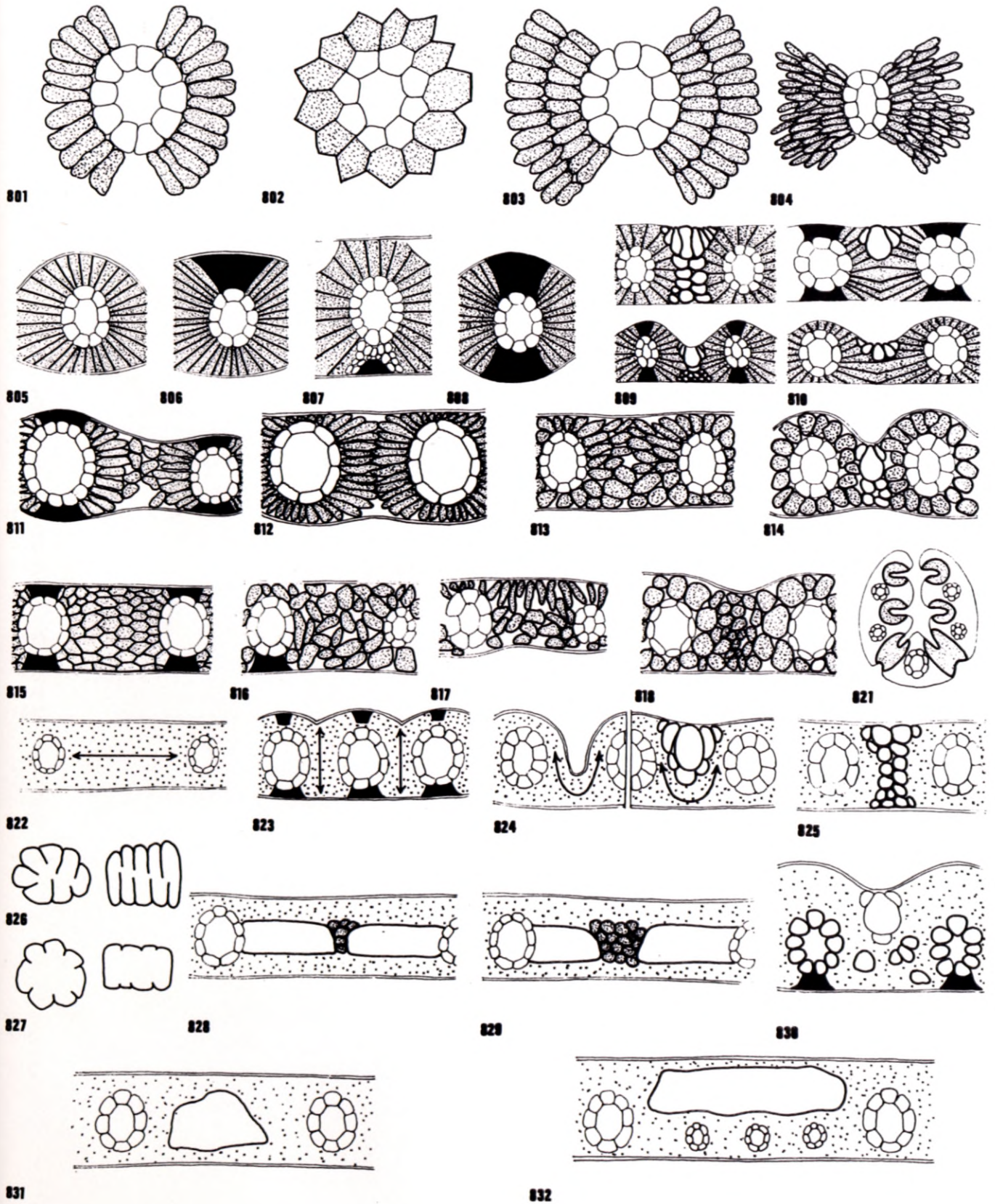
Irregularly defined air spaces in diffuse mesophyll with many intercellular spaces and chlorophyll-bearing aerenchyma; often subtending the stomata

835

Stellate cells representing a diaphragm interspersed by sclerotic strands present

836

CHLORENCHYMA



8. MESOPHYLL OF LEAF CONTINUED

COLOURLESS CELLS

Translucent cells constituting the remaining cells in the zones between successive vascular bundles, excluding the chlorenchyma, the sclerenchyma and the bulliform cells. They vary in size and may be larger or smaller than the bulliform cells but are always without chlorenchyma and never part of the epidermis i.e. not in contact with the surface.

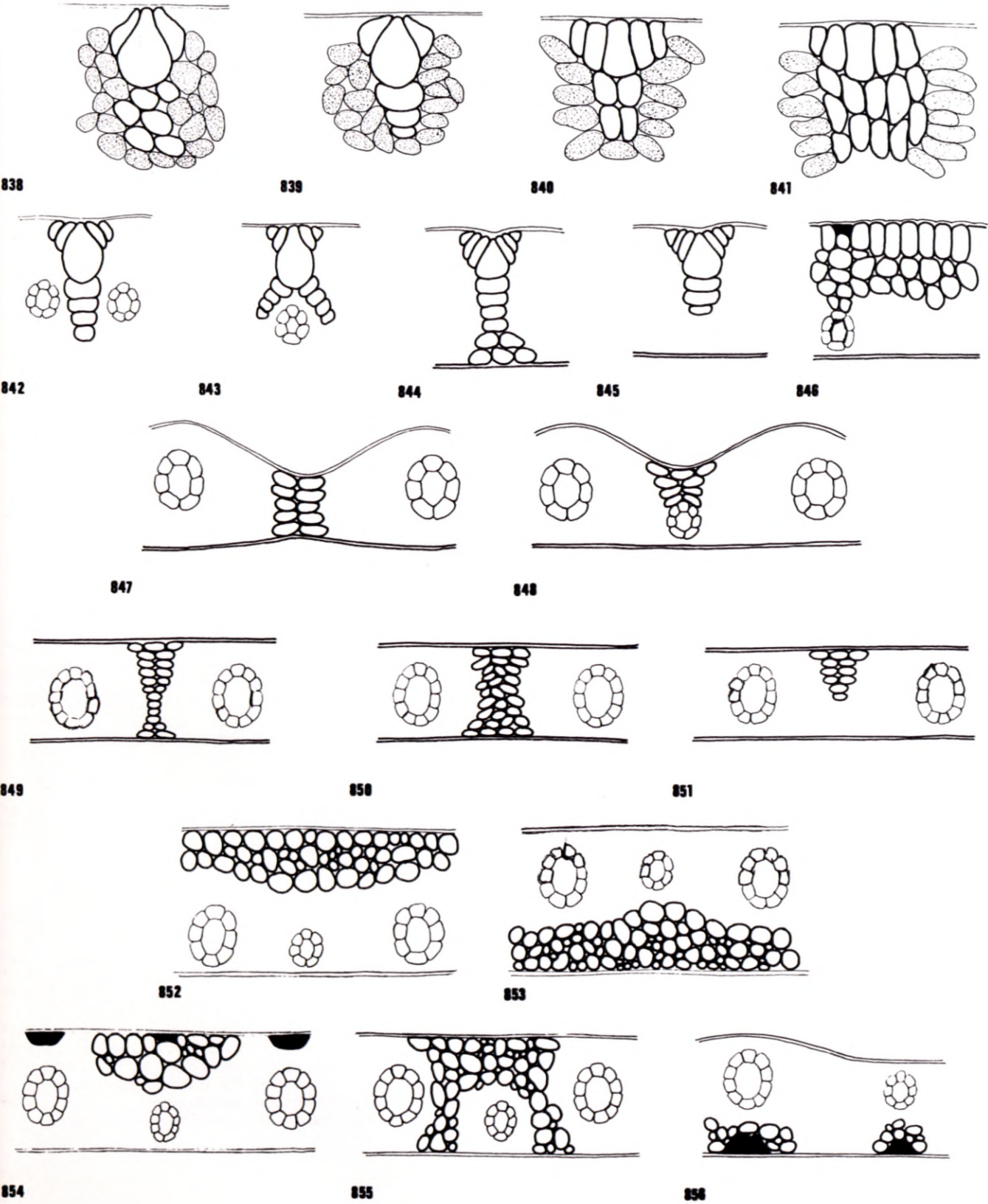
DESCRIPTION:

Absent , no colourless cells present	837
Well defined groups of colourless cells present:	
<i>Closely associated</i> with inflated bulliform cells or groups:	
Size of colourless cells:	
Smaller than bulliform cells; uninflated; often same size as chlorenchyma cells	838*
Similar in size or shape to bulliform cells; inflated	839*
Width of extension of bulliform cell group; composed of colourless cells:	
Narrower than bulliform cell group	840*
Same width as bulliform cell group	841*
Number of extensions from each bulliform cell group:	
1 extension from each group	842*
2 extensions from each group; one on either side of the vascular bundle	843*
Extent of intrusion into the mesophyll by colourless cells:	
Girders extend to the opposite epidermis	844*
Strands not extending to the opposite epidermis	845*
Band occupying the adaxial half of the leaf composed of colourless cells	846*
<i>Not closely associated</i> with large bulliform cells:	
Origin at the base of furrows:	
Girder-like extension to the opposite epidermis between the bundles	847*
Girder-like extensions to the bundle sheath or towards the bundle sheath	848*
Origin on flat epidermis or with slight furrows:	
Girder-like extension extends to the opposite epidermis:	
Column of girder only uni- or bi-seriate	849*
Girder multi-seriate	850*
Girder of colourless cells does not extend to the opposite epidermis	851*
III defined groups of smallish colourless cells; not associated with large or well defined bulliform groups; usually adaxial:	
Adaxial half of leaf consisting entirely of colourless cells	852*
Abaxial half of leaf consisting mainly of colourless cells	853*
Present on adaxial side of small vascular bundles only	854*
Form arches over third order bundles and extend to the abaxial epidermis	855*
Abaxial irregular groups; may be associated with sclerenchyma strands	856*

DESCRIPTION OF INDIVIDUAL CELLS:

Structure of colourless cells:	
Inflated, large parenchyma cells; resemble bulliform cells in size and shape	857
Smaller, thin-walled parenchyma cells; rounded in shape:	
Cells regular in size and shape; tissue uniform in appearance	858
Cells irregular in size and shape; tissue irregular in appearance	859
Thick-walled parenchyma or collenchyma cells	860

COLOURLESS PARENCHYMA CELLS



9. EPIDERMAL CELLS IN TRANSVERSE SECTION

Includes all cells and their appendages present in the epidermal layer. Can be subdivided into the bulliform cells and typical thickened epidermal cells.

ADAXIAL AND ABAXIAL EPIDERMIS of the leaf; cells of the upper and lower epidermis considered separately and the adaxial epidermis is fully described before commencing with the abaxial epidermis:

Cells of adaxial epidermis	901
Cells of abaxial epidermis	902

BULLIFORM CELLS

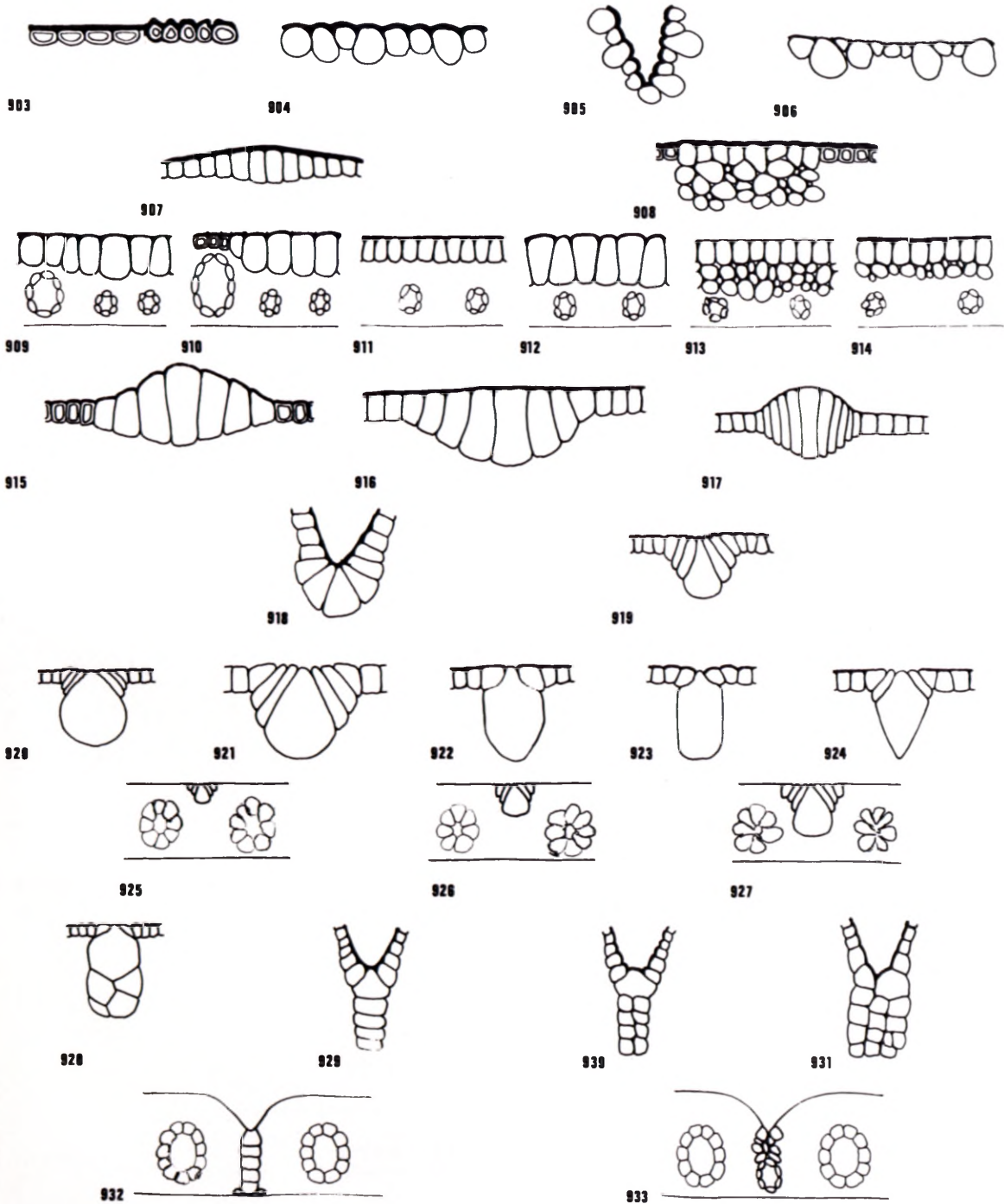
Colourless cells forming part of the epidermis but differing from the remaining epidermal cells by being markedly larger and inflated. Characteristically they are large and are restricted to groups often intimately associated with colourless cells but all gradations are found. Thus small solitary bulliform cells may be present or the epidermis may be comprised primarily of large, rounded and inflated cells. All these variations are included under bulliform cells for descriptive purposes. For bulliform cells of the keel see under midrib and keel.

DESCRIPTION: Adaxial and abaxial epidermides considered separately.

Absent , no bulliform cells in epidermis being described; consists of small cells; often thickened	903*
Bulliform cells only ; not arranged in regular groups; epidermal cells rounded and inflated the same size or slightly larger than the cells of the bundle sheath:	
All or most epidermal cells of this type	904*
Isolated cells larger; may comprise irregular small groups:	
On sides of deep furrows	905*
Not in furrows; usually in leaves with flat surfaces	906*
Groups of bulliform cells present in epidermis ; well-defined and regular; distinct from normal epidermal cells:	
Small bulliform cells; not conspicuously larger than the normal epidermal cells:	
Not associated with colourless cells	907*
Closely associated with colourless cells; indistinct	908*
Extensive groups of large, inflated bulliform cells extending over one or many vascular bundles:	
Not associated with colourless parenchyma:	
Distribution of extensive bulliform groups:	
Present throughout the epidermis; may be slightly reduced opposite the larger bundles	909*
Present in most of the epidermis but not present opposite the first order bundles and usually reduced over the second order bundles	910*
Size of constituent cells; taken in areas opposite the third order bundles:	
Occupy less than $\frac{1}{4}$ of the leaf thickness	911*
Occupy more than $\frac{1}{4}$ of the leaf thickness	912*
Associated with colourless parenchyma cells together with which it forms a zone of colourless cells above or below the chlorenchyma; may be disrupted over the first order bundles:	
Colourless zone comprises more than $\frac{1}{2}$ of the leaf thickness	913*
Colourless zone comprises less than $\frac{1}{2}$ of the leaf thickness	914*
Restricted groups of large, inflated bulliform cells; <i>Zea</i> type; parallel-sided cells i.e. inner tangential wall same length or only slightly shorter than the outer tangential wall:	
Projecting above the level of the epidermis; cf. cushion cells of macro hairs	915*
Level with the general epidermal surface	916*
Restricted groups of tall and narrow bulliform cells; not inflated; parallel-sided cells or with outer tangential wall slightly shorter than the inner wall; lateral walls long and straight	917*
Fan-shaped groups; each cell of group with outer tangential wall shorter than inner tangential wall; median cell of group appreciably larger than the remainder:	
Situated at bases of furrows	918*
Not found at bases of furrows or present in leaves without furrows; some cells parallel-sided	919*
Resemble fan-shaped groups due to central cell of group being larger and of a different shape than the rest which may be small by comparison; not necessarily with shorter inner tangential walls; <i>Sporobolus</i> type:	
Shape of central cell of group which is always narrow at epidermis and often recurved or straight at area of contact with lateral bulliform cells of group:	
Inflated, rounded; short area of contact with lateral cells	920*
Inflated, fan-shaped	921*
Narrower than deep; shield-shaped	922*
Elongated with parallel sides	923*
Elongated rather pointed base; diamond-shaped	924*
Size of central cell:	
Relatively small; not much larger than bundle sheath parenchyma	925*
Occupy $\frac{1}{4}$ - $\frac{1}{2}$ of the leaf thickness	926*
Occupy more than $\frac{1}{2}$ of the leaf thickness	927*
Narrow groups of bulliform cells and intimately associated large colourless parenchyma cells penetrating deep into the mesophyll; the contact with the adaxial epidermis made by the bulliform cell unclear; <i>Arundo</i> type	928*
Bulliform cells and closely associated colourless cells forming an extensive column or girder extending from the base of an adaxial furrow deep into leaf:	
Nature of column or girder:	
Column uni-seriate	929*
Column bi-seriate	930*
Column multi-seriate	931*
Position of column:	
Extends from base of furrow to abaxial epidermis	932*
Extends from base of furrow towards a vascular bundle	933*

CONTINUE DESCRIPTION OF ADAXIAL EPIDERMIS OVERLEAF AND THEN RETURN AND DESCRIBE THE ABAXIAL EPIDERMIS.

BULLIFORM CELLS



9. EPIDERMAL CELLS IN TRANSVERSE SECTION CONTINUED

TYPICAL EPIDERMAL CELLS AND APPENDAGES

Includes all epidermal structures as seen in transverse section excluding the bulliform cells. Incorporates the cuticle, macro-hairs, prickles and papillae as seen in transverse section.

DESCRIPTION:

Cuticle and Thickening of epidermal cell walls:

Outer tangential wall:

Outer walls not thickened and with a very thin cuticle 934*

Outer walls slightly thickened or with a thin cuticle 935*

Outer walls thickened and covered by a distinct, thick cuticle continuous over the epidermal cells:

Cuticle and cell wall equal to or greater than the depth of the average epidermal cells 936*

Cuticle and cell wall less than the depth of the average epidermal cells 937*

Outer tangential wall of each epidermal cell thickened individually:

Outer wall occupies half or more of the depth of the cells 938*

Outer wall occupies less than half of the depth of the cells 939*

Irregular; cells of different sizes comprise the epidermis 940*

Radial and *Inner* tangential walls of epidermal cells:

All walls of epidermal cells thickened 941

Only outer wall thickened 942

No walls thickened 943

Macro-hairs as seen in transverse section; nature of hair bases described:

No macro-hairs visible 944

Superficial bases; sunken into the leaf or embedded between inflated bulliform cells:

Epidermal cells not modified to form cushion cells associated with the base of the hair:

Swollen base not much larger than and situated between normal epidermal cells; included here are probably some elongated prickles:

Hair short and thick 945*

Hair slender and elongated 946*

Constriction above bulbous base embedded in normal epidermal cells:

Hair very thin and slender 947*

Hair thickened and stiff 948*

Epidermal and other cells modified to form a cushion base:

Base surrounded by small thickened cells which may extend a short way up the hair:

Hair with enormous swollen base 949*

Base of hair not exceptionally large and swollen 950*

Sunken bases; sunken in leaf or embedded between large, inflated epidermal cells or bulliform cells:

Constriction above bulbous base embedded between large epidermal cells:

Size and shape of hairs:

Hairs slender but relatively short 951*

Hairs thick and short 952*

Hairs long and very slender 953*

Hairs long and relatively thick 954*

Nature of bulliform or epidermal cells surrounding base:

Raised around hair forming a definite cushion 955*

Unraised bulliform cell groups 956*

Base bulbous and not constricted; embedded between bulliform cells 957*

Prickles and Hooks as seen in transverse section:

No hooks or prickles visible 958

Small hooks present in the epidermis; usually located in intercostal areas:

With short, curved barbs 959*

With straight, pointed and slender barbs:

Present between typical epidermal cells 960*

Present between inflated bulliform-like cells 961*

Thickened prickles present in epidermis; usually located opposite the vascular bundles:

Pointed broad prickle; base not bulbous 962*

Small with short barb 963*

Bulbous base; barbed 964*

Bulbous base; no barb; asperite 965*

Papillae on the epidermal cells as seen in transverse section:

Shape of outer tangential wall of the epidermal cells:

Outer walls of epidermal cells not arched to any marked degree; flattened 966*

Outer walls of epidermal cells arched but not papillose 967*

Arching of outer wall exaggerated; papillose; inflated papillae as wide as epidermal cells:

Thin-walled wide papillae scattered throughout the epidermis 968*

Entire or major part of epidermis composed of thin-walled wide papillae 969*

Papillae wide; as wide as or slightly narrower than epidermal cells; not inflated and thin-walled:

Distal, outer wall markedly thickened 970*

Papillus thickened and cuticular 971*

Papillae narrower than the epidermal cells:

Size of papillae:

Relatively broad but not much more than half the width of the epidermal cells 972*

Much less than half the width of the cell 973*

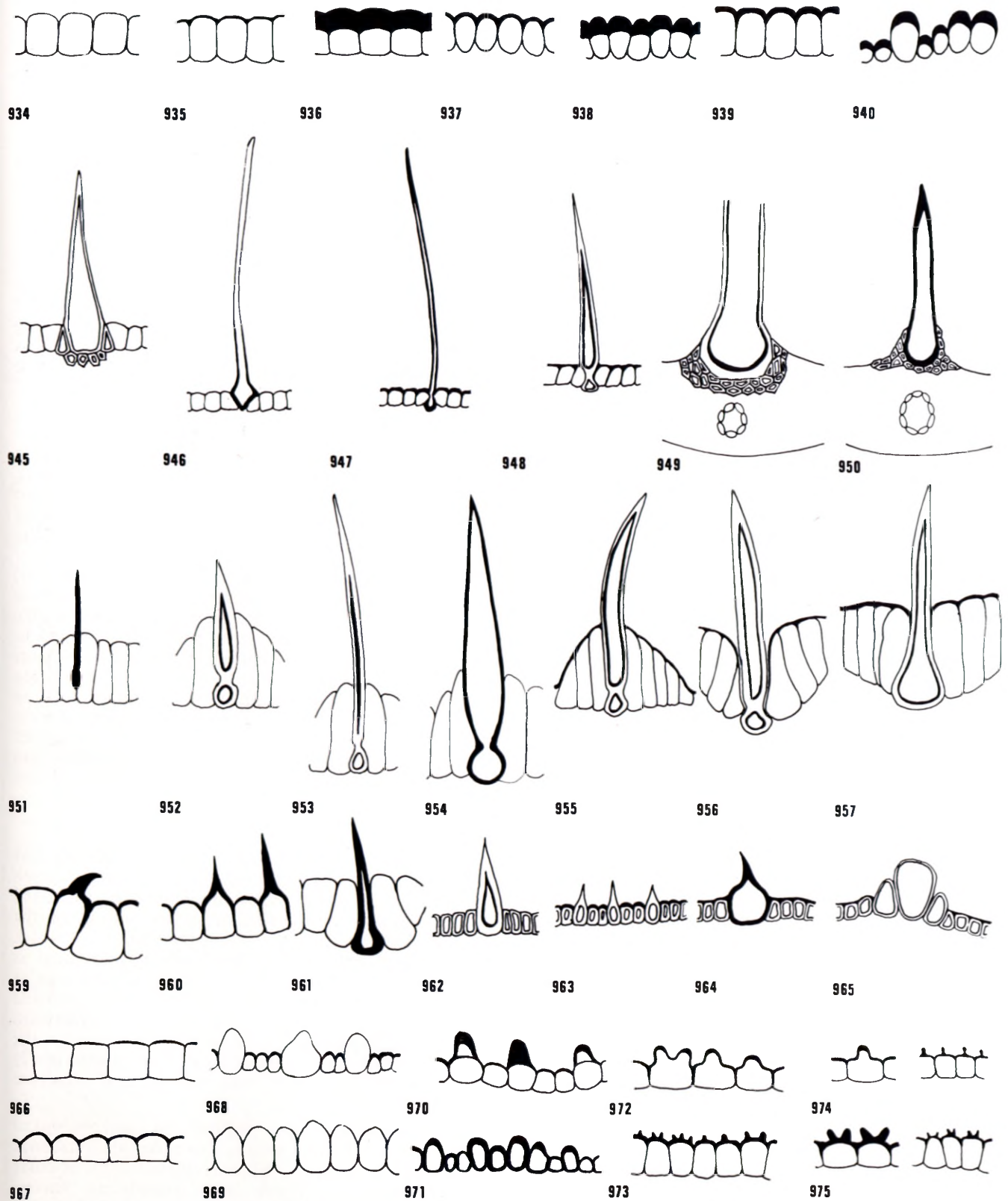
Number per cell as seen in transverse section:

One per cell 974*

Bifurcate; two per cell 975*

N.B. ADAXIAL AND ABAXIAL EPIDERMIDES DESCRIBED SEPARATELY.

EPIDERMAL CELLS



ANATOMICAL CHARACTERS

The following presents a brief conspectus of the nature of the various structures used in describing grass leaf anatomy, as well as their range of structural variation. Where relevant, an account is given of the function or development of these structures and the factors which contribute to their variability. Their importance as either taxonomic or diagnostic characters is stressed.

Outline of the lamina in transverse section

The foliage of the grass plant is comprised of the sheath, the ligule and the leaf-blade. In the following account, the leaf-blade, the expanded and free portion of the leaf as distinct from the sheath, is termed the lamina or the blade.

Outline of the lamina of open leaves

In many grasses with open leaf-blades the two halves of the lamina on either side of the median vascular bundle, midrib or keel, are relatively thin and wide, of equal width and usually symmetrically arranged about the median region. Infrequently, however, in certain species the portion of the lamina to one side of the midrib may not be symmetrical with that on the other half or perhaps the leaves may be relatively narrow and rather thick. These are exceptions to the general rule but are nevertheless useful for identification purposes.

The lamina of most grass leaves in transverse section appears flattened and expanded and a straight line connects both margins and the median bundle or keel. However, expanded laminae may become variously altered by involution usually by becoming inrolled or infolded. Infolding results in the appearance becoming V- or U-shaped because the two lateral halves of the lamina tend to become folded adaxially towards each other on either side of the median vascular bundle which may, or may not, be adaxially grooved or incorporated into a keel. Grooves or keels serve to accentuate the V- or U-shape which characterizes many species. Inrolled leaves may appear variously U-shaped or may show either convolute inrolling from one margin only, or involute inrolling where the leaf-blade rolls inwards from both margins.

The degree of infolding or inrolling varies with the environmental conditions and thus is not of much value diagnostically (Metcalfe, 1960). However, leaves of grasses that are fully expanded under optimum conditions invariably exhibit a characteristic type of involution in response to environmental stress. Therefore, it is the nature, and not the degree, of the infolding or inrolling that is of importance taxonomically and diagnostically. Furthermore, it must be noted that some leaves remain flat on drying and in others the leaf folds inwards a little near the keel but the margins remain reflexed. Other responses are shown by the corrugations becoming more pronounced in species with plicate blades, and the production of filiform leaves in species which normally possess expanded blades. Further details of the involution process are included under bulliform and colourless cell function.

Expanded leaves of different species thus exhibit various, but constant, movements in response to adverse environmental change, the resultant shape being of specific or generic diagnostic value. Various members of the Andropogoneae (*Hyparrhenia*, *Cymbopogon* and *Dichanthium*) often show limited infolding near the keel with the margins remaining reflexed and several species of *Cymbopogon* produce both expanded and filiform leaves (Vickery, 1935).

Some *Brachiaria* and *Oplismenus* species do not alter shape at all under conditions of water stress and remain flat and expanded. Certain grasses even show nyctinastic movements as described for *Leersia hexandra* by Goebel (Bor, 1960) in which the leaves are folded in dull weather but flatten in bright sunlight.

The variability exhibited by the leaf outline demonstrates the necessity for a sampling method which will adequately reveal the extremes of this variation. This should be achieved if adequate numbers of specimens of each species are collected, and fixed immediately, from different areas, over an extended period of time and from as many habitats as possible.

It appears reasonable to postulate that the type of folding adopted by the mature leaf in response to environmental conditions, as well as the symmetry of the leaf, may be correlated to the way in which the leaf is folded in the bud. Thus, Amedei (1932) has shown that *Chaetochloa palmifolia* (= *Panicum palmifolium*) is folded conduplicately in the bud and the mature leaf is more or less pleated depending on circumstances. The blades of grass leaves may be either folded, convolute or pleated in the bud (Bor, 1960). Therefore, the bud arrangements may simply be linked to the major types of involution exhibited in mature leaves except, perhaps, involute inrolling which is difficult to explain. Does it happen then, that a leaf which is convolute in the bud will exhibit definite V-shaped movements as a mature leaf instead of the expected convoluted inrolling? This possibility merits further investigation.

Apart from the characters of the shape of the outline, under optimum and adverse conditions, quantitative data of leaf width and thickness are also of value in describing and identifying leaves of different grasses. De Winter (1951) mentions the extreme thinness (about 0.05 mm) of the leaf of *Prosphytochloa prehensilis* (= *Potamophila prehensilis*) where the leaf is only about four cell layers thick. These quantitative characters are subject to considerable variation, and categories must be broadly defined to accommodate this variability. Detailed measurements have not been included in the keys and measuring is done by estimation of the leaf dimensions in relation to the known diameter of the field of view of the microscope used in the study. These broad categories are, nevertheless, useful for comparison with measurements given by other authors and can be easily modified as required.

Outline of permanently infolded leaves

In many grasses the lamina is very strongly and often permanently infolded or involuted. In such leaves the stomatal bands are invariably restricted to certain areas of the adaxial surface, usually on the sides of adaxial furrows within the channel. A thick cuticle and extensive sclerenchyma development is characteristic although this is not universally true as shown by Lewton-Brain (1904) for *Mibora minima* (= *Mibora verna*) which is entirely parenchymatous. However, these leaves can be distinguished by the fact that they are extremely narrow and permanently infolded to the extent that the internal structure is altered.

Numerous leaf-types defined on morphological criteria fall into this group. Examples are setaceous (wiry and bristle-like), filiform (thread-like), acicular (needle-like), junciform and cylindrical leaves. Anatomically these different types cannot be separated with any degree of certainty and a term inclusive of all permanently infolded leaves is required.

Acicular leaves invariably fulfill the requirements of the definition of being permanently infolded to the extent that the internal structure is altered. In filiform leaves, often produced under adverse conditions by species which normally have open, expanded leaves, however, the blade is reduced to an enlarged midrib structure, with a mass of colourless parenchyma above the bundles and the lateral laminae being reduced or absent. Some species of *Cymbopogon* exhibit this reduction regularly. The extreme case of this specialization is seen in *Miscanthidium teretifolium* which is cylindrical in transverse section and the adaxial surface can be recognised only by the presence of a minute groove on the adaxial side of the cylinder (Metcalf, 1960). In these cases, it must be stressed, the internal structural alteration of the leaf does not occur as a result of the leaf being permanently folded but rather as a result of loss of the lateral portions of the blade and a corresponding development of the midrib.

The cylindrical condition can probably arise by one of two different processes: the loss of the lateral regions and a concurrent development of the keel or by parenchyma development in a permanently infolded leaf excluding the adaxial channel. For convenience these two types of narrow leaf are included together under permanently infolded leaves. This further illustrates the need for a single term describing all reduced, permanently infolded and/or structurally altered grass leaves.

Transverse sections of these leaves often exhibit characters that are of taxonomic value because in assuming the permanently infolded, acicular form, the leaves of grasses of different affinities have not acquired precisely the same arrangement of vascular and mechanical tissue (Metcalf, 1960). The outline and the shape of the adaxial channel and ribs and furrows may be distinctive as well. Burbidge (1946a) was able to separate ten species of *Triodia*, all possessing acicular leaves, using characters of the outline, the adaxial channel and adaxial and abaxial furrows. For descriptive purposes it is, therefore, necessary to describe the shape of the blade as outlined by the abaxial surface, to count the number of vascular bundles present in the section, to describe the adaxial channel and to include leaf width and thickness dimensions. Ribs and furrows and sclerenchyma are described in later "keys". With these descriptive criteria the differences present in permanently infolded leaves are of significant specific diagnostic value.

It is absolutely essential to standardize on the portion of the blade to be sectioned in permanently infolded leaves as the shape can vary from the base to higher up the leaf. Arber (1923, 1934) noticed that folded leaves may approach radial symmetry and become cylindrical in the apical regions. In *Triodia* (Burbidge, 1946a), towards the apex of the lamina the lateral veins disappear one by one, commencing with the marginal pair. There is a corresponding progressive reduction in the amount of mesophyll so that finally the pungent point is formed of the median vascular bundle surrounded by sclerenchyma. This progressive change, of many characters, along a single blade is equally important in open, tapering leaves and is the prime reason for standardizing on material from the central region of the lamina from ligule to tip.

In the cases of some narrow, folded leaves it is often debatable whether they in fact should be described under open or under permanently infolded leaves. In such cases it is perhaps safest to describe them under both of these arbitrary categories.

Longitudinal ribs and furrows

The adaxial and abaxial surfaces of the grass leaf blade may be either flat or longitudinally ribbed. The ribs are usually developed in association with and adjacent to the larger vascular bundles. They are generally characteristic of and more fully developed on the adaxial than the abaxial surface (Metcalf, 1960). Ribs have also been termed ridges (Arber, 1934; Wilson, 1971) and if ribs are present there must be corresponding furrows between adjacent ribs. Furrow, in the context used here, is a term reserved exclusively for the depressed intercostal zone between adjacent ribs and which, with few exceptions, occur between the vascular bundles. If furrows are present in any other situation they are termed grooves to avoid possible confusion. Examples are the grooves present at the apex of the well-developed ribs of *Sporobolus artus* (Goossens, 1938) or those described immediately abaxial to the larger veins described by Sabnis (1921). In permanently infolded leaves the area bounded by the infolded lamina is characterized by being referred to as a channel.

In different species ribs and furrows may vary in height or depth, transverse shape, spacing and location. The ribs of a single grass blade can be of one or more distinct sizes or shapes, the ribs of each type being consistently associated with a specific order of vascular bundle. It is possible to measure the inclination of the ribs by measuring the angle formed by the sides of the two adjacent ribs at their base in the furrow (Wilson, 1971). Consequently, leaves with obtuse angles would have flatter surfaces than those with acute angles. The abaxial ribs may sometimes be taller than those of the adaxial surface. Where well-developed abaxial and adaxial ribs and furrows are similar and occur opposite each other the leaf section resembles a string of beads and is termed moniliform.

There are numerous examples of species of grasses possessing ribs or furrows that do not conform to the generalized definition outlined above. Lewton-Brain (1904) illustrates the enormous triangular ribs of *Aira caespitosa* where three vascular bundles are included in a single rib. *Glyceria fluitans*, another example illustrated by Lewton-Brain (1904), possesses low adaxial ribs situated between the vascular bundles which are situated beneath the 'furrows'. The ribs are occupied by copious air spaces. The flat-topped abaxial ribs of *Digitaria macroglossa* are exceptional because the ribs are in fact sclerenchyma caps and are not linked to the vascular bundles in any way.

Metcalf (1960) comments on the correlation between prominent ribs and a marked capacity for involution, and states that this ability has been evolved in response to ecological conditions. Furthermore, this has occurred independently in various grasses and consequently leaves of this type are found in grasses between which there are no close taxonomic affinities. Rib development is nevertheless, often useful for specific diagnostic purposes and Gordon-Gray and Ward (1970) have contrasted the consistent flatness of the leaf surfaces of *Phragmites mauritianus* with the slight ribs developed on comparable surfaces of leaves of the closely related *P. australis*.

A paper by Wilson (1971) shows that the adaxial ribs of *Lolium perenne* appear to be under the control of a few genes as indicated by the rapid and immediate response to selection achieved in this study. Thus, in a species which appears to exhibit a wide range of phenotypic variation with respect to rib development, this variation may in actual fact be the direct manifestation of the genotype. This fact should be borne in mind when variation patterns of adaxial ribs are studied.

Median vascular bundles, midribs and keels

In order to eliminate any possibility of ambiguity arising from the use of these terms it is necessary to define them in the context that they are to be used here. The median vascular bundle is so termed only if the centrally situated vascular bundle of the leaf is structurally indistinguishable from the other basic or first order vascular bundles of the leaf, and if this median bundle is not associated with any parenchyma development or thickening of the leaf. On the other hand, a midrib is centrally positioned but structurally distinct from the other first order bundles with respect to size, bundle sheath, sclerenchyma girder or vascular structure and lacks associated parenchyma. When parenchyma or bulliform cells are developed in association with the median bundle or bundles, the whole structure, often incorporating many bundles, is termed a keel. The median bundle of a keeled leaf may be a midrib, and thus distinguishable from first order bundles, or a median vascular bundle, being indistinguishable from the other first order vascular bundles. A keel usually projects either abaxially, adaxially or both, but this is not necessarily so and in V-shaped leaves, unless there is a marked thickening in relation to the rest of the lamina, the median bundle alone is considered to comprise the keel, if it is accompanied by parenchyma.

This distinction of the three distinct types is considered necessary because in the past these terms have been interchanged by various workers. Metcalfe (1960) considers a projecting midrib, whether associated with parenchyma or not, to constitute a keel. Thus, no distinction is made between leaves with or without parenchyma in association with the median vascular bundle.

The size of the keel in an individual leaf varies, usually becoming progressively larger towards the base of the blade so that, in transverse section, the extent to which a keel is prominent depends on the level at which the section is taken. Therefore, the central part of the leaf must be used for comparative purposes.

Vickery (1935) states that, in addition to variation at different points along a single leaf, the size and degree of development of the keel varies tremendously in different leaves of the same species, so that the appearance in transverse section can only be of importance when taken in conjunction with the macroscopic appearance of the keels of a number of leaves. This is definitely not necessary in some groups and Fisher (1939) found the keel-size, amount of parenchyma and sclerenchyma and the bulliform cells—to be a valuable diagnostic character in separating four species of *Chloris*.

Keel structure, although subject to variation, is important diagnostically because the various types of keel that are recognised are easily distinguished except that the inconspicuous keel with one vascular bundle merges into that with three bundles. Thus, size may vary but shapes are constant and distinguishable. In addition there are numerous associated characters such as sclerenchyma, parenchyma and bulliform cells of the keel, air spaces and the nature of the adaxial side of the keel.

Bambusoid and oryzoid grasses display distinct keel structure with prominent lacunae, with or without diaphragms (Holm, 1896), and with a complex system of bundle arrangement. The keel is comprised of two or more vascular bundles distributed near both the abaxial and adaxial surfaces and sometimes in the interior as well. This characteristic structure has been discussed by various authors such as Holm (1892,

1895, 1896), Arber (1934), Jacques-Felix (1955), Schweickerdt & Marais (1956), Tateoka (1965) and Launert (1965). In addition to the Bambusae & Oryzeae, the Streptochaetae and *Prosphytochloa prehensilis* (= *Potamophila prehensilis*) in South Africa (de Winter, 1951) possess superposed adaxial bundles in the keel. Air spaces developing in the keels of older leaves are common in many other genera. *Sporobolus artus* var. *lysigenatus* and *S. pyramidalis* (Goossens, 1938) are examples.

Vascular bundle arrangement

The vascular bundles, or parallel longitudinal veins, are usually arranged in a single row embedded at various positions in the mesophyll as seen in section. Certain grasses, such as *Oryza coarctata* (Tateoka, 1963) and *Porteresia* (Tateoka, 1965a) have a normal abaxial vascular bundle in each rib, but in addition, a superposed, adaxial, amphivasal bundle is situated immediately anterior to each of the abaxial bundles. This type of arrangement is also found in the keels of some bambusoid and oryzoid grasses.

The arrangement most commonly found in grasses is for all the bundles to be centrally positioned in the vertical plane of the blade. However, all bundles may be located closer to the abaxial or to the adaxial surface. Irregular arrangements, with bundles of different sizes or orders situated at different levels within the mesophyll are also found. In these instances, a regular pattern of arrangement of the different orders of bundles in the mesophyll is usually discernible. Cases where the bundles are located at irregular and inconsistent levels are rare, such as *Stipa tenacissima* (Metcalfe, 1960). This positioning of the vascular bundles in the blade appears to be a useful diagnostic character above the genus level that has been largely overlooked in the past.

The total number of vascular bundles in a section through a leaf blade taken halfway between the sheath and apex is inconsistent, varying with the width of the leaves. Little reliance can be placed on the total number of vascular bundles in a leaf as Vickery (1935) correctly stresses. She found that *Themeda avinacea* and *Cymbopogon exaltatus* may have flat expanded leaves 5–7 mm wide but under certain conditions filiform leaves consisting essentially of an enlarged keel are formed. This reduction in the width of the blade involves a reduction in the number of vascular bundles. Wider leaves do not necessarily have more vascular bundles than narrower leaves, however, as the bundles may be more or less crowded in different specimens of the same species. The total number of vascular bundles in a section is only considered to be useful in permanently infolded leaves, where there has been a reduction in the total number of vascular bundles.

The relative proportion and the alternation of the various sizes of vascular bundle along leaves of any one species of grass is remarkably constant. Slight variations in this respect may be found in leaves taken from different levels on a single plant, the variation being linked with differences in the width of the blades. For this reason basal leaves have been standardized upon for comparison. However, the proportion of first order bundles and smaller bundles in grasses belonging to different genera and species show marked differences.

The distribution patterns of bundles of different orders, and their variation, are important diagnostic characters. Thus, Holm (1891a) found a pattern of 1 first order bundle, 1 third order bundle, 1 second order bundle, 1 third order bundle, 1 first order

bundle, etc., in *Uniola latifolia*. He notes that this formula is not strictly constant but that it gives the general features concerning the relative number and arrangement of bundles. For other species of *Uniola* he was unable to give a formula because of excessive variation, especially in leaves from different localities. Leigh (1960, 1961), in a study of a few *Eragrostis curvula* strains distinguished two groups both with a basic pattern of 3 or 4 second order bundles between successive first order bundles repeating itself either four or eight times along the width of the leaf.

The use of a formula to describe the pattern of arrangement of the various orders of vascular bundle is only suitable when a regular pattern from median bundle to margin is present. Often no pattern is obvious with progressively fewer first order vascular bundles and more third order vascular bundles nearer the margin or more first order vascular bundles and fewer second and third order vascular bundles laterally situated. For this reason the use of formulae to describe these patterns is not suitable. On the other hand, if a constant part of the blade is described, e.g. the central part of the lamina between the median bundle and the margin, these observations on bundle alternation will be comparable, even for leaves where the pattern changes from median bundle to margin.

Vascular bundle structure

Metcalf (1960) visualizes the vascular bundle as consisting solely of the xylem and phloem elements, the bundle sheaths not being treated, for descriptive purposes, as if they constitute part of the bundle. For consistency and convenience this principle has been adopted in the present work and thus, when a bundle is described as being circular or angular in outline, these terms refer only to the outline of the xylem and phloem tissue.

The vascular bundles of the Poaceae are divided into three orders or ranks for descriptive purposes. These orders are not necessarily distinct but may intergrade and intermediate types are sometimes present. However, within a given leaf, three, and sometimes even four, classes of bundle are usually evident even although their structure may not conform exactly with that of the generalized definitions given below.

The three different orders in a single leaf are usually immediately evident because they differ in size. However, the relative diameters of the various orders cannot be used satisfactorily for the grass family as a whole, because equivalent orders of bundle differ in size and structure from one species to the next. Bowden (1964) classified bundles using the diameter as a criterion, but in species where all bundles are approximately the same size, but structurally different, this system is inadequate.

The first order bundles, or basic type (Metcalf, 1960) are characterized by having a metaxylem vessel on either side of the protoxylem which may be non-functioning, and replaced by a lysigenous cavity or lacuna. A few well-developed protoxylem vessels may be present in addition to the lysigenous cavity, or both cavity and vessels may be absent. In the second order bundles the xylem and phloem are easily distinguishable, but the large metaxylem vessels are lacking as is the lysigenous cavity. The third order vascular bundles are often small bundles in which the xylem and phloem is reduced to a few elements or may even be indistinguishable using light microscopy.

In first order bundles the metaxylem vessels may appear to be paired, but these are merely the oblique overlapping ends of otherwise solitary vessel elements. Thus the number of metaxylem vessels in an

individual bundle is unreliable as a diagnostic character because it varies with the level at which the sections are taken (Metcalf, 1960). For this reason, only the size and shape of these vessels are mentioned in the descriptive keys.

The phloem of the first order vascular bundles may exhibit varying degrees of sclerosis. This is especially common in grasses from dry localities (Metcalf, 1960). In *Triodia* (Burbidge, 1946a) and *Merxmuellera* (= *Danthonia*) (de Wet, 1960) the phloem may be divided into two or three groups by intrusion of small fibres. The phloem may adjoin the inner or parenchyma, bundle sheath or it may be completely surrounded by thick-walled fibres which isolate it from the xylem and the bundle sheath (Jefferies, 1916).

Bundles of the second order commonly resemble the first order bundles in shape and size and are only recognisable by the absence of the metaxylem vessels. Some authors have pointed out that second order bundles may show a tendency to develop into first order bundles (Vickery, 1935; Goossens, 1938).

In third order vascular bundles the metaxylem vessels are always lacking and in the smallest bundles of this order the xylem and phloem elements may be indistinguishable. These bundles, in fact, form a reduced conducting system and sometimes only a few lignified cells are present together with the phloem elements. Fisher (1939) notes that in these instances neither the protoxylem nor the metaxylem is present. In some genera the third order bundles are not conspicuously smaller than the basic type bundles, but are then distinguishable by the absence of sclerenchyma girders, or even strands, and/or the presence of bulliform cells adaxially.

The shape of the third order bundles may be classified as angular, inconspicuously angular, circular or elliptical in outline. They exhibit these shapes more clearly than do the larger bundles. This shape is taxonomically an important character, linked with the size and number of bundle sheath cells. Thus, small, angular bundles are, on the whole, characteristic of the panicoid, and non-angular bundles of the festucoid grasses.

Various classifications of the orders of vascular bundles employ the extent of development of the sclerenchyma strands and girders. Thus, Breakwell (1914, 1915) distinguishes primary bundles, in which the sclerenchyma is in direct contact with the bundle, and secondary bundles which possess an entire bundle sheath. Bowden (1964) also groups first and second order bundles as those possessing sclerenchyma girders and third and fourth order bundles as those with strands. As was pointed out above, the nature of the sclerenchyma developed in association with the various orders of vascular bundle is especially important in recognizing the larger types of third order bundle, but as bundles are defined here, bundle sheaths and sclerome are not included in vascular bundle descriptions.

Surprisingly few detailed studies have been conducted on the phloem of the Poaceae and these only recently. Thus, the mature phloem of *Avena* was studied by O'Brien & Thinman (1967), Buvat (1968) studied the sieve elements of *Hordeum* and Singh & Srivastava (1971) the phloem of *Zea*.

These studies have shown that the first-formed protophloem elements are located abaxially within a bundle and subsequent sieve elements differentiate successively in an adaxial direction to within a couple of cells of the differentiating xylem elements. Thus, in a mature bundle, all the major features of

differentiation of the sieve elements and companion cells are visible. The sieve elements of the protophloem often lack companion cells and may differentiate without lateral expansion. The later-formed sieve elements, as a rule, are accompanied by one or more companion cells and extend laterally as a first step in differentiation. Furthermore, the protophloem is usually thin-walled and may become crushed in mature bundles. The metaphloem has thicker walls and may even be thick-walled as in *Distichlis stricta* (Roy, 1969).

The xylem consists of primary xylem only. In first order bundles it can be divided into the protoxylem, which is that area in which the tracheary elements have either annular or helical thickenings or both, and metaxylem, which is the remaining primary xylem. This metaxylem can be sub-divided into the early and the late metaxylem; the late metaxylem being the two large vessels so characteristic of these bundles.

The structure of these late metaxylem vessels is an indication of their specialization. Cheadle (1955, 1960) has shown that specialization in the Angiosperms proceeds from long vessel members, that are angular as seen in transverse section, and with long scalariform plates with many bars and perforations on oblique end walls, to short vessel members that are circular in section, with simple plates, with single perforations on transverse end walls. In the Poaceae no great variation occurs in the late metaxylem which is very specialized. Thus, simple plates are common and scalariform plates rare. A character that is variable, and revealed in section, is whether or not the vessel members are circular or angular.

Vascular bundle sheaths

In the Poaceae every vascular bundle is surrounded, either completely or partially, by one or two single layered bundle sheaths. These bundle sheaths are taxonomically very important, and as a general rule single sheaths are characteristic of the panicoid and double sheaths of the festucoid grasses. Structural variations of the bundle sheath are also important for diagnostic purposes, as well as constituting evidence for important physiological differences between the various groups of tribes of the Poaceae. As would be anticipated in a tissue undergoing extensive changes, even within genera, to almost every generalization there are exceptions. Notwithstanding, the bundle sheath is undoubtedly of the utmost importance from a comparative anatomical viewpoint and fortunately numerous ultrastructural, physiological and taxonomic studies of the structure and functions of the sheath have been undertaken.

Recent terminology, to avoid reference to physiological activities and anatomical structure, as well as to eliminate the concept of specific functions for the two layers (Lommasson, 1957), has been restricted to the terms double (comprised of an inner and outer) and single sheaths. Various other terms have been used in the past, but none of them are applicable to the grass family as a whole.

The single sheath, and usually the outer sheath, when there are two, commonly consists of a single layer of large, thin-walled or slightly thickened cells. In section they are generally inflated and conspicuous, being larger than the adjoining mesophyll cells. Panicoid grasses, in general, have more inflated single or outer sheath cells than those of the outer sheath of the festucoid species (Metcalf, 1960). This sheath has often been referred to as the parenchyma bundle sheath but other terms include starch sheath, border

parenchyma as well as mestome sheath. None of these terms are generally applicable, as for example *Aristida* species have an inner bundle sheath consisting of larger cells than the outer sheath, which may be composed of thickened cells.

The typical single or outer sheath cells are either translucent, without chloroplasts, or they may contain green pigment in plastids similar to, or differing from, the chloroplasts of the chlorenchyma of the mesophyll. Many early workers ignored the contents of these sheath cells and represented them as being empty (Lewton-Brain, 1904; Lohaus, 1905; Prat, 1936; Vickery, 1936). However, the nature of these contents, whether typical chloroplasts or specialized plastids is undoubtedly a character of systematic importance as Brown (1958) correctly stresses. In this connection, it is important that in older leaves of wheat few or no plastids are present in the bundle sheaths (Percival, 1929). Thus, age of leaf sampled must be standardized for this character to be reliable. The detailed structure and function of these bundle sheath cells will be elaborated on later.

The arrangement of the chloroplasts in the bundle sheath cells has been used for diagnostic purposes—whether they display a horse-shoe shaped arrangement against the inner or outer tangential wall, or an even distribution in the cells. However, Roth (1968), has shown in xerophytic species such as *Sporobolus virginicus* that the horse-shoe shaped arrangements occur under low-intensity illumination, such as in the mornings, afternoons, at night and in rolled up leaves, while on sunny days the chloroplasts are evenly arranged all over the walls. This is probably an adaptation to efficient light utilization, but cannot carry much weight as a diagnostic character.

The single or outer sheath may possess girder-like extensions which are of diagnostic importance. The extensions either consist of colourless cells resembling those of the bundle sheath or there may be a gradual transition to the sclerenchyma fibres of the adjacent strand. The extensions may be uni-seriate to very wide, and sometimes flank narrow sclerenchyma strands or girders as in the first order bundles of *Garnotia scoparia* (Tateoka, 1958). Burbidge (1946) and Decker (1964) discuss the unique outer bundle sheath of *Triodia* leaves where the sheath of one bundle is continuous with those of adjacent bundles.

The adjacent sclerenchyma girders may interrupt the single, or outer, sheath to a greater or lesser extent, either adaxially, abaxially or both. Thus, the outer or single bundle sheath may be entire, horse-shoe shaped or even reduced to two lateral arcs. In *Arundinella leptochloa* (Tateoka, 1958) this sheath is only developed adaxially adjacent to the xylem, not being replaced by a sclerenchyma girder abutting on the lateral parts of the phloem.

Schweickerdt (1941) has used the number of cells comprising this sheath to distinguish the closely related genera of *Monelytrum* and *Tragus*. This cell number appears to exhibit excessive variability in most instances, but it is relatively constant for the third order bundles of groups such as the Paniceae.

Various degrees of possible reduction of the vascular bundles, as perhaps evidenced by relic bundle sheath cells have been observed in *Arundinella* (Vickery, 1935) and in *Garnotia* (Tateoka, 1958). In these grasses the first order vascular bundles have well developed outer sheaths, *Garnotia* even having abaxial extensions. In smaller bundles these extensions are reduced, and only a few cells, resembling those of the bundle

sheath, are irregularly present on the sclerenchyma girders or strands. The extreme situation is found between successive vascular bundles of some *Arundinella* and *Garnotia* species, where solitary, or groups of two to six cells, similar to those of the bundle sheaths are present, scattered in the mesophyll. Tateoka (1958) calls these groups of sheath-like cells, lacking associated vascular tissue, distinctive cells. Carolin, Jacobs & Vesk (1973) have shown that these distinctive cells are in fact isolated files of parenchyma sheath cells connecting with the bundle sheath proper. They consider this to be a specialization to the wide expanses of mesophyll between adjacent vascular bundles found in these species. These distinctive cells ensure that all mesophyll cells are ultimately in contact with the bundle sheath parenchyma cells.

The inner sheath normally consists of living, thick-walled, chloroplast-free cells which are elongated parallel to the vascular bundles. In section the cells of this sheath are nearly always smaller in diameter than the outer sheath and possess thickened walls, the thickening often being more conspicuous on the radial and inner tangential walls. Exceptions to these generalizations do occur as in *Aristida*, *Elytrophorus* (Schweickerdt, 1942) which has a thin-walled inner bundle sheath, and the Meliceae (Decker, 1964) where the inner sheath cells are uniformly thickened.

This sheath often resembles an endodermis and it has been shown to have similar physiological activity (van Fleet, 1942). It has been variously called the endodermis or mestome sheath in the past. Unfortunately, the term mestome sheath, has been used for parenchymatous sheaths as well and modern authors (Metcalf, 1960; Barnard, 1964), therefore, refer to it merely as the inner sheath.

The inner sheath is sometimes difficult to recognise, especially in cases where the cells are not distinct from those of the fibres of the vascular bundle ground tissue. However, cells of this layer are always in contact with the metaxylem vessels (Vickery, 1935). Thus, if large inflated cells are immediately adjacent to the metaxylem vessels it can be assumed that no inner sheath is present.

In addition to sometimes not being sharply differentiated from the bundle ground tissue, in some smaller bundles the inner bundle sheath is sometimes only developed adjacent to the phloem (Fahn, 1967). For this reason Metcalfe (1960) proposed the introduction of a third bundle sheath category—intermediate—in addition to single and double sheaths.

The inner bundle sheath cells of *Aristida* are anomalous in two ways: the diameter of the inner bundle sheath cells is greater than those of the outer sheath, and they contain numerous chloroplasts. These larger inner sheath cells may also have uniformly thick walls. Holm (1901) was the first to record this double bundle sheath, Vickery (1935) interpreted this condition correctly and Lommasson (1957) considered it to be of taxonomic importance, so much so as to warrant the placing of *Aristida* in a separate tribe. Theron (1936) attached little taxonomic importance to the sheaths of *Aristida*, probably having misinterpreted the specialized bundle sheath.

Lommasson (1957) was further able to show that, in the leaf sheath, where both bundle sheaths lack chlorophyll, on the adaxial side against the culm, the cells of the outer bundle sheath were larger than those of the inner bundle sheath. The transition to the opposite condition, as in the leaf blade, occurs where chlorophyll is formed in the bundle sheath cells and mesophyll. He advanced the explanation

that this anomalous condition represents the result of special activities occurring at the chlorophyll—vascular interface.

The structure and function of the parenchyma cells of the outer, or single sheath, and their dimorphic chloroplasts have been extensively studied. Zirkle (1929) recognised that the cells of the bundle sheath in *Zea* contained specialized chloroplasts concerned with starch storage. In 1944, Rhoades and Carvalho reported that in panicoid grasses, such as *Zea* and *Sorghum*, the single parenchyma sheath contained large specialized plastids, functioning in starch formation and storage. In the outer parenchyma sheath cells of the festucoid grasses, as represented by barley, wheat and oats, they noticed the plastids to be smaller than those in the mesophyll. In these species the plastids of the chlorenchyma, as well as those of the bundle sheath cells, form starch.

Rhoades and Carvalho further demonstrated that in festucoid grasses the mesophyll cells photosynthesise and accumulate starch during daylight. In these grasses the outer parenchyma sheath cells have numerous plastids which, however, are smaller than those of the mesophyll. Small amounts of starch, lying in the central region of the plastid were found in the sheath plastids as well as those of the mesophyll.

In *Zea* and *Sorghum* the chlorenchyma cells photosynthesise, but do not store starch at all. Thus, no trace of starch was found in the chloroplasts even if abundant starch was present in the bundle sheath plastids. The plastids of the single sheath appear to elaborate, and temporarily store starch, but have little photosynthetic activity. These workers showed, that the starch found in these plastids was derived from soluble carbohydrates made in the mesophyll plastids, and translocated to the sheath cells where starch synthesis occurred, and not from sugars synthesised in the plastids of the bundle sheath, which could be capable of photosynthesis since they contain a green pigment.

This transformation of soluble carbohydrates to starch in the bundle sheath plastids occurs only when the rate of movement of sugars into these cells is greater than the translocation from the bundle sheath cells into the vascular bundles. Starch, which accumulates throughout the day, is transformed back into soluble carbohydrates during the night, so that by morning the bundle sheath plastids are devoid of starch. Roth (1968) considers the possibility that these cells function in water storage as well, especially in xerophytic species.

Grasses with this specialized type of bundle sheath structure and function have been termed the eupanicoid subtype of the panicoid subfamily (Brown, 1961). The other subtype, the chloridoid grasses have a dark green parenchymatous sheath and a single layer of pale green radial chlorenchyma. Here the sheath cells photosynthesise in addition to accumulating starch. Brown (1961) postulates that the chlorenchyma may have assumed some unknown function to replace the activities of photosynthesis and starch storage which it has more or less lost. Otieno (1967) is of the opinion that the radiating mesophyll cell layer, of these chloridoid grasses, constitutes part of the bundle sheath which is then composed of three layers. This is not acceptable because all intermediate types between irregular and radiate chlorenchyma are found.

The starch-free bundle sheath plastid has numerous, small, colourless areas, resembling vacuoles, contained within a peripheral rim. It is in these vacuolar-like regions that starch is deposited. Thus, each plastid

contains numerous simple starch grains embedded in, but protruding from, the surface of the plastid.

Ultrastructural studies of these dimorphic chloroplasts of the panicoid grasses, by Laetsch & Price (1969) on *Saccharum*, Downton & Pylotis (1971) on *Sorghum bicolor* and Andersen *et al.* (1972) on *Zea* show, that the bundle sheath chloroplasts lose their grana during ontogeny. Young bundle sheath chloroplasts have well-developed grana, but mature plastids are agranal whereas mesophyll chloroplasts contain grana irrespective of their developmental stage. They conclude that the structure of the specialized chloroplasts in bundle sheath cells is a result of reduction and that this chloroplast dimorphism is a specialization of labour. There are various degrees of reduction in the sheath chloroplasts. *Zea*, *Coix* and various members of the Paniceae and Andropogoneae have rudimentary grana and Carolin, Jacobs & Vesk (1973) have shown that the Eragrostoideae in fact have well-developed grana. Johnson (1964) suggests the possibility that the degree of specialization of these chloroplasts has phylogenetic significance.

The chloroplast-containing sheath cells contain, in addition, a remarkably high concentration of additional cellular organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, but vacuoles are difficult to locate. The differences in the ultrastructural features of the cells of the bundle sheath and those of the chlorenchyma further suggest the existence of differences in the functions of these cells (Dobychina, 1970; Carolin, Jacobs & Vesk, 1973).

This detailed discussion of the chloroplasts and the bundle sheath has been considered necessary because in recent years this aspect has received considerable attention from the point of view of the Kranz syndrome. Thus, grasses with bundle sheaths containing specialized chloroplasts are typical of the high photosynthetic capacity grasses and those with no, or normal chloroplasts are characteristic of low photosynthetic capacity grasses (Black, 1971).

At present the advantages of highly developed sheath cells still remain unclear. Black (1971) infers that the anatomy of the high photosynthetic capacity plants results in more rapid rates of translocation, and perhaps higher concentrations of translocates, which may both prevent a feedback type of inhibition of photosynthesis by a product such as starch. Nutrients may, in addition, be supplied to non-photosynthetic parts of the plant such that high growth rates are facilitated.

The possibility of the inner bundle sheath, or the single sheath, when the inner sheath is lacking, having functions and characteristics similar to an endodermis has received considerable attention in the literature. In grasses with a single bundle sheath, the cells of this sheath do not have the anatomical characteristics associated with an endodermis, such as Casparian strips, thicker radial and inner tangential walls or suberized walls. This is applicable to a number of inner sheaths as well.

Van Fleet (1950) demonstrated, with a variety of histochemical reactions, however, that the cells of these sheaths share common substance reactions and may be induced to develop typical endodermal characteristics. Thus, normally the single sheath cells contain chloroplasts and do not exhibit characteristics of an endodermis. But in variegated and albino leaves, or in etiolated leaves, Van Fleet showed that those areas lacking chlorophyll show characters of a typical inner bundle sheath, i.e. endodermal characteristics.

In *Pennisetum villosum* and *Oplismenus hirtellus* he further showed that casparian deposits are developed on the radial walls prior to the unilateral disposition characteristic of the mature inner sheath. Endodermal characteristics, therefore, appear at varying stages in the development of the leaf in at least some of the cells of an atypical inner sheath. Thus, in any sheath bounding the vascular tissue, some or all, attributes of a true endodermis can be detected. Van Fleet points out that the endodermal characteristics are always more pronounced opposite the phloem than opposite the xylem. Once again for comparative work it is essential to standardize upon leaves of equivalent developmental stages.

Schwendener (1890) stated that the walls of the parenchymatous sheath of *Zea* contained suberized lamellae, something Van Fleet (1950) was not able to show for normal maize leaves. O'Brien & Carr (1970) also described a suberized lamella, which is probably the site of suberin deposition, in the walls of the inner sheath of *Triticum* and *Avena* as well as in the parenchyma sheath of maize. The suberized lamellae modify the plasmodesmata of the numerous pit-fields connecting the cells of the inner sheath with those of the outer sheath and vascular parenchyma, or between cells of the single parenchyma sheath.

Using these ultrastructural details O'Brien & Carr (1970) propose a function for the inner bundle sheath and a further function, in addition to photosynthesis and starch storage, for the single sheath. These workers are of the opinion that the suberized lamellae are relatively impermeable to water and thus there is the possibility that in grass leaves water loss is regulated at the vascular bundle as well as by the stomata. This control of water loss at the vascular bundles is probably essential for an adequate supply of solute to be maintained to the leaf tip, because, under conditions of water stress the lower part of the blade could conceivably transpire all the water available to the leaf. If the suberized lamellae restrict passive loss of water from the vascular bundles to the mesophyll, forcing the water to follow a symplastic route through the sheath cells, the water flow across the sheath could be regulated by the activity of the sheath cells.

Further circumstantial evidence for this possibility is that transfer cells, which are believed to help regulate solute exchange between tissue systems, are absent from the leaves of grasses. In dicotyledon leaves these transfer cells are thought to assist exchange between xylem and phloem, and between these and the adjacent mesophyll. O'Brien & Carr reason that if passive loss of water is restricted from the vascular bundles because of the suberized lamellae, and if the bulk of solute transfer must pass through the plasmodesmata, then transfer cells are unnecessary.

Sclerenchyma of the leaf

In the grass leaf blade, the sclerenchymatous tissue, or the sterome, includes all fibres as well as other thick-walled cells in certain instances. The sclerenchyma is commonly found in association with the vascular bundles, with the midrib or keel and in the margin.

Xylem and phloem fibres, and thickened vascular parenchyma, constitute part of the ground tissue of the vascular bundles. The sterome, or inner bundle sheath, is also fibrous, the fibres appearing, in some instances, ontogenetically the same as those of the hypodermal sclerenchyma, but differing in that they possess numerous pits which are lacking in the true

sterome (Artswager, 1925). This tissue is included in the description of vascular bundle structure and will not be discussed further here.

The sclerenchyma associated with the vascular bundles is in the form of sub-epidermal longitudinal bands following the course of each vascular bundle. In transverse section this circumvascular sclerenchyma may surround, be in contact with, or may be situated above or below, but not connected to the bundle or its sheath. This sclerenchyma tissue is termed a strand when it does not extend sufficiently deeply into the mesophyll to make contact with the bundle sheath cells (Metcalf, 1960). These strands, therefore, appear in section as small hypodermal "islands" of thickened tissue situated above and below, or on one side only of each vascular bundle. When the inner face of a group of fibres is in contact with, disrupts, or envelopes the bundle sheath, it is termed a girder (Metcalf, 1960). It resembles a girder in transverse section, extending from either or each epidermis to the bundle sheath. When girders are continuous from the vascular bundle to the epidermis on either side it is termed an I-beam construction (Gould, 1968).

All distribution patterns of the circumvascular sclerenchyma occur either as strands or girders. In addition, preliminary observations in the present study suggest that a further distinction between girders in contact with, and those disrupting the parenchymatous sheath, may be useful taxonomically.

In leaves possessing both strands and girders, the former are usually associated with the third order vascular bundles and the latter with larger bundles. Other leaves may have strands or girders only developed in association with all the vascular bundles of the leaf. The distribution and arrangement of the sclerenchyma associated with the bundles is, therefore, useful diagnostically. Furthermore, the amount of sclerenchyma present varies from species to species (Metcalf, 1960). This can be misleading, however, because marked intraspecific variation will be found in specimens from different localities or from different seasons as the work of Burduja & Toma (1970) on *Deschampsia flexuosa* and other species has shown. They found that on flowering tillers there was less sclerenchyma in the leaves than on non-flowering tillers where the fibre walls were also thicker with the lumen smaller. Taxonomically sclerenchyma distribution is seldom of more than specific diagnostic importance.

The distribution of sclerenchyma associated with the vascular bundles can be correlated with ecological factors as well. Grasses from arid areas are thus characterized by well developed sclerenchyma tissue while many tropical grasses often have a high proportion of the smaller bundles not accompanied by sclerenchyma.

The function of all the sclerenchyma present in the leaf is undoubtedly to provide mechanical support for the softer tissues. The development of sclerenchymatous tissues makes possible the withstanding of the physical stresses and strains imposed on an elongated, straplike leaf such as is predominant in the Poaceae. An additional function of the fibres is seen by their frequent silicification, especially in older leaves (Parry & Smithson, 1964) and they, therefore, can act as a depository for excessive silica. Silicified fibres result in elongated, pointed needles of silica.

The thickening of the fibre cell walls is usually by lignification, but in many specimens the staining reaction varies across a single sclerenchyma girder,

or in girders from different parts of the lamina. In these cases, when stained with safranin and fast green, there is a gradation from typically red-staining fibres to structurally similar fibres which attract the blue or green dye. Hoefer (1941-42) also found variations in the reaction of fibres in different parts of the leaf of *Stipa tenacissima* when treated with lignin stains. In addition, lignin tests with phloroglucin and hydrochloric acid and tests with Maules's reagent did not give identical results. Hoefer's tests showed, in addition that there is zonation in the degree of lignification in different layers of the cell wall of individual fibres as seen with transverse section. It appears, therefore, that the degree of lignification varies, but in addition, lignins of various chemical constituents appear at present to be grouped under the general term "lignin".

In certain grasses sub-epidermal longitudinal strands are found between successive vascular bundles, either alone or in addition to the circumvascular strands or girders. These supernumary strands are usually abaxial and situated opposite the bulliform cell groups or furrows (Lewton-Brain, 1904). When sclerenchyma is found between the bundles in expanded leaves it is of special importance diagnostically.

Continuous abaxial hypodermal bands are found in certain species, especially those with acicular leaves. These bands may be a regular, narrow strip, 2-4 cells deep, located immediately beneath the epidermis, or may result from lateral extensions of the fibrous tissue comprising strands or girders.

Mechanical tissue of the leaf margin may occur in the form of a cap or hood. When the marginal sclerenchyma is not in contact with the lateral bundle, it is termed a cap, and a hood is formed when this sclerenchyma extends above or below the lateral bundles. The fibres of the hood may, or may not, be in contact with the bundle sheath of the ultimate bundle, but always extend inwards from the margin, as far as, or further than, this bundle. This lateral fusion with the sterome tissue associated with the lateral bundles forms the hood-like structure (Goossens, 1938). In some instances, there is no sclerenchyma development at the extreme margin but the ultimate and penultimate lateral bundles may be intimately associated with specialized sclerenchyma development. Immediately interior to the fibrous cap may be situated normal mesophyll cells, small groups of enlarged, colourless parenchyma cells or a lateral intercellular duct may be developed.

Sclerenchymatous tissue is also found developed in association with midribs and keels. This has been described together with the median vascular bundles, midribs and keels.

In reduced, permanently infolded leaves the distribution of the sclerenchyma follows that outlined above in basic pattern, but appears symmetrical and distinctive because of the nature of the leaf blade. In these leaves this arrangement of the mechanical tissue is of taxonomic importance because, in assuming the permanently infolded form, the leaves of different grasses have not acquired precisely the same sclerenchyma tissue arrangements (Metcalf, 1960).

Mesophyll

In the Poaceae, the term mesophyll is generally applied to the ground tissue occupying all the space in the leaf not occupied by the vascular bundles, the bundle sheaths and the sclerenchyma. The mesophyll can be subdivided into the assimilatory chlorenchyma

and the colourless parenchyma which consists of translucent cells often in close association with the bulliform cells. For this reason further details of colourless parenchyma will also be found under the discussion of bulliform cells.

Grass leaf blade chlorenchyma seldom exhibits a distinct differentiation into palisade and spongy regions as seen in transverse section. In some species the adaxial chlorenchyma cells are more regularly and vertically arranged than the remainder. Metcalfe (1960) correctly stresses that this distinction is rare and at best unclear. Statements in the literature often imply that there is greater contrast between these zones than there actually is.

In dicotyledons, as Watson (1942) has shown with *Hedera helix*, the English ivy, the leaves respond to strong light by the production of palisade tissue. On the same plant shade-grown leaves have no palisade tissue at all. These cell differences are caused by factors affecting the processes of vacuolation and enlargement of the cells. Thus, the uppermost layer of mesophyll cells has an increased osmotic value due to a change in the starch-sugar ratio. This, according to Watson (1942), results in the absorption of water, increased vacuolation and expansion, with the resultant formation of palisade tissue.

In the Poaceae, the above does not appear to apply and in certain instances the opposite may be the case. In the genus *Panicum*, the shade-loving forest species often have the upper layer of chlorenchymatous cells more or less vertically arranged in a palisade manner in contrast to most other species of this genus where the assimilatory tissue is more of less radiate.

The arrangement of the chlorenchyma cells appears to be of fundamental taxonomic significance. Thus, in the festucoid type, the chlorenchymatous tissue is more or less homogenous without being arranged in any definite pattern in relation to the vascular bundles. In grasses of the panicoid type the assimilatory cells are arranged in a radiating manner around the vascular bundles as seen in transverse section. Each bundle is, therefore, situated in the centre of a regular circle, or partial circle of chlorenchyma. The former irregular arrangement is, in addition, generally associated with leaves in which the vascular bundles are widely spaced, whereas the radiate condition and closely placed vascular bundles are correlated (Vickery, 1935).

The division of the Poaceae into two sub-families with radiate or non radiate chlorenchyma is an oversimplification. Brown (1958) shows that within the panicoid grasses the Chlorideae and related tribes have the regularly radiate condition, whereas this radiate condition is less regular in other panicoid genera such as *Andropogon* and *Panicum*. Thus, the radiate condition is of more than one kind, and grasses with partially or incompletely radiate chlorenchyma also occur, as Metcalfe (1960) stresses. Nevertheless, the distinction between radiate and non-radiate chlorenchyma is important taxonomically provided that the limitations are recognized. For example, both conditions may occur in the same genus, as in *Sporobolus* where many species have regular, elongated chlorenchyma cells radiately arranged around the bundles, but in *S. panicoides* the cells are irregular in shape and arrangement (Goossens, 1938).

Early workers correlated the non-radiate condition with grasses from temperate regions, whereas radiate assimilatory tissue was linked with tropical grasses.

The geographical pattern is not so distinct, however, with many exceptions and Metcalfe (1960) remarks that the mesophyll arrangement is of more fundamental significance taxonomically than geographically.

In transverse section the individual chlorenchyma cells of most panicoid grasses appear elongated, narrow and tabular in shape. Their long axes are at right angles to the bundles. In festucoid grasses the cells are often irregular in shape and size. In some species, such as *Poa annua*, the largest cells are found nearest the vascular bundles (Bobrov, 1955) or the chlorenchyma cells abutting on both epidermides may be somewhat elongated and regular as in *Triticum vulgare* (Hayward, 1948). The irregular condition may be characterized by small, isodiametric cells tightly packed together or by irregularly shaped cells with many intercellular air spaces between them. The largest intercellular air spaces are commonly seen subtending the stomata and projecting deeply into the mesophyll. Pool (1923) illustrates such distinct sub-stomatal chambers in *Andropogon furcatus*.

Slade (1970) in a study of sun and shade-grown leaves of *Poa alpina* has shown that in sun leaves the mesophyll cells are more or less isodiametric and not longitudinally elongated as in shade leaves. The intercellular spaces are relatively inconspicuous in sun leaves but extensive in shade leaves as seen in longitudinal section. Slade also demonstrated that in transverse section there was no noticeable change in the horizontal widths of the intercostal zones of chlorenchyma between adjacent vascular bundles, but that the depth or thickness of the mesophyll was greatest in sun-grown leaves. This is as a result of reduction in cell size and intercellular spaces in shade leaves seen in transverse section in contrast to the situation in longitudinal section above.

The overall effect of these anatomical changes in sun and shade leaves is to physically weaken the leaf blade. Slade (1970) has shown, in addition, that in low light intensity the cell walls are thinner and a well defined cuticle may be absent.

It appears, therefore, that the light intensity under which a leaf develops has a profound effect on the arrangement of the assimilatory cells into palisade and spongy mesophyll, especially in dicotyledons (Watson, 1942), as well as the size and shape of the individual cells as shown by Slade (1970). The applicability of these findings to the chlorenchyma cell structure and arrangement in the Poaceae as a whole does not appear clear at present. Thus, if the radiate and non-radiate conditions are of fundamental phylogenetic significance it appears unlikely that major differences of tissue arrangement will occur under varying light intensities. Some generalizations do seem to apply and most forest dwelling species examined are characterized by very thin mesophyll tissue. *Prophytochloa prehensilis* is an extreme example with the whole leaf being only four cell layers thick (de Winter, 1951).

Intercellular air spaces occur between the chlorenchyma cells of many grasses belonging to tribes of both the major subfamilies. These spaces may be very conspicuous in hygrophilous species (Arber, 1934; Vickery, 1935). In other aquatic species there are, in addition, distinct air cavities or lacunae present. All the species of *Elytrophorus* have these lacunae situated between adjacent vascular bundles (Schweickerdt, 1942) and in *Vetiveria* they occur over the smaller bundles (Kanmathy, 1969). The lacunae are traversed by colourless aerenchyma cells which are

often stellate in shape and represent diaphragms in the air cavities. Kanmathy (1969) reports sclerotic strands interspersed in the stellate cells.

Cavities developing as a result of breakdown of parenchymatous tissue, especially in the region of the keel, and in the keel itself, are found in many grasses. These differ from lacunae in being indistinct with the breakdown of the cells continuing. This type of cavity has been used diagnostically for *Molinia caerulea* (Lewton-Brain, 1904) and *Sporobolus artus* var. *lysigenatus* (Goossens, 1938).

A further modification of the mesophyll tissue is found in certain markedly infolded leaves where the chlorenchyma is confined to small bands adjacent to the adaxial and abaxial cleft-like furrows which contain the stomata. The tissue between the groups of assimilatory tissue is made up of colourless parenchyma. This unique type of anatomy is discussed by Burbidge (1946, 1946a) for *Triodia* species. Burbidge believes that this reduction of the chlorenchymatous tissue results in a corresponding narrowing of the width of the furrows and consequent protection of the stomata. De Wet (1956) found localized bands of chlorenchyma cells around the abaxial grooves in species of *Merxmüllera* (= *Danthonia*) and suggests that this may indicate relationships.

In some species, groups or isolated, bundle sheath-like cells with chloroplasts, but lacking associated vascular tissue are found scattered in the mesophyll. These are termed distinctive cells (Tateoka, 1958) and are described under vascular bundle sheaths.

Bamboos, and a few other grasses, are characterized by the presence of fusoid cells in the mesophyll of their leaves. These cells have been termed enlarged parenchyma cells (Page, 1947) and in transverse section have a fusiform or pyriform outline, being elongated transversely and alternating with chlorenchyma cells and the vascular bundles. Adjacent fusoid cells may be separated by a single vertical column of chlorenchyma or many chlorenchyma cells may separate them. The bulliform cells are located immediately adaxially to these chlorenchyma cells separating adjacent fusoid cells.

These conspicuous colourless cells have been incorrectly interpreted as intercellular spaces (Jacques-Felix, 1955). Their cellular nature was confirmed by Page (1947) and Metcalfe (1956).

The fusoid cells are, in fact, narrow and plate-like with the long axis of the cells lying at right angles to the long axis of the lamina. Longitudinal sections of the blade show these cells to have very narrow lumina in transverse section, the cells having collapsed in such a way that the tissue resembles a row of "I's". Between the dead, collapsed, mature cells there are large spaces which connect with the intercellular spaces of the chlorenchyma. In certain species the fusoid cells fail to collapse, but become rounded and separated from one another. The regularity and form of these collapsed cells seems to indicate that some force operates on the whole leaf at the same time (Page, 1947).

In addition, the Bambusae, as well as the Oryzeae, are characterized by having chlorenchyma comprised of what, in the grass anatomy literature, have become known as arm cells. These have also been called irregularly lobed or cleft cells (Page, 1947) or plicate mesophyll (Esau, 1960). These so-called arm cells possess inwardly directed projections or folds which may be continuous across the cell as seen in section and completely divide the cells into elongated compartments. The infoldings may end blindly in the

lumina as well. These projections may be developed from the upper or the lower wall of the chlorenchyma cell, or from both the upper and lower, or from all walls (Brandis, 1907).

Chih-Ying Wu (1958) has shown that in some bamboo species the arm cells located above the fusoid cells have projections from the lower walls, whereas the cells below the fusoid cells and cavities have folds projecting from the upper walls. This phenomenon is mentioned by Arber (1934), but in the Oryzeae, even when fusoid cells are present, the arm cells illustrated by Tateoka (1963) have infoldings from all directions and of equal length.

It is not certain how these projections arise. They could be infoldings of the cell walls as an illustration of Carolin, Jacobs & Vesk (1973) indicates, but in some grasses their appearance suggests that they arise as fine partitions that at first traverse the cell completely, but subsequently become broken as the cells become enlarged (Metcalf, 1960). Haberlandt (1884) called these "armpalisadezellen" and explained the significance of the infoldings as increasing the inner surface area of the cell, thereby creating space for more chloroplasts. Infoldings are common in many grass genera, and are termed arm cells or peg cells, and in the bamboos and their relatives it is possible that the pegs become "fused", eliminating the air spaces.

Fusoid and arm cells are important taxonomic characters. Thus, fusoid cells are especially characteristic of the Bambusae (Metcalf, 1960), and when they occur in other genera, such as *Oryza* (Tateoka, 1963), affinities between these genera and the bamboos may be indicated. Arm cells, although characteristic of the Bambusae and Oryzeae, may also show variations in their occurrence. Thus, *Oryza tisseranti* is exceptional amongst the Oryzeae in not having arm cells (Jacques-Felix, 1958). Gordon-Gray & Ward (1971) noticed mesophyll cells with invaginated walls in *Phragmites* and suggest that this may reflect closer relationships to the Bambusae and Oryzeae than was previously thought possible. The extreme case is that of *Saccharum*, where Merida (1970) found cells with internal folds situated below the stomata in some varieties. This apparent occurrence of arm cells in a member of the Andropogoneae is certainly surprising.

Bambusoid and oryzoid grasses are very poorly represented in South Africa. For this reason, the accompanying descriptive key is not concerned with the details of arm and fusoid cells to any great degree. As presently constituted it cannot be expected to differentiate between the various bamboo genera.

The colourless parenchyma constituent of the mesophyll, especially that intimately associated with the bulliform cells is described, together with these bulliform cells, under the description of bulliform cells.

Bulliform and colourless cells

Bulliform cells are single, translucent cells, or groups of colourless cells, constituting part of, or the entire epidermis, but differing from other epidermal cells in being larger and more inflated. These cells occur most commonly, but not exclusively, at the bases of adaxial furrows (Metcalf, 1960). Shields (1951) restricts the term bulliform cell to inflated epidermal cells present in the adaxial furrows. However, structurally similar cells may be present in the abaxial epidermis as well as, or instead of, the adaxial epidermis. Other terms used to describe these cells are hinge cells and motor cells, but the

descriptive term bulliform, denoting their inflated appearance, is preferred to these terms referring to their disputed function in leaf involution.

Bulliform cells often form longitudinal, parallel, intercostal bands, and are commonly associated with underlying groups of structurally similar cells, which usually form vertically elongated units in the mesophyll when viewed in transverse section. Shields (1951) designated these associated cells, hinge cells. Here they are grouped with the colourless cells, a term incorporating all the translucent cells present in the mesophyll excluding bundle sheath cells devoid of chloroplasts, fusoid cells of bamboos and epidermal cells. The bulliform and associated colourless cells may appear in transverse section as deep girders, or may be restricted and superficial. The colourless cells associated with the bulliform cells are variable in shape and size, but all colourless cells, whether associated with the bulliform cells or not, are always without chloroplasts.

Bulliform cells have long been recognised as valuable taxonomic characters. As early as 1907, Brandis reviewed the distribution of bulliform cells in the Poaceae as a whole. The major character subdivisions he used were the presence of bulliform cells in the adaxial epidermis only, the presence of bulliform cells in both adaxial and abaxial epidermides, and the absence of distinct bulliform groups. The adaxial bulliform cell distribution was further subdivided into those grasses where bulliform cell groups alternated with all vascular bundles, those where the bulliform cells were present between successive first order bundles, but located over the third order bundles, those with bulliform cells on either side of the midrib and at the margins, those with bulliform cells only located on either side, or above the midrib. Using these distributional criteria of bulliform cells Brandis (1907) found uniform distribution in the tribes Bambuseae and Maydeae only. In all the other tribes studied various genera had different bulliform cell arrangement in some of their species.

The arrangement, frequency, distribution, as well as the relative size and shape of the bulliform cells and colourless cells are of taxonomic importance especially at the specific level. Thus, in *Elytrophorus*, Schweickerdt (1942) found the presence of conspicuous bulliform cells flanking the midribs only, to be the outstanding diagnostic character of *E. africanus*, whereas in *E. interruptus* bulliform groups are present throughout the leaf width, but decrease in size towards the margin. Furthermore, in one species, the shape of the individual bulliform cells in transverse sections is rectangular, whereas in the other species they are trapezoidal with the outer wall being shorter than the inner wall. In *Leersia hexandra* there are groups of bulliform cells between all the vascular bundles and on both epidermides but other *Leersia* species have only two groups of bulliform cells on either side of the midrib (Holm, 1895). Goossens & Theron (1934) found the bulliform cells to be of significance in classifying the different varieties of *Themeda triandra*. The arrangement of the colourless cells in relation to the bulliform cells can be of specific diagnostic importance. In *Imperata cylindrica* (Vickery, 1935), where the bulliform cell groups occur over third order bundles, two rows of colourless cells are produced towards the abaxial surface, one on either side of each bundle.

The function of the bulliform cells has been the subject of much controversy in the literature and a totally satisfactory explanation of their function has, as yet, not been forthcoming. Duval-Jouve (1875) was

the first to describe these cells and believed that the opening and inrolling of the blade resulted from changes in the turgor pressures of these cells. Some workers, such as Breakwell (1915), have carried this even further by postulating that rolling occurs only at times of water shortage and results in the leaf becoming tubular, thus protecting the greater number of adaxial stomata from excessive transpiration. This teleological viewpoint is not supported by the fact that many grasses, including drought resistant types which exhibit inrolling, have more stomata on the exposed abaxial surface than the protected adaxial surface (Vickery, 1935). Hayward (1948) mistakenly states that the outer surfaces of the bulliform cells are not cutinised, and thus readily lose water through diffusion. Metcalfe (1960) points out that in many grasses exhibiting involution the outer wall of the bulliform cells may be considerably thickened.

The conventional explanation of bulliform function thus accepts that the bulliform cells regulate the movement of the blade when it opens and closes. Goossens & Theron (1934) draw a comparison between bulliform cells and stomatal guard cells and the changing of cell shape and turgidity with turgor changes. However, whether the bulliform cells actually cause the rolling movements by collapse due to water loss, or whether their size and plasticity merely permit them to be compressed and so allow rolling to occur, has been the subject of some controversy.

Numerous facts point to caution in explaining the function of these cells. Brandis (1907) remarks on the fact that in mature leaves the bulliform cells may become entirely filled with solid silica making the leaf rigid. Bor (1960) quotes Goebel (1926) as describing nyctinastic movements in *Leersia hexandra* in which the leaves fold up in dull weather and flatten again in full sunlight. These movements obviously are not dependant on different water regimes within the bulliform cells. In addition, involution may occur in the absence of bulliform cells and often the thin lateral walls of bulliform cells are not collapsed or distorted in naturally rolled or wilted grass leaves (Shields, 1951).

Tschirch (1882) challenged this theory that movements depended only on turgor changes in the bulliform cells. While he agreed that rolling and unrolling of grass leaves was influenced by the loss and uptake of water, he thought that these movements were due, at least in some cases, to changes in the leaf fibres rather than the epidermal bulliform cells. He explained that the adaxial, subepidermal sclerenchyma fibres have a high capacity for imbibition and a marked tendency to shrink when dehydrated. This results in contraction of the adaxial ribs causing inrolling because the inflexible lower epidermis, reinforced by sclerenchyma gives the abaxial surface an unbroken rigidity. This contraction, coupled with cohesion among the chlorenchyma cells and among the bulliform cells may result in leaf involution. Thus, involution has also been defined as a cohesion and shrinkage phenomenon, the result of a differential contraction of the mesophyll and the rigid lower epidermis. As the mesophyll cells shorten in drying, they become stretched around the vascular bundles, causing the leaf to roll.

Shields (1951), in a detailed study of grass leaf involution, has shown that differential shrinkage of the adaxial and abaxial rib surfaces occurs. This shrinkage was more pronounced in all the species studied on the adaxial rib surface. This illustrates the lateral shrinkage in the adaxial sclerenchymatous

fibres, where present, and in the mesophyll. In leaves lacking adaxial sclerenchyma girders or strands, large adaxial mesophyll cells collapse in the wilted leaf and the bulliform cells show varying degrees of buckling. Shields found that lateral and vertical contraction in the bulliform and associated colourless cells was no greater than in the adjacent mesophyll. In wilted leaves the abaxial ribs appear more prominent as a result of the smaller amount of shrinkage in the vascular tissue than in the surrounding cells. Shrinkage of the bulliform and colourless cells appears to play no part in involution except to facilitate the turning inward of the upper leaf surface.

The fact that the bulliform cell walls are hygroscopic and, therefore, may assist in movement was reported by Goossens & Theron (1934). This was supposedly shown by the fact that dead, detached leaf segments roll more tightly than in normal reversible involution and, conversely, increasing their water content leads to the partial unfolding of the dead leaf segment. Shields (1951) comments that this partial expansion of a dried leaf through adsorption by all the cell walls (which are also dead in the living blade) suggests that decreased hydration of the cell wall may play a passive part in reversible wilting. Since the bulliform cells are dead in this case, absorption of water by the protoplasm and large central vacuole cannot occur.

Involution does not result from plasmolysis. Plasmolysis, through vertical contraction of the mesophyll, may cause an increase in width, probably by relieving tissue strains through shrinkage of the protoplasts (Burstrom, 1942). As a result vascular bundles become more widely separated, and the thick-walled, stiff abaxial epidermis, which does not change in width, tends to curve backwards in plasmolysis so that the underside of the leaf becomes concave. Since plasmolysis involves turgor change the diametrically opposed characteristic of inrolling in wilted leaves implies that involution does not result from turgor changes.

Loss of water from the bulliform cells to the chlorenchyma in particular, may further contribute to the collapse of these cells. With a cuticle on their outer surface these epidermal cells are more resistant than the mesophyll cells to the drying effects of the atmosphere. However, Haberlandt (1928) showed that when an organ is transpiring rapidly, the epidermis loses water to photosynthetic tissue with its higher osmotic pressure.

Shields (1951) examined the bulliform cells in unwilted but tightly rolled leaf buds. They are small but turgid, rather than flaccid and collapsed, as in the wilted blades of mature leaves. Furthermore, unfolding from the developing bud involves general growth, particularly in the adaxial mesophyll, and does not result primarily from the enlargement of the bulliform cells, although these enlarge by stretching growth due to turgor changes. The rolling mechanism is, therefore, not related to the unfolding of the bud. Rolling involves cohesion and shrinkage in both living and non-living parts, and bud unfolding, turgor and stretching movements at a time when all cell walls are highly elastic.

To summarize, it has been shown that structural elements other than bulliform cells contribute to involution. 1. Rolling in drying is characteristic of certain grass leaves entirely lacking in bulliform cells, and if bulliform cells are present they may show no buckling or other distortion in the wilted leaf. 2. Since average measurable lateral shrinkage (which is negligible on the abaxial surface of wilted leaves)

amounts to 7–12 per cent on the adaxial rib face (Shields, 1951) in naturally wilted leaves, subepidermal sclerenchyma and other adaxial elements of the mesophyll must contribute to involution. 3. Since dried leaves unfold partially when placed in water, involution must result in part from decreased water content in non-living cell walls which may or may not contain protoplasts. Rolling cannot result entirely from turgor movement, which, by definition, is a reversible change in the water content of living cells. Water loss by protoplasts alone is insufficient to cause involution in a leaf composed largely of non-living mechanical tissue. The form of the wilted leaf is determined by a number of elements in the mesophyll, the buckling of bulliform and colourless cells, where it occurs, being in part from passive compression.

Anatomical variations in the structure and distribution of bulliform cells in different genera exhibiting involutory movements preclude the universal application of any one explanation of this phenomenon in wilting grass leaf blades. What is important from a descriptive point of view is the fact that the outline of the lamina can be markedly altered by this phenomenon. It appears to be constant within a species and, therefore, it is not the degree of infolding or unrolling that is important but rather the type of movement.

This is confirmed by the work of Dunlop (1913) on the curling of the leaves of different varieties of sugar cane. Involute curling, where the upper surface is protected, is characterized by the following anatomical structures: the bulliform cells are not prominent and the cells immediately interior to these cells are comparatively large and thin walled; the upper epidermis is not greatly lignified. Varieties of sugar cane exhibiting revolute curling, whereby the lower surface is protected, have large prominent bulliform cells attached to the vascular bundles by lignified cells. The vascular bundles are closer together and the upper epidermis is greatly lignified. Dunlop (1913) is of the opinion that this revolute curling is a permanent characteristic of the varieties showing it.

A further possible function of the bulliform and colourless cells has been advanced by Breakwell (1915). He states that the arrangement and distribution of the colourless cells allows light to penetrate to the chlorenchyma cells, even in the rolled up position of the leaf bud and thus aids in the development of the leaf. The rapid development of leaves with large bulliform groups and colourless parenchyma, such as *Astrebla pectinata*, is cited as circumstantial evidence for this possible function.

Epidermal cells in transverse section

Typical epidermal cells in transverse section normally do not exhibit any particularly important diagnostic characters, although examination of them in section can be of assistance in the interpretation of many epidermal structures as seen in surface view. Sections through the epidermis can aid in establishing the nature of the papillae, the attachment and structure of other epidermal appendages, the positioning of the stomata or the cuticle form and thickness.

Epidermal cells are usually square to rectangular in section, or the outer wall may be more or less arched or even papillate. The papillate appearance, as seen in surface view, may be due to a pronounced arching of the whole outer tangential wall, or more commonly (Vickery, 1935), due to the presence of a number of separate papillae arranged in a longitudinal row or rows. However, when these papillae are in a single row, and as wide as the epidermal cell, the outer wall

also appears as strongly arched in section. Narrower papillae appear as distinct papillae on the outer tangential wall surface. Where more than one row of papillae are present on each cell they appear as bifurcate or multiple papillae. The papillae may be inflated and thin-walled, resemble conical warts or may be sharp-pointed expansions of the outer wall (Holm, 1891a) or teeth (Sabnis, 1921). It is also common for the distal ends of the papillae to be thickened.

The level of the stomata in different varieties of sugar cane has been correlated with differences in drought resistance by Merida (1970). They may be located at the same level as the epidermal cells or sunken below them as seen in transverse section. In certain grasses, such as *Spinifex hirsutus*, the stomata are present in depressions formed by the surrounding cells being enlarged and raised above the general level of the epidermal surface (Breakwell, 1915). The presence of stomata on both surfaces, or only the adaxial or abaxial surfaces can also be confirmed.

Micro-hairs are rarely seen in leaf sections but prickles and macro-hairs are commonly sectioned. The nature of the cushion cells surrounding the base of many macro-hairs, whether raised or not, can easily be determined. Interlocking prickles forming arches over the stomata can sometimes be seen as in certain *Danthonia* species (De Wet, 1960). Burbidge (1946a) found hairs consisting of a bulbous-based cell and a small apical cell imbedded in the epidermal cells at the base of grooves of leaves of all species of *Triodia*.

The size of the epidermal cells may vary over and between successive bundles as well as the cells of the adaxial and abaxial epidermides being of different sizes. In many grasses, especially members of the tribe Andropogoneae, the epidermal cells are exceptionally large, and can occupy up to half the leaf thickness. These are included under bulliform cells.

Cuticle thickness can be determined in leaf sections. Thus, it may be seen in many species that the lower epidermis is more strongly cuticularized than the upper. Slade (1970) has demonstrated the lack of a well defined cuticle in shade-grown leaves of plants which have distinct cuticles on the sun-grown leaves.

The thickness of this cuticle is of the utmost importance in faecal analysis studies, because during the digestion process all the cellulose cell-walls are dissolved away leaving the cutinized cuticular membrane behind. Thus, it is the relative thickness of the cuticular membrane, and not of the entire outer tangential wall, that is of importance in these studies of the diet of grazing animals. It should be stressed that the outer wall may be markedly thickened but lack a thick cuticle. The cuticle proper, therefore, consists of a layer of adcrusted cutin continuous over the entire leaf surface. Each cell does not have an individual cuticle but it may be individually thickened. This difference can readily be determined in transverse sections of these epidermal cells.

CONCLUSION

It is hoped that this attempt to introduce uniform standards to the description of grass leaf blades, as seen in transverse section, will stress the urgent need for standardization in these studies. If this can be achieved, anatomical descriptions will have a much wider applicability in the fields of comparative leaf anatomy and grass systematics in general.

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UITTREKSEL

Omskrywende sleutels, definisies en diagramme, vir die standaardisering, vereenvoudiging, en beskrywing van die grasblaar struktuur soos beskou in dwarsnit, word aangegee. Meer as 500 eienskappe is ingesluit met die moontlikheid vir uitbreiding tot 999. Aantekeninge van die variasie en die taksonomiese waarde van die eienskappe word ook verstrek.

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