POACEAE

TAXONOMIC SIGNIFICANCE OF EPIDERMAL STRUCTURE IN SOUTHERN AFRICAN MEMBERS OF *HELICTOTRICHON*

INTRODUCTION

Helictotrichon Besser ex Schult. & Schult.f. (excluding *Avenula* (Dumort.) Dumort. and *Amphibromus* Nees) is a genus of temperate C_3 grasses with about 40 species (Gibbs Russell *et al*. 1990; Mabberley 2008). The genus is most diverse in the temperate regions of the northern hemisphere, especially in Europe, from where it extends southwards along the African mountains (Afromontane Region). This paper deals only with those species occurring in southern Africa, a secondary centre of diversity for the genus.

In a taxonomic revision based mainly on macromorphology, Schweickerdt (1937) recognized 12 species of *Helictotrichon* in southern Africa. A new taxonomic revision of the group (Mashau *in progress*), which also considered evidence from leaf anatomy, applied to all these species as well as two newly described ones (Mashau *et al.* 2010), thus bringing the total number of species on the subcontinent to 14. The anatomical attributes of the leaf epidermis of these species were studied for the first time and proved to be particularly useful for distinguishing among species. The purpose of the present paper is to report on the taxonomic significance of leaf epidermal characters in the southern African members of *Helictotrichon*.

Helictotrichon quinquesetum (Steud.) Schweick. is only known from two collections from the Cape Peninsula, both dating from the 19th century. The species is currently considered critically rare and is probably extinct (Helme & Raimondo 2010). Only fragments of the inflorescence from the type, *Ecklon 929* (removed from the holotype in OXF and isotype in K), are available at the National Herbarium (PRE). The original collections are also very poor and have hardly any leaves (seen on Aluka website). Hence, due to the limited material available, this species was not studied anatomically.

MATERIALS AND METHODS

The epidermal structures of 13 *Helictotrichon* species were studied (see Table 1 for a list of species and voucher specimens). *H. quinquesetum*, a very rare species, was not studied (see above).

Portions of dried leaf blades were cut from herbarium specimens, transferred to test tubes containing distilled water and heated in a water bath at 50°C for about 24 hours. After allowing cooling for about 12 hours, the rehydrated leaves were fixed in formalin-acetic-acid-alcohol [FAA] (Johansen 1940) for at least 48 hours. Before further processing, the pieces of leaf blade were thoroughly washed in water to remove all traces of the fixative.

To obtain the epidermal peels, leaf blades were transversely cut into pieces of about 10 mm long, and one margin removed by cutting it off. The pieces were placed in stopper glass tubes, covered with Jeffrey's solution [equal volumes of 10% aqueous chromic acid (CrO_3) and 10% nitric acid (HNO_3)] and left at room temperature for about 24 hours until the unwanted tissue between the upper and lower leaf epidermis had dissociated and was easily freed from the epidermis (Kiger 1971). The sufficiently macerated pieces of leaves were thoroughly washed in water and then stained with 1% safranin in 50% ethanol for about 30 minutes to 1 hour. The stained material was then dehydrated in a graded ethanol series: 50% ethanol; 70% ethanol; 94% ethanol; 100% ethanol followed by 50:50 ethanol and xylol; and finally 100% xylol (10 minutes in each solution). The leaf segments were finally opened like a book (abaxial and adaxial surfaces parted) and any remaining mesophyll removed and mounted in entellan (Product 7961, E. Merck, Darmstadt) under cover slips on microscope slides. The descriptive terminology for the leaf epidermal anatomy follows Ellis (1979).

TABLE 1.—List of species and specimens examined

Species	Specimens examined
H. barbatum (Nees) Schweick.	*Acocks 18632*
H. capense Schweick.	*Story 3803; Tyson 20564*
H. dodii (Stapf) Schweick.	*Pole-Evans 499, 518*
H. galpinii Schweick.	*Ellis 1389; Hilliard & Burtt 16478*
H. hirtulum (Steud.) Schweick.	*Smook 5859; Spies 1695A*
H. leoninum (Steud.) Schweick.	*Ellis 225; Cleghorn 3130*
H. longifolium (Nees) Schweick.	*Smook 1184; Davidse 6962*
H. longum (Stapf) Schweick.	*Acocks 20691; Ellis 5432*
H. namaquense Schweick.	*Ellis 5997*
H. natalense (Stapf) Schweick.	*De Wet 1722; Rennie 1534*
H. rogerellisii Mashau, L.Fish & A.E.van Wyk	*Ellis 4663*
H. roggeveldense Mashau, L.Fish & A.E.van Wyk	*Acocks 17178; Ellis 5117*
H. turgidulum (Stapf) Schweick.	*Smook 5888; Spies 3953*

RESULTS AND DISCUSSION

The epidermis of the *Helictotrichon* leaf blade exhibits taxonomically significant variation in the structure and shape of the silica bodies, costal cells, intercostal short cells, stomata, intercostal long cells, prickles and macro-hairs. Examples of the variation in these structures are illustrated in Figures 1 and 2. The differences between species are summarized in Table 2, and below in the form of a dichotomous key.

Leaf anatomical key to the species of *Helictotrichon* in southern Africa (excluding *H. quinquesetum*) based on epidermal structures as seen in surface view

1a Macro-hairs (including crozier hairs) and prickles present:

2a Silica bodies equidimensional (vertical and horizontal dimensions approximately equal), round (circular in outline), fitting into concavities of cork cells:

3a Macro-hairs straight; square to rectangular paired intercostal cork-silica short cells situated between long cells . . . . . *H. roggeveldense* (Figure 2B)

3b Macro-hairs with apices hooked (crozier type); crescentic (enfolding the silica cell) paired intercostal cork-silica short cells situated between long cells . . . . . *H. rogerellisii* (Figure 2A)

2b Silica bodies horizontally elongated (horizontal dimensions greater than vertical dimensions), ends rounded and outlines sinuous:

4a Macro-hairs with many, usually smaller, specialized epidermal cells associated with the base of the hair; intercostal long cells with moderately thickened walls and cuticular flanges well-developed . . . . *H. longum* (Figure 1F)

4b Macro-hairs with one specialized epidermal cell associated with the base of the hair; intercostal long cells with unthickened walls and no cuticular flanges present . . . . . *H. galpinii* (Figure 1C)

1b Macro hairs absent; prickles present:

5a Prickle base angular:

6a Prickle barb short (less than the length of the base); costal short cells are silico-suberose couples (cork-silica cell pairs) with cork cells tall and narrow, and silica bodies not tall and narrow . . . . . *H. hirtulum* (not illustrated)

6b Prickle barb medium (as long as or slightly longer than the base); costal short cells are silico-suberose couples (cork-silica cell pairs) with cork cells crescentic and enfolding the silica bodies . . . . . *H. longifolium* (Figure 1E)

5b Prickle base elongated, oval or elliptic:

7a Costal short cells silico-suberose couples with cork cells crescentic and enfolding the silica bodies:

8a Intercostal long cells straight-walled without undulations:

9a Prickles small (base shorter than the stomata) . . . . . *H. turgidulum* (Figure 2C & D)

9b Prickles medium (base as long as or slightly longer than the stomata) . . . . . *H. dodii* (Figure 1B)

8b Intercostal long cells irregular (slightly undulating), with moderately, or deeply undulating walls:

10a Prickle barbs (shape in relation to base) not developed from the apex of the conical base and raised . . . . . *H. capense* (Figure 1A)

10b Prickle barbs developed basally from the apex of the base, and slightly raised:

11a Silica bodies elongated (horizontal dimension greater than vertical dimension), ends rounded and outlines smooth . . . . . *H. natalense* (Figure 1H)

11b Silica bodies equidimensional (vertical and horizontal dimensions approximately equal), ends squarish or angular and outlines irregular . . . . . *H. leoninum* (Figure 1D)

7b Costal short cells silico-suberose couples with cork cells tall and narrow but silica bodies not tall and narrow:

12a Silica bodies equidimensional (vertical and horizontal dimensions approximately equal), ends square, outlines (shape) cubical or slightly rectangular . . . . . *H. barbatum* (not illustrated)

12b Silica bodies elongated (horizontal dimensions greater than vertical dimensions), ends rounded, outlines (shape) sinuous or nodular:

13a Silica bodies ends rounded and outlines (shape) nodular . . . . . *H. namaquense* (Figure 1G)

13b Silica bodies ends rounded and outlines sinuous . . . . . *H. galpinii* (Figure 1C)

The variation in the characters used in describing the leaf epidermal anatomy of *Helictotrichon* and their taxonomic importance are highlighted below. Structures such as intercostal long cells, stomata, intercostal short cells, prickles, macro-hairs, silica bodies and costal cells are particularly useful for distinguishing between species (Table 2).

Intercostal long cells (Figure 2D)

The intercostal zone of the epidermis consists of two distinct sized cells. The larger cells are elongated horizontally and are relatively narrow vertically. These intercostal long cells can vary, in size, shape and undulations, vertically across a single intercostal zone (Figure 2D). Therefore the intercostal long cells adjoining the adjacent costal zone are often comparatively short and wide with markedly undulate walls and the long cells located centrally are longer and narrower with straighter walls (Ellis 1979).

The intercostal long cells of *H. turgidulum* and *H. dodii* have straight, not undulating walls. In *H. capense*, *H. leoninum* and *H. natalense* these cells are either irregular (slightly undulating), moderately, or deeply undulating. In *H. longum* walls of the intercostal long cells are moderately thickened and cuticular flanges are developed, whereas in *H. galpinii* the cell walls are unthickened with no cuticular flanges present.

Intercostal short cells (Figure 2C)

These cells, which generally alternate in horizontal rows with the much longer intercostal long cells are usually nearly equidimensional in shape. The silico-suberose couples or cork-silica cell pairs are intercostal short cells that occur in pairs; other short cells are solitary. For diagnostic purposes the frequency of the occurrence of short cells and the contrasting of their presence in the costal and intercostals zones are worth considering (Ellis 1979).

The basal member of the short cell pair is always the cork cell or suberous cell, whether it is a silico-suberose couple or not. A brief description of the changes that characterize the primary stages of differentiation of the cork-silica cell pairs was not studied in *Helictotrichon*, although these microscopic changes give a better understanding of the development of these taxonomically very important silica bodies (Ellis 1979).

Intercostal short cells in the material studied are paired short cells situated between long cells, and show some interspecific variation. The paired short cells in *Helictotrichon capense*, *H. dodii*, *H. longifolium*, *H. natalense* and *H. rogerellisii* are silico-suberose couples or cork-silica cell pairs and are crescentic, enfolding the silica cell, whereas those of *H. barbatum*, *H. leoninum* and *H. roggeveldense* are square to rectangular in shape.

Stomata (Figure 2D)

The subsidiary cells, as seen in surface view, can be used to classify grass stomatal complexes, which are located in the intercostals zones. To describe the stomatal complexes of Poaceae, the following terms are needed (Stace 1965; Ellis 1979).

The dome-shaped subsidiary cell type is referred to as ovoid, while the parallel sided type is rectangular. In Poaceae, the stomata are confined to the intercostal zones, which may, depending on the species, include one or more stomatal bands and each band may include one or more rows of stomata.

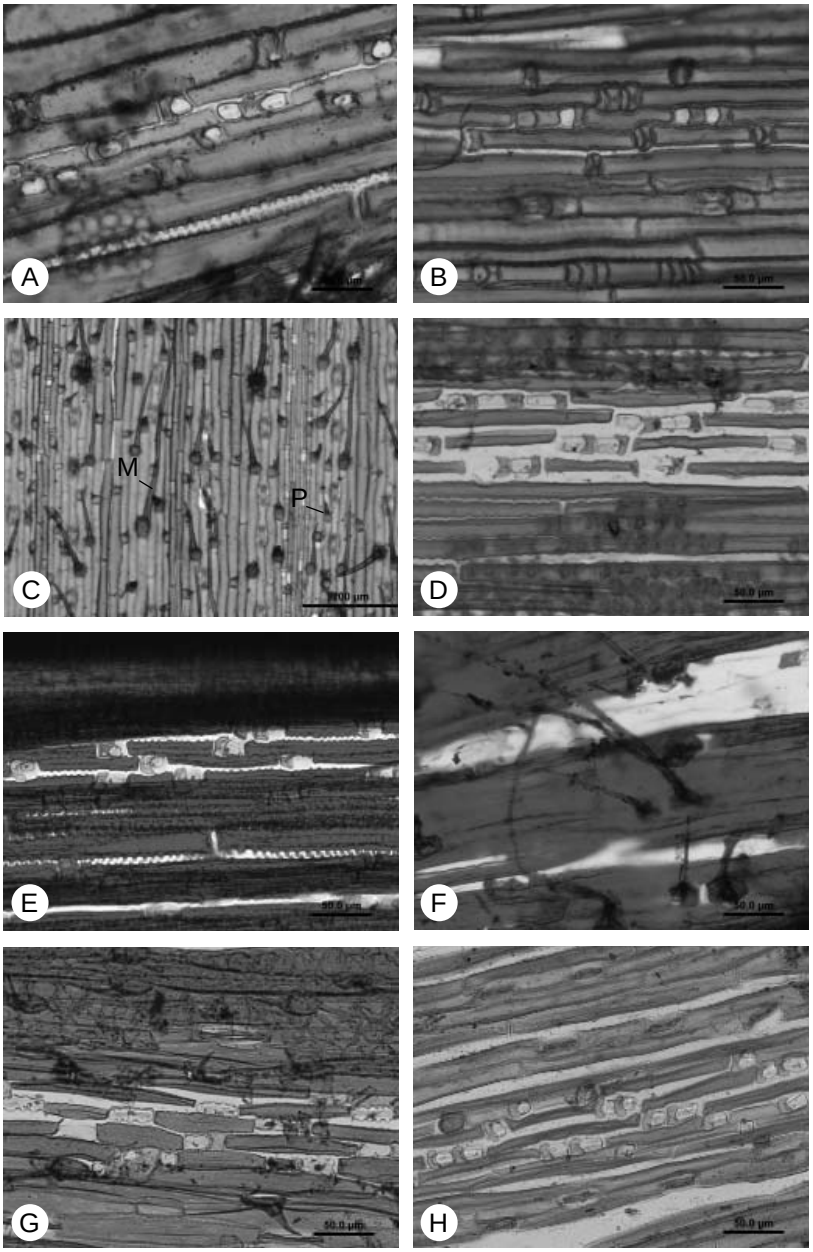

FIGURE 1.—Epidermal structures in *Helictotrichon*. A, *H. capense* (*Story 3803*); B, *H. dodii* (*Pole-Evans 499*); C, *H. galpinii* (*Ellis 1389*), P, prickle, M, macro-hair; D, *H. leoninum* (*Ellis 225*); E, *H. longifolium* (*Davidse 6962*); F, *H. longum (Acocks 20691)*; G, *H. namaquense* (*Ellis 5997*); H, *H. natalense* (*Rennie 1534*).

In all the *Helictotrichon* species studied, the stomata are either dome-shaped, that is the subsidiary cells are rounded, or have parallel-sided subsidiary cells so that they are rectangular in outline; or the stomatal complex is long and narrow. *Helictotrichon capense* and *H. dodii* stomata are tall dome-shaped as the vertical width of the subsidiary cells is greater in relation to the horizontal length. The rest of the species investigated have parallel-sided subsidiary cells.

Costal short cells (Figure 2C)

Usually the costal short cells are not the same shape or arranged in the same manner as those of the intercostal zones. It is therefore important in comparative work to ensure that there is no confusion by comparing intercostal short cells with costal short cells. Although the morphology of the costal short cells appears to be generally taxonomically more important than that of the intercostal short cells (Ellis 1979), both were examined in the present study.

When comparing the horizontal arrangement of the costal short cells, it was found that *H. capense*, *H. dodii*, *H. leoninum*, *H. longifolium*, *H. natalense*, *H. rogerellisii*, *H. roggeveldense* and *H. turgidulum* have silico-suberose couples with cork cells crescentic and enfolding the silica bodies. On the other hand, *H. barbatum*, *H. galpinii*, *H. hirtulum*, *H. longum* and *H. namaquense* have costal short cells where the silico-suberose couples have cork cells tall and narrow but the silica bodies are not tall and narrow (in comparison to those where the cork cells are the same shape as the silica bodies).

Prickles (Figure 1C)

These arise directly from, and form an integral part of the epidermis and are tough, shortly pointed structures with swollen bases and short, sharp spines or barbs. The relative differences in size and length of their barbs and bases are mainly used to distinguish different types of prickles:

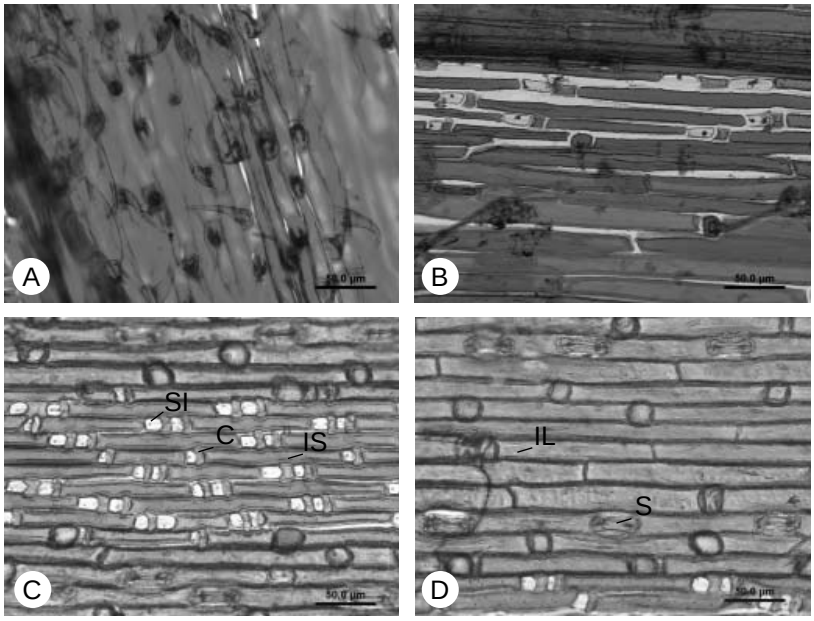

FIGURE 2.—Epidermal structures in *Helictotrichon*. A, *H. rogerellisii* (*Ellis 4663*); B, *H. roggeveldense* (*Acocks 17178*); C, *H. turgidulum* (*Spies 3953*); D, *H. turgidulum* (*Spies 3953*). SI, silica body, C, cork cell, IS, intercostal short cell, S, stoma; IL, intercostal long cell.

TABLE 2.—Characters used in describing the leaf epidermal anatomy of *Helictotrichon*

Species	**Leaf epidermal characters**													
	1 Intercostal long cells		2 Intercostal short cells		3 Stomata		4 Prickles		5 Macro-hairs		6 Silica bodies		7 Costal short cells	
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b
H. barbatum	X			X		X		X				X		X
H. capense		X	X		X			X				X	X	
H. dodii	X		X		X			X				X	X	
H. galpinii	X					X		X		X	X			X
H. hirtulum		X				X	X				X			X
H. leoninum		X		X		X		X				X	X	
H. longifolium		X	X			X	X					X	X	
H. longum	X					X				X	X			X
H. namaquense	X					X		X			X			X
H. natalense		X	X			X		X			X		X	
H. roggeveldense		X		X		X				X		X	X	
H. rogerellisii		X	X			X			X			X	X	
H. turgidulum	X					X		X				X	X	

1 = Intercostal long cells:
1a = walls straight not undulating.
1b = walls either irregular (slightly undulating), moderately, or deeply undulating.
2 = Intercostal short cells:
2a = silico-suberose couples or cork-silica cell pairs are crescentic, enfolding the silica cell.
2b = silico-suberose couples or cork-silica cell pairs are square to rectangular in shape.
3 = Stomata:
3a = are tall dome-shaped as the vertical width of the subsidiary cells is greater in relation to the horizontal length.
3b = subsidiary cells are parallel-sided.
4 = Prickle base:
4a = base angular.
4b = base elongated, oval or elliptic.
5 = Macro-hairs:
5a = apices hooked and known as crozier hairs.
5b = with single or many, usually small specialized epidermal cells associated with the base of the hairs.
6 = Silica bodies:
6a = elongated (horizontal dimension greater than vertical dimension).
6b = equidimensional (vertical and horizontal dimensions approximately equal).
7 = Costal short cells:
7a = have silico-suberose couples with cork cells crescentic and enfolding the silica bodies.
7b = the silico-suberose couples have cork cells tall and narrow but the silica bodies are not tall and narrow.
X = *Character state present.*

Prickles have elongated, oval or elliptical bases and are usually larger than hooks.

The angular prickles of the leaf margin comprise a distinct group and usually differ from the typical prickles occurring elsewhere on the same leaf.

The relative proportions of the barb length to the base size are gauged by projecting parallel lines through the apex of the barb and the opposite ends of the prickle hair base. The base size is assessed, in this study, by comparing it with the length of the stomata on the same leaf (Ellis 1979).

Barb size estimated by comparison with the length of the base: *Helictotrichon hirtulum* and *H. longifolium* both have the prickle base angular, but differ from one another in that the prickle barbs are shorter than the length of the base (short barb) in *H. hirtulum*, whereas in *H. longifolium* the prickle barbs are as long as or slightly longer than the base (medium barb length).

Base size estimated by comparison with the length of the stomata: *H. turgidulum* has small prickles (the base is shorter than the stomata), while in *H. dodii* the prickles are medium-sized (the base is as long as or slightly longer than the stomata).

Barb shape in relation to the base: When using this character it was found that *H. capense* has prickle barbs that are not developed from the apex of the conical base and are raised, while *H. leoninum* and *H. natalense* have prickle barbs developed basally from the apex of the base and slightly raised.

Macro-hairs (Figure 1C)

These are much longer than micro-hairs and prickles, varying considerably in length, even on a single leaf, in flexibility, wall thickness and how far the bases are sunken between the surrounding epidermal cells. The different macro-hair types, such as the crozier and the type of epidermal cells associated with the base of the hair, are taxonomically significant. These hairs arise from conical bases in the intercostal zones (Ellis 1979).

Helictotrichon rogerellisii has a specialized type of macro-hair with hooked apices known as crozier hairs, while the following have straight macro hairs and are distinguished from each other as follows: in *H. longum* the macro-hairs have many, usually small, specialized epidermal cells associated with the base of hair; *H. galpinii* and *H. roggeveldense* have macro-hairs with a single specialized epidermal cell associated with the base of the hair.

Silica bodies (Figure 2C)

Silica bodies, found in specialized epidermal cells, are discrete deposits of hydrated silica. This term is distinct from the term opal phytolith, which includes silica deposits sometimes present in other epidermal cells (Ellis 1979).

The shapes of typical silica bodies are very important taxonomically as they are characteristic for species (Ellis 1979). However, note that the shapes of the silica cells are not necessarily the same as the outlines of the silica bodies in which they are located. Various shapes of silica bodies have been recognized such as round, oval, oblong, linear, squarish or rectangular, as well as cresent-shaped or half-moon (Ellis 1979). In *Helictotrichon*, the shapes of silica bodies, which are normally described as seen in surface view, are round, squarish or rectangular.

The *Helictotrichon* species investigated here show a variety of silica bodies. *H. galpinii*, *H. hirtulum*, *H. longum*, *H. namaquense* and *H. natalense* have silica bodies that are elongated (horizontal dimension greater than vertical dimension); but they differ from one another in that those of *H. natalense* have rounded ends and smooth outlines; in *H. namaquense* the ends are rounded and the outline or shape nodular; and in *H. galpinii*, *H. hirtulum* and *H. longum* they have rounded ends and a sinuous outline.

Helictotrichon barbatum, *H. leoninum*, *H. rogerellisii* and *H. roggeveldense* all have silica bodies that are equidimensional (vertical and horizontal dimensions approximately equal). Those of *H. leoninum* have squarish or angular ends with irregular outlines, *H. barbatum* has square, cubical or slightly rectangular ones, whereas those of *H. rogerellisii* and *H. roggeveldense* are round (circular in shape), fitting into the concavities of the cork cells.

CONCLUSIONS

New studies of the genus *Helictotrichon* in southern Africa were necessary because much work is being done worldwide on the delineation of the genus, but hitherto little has been done on the southern African species (Mashau *et al*. 2010). Members of the genus in southern Africa are difficult to separate into species based on morphological characters alone. The anatomical features of the grass leaf epidermis have proved to be very useful in helping delimit the species and should be used in addition to the morphological characters.

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