Panicum ecklonii, a new record of a C_4 photosynthetic variant

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The anatomy of the leaf blåde of P. ecklonii Nees collected in the eastern Cape was investigated with light and transmission electron microscopes. The leaf blades of this material contain three orders of closely spaced vascular bundles, each of which is surrounded by a radiating mesophyll, and an underlying bundle sheath, which consists of relatively thick-walled, chlorenchymatous cells. Bundle sheath cells contain large, centrifugally arranged, agranal chloroplasts. The longitudinal bundles are separated by one layer of achlorophyllous mesophyll. The outer tangential and radial walls of the bundle sheath cells contain a suberized compound middle lamella. Few mitochondria occur in bundle sheath cells and are usually closely associated with plasmodesmatal pits in the outer tangential walls of the bundle sheath cells. Large bundles are subtended by ad- as well as abaxial hypodermal sclerenchyma strands. Intermediate bundles are connected to the adaxial hypodermal sclerenchyma strands by a single-layered bundle sheath extension, and the bundle sheath is in direct contact with hypodermal sclerenchyma strands abaxially. Small bundles are embedded in mesophyll tissue. Specimens of P. ecklonii from the eastern Cape thus display typical C_4 NADP anatomy.

Die anatomie van die blaarskyf van P. ecklonii Nees wat in die Oos-Kaap versamel is, is met behulp van lig- en transmissie-elektronmikroskope ondersoek. Die blaarskywe van hierdie materiaal bevat drie tipes van digopmekaargerangskikte vaatbundels, elk omring deur 'n uitstralende mesofiel en 'n onderliggende bondelskede wat bestaan uit redelik dikwandige chlorenchiemselle. Bondelskedeselle bevat groot sentrifugaal-gerangskikte korrellose chloroplaste. Die lengteverlopende bondels is geskei deur een laag mesofiel sonder chlorofil. Die buitenste tangensiale en radiale wande van die bondelskedeselle bevat 'n kurkagtige saamgestelde middellamella. Min mitochondria kom voor in die bondelskedeselle en is gewoonlik nou geassosieer met plasmodesmale holtes in die buitenste tangensiale wande van die bondelskede.

Keywords: C_4 photosynthesis, grass leaf anatomy, Panicum ecklonii

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Introduction

Panicum ecklonii Nees was described by Chippindall (1955) as being a densely tufted perennial which occurs in open grassland and disturbed ground in montane regions from the eastern Cape to the Transvaal in southern Africa, and northwards into tropical Africa. The plant is typically a constituent of mountain sourveld, and is especially frequent in valleys and on hillsides that are subjected to burning. *P. ecklonii* can apparently be confused with *Alloteropsis semialata* in the vegetative state (Chippindall 1955).

P. ecklonii was collected as part of the leaf structure/function studies being undertaken in our laboratories on veld grasses of the eastern Cape. Herbarium voucher specimens (*Phillipson 1155*, UFH) were collected in the Amatole Mountains, and are housed in the herbarium at the University of Fort Hare, Republic of Ciskei (duplicates at Rhodes University, Grahamstown). Routine investigation of transections of the leaf blade indicated that the grass appeared to be a C_4 photosynthetic type. This was surprising, in that previous studies on material from the Transvaal and Natal clearly showed such specimens to be C_4, non-Kranz types, lacking specialized bundle sheath chloroplasts (Ellis 1977). We thus decided to investigate the anatomy of the leaf in detail, in order to determine if the species which we had collected differed significantly from that described previously by Ellis (1977).

The results reported in this paper deal specifically with features which demonstrate conclusively that *P. ecklonii* collected in the eastern Cape, is a C_4 species, and possibly NADP-me.

Materials and Methods

Light and electron microscope studies

Plant material was collected near Menziesburg (30° 37′ 10″ S, 26° 53′ 35″ E) on recently burnt veld and established in pots in the greenhouse. Four weeks after establishment, mature leaf material was removed and diced into small (approximately 5×6 mm segments) and fixed in 6% glutaraldehyde in 0,05 M sodium cacodylate buffer at 4°C. The fixative was changed three times in a period of 8 hours. Leaf segments were subsequently diced into smaller segments (approximately 2×3 mm), washed in three changes of 0,1 M cacodylate buffer, and postfixed in 1% OsO_4 in 0,1 M cacodylate in a refrigerator overnight. Dehydration was accomplished using a cold graded ethanol series, followed by propylene oxide. Embedment was in Spurr's (1969) hard resin.

Thick (0,5 to 2,0 μm) monitor sections were cut with glass knives using an LKB V ultramicrotome. Sections were dried down on glass slides at 60°C and contrasted with 0,05% (w/v) toluidine blue at pH 7,2. Thin sections were cut with diamond knives (du Pont), picked up on copper grids and contrasted with uranyl acetate, followed by lead citrate and viewed using an Hitachi H-600 electron microscope at 75 and 100 kV.

Results

Figure 1 shows the distribution of *P. ecklonii* in southern Africa according to Chippindall (1955), amended to include more recent collecting sites (Ellis 1977, and this paper).

Brief description of the leaf blade

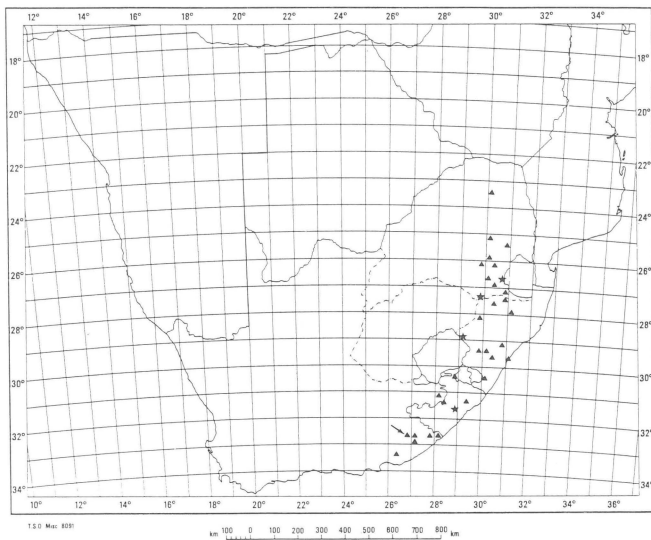The mature leaves of *P. ecklonii* are simple and slender and

Figure 1 Distribution of *Panicum ecklonii* Nees, modified after Chippindall (1955). Solid stars = Ellis (1977) records added. Arrow points to locality of specimens reported in this paper (*Phillipson 1155*, UFH).

are usually loosely to densely hairy. The leaves exhibit the Kranz structure typical of C_4 grasses — a concentric radial layering of the mesophyll and chloroplast-rich bundle sheath cells around the vascular bundles. The longitudinal bundles, together with their associated Kranz layers (the bundle sheath and the Kranz mesophyll) are separated radially from one another by no more than two layers of mesophyll, which, in some instances, may lack chloroplasts and are thus termed 'translucent cells' (Ellis 1976).

In addition to the single median bundle, three orders, or classes of veins can be recognized:
(a) Large bundles (Figure 2). These bundles are characterized by the presence of large metaxylem vessels which lie on either side of the protoxylem. The protoxylem is often represented by a lacuna. These bundles are subtended by a large sclerenchyma girder on the adaxial side and a prominent hypodermal sclerenchyma strand on the abaxial side of the vascular bundle.
(b) Intermediate bundles (Figures 2–4). These bundles lack large metaxylem vessels and protoxylem lacunae. Hypodermal sclerenchyma strands occur on both ad- and abaxial sides, or are associated with the abaxial side of the vascular bundle only.
(c) Small bundles (Figures 2 & 3). Apart from the lack of large metaxylem vessels and protoxylem, these bundles are not associated with either hypodermal sclerenchyma strands or girders.

It is immediately apparent that the small interveinal distances, (Takeda & Fukuyama 1971; Kawamitsu *et al.* 1985) with a maximum lateral cell count of 4 or less (Hattersley & Watson 1975) indicates that *P. ecklonii* is a C_4 grass. In contrast, *P. ecklonii* as described by Ellis (1977, see Figure 5) has a maximum lateral cell count of 8, which is correctly described as a C_3 variant based on Hattersley & Watson's (1975) criteria.

A conspicuous feature of transections of the leaves examined during the course of this study, is the presence of a number of intermediate longitudinal bundles which are associated with adaxial parenchymatous bundle sheath extensions (Figures 2 & 4). Similar bundle sheath extensions occur in the C_3 variant reported by Ellis (1977). These bundle sheath extensions are suspended by ad- as well as abaxial

hypodermal sclerenchyma strands (SS, Figure 4). The Kranz mesophyll appears to be more closely packed in this type of intermediate bundle, compared with intermediate bundles which lack bundle sheath extensions.

Light and electron microscope investigations clearly demonstrate the chloroplast dimorphism associated with C_4 plants. The chloroplasts in all bundle sheath cells examined are centrifugally arranged and agranal (Figures 3–4, 5 & 7). In contrast, Kranz mesophyll chloroplasts are generally smaller and contain conspicuous thylakoid stacks (Figure 7). A suberin lamella (SL, Figures 5–7) occurs in the outer tangential and radial walls of the bundle sheath cells. These lamellae take on a double 'tramline' appearance in concomitant radial bundle sheath walls (Figure 7). The suberin lamella is conspicuously swollen and polylamellate where plasmodesmata traverse the interface between bundle sheath and Kranz mesophyll cells (Figure 7). Plasmodesmata aggregates occur in deep pits (Figure 7) in the outer tangential walls of bundle sheath cells. Recent infra-red gas analysis studies conducted on *P. ecklonii* (Botha & Russell 1988) clearly lend support to the anatomical features described above and strengthen our contention that the *P. ecklonii* specimens collected in the eastern Cape are C_4 and possibly NADP-me.

Discussion

Apart from *Alloteropsis semialata*, which was previously described by Ellis (1974) and investigated further by Frean *et al.* (1983) *P. ecklonii* represents the only other grass species in which C_3 and C_4 forms have been identified, within what was previously reported to be a C_3 species. According to Ellis (1977) *Panicum* is the only genus within the Poaceae with representatives of all three sub-groups of the C_4 plants. As mentioned earlier, it is interesting to note that *P. ecklonii* and *A. semialata* can be confused in the vegetative state, more so since they grow in similar areas.

What is of relevance to the present authors is the fact that of the 20 *Panicum* species investigated by Ellis (1977), 8 were C_3, 8 were PCK, 4 were NAD-me, no NADP-me species being encountered during his study. *P. ecklonii* is thus a significant find, as it is the first record of a species which contains C_3 and C_4 forms within this genus in southern Africa. Based solely on anatomical characteristics, the C_4 variant reported here appears to be NADP-me. The C_4 *P. ecklonii* on which we are reporting, has however, not been biochemically typed yet.

Ellis (1974) has commented on the ecological significance of differing photosynthetic capacities (rates of CO_2 fixation) and differing abilities to withstand moisture stress in C_3 and C_4 plants. C_4 plants have higher photosynthetic capacities and are about twice as efficient in their water use compared to C_3 plants. C_4 plants appear to be better adapted to arid regions, which have high temperatures and light intensities (Smith & Brown 1973). The presence of a suberin lamella in C_4 NADP-me and C_4 PCK types — in outer tangential and radial walls of bundle sheath cells in NADP-me types, and in the outer tangential walls of bundle sheath cells only in PCK types, is thought to further adapt these plants to arid conditions in which they are commonly found (Botha *et al.* 1982; Carolin *et al.* 1973; Ellis 1974; Hattersley & Browning 1981; Hattersley & Perry 1984).

O' Brien & Carr (1970) suggested that if the suberin lamella between bundle sheath and mesophyll cells acted as a barrier to water transport, its presence would thereby enable the plant to survive dry conditions more easily than plants which lacked this structure. It has been shown that the suberin lamella may

force water transport to take a symplastic pathway through the plasmodesmata, from the xylem to the mesophyll in *Themeda triandra* (Botha *et al.* 1982). The suberin lamella appears to direct at least some water from the xylem, through the compound middle lamella between concomitant bundle sheath cells in *Zea mays* (Evert *et al.* 1985), *Saccharum*
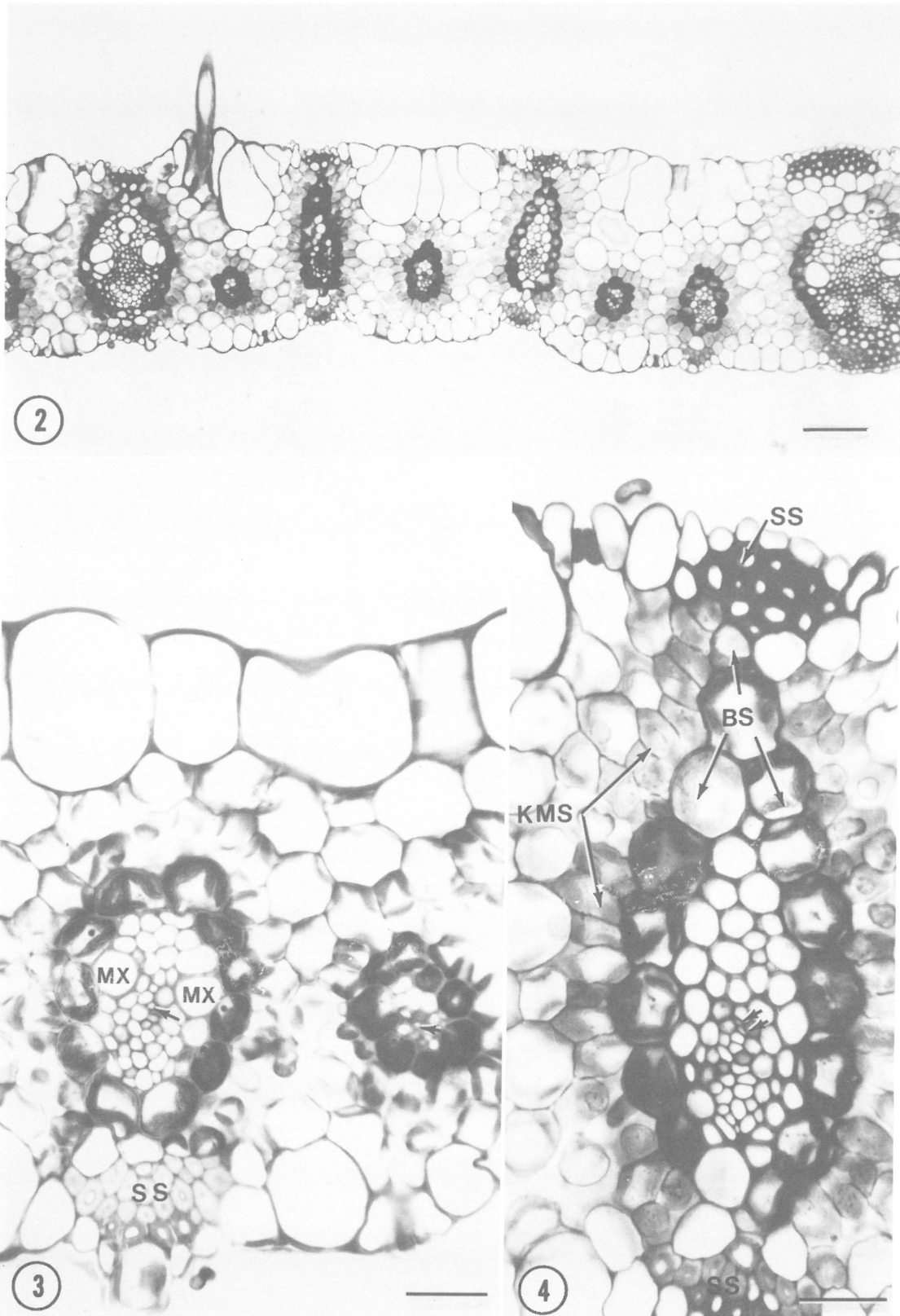
Figures 2 – 4 Photomicrographs of transections through the blade of *Panicum ecklonii* leaf, showing spatial arrangement of the vascular bundles. **2.** Low power light micrograph, showing median (extreme right), large (extreme left) as well as two intermediate bundles with prominent bundle sheath extensions. Note bulliform cells in adaxial epidermis, which overlie small veins. Small veins are completely embedded in mesophyll. Bar = 50 μm. **3.** Shows a small intermediate (left) and a small vein (right) and associated Kranz mesophyll tissue. Note hypodermal sclerenchyma strand on abaxial side of the intermediate vein. Large and intermediate veins lack a mestome sheath, metaxylem vessels (MX) abut bundle sheath cells. Protoxylem is lacking from this vein. Unlabelled arrows point to thick-walled sieve-tubes. Bar = 20 μm. **4.** Transection of an intermediate vein with a prominent bundle sheath extension, which abuts hypodermal sclerenchyma strand on the adaxial side of the leaf (SS). Kranz mesophyll cells (KMS) appear more closely packed in this vein than the intermediate vein shown in Figure 2. Paired arrowheads point to thick-walled sieve tubes. Bar = 20 μm.

officinarum (Botha & Evert 1986) and between the mestome sheath cells in *Bromus unioloides* (Botha & Evert 1986). Recently Canny (1986) suggested that the role of the suberin lamella is to keep separate the oppositely directed fluxes of water and assimilate that pass through the interface between

Kranz mesophyll and bundle sheath. It is possible however, that the suberin lamella may in addition create a CO_2-tight compartment which would prevent leakage in those species which have numerous intercellular spaces between bundle sheath and Kranz mesophyll cells. Thus the role of the suberin
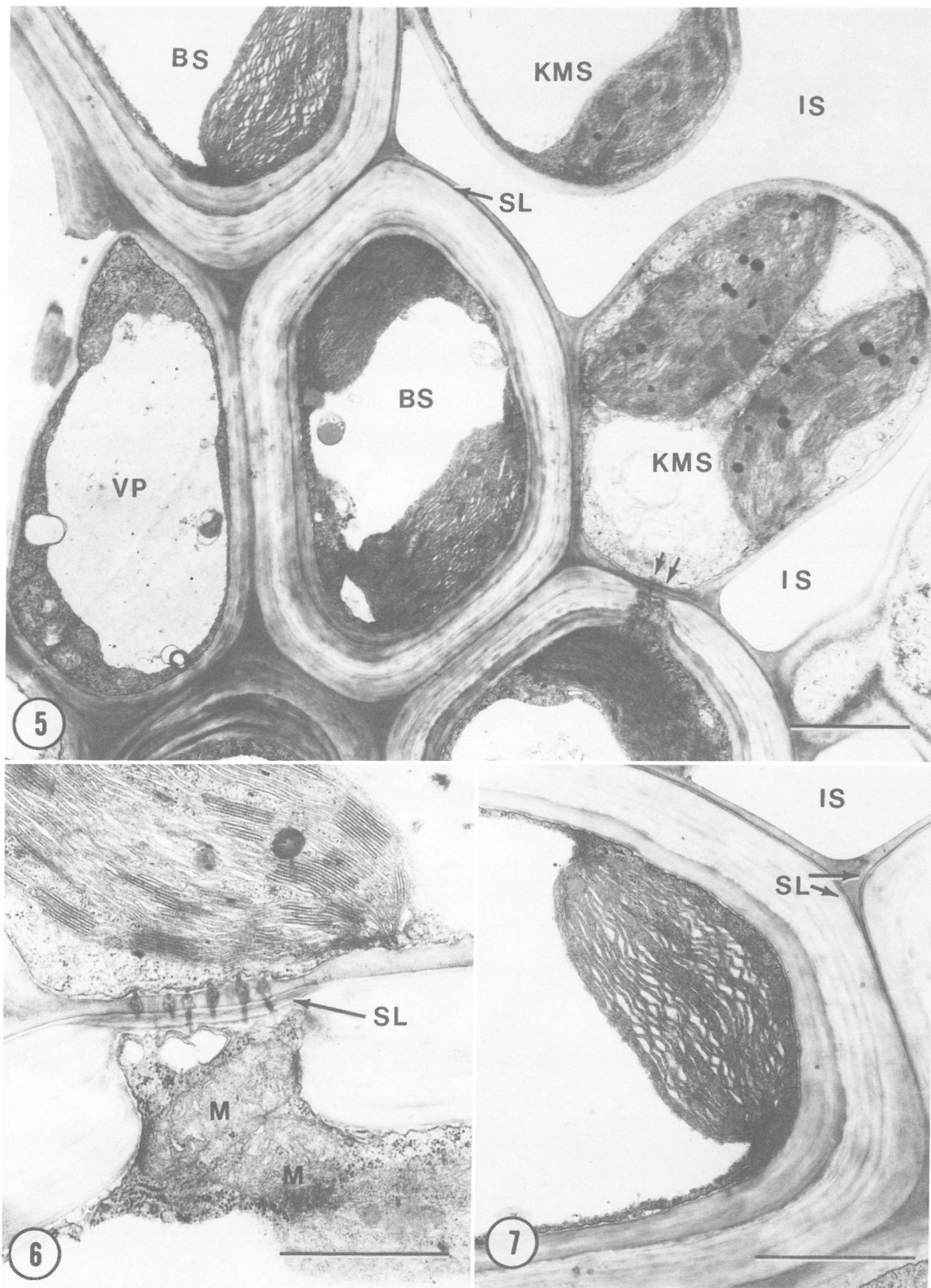
Figures 5 – 7 Show aspects of the ultrastructure of the longitudinal bundles in *P. ecklonii* leaf blades. **5.** Transection through part of the intermediate vascular bundle shown in Figure 3. Kranz mesophyll and bundle sheath chloroplasts are approximately the same size. Note the suberin lamella (SL) associated with the outer tangential and radial walls of bundle mestome cells. Large intercellular spaces (IS) occur between Kranz mesophyll cells. Paired arrows point to a plasmodesmatal pit-field at the Kranz mesophyll – bundle sheath interface. VP = vascular parenchyma. Bar = 2 µm. **6 & 7.** Transections from the same bundle as illustrated in Figure 5, sectioned through the Kranz mesophyll – bundle sheath interface, showing distinct polymorphism associated with the Kranz mesophyll (Figure 6) and bundle sheath (Figure 7) chloroplasts. Note almost complete lack of thylakoid membranes in bundle sheath cell chloroplast. Mitochondria (M, Figure 6) are spatially closely associated with the plasmodesmatal aggregate between bundle sheath (below) and Kranz mesophyll (above). Plasmodesmata are constricted where they pass through the swollen, shell-like suberin lamella (SL). Figure 7 shows 'tramline' appearance of the suberin lamellae in the radial walls between concomitant bundle sheath cells. Bars = 1 µm.

lamella may be more complex than it is presently thought to be.

Data presented by Ellis *et al.* (1980) in their analysis of the distribution of C₄ types in South West Africa/Namibia, presents a different picture. Ellis *et al.* (1980) clearly demonstrated that the relative frequency of NADP-me types increases with increasing rainfall. These authors showed that NAD species are dominant in drier areas, and that PCK species are intermediate in distribution. Ellis *et al.*'s (1980) results are supported by a more recent survey of Australian *Eragrostis* C₄ species (Prendergast *et al.* 1986). The latter authors reported that most of the dryland grasses investigated during the course of their study were NAD-me types.

Broader studies of ecological correlations with C₄ subtype distribution in the vascular plants are therefore necessary before firm generalizations can be made concerning the adaptive significance of the C₄ biochemical/anatomical subgroupings.

Acknowledgements

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