

Photosynthetic Pathways and the Geographical Distribution of Grasses in South West Africa/Namibia

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Analysis of floristic lists for South West Africa/Namibia shows that, throughout the territory, more than 95% of the grass species occurring in any given area display the C₄ photosynthetic pathway. Exceptions are areas in the north-east and south-west where between 5% and 18% of the grass species are of the C₃ type. The south-western district of Lüderitz falls within the winter rainfall area and it is only here that temperate C₃ genera are found. The C₃ species in the north-east belong to tropical groups. Most of the South West African C₃ grasses grow in specialized habitats and are either hydrophytes or sciophytes. Subdivision of the C₄ grasses into the three subtypes of the C₄ pathway reveals distinctive distributional trends. Malate formers or NADP-me species clearly become more abundant with increasing rainfall, whereas the aspartate formers show the opposite tendency. However, within the aspartate forming group the results show that it is specifically the NAD-me type of species which dominate in areas of very low precipitation, notably in the Namib and pre-Namib areas where rainfall is less than 200 mm/yr. The PEP-ck species form a group intermediate between the malate formers and the NAD-me grasses, especially as far as their water requirements are concerned.

'n Ondersoek van plantlyste van Suidwes-Afrika toon dat minstens 95 persent van die grasspesies in enige gegewe gebied die C₄-fotosinteseweg volg. Die noordoostelike en suidwestelike gebiede is uitsonderings waar tussen 5 persent en 18 persent van die grasspesies van die C₃-soort is. Die suidwestelike gebied van Lüderitz is 'n winterreënvalgebied en slegs hier word die gematigde C₃-spesies gevind. Die C₃-spesies van die noordoostelike gebied behoort tot die tropiese groep. Die meeste van Suidwes-Afrika se C₃-grasse groei in gespesialiseerde habitats en is òf hidrofiete òf skiofiete. Onderverdeling van die C₃-grasse in die drie subtypes vertoon opvallende verspreidingspatrone. Die groep wat hoofsaaklik appelsuur produseer, en ook NADP-mespesies genoem word, neem toe met toenemende reënval, terwyl die groep wat hoofsaaklik aspartiensuur produseer volop word met afnemende reënval. Binne die aspartaatgroep is dit egter die NAD-me-tipe wat domineer in gebiede met 'n baie lae reënval, veral in die Namib-woestyn en in die voor-Namib met 'n reënval van minder as 200 mm/jaar. Die PEP-ck-grassoorte is 'n groep tussen die malaatproduseerders en die NAD-me-spesies wat hul waterbehoefte betref.

It has been shown previously that South African grasses exhibiting either the C₃ or the C₄ photosynthetic pathway are, to a marked degree, geographically separated.¹ This large-scale distribution pattern indicates that C₄ grasses predominate over most of South Africa, and it is only in the winter rainfall region of the western Cape, and along the summits of the eastern Cape and Drakensberg mountains, that C₃ species increase in abundance. This distribution pattern suggests that low minimum temperatures during the growing season favour species with the C₃ pathway and that C₄ grasses were more successful when temperatures are higher. Similar conclusions are arrived at independently in an analysis of the North American grass flora.² Large-scale patterns of grass distributions, therefore, indicate that minimum temperature during the growing season is the environmental parameter having the strongest correlation with the relative abundance of C₄ grasses in a particular region.

Floristic surveys in Costa Rica³ and Kenya⁴ reveal that the highest proportion of C₄ grasses is found at low altitudes with high temperatures, high insolation and with a low incidence of available soil moisture. At moister, higher altitudes with no water stress, C₃ species replace the C₄ grasses. The data from these and other studies,^{5,6} as well as observational and experimental information on a few C₄ species,⁷⁻¹⁰ thus support the hypothesis that the C₄ pathway is adapted to arid areas.¹¹⁻¹⁷ These deductions are apparently contradicted by our observation that C₃ grasses dominate in the most arid regions along the west coast of South Africa.¹ For this reason we suggested that the prevailing temperature during the growing season was the most significant environmental factor limiting the relative success of the C₃ or C₄ pathway. The presumed dependence of C₄ plants on arid environments is further questioned by the observation that C₃ plants predominate in the non-grass component of the flora of the central Namib Desert.¹⁸ We are of the opinion, therefore, that large-scale distribution patterns do not corroborate extrapolations from limited studies of particular species or local habitats and that the role of water stress in the success of the C₄ pathway has not been adequately demonstrated. In order to investigate this apparent discrepancy further and to obtain more information on the optimum environmental requirements of the C₃ and C₄ pathways, we have undertaken a survey of the distribution of grass species in South West Africa/Namibia.

South West Africa has specific advantages in this respect. It covers a large area, extending from 17° to 29° south latitude. Temperatures throughout the territory are relatively uniform and fairly high with average summer maximum temperatures of about 30°C experienced throughout the region except for a narrow strip along the coast where maximum summer temperatures of about 20°C prevail.¹⁹⁻²³ The principal climatic variable is precipitation, which increases from less than 50 mm/yr in the south-west and

along the Atlantic coast to over 500 mm/yr in the extreme north-east.²⁴ The vegetation ranges from true desert communities to tree savanna and woodland in the north-east.²⁵

The climate of South West Africa is less complex than that of South Africa, which includes a wide range of climatic zones and experiences abrupt climatic changes over relatively short distances.²⁴ It was therefore considered that the gradual climatic changes in South West Africa, reflected mainly by a shallow moisture gradient, would be ideal for investigating the ecological requirements of the C₃ and C₄ pathways in the grasses. In addition, the distribution patterns of the three subtypes of the C₄ pathway that are now generally recognized, could also be examined and related to geographic patterns in precipitation.

Plants with the C₄ pathway, or Kranz syndrome, possess a wide range of physiological, biochemical, anatomical and ultrastructural characters that have been extensively studied and shown to be consistently related.^{26,27} It has therefore been possible to demonstrate that the detection of any one of a wide spectrum of characteristics is sufficient to identify the entire syndrome. The C₃ and C₄ photosynthetic pathways are also related to the taxonomy of the grasses and are not randomly distributed in the Poaceae, but are restricted, with few exceptions, to certain subfamilies and tribes.²⁶⁻³² The relationship of the C₃ and C₄ pathways to grass systematics is, therefore, well appreciated and the classification of the Poaceae can be helpful in predicting photosynthetic and other physiological characteristics.^{33,34}

This association of functional and structural features also applies to the three subtypes of the C₄ pathway.^{31,32} Since the diagnostic criteria of these groups are not widely appreciated, a brief discussion of their distinguishing characteristics is given below.

All C₄ grasses initially fix CO₂ in the mesophyll cells, which results in the formation of oxaloacetate which is subsequently converted into either malate and/or aspartate.³⁵⁻³⁷ Depending on the

relative quantities of malate and aspartate formed, two distinct subgroups of C₄ plants are recognized: malate and aspartate formers.

This biochemical distinction is associated with structural differences reflected in the leaf anatomy as illustrated in Fig. 1. Malate formers are characterized by a single chlorenchymatous, or Kranz, sheath, with centrifugal chloroplasts present around the larger vascular bundles (Fig. 1, 1-3).^{26,27,31,32} This condition is readily recognized as no cells are present between the metaxylem vessel elements and the laterally adjacent Kranz sheath cells of the primary, or first order, vascular bundles. In aspartate formers (Fig. 1, 4-9) a mestome, or inner bundle, sheath is situated between the metaxylem elements and the Kranz sheath.^{31,32} Ultrastructurally, the malate formers lack well-developed grana in the chloroplasts^{35,38,39} and have a low mitochondrial frequency.⁴⁰ In addition, malate formers do not exhibit a post-illumination CO₂ burst³⁶ as the aspartate formers do.

The malic and aspartic acids, formed by the first carboxylation reaction of C₄ photosynthesis, are transferred to the Kranz sheath cells where decarboxylation occurs, by the NADP-malic enzyme in the case of the malate formers.⁴¹ In the aspartate formers it has been shown that 70% or more of the decarboxylation activity is due to one or other of two enzymes, either PEP-carboxykinase or NAD-malic enzyme.^{41,42} Two subtypes within the aspartate formers can, therefore, be recognized. For convenience these are termed PEP-ck type and NAD-me type species, respectively. The predominance of either of these decarboxylating enzymes is also associated with anatomical differences in the plants.³² PEP-ck species have chloroplasts located centrifugally against the outer wall of the Kranz sheath cells (Fig. 1, 4-6), whereas the chloroplasts are centripetally located in NAD-me species (Fig. 1, 7-9). In addition the mesophyll of NAD-me plants consists of a single layer of distinct, radially arranged, narrow tabular cells (Fig. 1, 7-9), whereas in

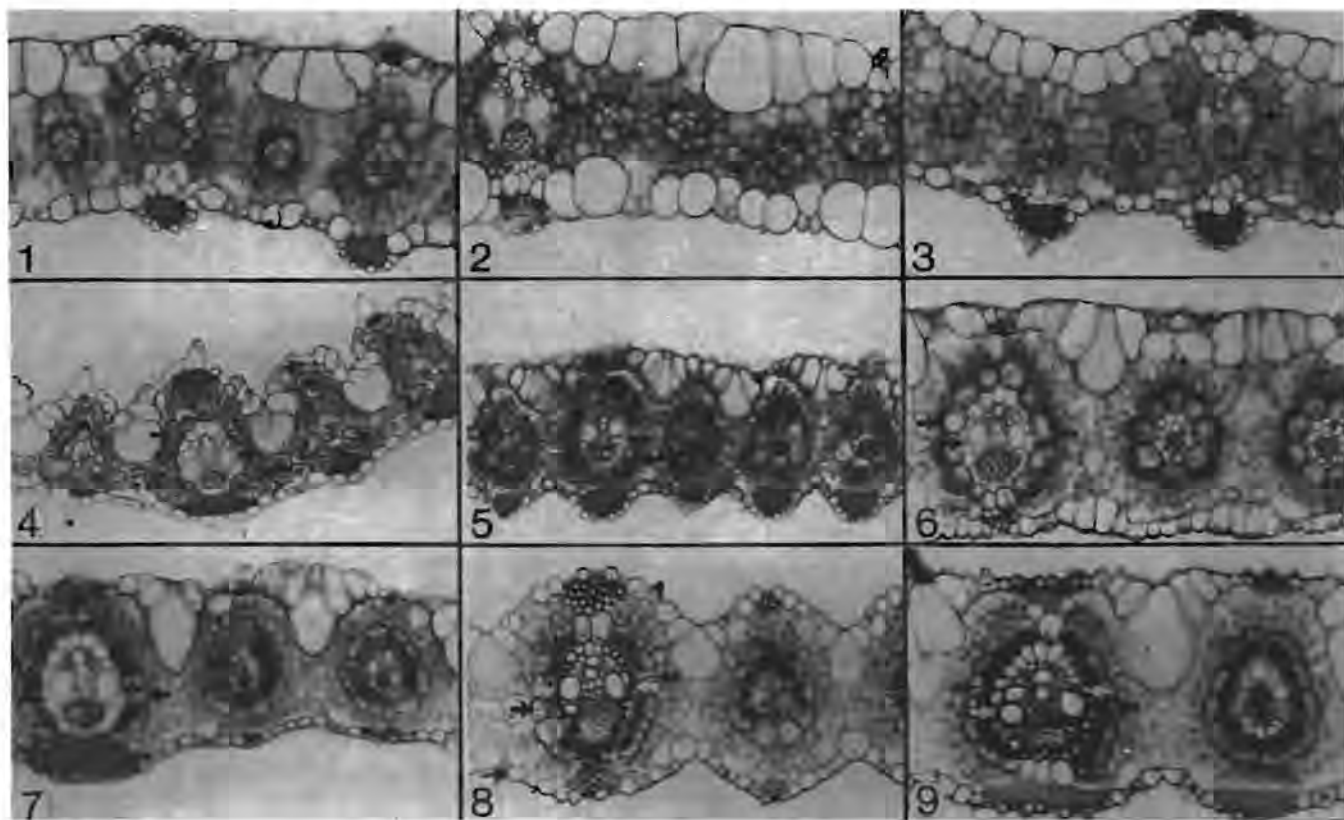


Fig. 1. C₄ leaf anatomy of selected South West African grasses. Note that all have a Kranz bundle sheath and a single layer of radially arranged mesophyll cells. 1-3, Malate formers with single, Kranz bundle sheath (arrowed). 1, *Antheophora pubescens*; 2, *Setaria sphacelata*; 3, *Digitaria eriantha*. 4-9, Aspartate formers with inner mestome sheath and outer Kranz sheath, i.e. double bundle sheath (indicated by arrows). 4-6, PEP-ck type species with specialized chloroplasts in centrifugal position: 4, *Eragrostis viscosa*; 5, *Rhynchelytrum repens*; 6, *Leucophrys mesocoma*. 7-9, NAD-me type species with centripetal chloroplasts in Kranz sheath cells. 7, *Eragrostis porosa*; 8, *Panicum arbusculum*; 9, *Monelytrum lüderitzianum*. Magnification $\times 150$.

Table 1. Relative ¹³C content with respect to the SMOW standard, δ, of some South West African grass species.

Species	Collector and specimen no.	Locality	Sample no. (M-)	Analysis no. (MC-)	δ ¹³ C (‰)
<i>Panicum brevifolium</i>	O. H. Volk 1870	Okavango R, SWA	1060	1642	- 27.2
<i>P. impeditum</i>	M. G. L. Mills 74	Kalahari Gemsbok Park, Cape	1055	1643	- 15.2
<i>P. lanipes</i>	W. Giess & M. Müller 13993	Okaukuejo, SWA	1059	1641	- 12.1
<i>P. pansum</i>	H. Merxmüller & W. Giess 1875	Grootfontein, N. SWA	1058	1639	- 11.2
<i>P. pearsonii</i>	K. L. Tinley 1391	Tsumeb, SWA	1057	1640	- 12.1
<i>P. schinzii</i>	W. Giess 8435	Karibib, SWA	1061	1645	- 12.1
<i>P. trichonode</i>	W. Giess 14954	Tsumeb, SWA	1056	1644	- 12.3
<i>Acrachne racemosa</i>	W. Giess 3187	Kaokoveld, SWA	1062	1646	- 12.9

PEP-ck species the radial arrangement is less regular and the chlorenchyma cells are larger and less elongated (Fig. 1, 4 - 6).

Our knowledge of the structural and functional features of these subdivisions of the C₄ group of plants is considerable but little or no information is available on the possible ecological implications of these differences in the mechanism of photosynthesis. No attempt has been made to examine the environmental significance of these structural and functional differences, although it is relatively simple to classify grasses into the three categories on anatomical grounds alone. We have made certain taxonomic deductions from published reports. Thus, from Tieszen *et al.*'s data it can be inferred³² that malate formers (all the Andropogoneae) require higher soil moisture than the aspartate formers (the Chlorideae, Eragrosteae and Sporoboleae). However, taxonomic criteria cannot be used to distinguish the subtypes of the aspartate group and anatomical or biochemical distinctions are required. This is because many genera include NAD-me as well as PEP-ck species.³²

Methods

The most recent floristic description of the grasses of South West Africa, in the *Prodromus einer Flora von Südwestafrika*,⁴³ was used as a basis for the present study. For each of the 347 grass species listed as occurring in the territory, the magisterial districts in which they were collected are given according to the Farm Map of 1950.⁴³ The lists of grasses compiled for each of the magisterial districts in this way were augmented with detailed checklists for specific areas.⁴⁴⁻⁴⁸ Collectors' lists of collections from Etosha Pan submitted for identification to the Botanical Research Institute, Pretoria, were also consulted because the *Prodromus* includes very few grasses from this district. The Lüderitz-North district (LUN) is a restricted diamond-mining area in which very little plant collecting has been undertaken, and consequently the species lists from here are incomplete. The Caprivi Strip was ignored as it falls within the region covered by the Flora Zambesiaca and was not included in the *Prodromus*.

Classification of the South West African grasses as being either C₃ or C₄ was based on either the leaf anatomy or the ratio of the carbon isotopes, ¹³C/¹²C. The relative isotope ratios of *Acrachne racemosa* and several *Panicum* species, for which data have not been published previously, are given in Table 1. The percentage of C₄ species in the total number of grasses was thus calculated for each of the magisterial districts (Fig. 2).

The C₄ grasses were further classified as being either malate or aspartate formers, and, if the latter, as either PEP-ck or NAD-me types. This subdivision was based solely on the anatomy and cytology of the bundle sheath and mesophyll cells,^{26, 27, 31, 32} of freshly fixed leaf-blade material or on published biochemical analyses.^{37-40, 42, 49, 50} The division into malate and aspartate formers therefore, was made on the basis of the presence of a single or a double bundle sheath, respectively. All the C₄ species could be classified as being either malate or aspartate formers even if anatomical material was not available in every case. This was because all grass genera indigenous to South West Africa comprise only aspartate or

only malate forming species. The genera which appear to include both malate and aspartate species (*Alloteropsis*, *Digitaria* and *Panicum*)³² are represented in the territory by species of one C₄ subtype only. The frequency of occurrence of species representing the malate and aspartate C₄ photosynthetic subtypes is expressed as a percentage of the total of C₄ grasses for the various districts and presented graphically in Fig. 3.

In this study the assignment of the aspartate formers to the PEP-ck and NAD-me subtypes was based on anatomical criteria such as Kranz chloroplast position, Kranz cell shape and mesophyll cell structure and arrangement,³² as well as on published biochemical findings.^{38-40, 42, 50} Taxonomically, these aspartate subtypes are not separated as clearly as are the malate and aspartate forming species and, therefore, extrapolation based on the classification of the grasses cannot be used to determine whether a given aspartate forming species is a PEP-ck or NAD-me type or not.³² Thus, *Eragrostis*, *Sporobolus* and *Panicum*, genera with many South West African representatives, have both PEP-ck and NAD-me representatives. Species for which no anatomical material was available could not therefore be classified beyond the aspartate

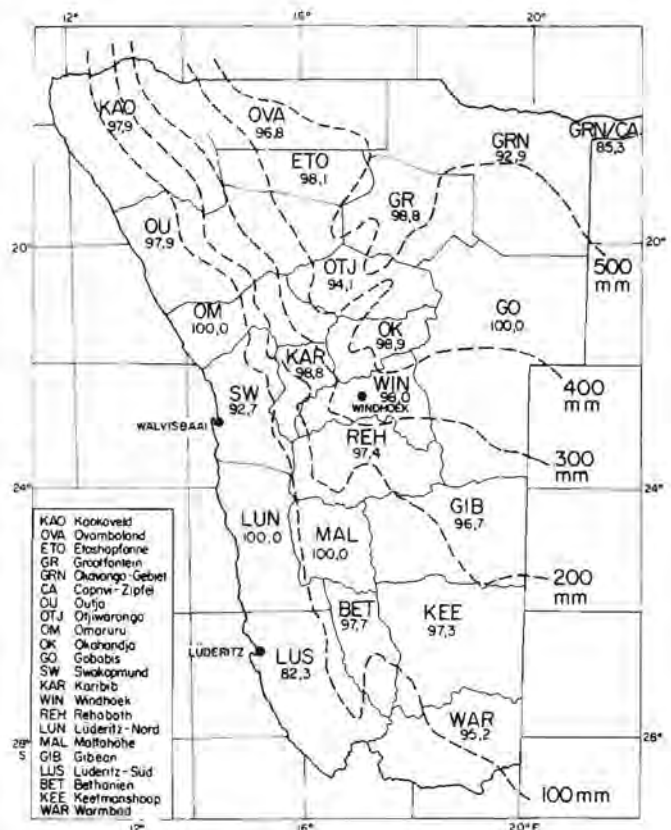


Fig. 2. Isohyets and magisterial districts of South West Africa. The numbers on the map refer to the percentage of C₄ grass species recorded for each magisterial district.

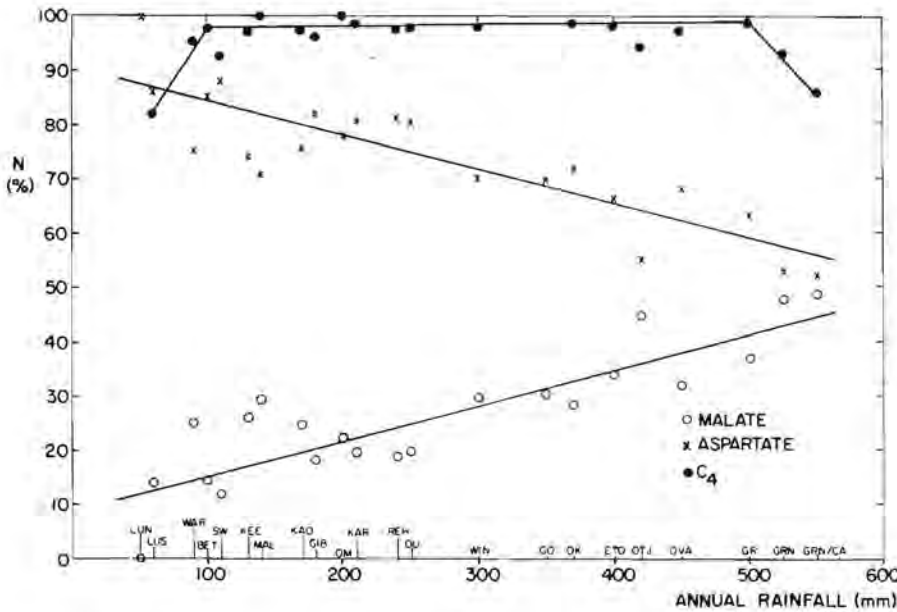


Fig. 3. The frequency (N) of C_4 grasses (●) expressed as a percentage of the total number of grass species, and the frequency (N) of malate (○) and aspartate (x) forming species expressed as a percentage of the total of C_4 grass species and plotted as a function of the estimated annual rainfall for each magisterial district of South West Africa. For abbreviations used refer to Fig. 2.

group. These species comprised 19% of the total of aspartate-forming grasses; the bars in Fig. 4 represent this uncertainty.

The average rainfall figures given for the magisterial districts in Figs 3 and 4 are of necessity only approximate, especially where a steep precipitation gradient is present. They nevertheless reveal the general rainfall pattern with sufficient accuracy for the present purpose.

Results and discussion

Distribution of C_3 grasses

Of the 347 grass species listed for South West Africa,⁴³ only 31 (8.9%) are of the C_3 type. As is evident from Figs 2 and 3 these C_3 species are concentrated in two widely separated areas—the extreme south-west and the extreme north-east—where they constitute between 5% and 18% of the grass flora. In the remainder of the territory less than 5% of the grass species are of the C_3 type.

The annual rainfall in the south-western Lüderitz-South (LUS) district is less than 100 mm, while that in the north-east exceeds 500 mm.²⁴ The rainfall, therefore, does not appear to influence the occurrence of C_3 grasses in the country. This observation corroborates our previous conclusions that precipitation is not an important factor in determining the relative success of C_3 grasses.¹ A more detailed examination seems warranted.

The C_3 grasses found in South West Africa are taxonomically ex-

tremely diverse. They are classified into 21 genera, eight tribes and five of the six subfamilies of the Poaceae—the sixth, the Eragrostoideae, being the only subfamily without any C_3 representatives. Three tribes, the Danthonieae, Paniceae and Aristideae, and one genus, *Panicum*, include both C_3 and C_4 species, as shown in Table 2.

This broad taxonomic spread of C_3 species is exceptional, as normally their representatives in any local grass flora reflect either a strong temperate or a tropical bias.¹ Thus, either tropical panicoids, aristidoids, bambusoids and oryzoids or the more temperate festucoids and danthonoids usually predominate. In South West Africa both temperate and tropical C_3 grasses are found (Table 2).

Our analysis shows, however, that these temperate and tropical species are separated geographically—the temperate grasses grow in the Lüderitz-South district, while the tropical C_3 species are present in the north-eastern districts of Okavango (GRN) and Ovamboland (OVA).

The extremely arid south-western district falls almost entirely within a winter rainfall region. The growing season therefore corresponds with the cooler months of the year, with an average daily maximum temperature of 17.5°C at Lüderitz in July.¹⁹⁻²³ These temperatures are below the optima for most C_4 plants³ and the winter minimum temperatures (Lüderitz: July average daily minimum 10.1°C) are probably sufficient to produce pathological

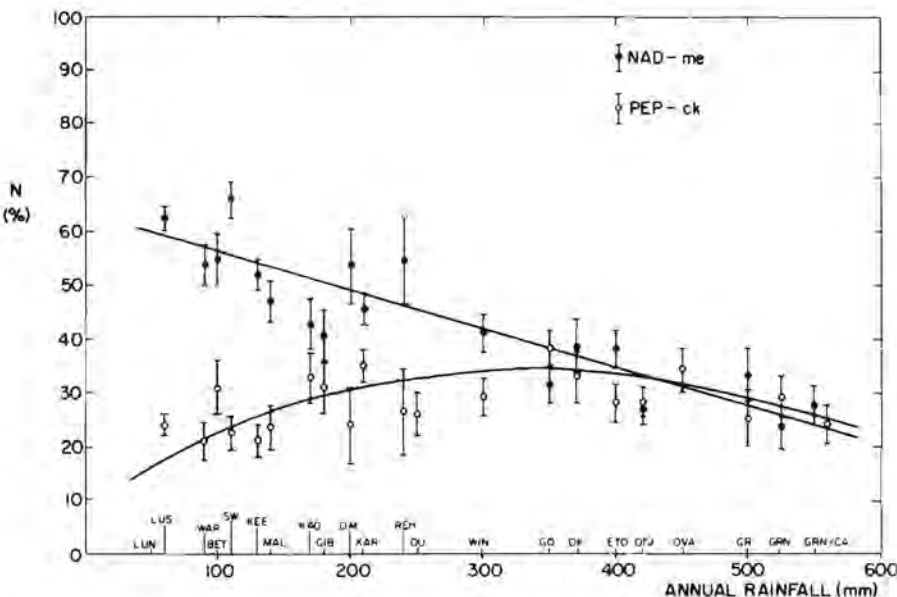


Fig. 4. The frequency (N) of NAD-me (⊥) and PEP-ck (○) species expressed as a percentage of the total of C_4 grass species and plotted as a function of the estimated annual rainfall for each magisterial district of South West Africa. For abbreviations used refer to Fig. 2.

Table 2. Taxonomy and ecology of the C₃ grasses of South West Africa/Namibia

Species	Tribe	Sub-family	Ecological notes
<i>Dregeochloa pumila</i>			Drought-tolerant species
<i>Merxmüllera rangei</i>			
<i>Pentaschistis patula</i>			
<i>Chaetobromus involucreatus</i>	Danthonieae		
<i>Schismus barbatus</i>			
<i>Karroochloa schismoides</i>		Arundinoideae	
<i>Ehrharta delicatula</i>			Restricted to the winter rainfall area in the south-west. Temperatures during growing season relatively low.
<i>E. pusilla</i>			
<i>E. triandra</i>	Ehrharteae		
<i>E. virgata</i>			
<i>E. longifolia</i>			
<i>Polypogon monspeliensis</i>			Annuals with short life-cycles and restricted to ephemeral but moist localities or perennials from wet habitats
<i>P. semiverticillatus</i>			
<i>P. minutiflorus</i>	Agrosteae		
<i>Agrostis lachnantha</i>		Festucoideae	
<i>Puccinellia angusta</i>			HYDROROPHYTES
<i>Poa heterogama</i>	Festuceae		
<i>Elytrophorus globularis</i>			HYDROPHILUS
<i>E. spicatus</i>	Danthonieae(?)	Arundinoideae	
<i>Phragmites australis</i>			TROPICAL Summer growing season with high temperatures. Primarily in the north-eastern districts
<i>P. mauritianus</i>	Arundineae		
<i>Arundo donax</i>			
<i>Oryza longistaminata</i>			TROPICAL
<i>Leersia hexandra</i>	Oryzeae	Oryzoideae	
<i>Acroceras macrum</i>			PANICEAE
<i>Sacciolepis typhura</i>			
<i>S. africana</i>			
<i>S. auriculata</i>			
<i>Oplismenus burmannii</i>	Paniceae	Panicoideae	SHADE-LOVING SPECIES
<i>Panicum brevifolium</i>			
<i>Sartidia angolensis</i>	Aristideae	Aristidoideae	GRASSLAND SPECIES

symptoms in C₄ species.² It is therefore not surprising that several perennial C₃ species are found in exposed positions in this district but nowhere else in the country. These are drought-tolerant plants and many exhibit specialized growth forms and leaf anatomy, as does *Dregeochloa pumila*, for instance.³¹ Such adaptations may enable these species to make use of moisture condensed from the frequent fogs which occur in the region.

All the C₃ grasses of this south-western winter rainfall area belong to temperate tribes and represent northerly extensions of the flora of the Cape Province where representatives of the Danthonieae, Ehrharteae, Agrosteae and Festuceae are common. Apart from a few members of the Danthonieae, which are perennials, the remainder are annuals with short life cycles. All the representatives of the Festuceae, Agrosteae and Ehrharteae belong in this category and, in addition, appear to avoid the harsh climate by occupying protected, often shady positions where moisture conditions are more favourable. This is so evident that all these species have been classified as hydrophytes, as they all may possess lacunae in the mesophyll of their leaves. If these plants are not found in association with open water, they appear to grow wherever sufficient water accumulates, when it rains, to enable them to complete their life cycles before the water has evaporated. Most C₃ species therefore occupy sites with mesic microclimates in this otherwise

arid region. Whether the increase in soil moisture or the lower temperatures of the growing season favour the occurrence of these C₃ grasses is not clear from the South West African evidence. Distribution data on temperate C₃ plants from South Africa¹ and elsewhere^{32,33} indicate that it is indeed the minimum temperatures which are the major selective factor in the success or otherwise of the C₃ pathway in grasses.

In the north-east, in the Okavango and Ovamboland districts, the C₃ species comprise more than 5% of the grasses (Figs 2 and 3). The C₃ grasses in this hot, summer rainfall area occupy specialized habitats and are either hydrophytes or obligative sciophytes (Table 2). The hydrophilous species are either perennials associated with permanent water bodies or ephemerals which grow in seasonally inundated sites. The perennials belong to the tropical tribes Oryzeae and Paniceae as well as the Arundineae. The classification of the annual, *Elytrophorus*, is uncertain. This genus has been placed in the Danthonieae,³⁴ although some authors consider it to be typically chloroid.³³ If the genus were to be placed in the Eragrostoideae, then it would be the only one of C₃ type in an otherwise completely C₄ subfamily.^{27,32}

The restricted and specialized habitats occupied by these tropical C₃ grasses help provide insight into the environmental conditions which limit the distribution of C₃ grasses, or, conversely, in

response to which the C_4 pathway has evolved. In a hot tropical region the habitats occupied by C_3 grasses have three factors in common: relatively high moisture regimes, reduced solar radiation and low leaf temperatures. In some of the aquatic communities where C_3 grass species predominate, for example *Phragmites*, this diminished irradiance may be due to self-shading effects.⁴

The oryzoid, bambusoid and tropical arundinoid C_3 grasses are characterized anatomically by the presence of arm cells in the mesophyll. They also have an outer bundle sheath of large, thick-walled cells, a feature shared with the C_3 panicoid species. This structure differs considerably from that of the temperate festucoid C_3 grasses, which have diffuse, irregularly arranged mesophyll and thin-walled parenchyma sheath cells, and may imply physiological and ecological differences between the two kinds of grasses. This hypothesis appears to be supported by the spatial separation of the tropical and temperate C_3 species into areas of different climate in South West Africa. Caution is needed when extrapolating data on temperate species to tropical C_3 plants such as the bamboos and rice.

The above discussion shows that, although the average annual precipitation does not constitute the limiting factor for the success or otherwise of C_3 grasses, they are nevertheless apparently restricted to environments where water stress is not severe. This is especially noticeable in an arid territory like South West Africa but, where moisture availability is not a restriction, low minimum temperatures during the growing season undoubtedly provide temperate C_3 grasses with a competitive advantage. Water availability combined with reduced radiation and leaf temperatures appear to be strongly associated with the relative abundance of tropical C_3 species in regions with hot, tropical climates.

Distribution of C_4 grasses

C_4 species dominate the grass flora throughout South West Africa (Fig. 2) and comprise more than 80% of the grass species present in any district. It is only in the north-eastern Okavango and in the south-western Lüderitz-South districts, that the abundance of C_4 species drops below 95% (Fig. 3). It appears, therefore, that C_4 grasses have considerable advantages over their C_3 counterparts throughout the territory and the prevailing conditions are undoubtedly more favourable for the C_4 photosynthetic pathway everywhere except in certain habitats of deep shade and abundant water supply as discussed above. The uniformly high summer temperatures in the territory are considered to be in the optimum range for C_4 photosynthesis ($30^\circ - 45^\circ\text{C}$)³⁵ and the high proportion of C_4 grasses found throughout the region (Figs 2 and 3) appears to confirm this hypothesis. Their abundance further suggests that C_4 grasses exhibit a wide tolerance of moisture conditions, from extremely arid (less than 100 mm/yr) to reasonably mesic (about 550 mm/yr)²⁴ in the woodland communities. This negligible effect of rainfall can be illustrated further by the high percentage of C_4 species along the Natal Coastal Belt,¹ an area experiencing precipitation in excess of 1000 mm/yr.

Restricted water availability in hot, tropical climates has been proposed as the primary environmental factor determining the relative success of plants possessing the C_4 photosynthetic pathway in these particular circumstances. Our results indicate, however, that C_4 grasses as a whole are common in areas with widely differing precipitation regimes. In order to examine more critically the role of moisture in determining the relative success of C_4 grasses, we have studied the distribution patterns of the subtypes constituting the C_4 pathway.

Distribution of malate and aspartate forming species

It is evident from Fig. 3 that the malate forming C_4 grasses are uncommon in the districts experiencing the least rainfall but steadily grow in number with increasing precipitation. The north-eastern districts, which receive the highest rainfall in South West Africa, have the greatest proportion of malate forming species—45% of

the total of C_4 grass species. Extrapolating from Fig. 3, a continuation of this trend can be predicted. Hence, to the north and east of the Okavango district, where the rainfall exceeds 750 mm/yr, malate forming grasses should be dominant. Floristic lists for the Lengwe National Park in Malawi⁵⁶ appear to confirm this prediction: 55% of the grass species in this area of 850 mm/yr rainfall are malate formers.

It has been demonstrated that the Andropogoneae in Kenya attain their highest development at intermediate levels of soil moisture.⁴ We assume that all andropogonoid grasses are malate formers.³² In Kenya it has been shown that they are replaced by C_3 species at higher moisture levels⁴ which, in this instance, are associated with high altitudes and thus lower temperatures. Malate forming C_4 grasses, therefore, appear to be most successful in conditions of minimal water stress but with high temperatures and irradiance. In South West Africa, where the temperature lowering effects of increasing altitude are not present, this is illustrated by the relatively large number of aquatic malate forming grasses in open water conditions in the north-eastern districts. Thus, 16 aquatic malate forming species are found there, belonging to the following genera: *Paspalidium*, *Paspalum*, *Echinochloa*, *Eriochloa*, *Miscanthidium*, *Vossia* and *Vetiveria*. These are all emergent hydrophytes in contrast to seasonally inundated soak-zone species⁵⁷ and occur in addition to 12 C_3 grass species and only a single aspartate former.

It appears that, given suitably high temperatures, malate forming grasses generally attain their greatest abundance in conditions of reduced water stress. The malate subtype of C_4 plants is undoubtedly not well represented in arid and semi-arid tropical regions—an environmental requirement generally associated with C_4 plants.

Aspartate forming C_4 grasses attain their maximum frequency in areas of low rainfall and decrease in abundance with increasing moisture availability (Fig. 3). This trend is also evident in Kenya, since the Chlorideae, Eragrosteae and Sporoboleae, all species which we can predict to be aspartate formers,³² are most abundant in conditions of low soil moisture availability.⁴ It is interesting to note that, where aspartate forming plants are found in aquatic habitats in South West Africa, they are invariably associated with high levels of salinity, for example *Odyssea*, *Oryzidium*, *Sporobolus* and *Diplachne*.⁵⁷

Distribution of NAD-me and PEP-ck type species

As the aspartate subtype of C_4 plants can be subdivided into NAD-me and PEP-ck categories, it is necessary to examine the distribution of each of these before generalizations can be made for the aspartate formers as such. Figure 4 illustrates the geographical distribution of PEP-ck and NAD-me grasses in South West Africa.

There are significant differences between the distribution patterns of NAD-me and PEP-ck species which are not evident in that for the combined aspartate formers. The frequency of NAD-me grasses increases from 25% of the total of C_4 species where rainfall is 550 mm/yr to 55% where the rainfall is less than 100 mm/yr. NAD-me grasses dominate in arid areas with low and unpredictable rainfall and high temperatures and radiation. It is, therefore, specifically this subtype which substantiates the belief in the adaptation to aridity of the C_4 pathway.

The PEP-ck subtype appears to be intermediate between the NAD-me type and the malate forming NADP-me type (Figs 3 and 4) with respect to moisture requirements. In South West Africa they achieve their maximum abundance in areas receiving rainfall of 350 mm/yr and decline when the precipitation departs from this level. Where rainfall is less than 350 mm/yr, PEP-ck species are generally found in relatively moist habitats such as along drainage lines or amongst boulder outcrops. In areas with rainfall in excess of 350 mm/yr, PEP-ck grasses once again are found primarily in more specialized habitats. *Panicum maximum* is a good example of a common PEP-ck species found in the north-eastern savannas or woodlands.⁴³ In these districts, this grass is almost exclusively restricted to growing beneath the canopies of the savanna trees.⁵⁸

From the results presented in this paper, the implications of this observation are that such semi-shade habitats must be more xeric than the open grassland. The reason, however, may be due to a factor completely unrelated to moisture.

In this study it was not possible to examine the undoubtedly important effects of nutrients on the distribution of grasses. Thus, nutrient drop from the tree canopy may also account for the relative abundance of aspartate forming PEP-ck species beneath the trees in a grass cover otherwise dominated by malate forming grasses.

It is of interest to note that the major anatomical difference between the PEP-ck and NAD-me aspartate forming grasses is the location of the specialized chloroplasts in the Kranz sheath cells. They are centripetal in NAD-me species and centrifugal in the PEP-ck group (Fig. 1).³² In this respect the PEP-ck species resemble the malate formers more closely than the NAD-me kind. Ultrastructurally, however, these centrifugal chloroplasts differ in that they are agranal in the malate forming grasses but contain grana in the aspartate formers.^{38,39} Mesophyll cell arrangement and structure in PEP-ck species bear strong similarities to the structure of the mesophyll in malate formers (Fig. 1). Except for the presence of a single or double bundle sheath, malate and PEP-ck grasses exhibit many anatomical similarities, so it is not surprising that these two subtypes initially become more abundant with increasing rainfall.

Summary and conclusions

Summer temperatures throughout most of South West Africa are remarkably uniform except for the narrow Atlantic coastal belt. These temperatures are relatively high and well within the optimum range for the C₄ photosynthetic pathway. Rainfall, on the other hand, exhibits a shallow gradient and increases in a northerly and easterly direction from less than 100 mm/yr to over 500 mm/yr.

Throughout South West Africa, C₃ species constitute a minor component of the grass flora. In the south-west, with winter rainfall and consequently relatively low temperatures during the growing season, temperate C₃ plants comprise up to 18% of the grass species present. In the north-east, with high temperatures during the growing season, the C₃ type once again makes up about 15% of the grass species. In the latter districts, however, tropical C₃ grasses are present, so that there is a distinct spatial separation of the temperate and tropical C₃ grasses in the territory. Growing season temperatures are, therefore, important in determining whether C₃ grasses with tropical or temperate characteristics are present in a particular area. In addition, high temperatures favour the presence of C₄ grasses.

C₃ grasses reach their maximum relative abundance in the most arid, south-western district of Lüderitz-South and, hence, it appears as if precipitation has no influence on the presence of these C₃ plants. However, closer examination of the C₃ grasses of this district showed that they favour relatively moist micro-environments. In the north-east, where C₃ grasses once again increase in abundance, the same high moisture requirements were observed for the tropical C₃ species. Thus it appears that grasses exhibiting the C₃ photosynthetic pathway are limited, to a large extent, to habitats with minimal water stress.

C₄ grasses are dominant throughout the territory, whether the climate is extremely arid or relatively mesic. In an environment of consistently high temperatures and irradiance during the growing season, the amount of rainfall has very little effect on the presence of C₄ species as a whole. However, the three subtypes of C₄ grasses show quite different distribution patterns which appear to depend on the rainfall. Malate formers are found to increase in abundance with increasing precipitation, whereas NAD-me species decrease. PEP-ck aspartate forming species are intermediate in their moisture requirements. In hot tropical climates, therefore, it can be predicted that NAD-me species will be more successful in truly arid areas, whereas malate forming NADP-me grasses will be more common in areas with reduced water stress.

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Research Letters

Effect of Silver Thiosulphate on the Senescence of Emasculated Orchid (*Cymbidium*) Flowers

Application of ethylene to orchid flowers induces anthocyanin formation in their gynostemium (columns) and labella (lips).¹ The production of this pigment, which signifies the onset of flower senescence, can also be observed after pollination;² emasculat-³ and the application of auxin;⁴ all of which stimulate the evolution of ethylene by orchid flowers. During shipment, the dislodging of pollinia (emasculatation) could result in sufficient quantities of ethylene being evolved either to damage flowers directly or to initiate the production of this hormone by intact flowers. This could result in all the flowers in a container being lost.⁵ With carnations, ethylene plays an equally important role with regard to the senescence and keeping quality of the cut flowers. Whether produced by or applied to these flowers, ethylene accelerates their senescence and brings on such symptoms as 'sleepiness'.^{6–8}

The longevity and keeping quality of cut carnations can be doubled, however, by treatment with silver thiosulphate.^{9,10} The advantage of using this compound, in which the silver is chelated to an anionic complex [Ag(S₂O₃)₂]³⁻, is that its mobility within plant tissue is greatly increased.¹⁰ Silver acts as an anti-ethylene agent¹¹ and this behaviour is retained by the silver thiosulphate complex, which completely blocks the ethylene surge normally preceding the onset of senescence in carnations.¹² In view of the similar effects of ethylene on the senescence of carnations and orchid flowers, the investigation reported here was carried out to

establish whether or not pretreatment with silver thiosulphate could increase the longevity of emasculated orchid flowers as it does for the carnation.

Flowers of a cultivar of *Cymbidium* (Jollity 'Golden Heritage'), derived by hybridisation, were used in this experiment. Large plants were left until the first 12 flowers on the spikes had opened fully. Flowers were severed from the spikes at the base of the pedicel and selected for uniformity of age and appearance. For each experiment the flowers were divided into four

Table 1. Senescence of *Cymbidium* flowers both with and without exposure to silver thiosulphate

Time (days after treatment)	Untreated flowers		Silver thiosulphate-treated flowers	
	Intact	Emasculated	Intact	Emasculated
0	-	-	-	-
3	0	2	1	1
6	3	4	1	2
9	4*	4*	2	3
12	4*†	4*†	3*	3*†

The degree of senescence was determined by evaluating the extent of anthocyanin accumulated (blushing) in the labella (lips) and the curvature of the gynostemium (columns). 0 = no effect; 1 = slight blushing, no curvature; 2 = moderate blushing, no curvature; 3 = moderate blushing, slight curvature; 4 = intense blushing, pronounced curvature. *Indicates the point at which the petals turned pink. †Indicates the point at which the petals began to curl.