A COMPARATIVE STUDY OF THE DISTRIBUTION OF C_3 AND C_4 SPECIES OF CYPERACEAE IN SOUTH AFRICA IN RELATION TO CLIMATE.

David Chuba

SUPERVISOR: Prof. W. D. Stock

Botany Department
University of Cape Town
Rondebosch, 7701, South Africa.

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ABSTRACT

In this study the contribution of the climatic factors such as temperature, altitude, and mean annual precipitation as well as phylogeny to the determination of the distribution of C_3 and C_4 genera of Cyperaceae in South Africa has been investigated. Multiple regression analysis was used to determine the climatic factor that best predicted percent C_4 distribution. To see if the C_4 syndrome was influenced by taxonomy, the percentages of C_4 species was determined for each of the subtribes and genera. The results show that the relationship between the climatic factors and percentage of C_4 Cyperaceae species is similar to what has been found for the grasses (Vogel et al., 1978). However C_3 species of Cyperaceae seem to be abundant in most parts of South Africa. Only minimum growing season temperature showed significant correlation ($P = 0.000391$) with percentage C_4. The presence of the C_4 syndrome is shown to have some consistency within subfamilies, tribes and genera.

INTRODUCTION

The family Cyperaceae has a worldwide distribution, consisting of more than 4000 species (Ueno and Takeda, 1992). These species occur under a wide range of ecological conditions growing in many different environments, with the majority being found in wet and aquatic habitats (Ueno and Takeda; 1992, Gordon-Gray, 1995, Sage et al, 1999). According to Gordon-Gray (1995), they are associated with estuarine, riverine lacustrine, bog (vleis) and other temporarily moist areas. Some occur in drier areas and are present in grasslands competing with grasses or colonising bare exposed hillocks (Gordon-Gray, 1995). A few others occupy shady niches in the transition zone from woodland to grassland or occur mainly as summer annuals in the thin, temporarily moist soil overlying rock outcrops (Ueno and Takeda, 1992, Gordon-Gray, 1995). There are also those that favour open forests glades as under storey plants (Gordon-Gray, 1995). A few are notorious weeds, among the worst being the 'water grass', *Cyperus esculentus* L and red nut grass, *C. rotundus* L (Gordon-Gray, 1995). Cyperaceae also grow in environments with abundant water (e.g. *Eloecharis*, Ueno et al, 1989). Cyperaceae therefore have significance in land use and management, for they are indicator plants of wetland areas which are now beginning to receive some attention in relation to water conservation (Gordon-Gray, 1995).

The distribution of the different species of Poaceae in different environments has been associated with their different photosynthetic types (or the ways in which they assimilate carbon dioxide) (Tieszen, et al., 1979, Teeri et al., 1980, Ueno and Takeda, 1992, Ehleringer et al., 1997). There are a number of ways in which plants assimilate carbon dioxide. One way is via the Calvin Benson photosynthetic pathway (C_3). Another way is through the Hatch-Slack pathway (C_4) (Kortschak et al., 1965). In the Calvin cycle the initial product of carbon dioxide fixation is the three carbon substance phosphoglyceric acid (PGA), whereas in the Hatch Slack pathway, a four carbon molecule oxaloacetic acid is the first carboxylation product. Because of this difference in the number of carbon atoms in the first carboxylation product, the two modes of photosynthetic pathways are termed C_3 and C_4 metabolism respectively. The C_3 and C_4 photosynthetic pathways are species specific and C_4 and C_3 (with a few distribution of C_4 and C_3 grasses in South Africa have yielded interesting results suggesting that it is the low temperatures during the growing season that favours C_3 species and high temperatures during the growing season that favours C_4 species. The C_4 or Kranz plants show a marked tropical affinity, a fact which according to Kortschak et al. (1965), has given rise to the belief that the Kranz anatomy is an adaptation to a tropical environment.

According to Ehleringer, et al. (1997), the quantum yield for CO_2 uptake (light use efficiency) of C_3 and C_4 species depends on temperature. The differences in this dependence on temperature are related to the observed plant distributions. So far light use efficiency is the only mechanism proposed as determining the distributional differences in C_3 and C_4 monocotyledonous plants (monocots) (Ehleringer, et al., 1997). From models of C_3 and C_4 light use efficiencies Ehleringer et al. (1997) proposed that C_4 dominated ecosystems expanded when CO_2 concentrations reached very low levels that existed during the Quaternary. Evidence from bog and lake sediment cores and pedogenic carbonates support the hypothesis that C_4 ecosystems were more extensive during the last glacial maximum in the Quaternary and then decreased in abundance following deglaciation as CO_2 levels increased (Ehleringer, et al., 1997).

Their high photosynthetic efficiency together with economy in water utilisation have led to the proposal that aridity was also an important adaptive advantage of C_4 plants (Vogel et al., 1978, Ehleringer et al., 1997). Bjorkman and Berry (1973) for example demonstrated experimentally that C_4 species of *Atriplex* were more tolerant to drought than the C_3 species of the genus. A strong correlation has also been found between the C_4 pathway and summer annuals in the desert environments of the South Western United States and Mexico (Mulroy and Rundel, 1977). However earlier, Kortschak et al. (1965), noted that the wide spread occurrence of C_4 taxa in Poaceae did not seem to be accompanied by a similar predominance of C_4 species in other families. For example they found that while all the grasses of the Namib desert were of the C_4 type and only 20% of the other plants investigated were Kranz or CAM, most of them being succulent CAM species (Kortschak et al., 1965). Other surveys of the exceptions) plants generally differ in their leaf anatomy (Kortschak et al., 1965). For C_4 plants, the vascular bundle is surrounded by a distinct thick-sheath (Kranz) of cells containing specialised chloroplasts which differ in size, shape, or number, from the chloroplasts of the mesophyll. The mesophyll cells are radially arranged and are either in direct contact with a Kranz cell or at the most one cell removed from it (Kortschak et al., 1965). In the Kranz cell, the malate and aspartate are decarboxylated and the resulting carbon converted to sugars and starch via the normal Calvin cycle. In C_3 plants there is no specialised sheath and the mesophyll cells show no regular pattern of arrangement. The two photosynthetic pathways are therefore designated Kranz and non-Kranz for C_4 and C_3 respectively (Kortschak et al., 1965). Both of these two modes of photosynthesis have been identified in about 14 different families including the family Cyperaceae, species of which exhibit both C_3 and C_4 types of photosynthesis.

In a bid to investigate what natural habitats where originally responsible for the evolution of the C_4 pathway, Kortschak et al. (1965) considered that a possible approach was to investigate the distribution frequency of C_3 and C_4 species over a large region covering a wide range of climatic zones. Since then a number of studies (Ellis, 1974; Teeri and Stowe, 1976, Teeri et al., 1980; Ueno and Takeda; 1992, Ehleringer et al., 1997) have been conducted to try and determine the environmental factors that influence the distribution of C_4 and C_3 plants. Vogel et al. (1978) also conducted a survey in which they found that C_3 and C_4 species of Poaceae, in South Africa, are separated geographically to a marked degree. They also found that C_4 species where more abundant than the C_3 species in many parts of the country. This study (Vogel et al. 1978) also showed that C_3 grasses are predominant in the winter rainfall areas of the Western Cape and the Drakensberg and other mountain ranges in the Eastern Cape. They also suggested that C_3 species grow better under low temperature conditions, while C_4 species grow well under high temperature conditions. Related to these findings, is the observation that the non Kranz form of the grass species *Alloteropsis semialata* (R. Br.) is restricted to higher and cooler areas of South Africa, whereas the Kranz form of the species occurs mainly at lower warmer altitudes (Ellis, 1974). The studies indicated above (Vogel et al., 1978) on the vegetation in certain desert environments however have shown C_4 species to be rare in comparison with C_3 or CAM (Crassulacean Acid metabolism) plants (Vogel et al. 1978). Many CAM plants are succulents capable of utilising both the C_3 and CAM modes of photosynthesis (Vogel et al., 1978). The distribution map from Vogel et al. (1978) suggests that high temperatures and aridity, rather than a tropical environment, are the most likely factors that provide the selective pressure in the development of C_4 pathway.

Such studies as the ones above have not been done for sedges in South Africa. Because of their habitat preferences, the sedges need to be better known (Gordon-Gray, 1995), and it is interesting to see whether their distribution pattern in South Africa is also under the influence of similar environmental factors as those influencing the distribution C_3 and C_4 species of grasses (Vogel et al., 1978). It is also interesting to see whether the widespread occurrence and predominance of C_4 taxa of Poaceae, in South Africa, is also manifested by Cyperaceae species. The aim of this study is to investigate whether the distribution of C_3 and C_4 genera of Cyperaceae in South Africa, is determined by climatic factors such as temperature, altitude, mean annual precipitation and rainfall seasonality pattern or merely by phylogenetic relationships.

Such studies on the whole would contribute to the understanding of the environmental conditions that determine the distribution of C_4 and C_3 species of plants and also the natural habitats that were responsible for the evolution of the C_4 pathway (Vogel et al., 1978). This understanding of C_3/C_4 plant distribution will also be basic knowledge for studies on the distribution, evolution and migration of both invertebrates and vertebrates feeding on these plants (Ehleringer et al., 1997).

METHODOLOGY

Plant materials

Herbarium specimens from the Bolus Herbarium were used to determine the C_3/C_4 status of 68 species of Cyperaceae. In an initial survey, the geographic distributions of all the available specimens of Cyperaceae in the Bolus Herbarium were ascertained from the literature (Bond and Godblatt, 1984; Arnold and de Wet, 1993) to determine which species occur either in winter rainfall, summer rainfall or both rainfall regions, in South Africa (including Lesotho and Swaziland). All specimens for which material could be obtained in the Bolus Herbarium (i.e. those with more than one specimen) were selected for the study. All the available specimens shown from the initial survey to be winter rainfall species, were used for the study since they were few (only about 16). Samples were obtained by clipping off a small part of the leaf (approximately 9mm^2) or a small part of the culm in leafless species. The specimens were placed in small plastic vials, on a specimen tray. The specimens used in the study, with the specimen vouchers are listed in table 1.

Table 1: Specimens used in the current study. The tribal classification is after Ueno and Takeda, (1992) and Bruhl (1995). All the specimens are deposited in the Bolus Herbarium, Department of Botany, UCT.

TRIBE	**GENERA AND SPECIES**	**Specimen Voucher**
Schoeneae	*Chrysithrix junciformis* Nees	Stokoe s.n.
Schoeneae	*E. cernuus* Levyns	Levyns R.M., 8873
Schoeneae	*Epischoenus complanatus* Levyns	Esterhuysen, E., 11575
Schoeneae	*E. dregeanus* (Boekeler) Levyns	Levyns R.M., 9379
Schoeneae	*E. lucidus* (C.B. Clarke) Levyns	Esterhuysen, E., 11312
Schoeneae	*E. villosus* Levyns	Esterhuysen, E., 16927
Scirpeae	*Ficinia gydomontana* Arnold	Esterhuysen, E., 27706
Scirpeae	*F. angustifolia*	Esterhuysen, E., 26427
Scirpeae	*F. acuminata* (Steudel) Nees	Levyns R.M., 11212
Schoeneae	*Neesenbeckia punctoria* (Vahl) Levyns	Levyns R.M., 8328
	Trianoptiles capensis (Steudel) Harvey	Levyns R.M., 7762
	T. Solitaris (C.B. Clarke) Levyns	Levyns R.M., 34682
	T. stipitata Levyns	Levyns R.M.,7678
Schoeneae	*Tetralia compacta* Levyns	Levyns R.M., 8726
Schoeneae	*T. exilis* Levyns	Levyns R.M., 6229
Cypereae	*Lipocarpha chinensis* (Obs.) Kern	Jeague 520
Cypereae	*L. hemisphaerica* (Roth) Goetgebeur	Rev. F. A. Rogers, 6024
Cypereae	*L. nana* (A.Rich) Cherm.	C. S. Clarke, 6025
Cypereae	*L. rehmannii* (Ridgley) Geotghebeur	Bolus, H., 4529
Cypereae	*Ascolepis capensis* (Kunth) Ridgley	Bolus, H., 3944
Cypereae	*Cyperus amabilis* Vahl.	Schupers, J.J.,893
Cypereae	*C. compressus* L.	de Winter, B. & Giess 6889
Cypereae	*C. corymbosus* Rottb	Maputaland Expedition, 14319
Cypereae	*C. fulgens* CB Cl. var. fugens	Rev. Lawson, s.n.
Cypereae	*C. leptocladus* Kunth	Maputaland Expedition, 14316
Cypereae	*C. natalensis* Hoscht	Hood, 860
Cypereae	*Pycreus flavescens* (L.) Reichb	Pegler, A., 1089
Cypereae	*P. polystachyos* (Rottb.) Beau. Var. laxiflorus	H. Bolus, 1326

Cypereae	*P. pelophilus* (Ridgley) C.B. Cl.	Gibbs Russell, G.E., 2076
Cypereae	*M. keniensis* (Kuenketh.) Hopper	J. Burtt-Davy, s.n.
Cypereae	*M. laxiflorus* Turrill	Rev. F. A. Rogers
Cypereae	*M. vestitus* (Hochst. ex Krauss) C.B. Cl.	
Cypereae	*Alinula paradoxa* (Cherm.) Goetghebeur &Vorster	Joseph Burtt-Davy, 1749
Cypereae	*Kyllinga elatior* Kunth	Hood, 3993
Cypereae	*K. nemoralis* (J. R. & G. Forst.) Dandy	
Cypereae	*K. pulchella* Kunth	
Scirpeae	*Fuirena ciliaris* (L.) Roxb. var ciliaris	Rev. Rogers, F.A., 13216
Scirpeae	*F. leptostachya* Oliv. var nudiflora C.B. Cl.	C. F Swynnerton, 16027
Scirpeae	*F. abcordata* O.L. Forbes	Maputaland Expedition, 14313
Scirpeae	*F. pachyrrhiza* Ridley var. pachyrrhiza	Flanagan, H.G., 970
Scirpeae	*F. stricta* Steud	Schlecter, 3194
Scirpeae	*F. umbellata* Rottb	Rev. Rogers, F.A., 13277
Scirpeae	*Schoenoplectus confusus* (N.E. Br.) K. Lye subsp. natalitus J. Browning	L. Gibbs, 106
Scirpeae	*S. corymbosus* (Roth. ex Roem & Schult.) J. Raynal var. brachyceras (A. Rich) K. Lye	Rev. Rogers, F.A., 6431
Scirpeae	*S. praelongatus* (Poir.) J. Raynal	H. Bolus, 9476
Scirpeae	*S. pulchellus* (Kunth) J. Raynal	Geo Potts, 1076
Scirpeae	*Eleocharis atropurpurea* (Retz.) Presl	H. J. Schlienben, 6398
Scirpeae	*Fimbristylis longiculmis* Steud.	Rev. F. A. Rogers, 4563
Rhyncosporeae	*Rhyncospora rubra* (Lour) Makino subsp. africana J. Raynal	
Schoeneae	*Tetraria natalensis* (C.B.Cl.) Koyama	Rev. Rogers, F.A., 19183
Trilepideae	*Coleochloa setifera* (Ridley) Gilly	Esterhuysen, E., 21464
Sclerieae	*Scleria angusta* Nees ex Kunth	J. M. Wood 3863
Sclerieae	*S. greigifolia* (Ridley) C.B. Cl.	F. OO. Stohr, 427
Sclerieae	*S. rehmannii* C.B. Cl.	H. Bolus, 1893
Sclerieae	*S. transvaalensis* E.F. Franklin	Meyer, N. L., 15
Cariceae	*Carex spicato-paniculata* C.B. Cl. x C.zuluensis C.B. Cl.	Schlecter, R. 4759
Cariceae	*C. subinflata* Nelmes	Wolley Dod 3467
Cypereae	*Cyperus longus* L. var. longus	Schelchter, R., 3925
Cypereae	*Cyperus longus* L. var. lenuiflorus (Rottb.) Boeck	H. Bolus, 3926
Cypereae	*C. maginatus* Thumb.	Ward, C.J., 12292
Scirpeae	*Eleocharis limosa* (Schrad.) Schult.	Flanagan, H.G., 903
Scirpeae	*Fucinia stolnifera* Boeck	Levyns, R.M., 6563
Scirpeae	*Isolepis fluitans* (L.) R. Br.	Dummer, R.A., 1620
Scirpeae	*I. tenuissma* Nees	Esterhuysen, E., 28973
Cypereae	*Cyperus laevigatus* (L.) C.B. Clarke	Bolus, H., 715
Cypereae	*Mariscus congestus* (Vahl.) C.B. Cl.	A. O. D. Mogg, 11683
Cypereae	*Pycreus nitidus* (Lam.) J. Raynal (P. Lanceus)	Tyson, W., 16011
Cariceae	*Schoenoxiphium sparteum* (Wahlenb.) C.B. Cl.	Pegler Alice, 1196

Photosynthetic status

All the species selected for the study, from different areas of South Africa, were examined for their photosynthetic (C_3/C_4) status. To do this the ratios of the carbon isotopes ^{13}C and ^{12}C were determined (Vogel et al., 1978). The use of this method is based on the existence of carbon in three isotopic forms ^{12}C, ^{13}C, and ^{14}C (Van der Merwe, 1982), and their strong fractionation during photosynthesis when plants metabolise carbon dioxide (Park and Epstein, 1960). The first two isotopes (^{12}C and ^{13}C) are stable, but ^{14}C disintegrates radioactively over time (Van der Merwe, 1982). All three isotopes react chemically in the same way but because their atoms differ in atomic weights and are of different sizes, they react at different rates (Van der Merwe, 1982). Because of this different chemical and metabolic processes change the ratios between the atoms in ways that are unique and characteristic for each photosynthetic type (Van der Merwe, 1982). To specify the differences in the isotopic composition between different photosynthetic types, the ^{13}C /^{12}C ratio of a given sample is compared in a mass spectrometer with the ratio of an agreed standard (Van der Merwe, 1982). The difference between the standard and the sample is known as the relative ^{13}C content, designated by δ, and is measured in parts per thousand, or per mil (‰) (Van der Merwe, 1982). Thus if a sample of carbon proves to have a ^{13}C /^{12}C ratio which is less than that of the standard by 5 per mil, it is said to have a $\delta^{13}C$ value of –5‰ (minus 5 per mil). The commonly agreed reference for the $\delta^{13}C$ measurements (the Chicago PDB marine carbon standard) is derived from a piece of cretaceous marine fossil, *Belemnitella americana,* obtained from the peedee formation in South Carolina (Craig, 1957). However this piece of carbonate has long since been used up and current measurements are only related to this fossil through secondary standards prepared by the National Bureau of Standards. Samples with $\delta^{13}C$ values more negative than -20‰ are all C_3 plants while those more positive than –16‰ are C_4 (Vogel et al., 1978).

For the current study, carbon isotope analysis was done on two duplicates from each of the 68 Cyperaceae species studied. The materials were ground in liquid nitrogen in small plastic vials using a glass rod and a small metal wire of about 1mm diameter. Samples between 0.05 and 0.08mg were weighed out in 8 x 5mm tin foil cups, using a Sartorius 7-place microbalance. These were then rolled into balls and placed on a sample tray. Standards were also weighed (0.05- 0.08mg for Nasturtium standard (NASTD) and 0.04- 0.06mg for Australian National University sucrose (ANUsuc)) and examined after about every 7 samples. One hundred and sixty six samples, including standards, were sequentially combusted in a Carlo Erba NA1500 elemental analyser and interfaced via an open slit to a Finnigan MAT 252 mass spectrometer (Bremen Germany).

The discrimination values were standardised and expressed in ‰, using the following equation:

$$\delta^{13}C = (R_{sample}/R_{std} - 1) \times 1000$$

where $\delta^{13}C$ is the isotope ratio in delta units to the PDB standard and R_{sample} and R_{std} are the ratios of the sample and standards respectively. The expression $(R_{sample}/R_{std} - 1)$ is multiplied by 1000 to allow expression in ‰. The main advantage of adopting the carbon isotope composition method is that dried herbarium specimens can be used whereas other methods depend upon the availability of fresh leaf material.

Geographical distribution of C_4 and C_3 species

To determine the geographical distribution of C_4 and C_3 species of Cyperaceae in South Africa, distribution maps for the species under study were generated using the PRECIS database and MAPPIT software of the National Botanical Institute, Pretoria, South Africa. Maps of South Africa showing the grid squares (Fig. 1) and the provincial boundaries were photocopied on A4 sized transparencies and distribution points for at least six species, were marked on each of eight maps, by placing these overheads on top of the distribution maps for the species and tracing the points. The resulting combined species distribution maps in conjunction with climatic and rainfall maps (Schulze et al., 1997) were used for determining the geographical distribution of C_4 and C_3 species of Cyperaceae. These maps were also used for determination of rainfall pattern, altitude, temperature during the growing season, and mean annual precipitation values for each of the species as explained below. Numbers and percentages of C_4 and C_3 species where calculated for each grid square on the map and for each of the different rainfall regions. In order to determine the climatic factors that influence the relative floristic abundance of C_4 and C_3 species of Cyperaceae in South Africa, the ratios of the number of C_4 species to the total number of members of Cyperaceae (C_4 percentage), in the different parts of South Africa (i.e. following grid squares), were examined in terms of various climatic variables as explained below.

Rainfall Seasonality

Rainfall seasonality was determined using maps from Schulze et al. (1997). To confirm which species fall into particular rainfall seasons, The combined species distribution maps made above were successively placed over the rainfall seasonality map and the range of each species with regard to rainfall regime determined. The seasons were divided into All year, winter (June, July August, September) early summer (December) mid summer (January), late summer (February) and very late summer (March to May) rainfall regions (Fig 1) according to Schulze et al. (1997).

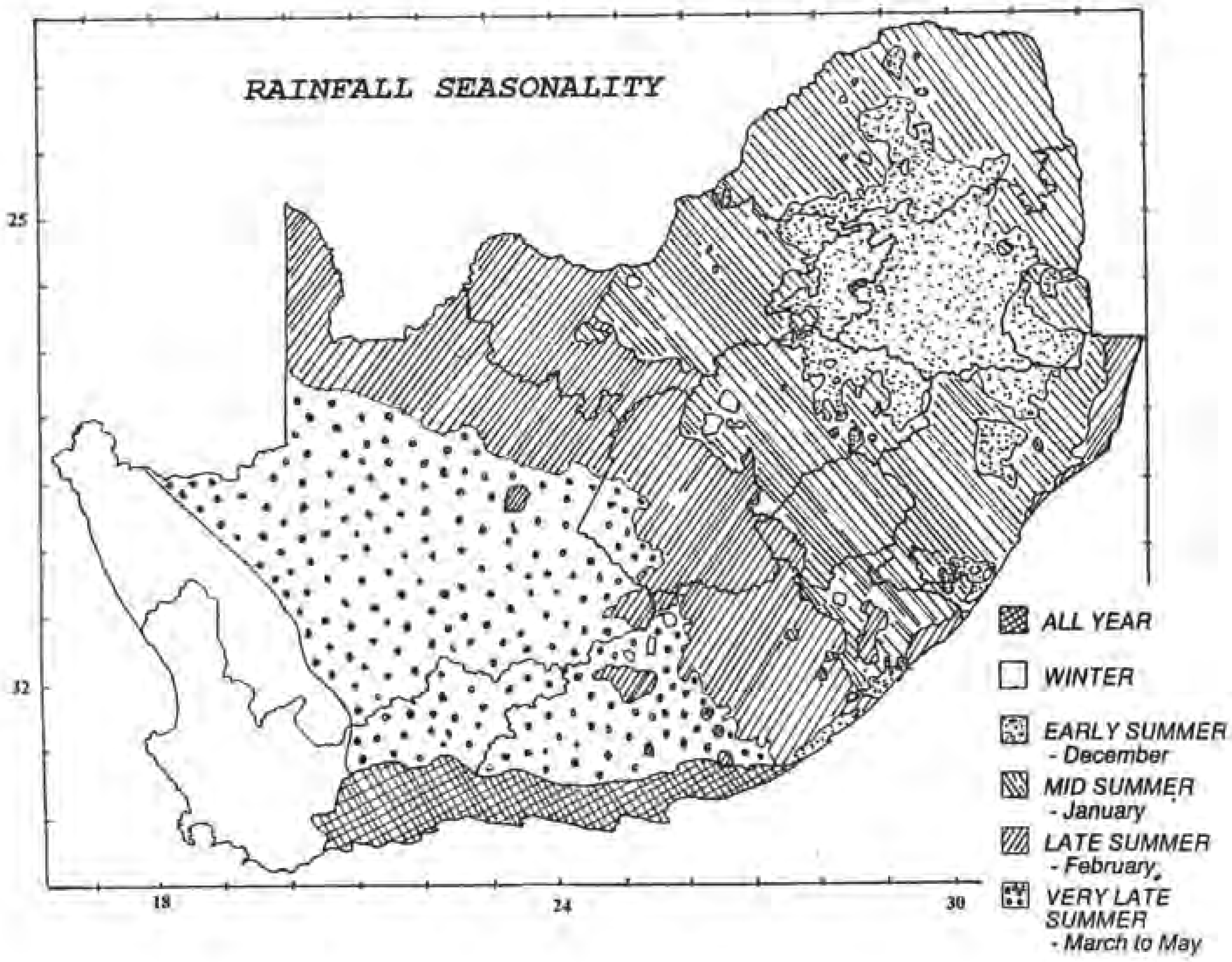

Fig. 1: Map of South Africa showing the different rainfall seasons. The months given are months with peak rainfall concentration.

Mean annual precipitation

The mean annual precipitation was determined by placing distribution maps of species over the map of mean annual precipitation (Schulze et al., 1997) and noting the maximum and the minimum values for each species according to species distribution. The mean annual precipitation in South Africa ranges from less than 100mm to greater than 1200mm. The maximum and minimum mean annual precipitation was also determined for each of the 2-degree grid squares within the map.

Temperature

To determine the effect of growing season temperature, the means of daily maximum and daily minimum temperatures were obtained using the climate maps from Schulze et al. (1997). For the particular distribution of each species, the appropriate growing season months as indicated above were used for determining the temperatures (i.e. June to August for winter rainfall season and between December and May for the different types of summer rainfall regions). This was done by placing the combined distribution maps over the temperature maps, and noting the maximum and minimum temperatures for each species. The numbers and percentages of C_4 and C_3 species in each of the grid squares was also determined using the combined species distribution maps.

Minimum temperatures for C_4 and C_3 Transition:

With reduced CO_2 levels (i.e. current levels) the process of photorespiration, which is a temperature dependent process, reduces the quantum yield for CO_2 uptake to levels below maximal values. Therefore we would expect, under normal conditions, that at higher temperatures C_3 plants would show reduced quantum yield. Theoretically as temperature increases, the percentage C_3 is expected to decrease and at a certain level it will be 50% (i.e. equal numbers of C_3 and C_4 species). The temperature at which the percentage C_4 is just below 50% is the switch over or transition temperature. Ehleringer et al. (1997) defined transition temperature as the point at which C_4 abundance fell below 50%. For the South African species of Poaceae (Vogel et al., 1978), this was estimated to be 25°C. In this study the minimum growing season temperature was significantly correlated with C_4 percentage and therefore this was examined by plotting a regression line of minimum temperature versus percentage C_4 to see if a transition temperature was clearly defined for the C_4 species of Cyperaceae.

Altitude

To determine the altitudinal distribution for each species the combined distribution maps were placed over the altitude map from Schulze et al. (1997) and the maximum and minimum elevation noted for each species. The altitude distribution in South Africa ranges from 0 to greater than 2500 m above sea level. The minimum and maximum altitude was determined for each of the grid squares within the map. In certain cases the altitude range within one grid square was very wide due to the occurrence of a number of altitude different elevations within the same Grid Square. In some therefore it was necessary to check which part of the grid square was occupied by the species present within the grid square.

Phylogeny.

The phylogeny of suprageneric groups in Cyperaceae has recently been examined by Muasya et al. (1998). Their study while supporting the existing monophyletic groups indicated a broader circumscription of Cyperus, which was found to form a polytomy with *Juncellus*, *Kyllinga* and *Pycreus*. Muasya et al.'s (1998) study also showed uncertainty in the generic and subtribal status of the Scirpeae, which appeared as polyphyletic. They did not however make any taxonomic changes and therefore in this study, the taxonomy used is that of Ueno and Takeda (1992) and Bruhl (1995). To see if the C_4 syndrome was determined by taxonomy, the numbers of C_4 species was determined for each of the subtribes and genera. The species of *Trionoptiles* for which the subtribal status could not be ascertained were excluded.

Data analysis

To determine the climatic variable(s) which contribute most to the prediction of the percentage C_4 species of Cyperaceae in the different parts of South Africa, Statistical analysis was done using a stepwise multiple regression analysis as implemented in STATISTICA software (StatSoft, Inc., 1996). The percentage C_4 was the dependent variable while the minimum and maximum temperature, altitude, and maximum mean annual precipitation were the independent variables. Previous studies have suggested correlation of these factors with percentage C_4 (Teeri et al., 1980; Ueno and Takeda; 1992; Ellis, 1974; Ehleringer et. al., 1997; Teeri and Stowe, 1976.). Multiple regression was done using the forward stepwise regression method. The Forward stepwise regression proceeds by selecting the variable that shows the highest correlation with the dependent variable, then it includes in the regression, the variable that gives the highest F statistic, provided that F statistic is greater than the chosen limit in this case (F = 0.0001). As variables are added, any variable whose partial F falls below this limit is removed from the regression. This process continues until none of the remaining variables provides a substantial (partial $F > 0.0001$) improvement in fit (StatSoft, 1984-1995).

The regression equation is in the form

$$\mathbf{Y = B_0 + B_1 * X_3 + B_2 * X_1 + B_3 * X_4} \textbf{ e.t.c.}$$

where B_0 = the intercept,

B_1, B_2 e.t.c. = raw regression coefficients.

X_1, X_2, e.t.c. = independent variables contributing to the regression.

Further analysis (Linear regression analysis) was done for the variable that showed significant correlation with percentage C_4.

RESULTS

Geographical distribution of C_4 and C_3 species

There was a clear gap in the $\delta^{13}C$ values, showing the distinct difference between the C_3 and the C_4 species (Fig 2). Of the species studied 38% had C_4 photosynthesis. Interestingly C_3 species of Cyperaceae seem to be abundant in most parts of South Africa with the percentage of C_4 ranging from 14% in grid square 3 (part of CapeTown) to 67% in Grid square 8 (Near Umtata) and gird square 25 (parts of Richards Bay and Ulundi). The frequency distribution of C_3 and C_4 species in each of the grid squares is shown in Fig. 3. The lowest percentages of C_4 were therefore recorded in the winter rainfall areas of the western Cape and the highest in some parts of the summer rainfall areas of the Kwazulu Natal and the eastern Cape. In the summer rainfall region of South Africa the percentages of C_4 showed a range of 22 to 67% whereas in the winter rainfall areas it ranges from 14 to 40%. The species that were confined to the winter rainfall areas were all C_3 species whereas those that were confined to the summer rainfall regions included both C_3 and C_4 species.

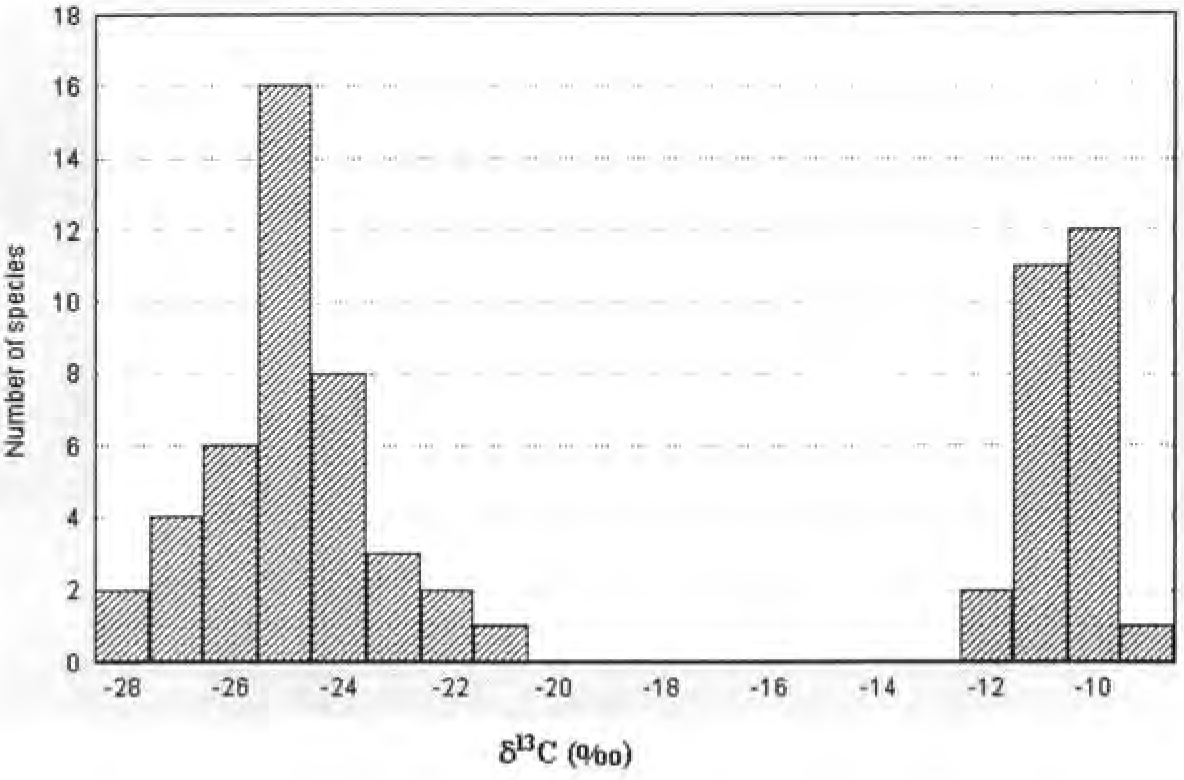

Fig 2: Histogram of relative ^{13}C content (δ^{13}C values) of 68 species of Cyperaceae indicating a clear distinction between C_3 and C_4 species. The δ^{13}C values for C_4 species range from –8.16 to – 12.31 ‰ whereas those of C_3 species range from –29.24 to –21.20 ‰

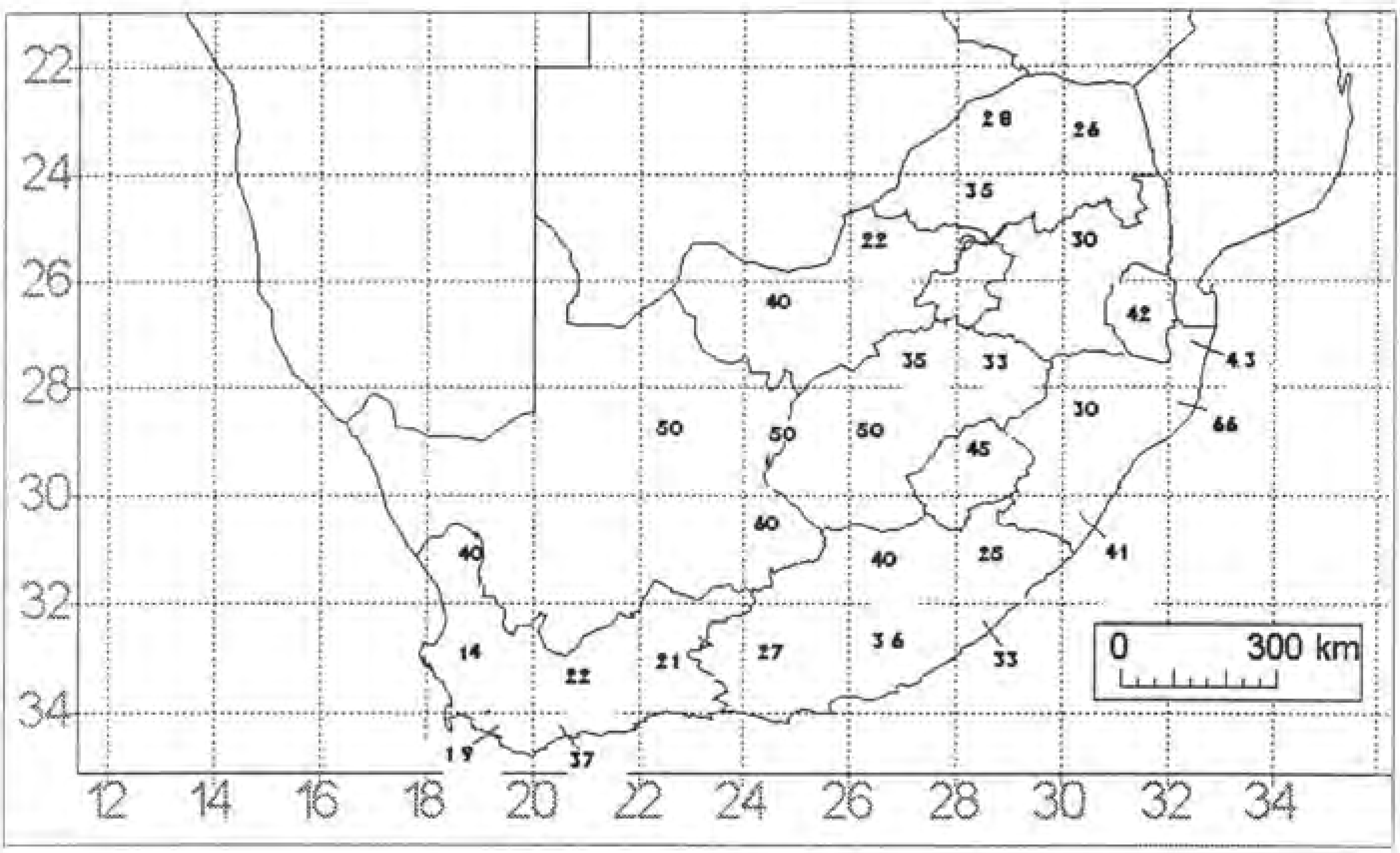

Fig.3: Map showing the distribution of C_3 and C_4 species of Cyperaceae in South Africa. The numbers are percentages for each particular grid square. Percentages for grid squares with less than 5 species have been excluded.

The percentages of C_4 species in each of the categories of species studied (i.e. winter, summer and all year rainfall regions), are given in table 2. Of the 24 species found to occur in the winter rainfall region, in this study, only 4 were C_4 species. C_3 species were mostly predominant even in the summer rainfall regions and of the species that were wide spread 55.1% were C_3 species. C_3 species therefore seems to predominate in the entire country which is unlike the case of the grasses where C_4 species predominate in most areas except the winter rainfall region (Vogel et al., 1978).

Table 2: Abundance of C_4 species of Cyperaceae in different rainfall regions of South Africa.

Rainfall regime of species	**Number of C_3 species**	**Number of C_4 species**	**Percentage C_4**
Winter rainfall region	20	4	16.67%
Summer rainfall region	27	22	44.9%
All year rainfall region	11	4	26.67%

Multiple regression analysis

Mean annual precipitation and minimum altitude showed slight but not significant correlation ($p = 0.7497$ and 0.1454 respectively) with $\%C_4$ sedges. Maximum altitude and maximum growing season temperature both showed no correlation with percentage $\%C_4$ and were therefore not added to the regression equation by the stepwise multiple regression analysis. Only minimum growing season temperature showed significant correlation ($P = 0.000391$) with percentage C_4. The multiple regression analysis yielded a coefficient of determination (R^2) of 0.41378.

The regression equation is given by

$\%C_4 = 36.47 + 1.54660 \times GMT + -0.01393 \times MMAPT + -0.00774 \times MA.$

Where GMT = minimum growing season temperature

MMAPT = Maximum mean annual precipitation

MA = Minimum altitude

36.47 = the intercept

Values 1.54660, 0.01393 and 0.00774 = raw regression coefficients

Linear regression analysis

Linear regression analysis (Fig. 4) of growing season minimum temperature showed a significant correlation with $\%C_4$ (coefficient of determination, $R^2 = 0.3148$). Minimum temperature during the growing season therefore seems to be the best predictor of C_4 percentage amongst the parameters examined. The estimated linear regression equation is as follows:

$$\%C_4 = 1.29 \times MT + 23.94$$

where $\%C_4$ = percentage of C_4 species of Cyperaceae
MT = minimum growing season temperature
23.94 = intercept and
1.29 = slope of the regression line

Minimum temperature for C_4 and C_3 transition

From the regression equation the minimum growing season temperature at which the percent C_4 would be 50% was determined to be 20.2°C. This is however above the highest minimum temperatures recorded.

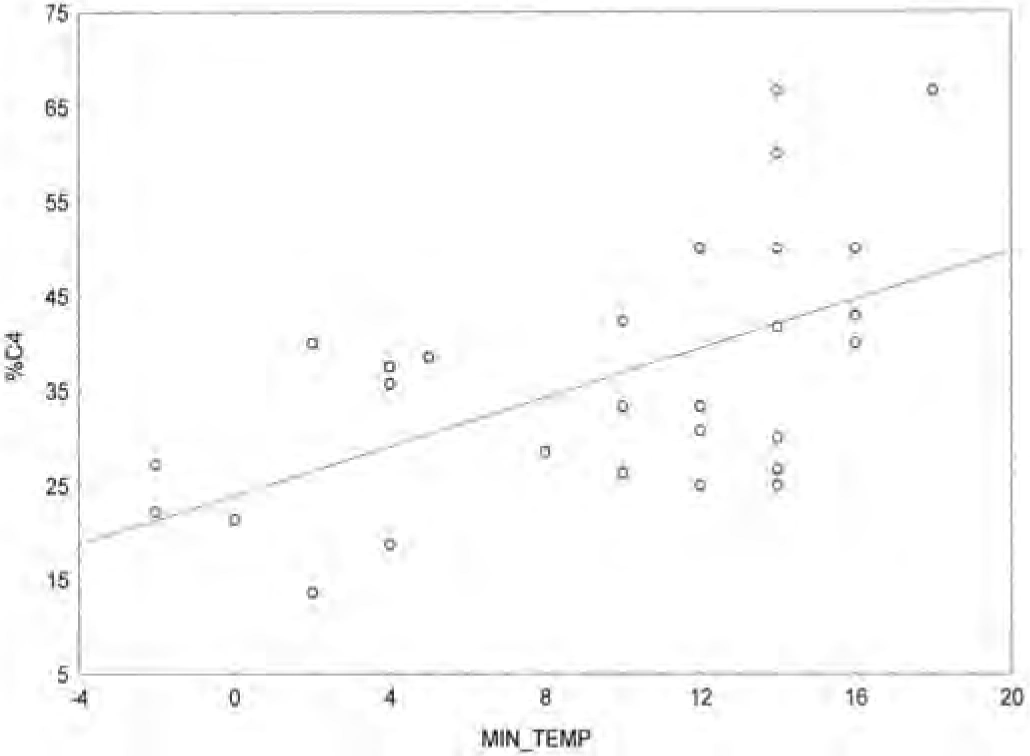

FIG.4: **Graph of percent C_4 versus minimum growing season temperature. There is a significant correlation between temperature and %C_4. The regression equation is: %C_4 = 1.29 x MT. + 23.94, where 1.29 = slope of the regression line, MT = minimum growing season temperature and 23.95 = intercept.**

Phylogeny

Twenty six species of Cyperaceae (Table 3) were C_4 and these were scattered among 9 genera of 4 tribes (Cypereae, Scirpeae and Abildgaardieae of the subfamily Cyperiodeae, and Rhyncosporeae of the subfamily Caricoideae) (Table 3). Forty-two species were C_3 and these were scattered among 16 genera of 6 tribes (Trilepideae, Schoeneae, Cariceae and Sclerieae of the subfamily Caricoideae and Cypereae and Scirpeae of the subfamily Cyperiodeae). Genera that were found to include both C_4 and C_3 species were *Cyperus* and *Lipocarpha* (tribe Cypereae), and *Schoenoplectus* (tribe Scirpeae). The rest had either exclusively C_3, or exclusively C_4 species. Genera that had exclusively C_4 species were *Kyllinga*, *Mariscus* and *Pycreus*. *Rhyncospora* of the tribe Rhyncosporeae, *Ascolepis* of the tribe Cypereae and *Fimbristylis* of the tribe Abildgaardieae were all C_4 but only one species was included in the present study and there is therefore need to examine more species of these genera. Genera that had exclusively C_3 species were *Carex* of the tribe Cariceae, *Tetraria* and *Epischoenus* of the tribe Schoeneae, *Ficinia*, *Eleocharis* and *Fuirena* of the tribe Scirpeae, *Isolepis* of the tribe Schoeneae and Sclera of the tribe Sclerieae. *Alinula*, of the tribe Cypereae, *Chrysithrix*, *Neesenbeckia* of the tribe Schoeneae, *Coleochloa*, of the tribe Trilepideae and *Schoenoxiphium*, of the tribe Cariceae were all C_3 but only one species was used in the study. The tribes Cariceae, Schoeneae and Sclerieae of the subfamily Caricoideae did not contain C_4 species. For the tribes Abildgaardieae of the subfamily Cyperiodeae and Rhyncosporeae of the subfamily Caricoideae only one species was used in the study and they were all C_4 species.

Table 3: Numbers and percentages of C_4 species in different subfamilies, tribes and genera studied. * = values for subfamilies, ** = values for tribes.**

Subfamily	Tribe	Genus	Total number of species	Number of C_4 species	%C_4
Cyperoideae			45***	25***	55.6***
	Cypereae		27**	23**	85.2**
		Cyperus	10	8	80
		Lipocarpha	4	3	75
		Kyllinga	3	3	100
		Mariscus	4	4	100
		Pycreus	4	4	100
		Ascolepis	1	1	100
		Alinula	1	0	0
	Scirpeae		18**	1**	5.5**
		Eleocharis	2	0	0
		Isolepis	2	0	0
		Schoenoplectus	4	1	25
		Ficinia	4	0	0
		Fuirena	6	0	0
	Abildgaardieae		1**	1**	100**
		Fimbristylis	1	1	100
Caricoideae			21***	1***	4.8***
	Rhynchosporeae		1**	1**	100**
		Rhyncospora	1	1	100
	Trilepideae		1**	0**	0**
		Coleochloa	1	0	0
	Schoeneae		12**	0**	0**
		Tetraria	3	0	0
		Epischoenus	5	0	0
		Chrysithrix	1	0	0
		Neesenbeckia	1	0	0
	Cariceae		3**	0**	0**
		Carex	2	0	0
		Schoenoxiphium	1	0	0
	Sclerieae		4**	0**	0**
		Scleria	4	0	0

DISCUSSION

Because plants with different photosynthetic pathways possess different biochemical, physiological and anatomical features, their distribution is likely to be under differential influence of environmental factors such as temperature aridity, precipitation and altitude. As mentioned earlier, the influence of these environmental factors has already been reported in a number of studies (e.g. Teeri and Stowe, 1976; Stowe and Teeri, 1978, Tieszen et al, 1979, Laetsch, 1974, Vogel, et al., 1978, Teeri et al., 1980, Ueno and Takeda, 1992, Elheringer, et al., 1997). These studies have shown a clear difference in the distribution of grasses and sedges related to their photosynthetic systems.

C_4 species concentrate CO_2 at the site of RuBP carboxylation (Ehleringer et al., 1997) and it is generally agreed that this is of selective advantage whenever internal CO_2 concentrations become low. Conditions of low internal CO_2 concentrations would occur under high light intensities as well as during periods of water stress (Laetsch, 1974, Elheringer, et al., 1997) and increased stomatal resistance. This apparent selective advantage of C_4 species would definitely be expected to predictably influence the differential distribution of species with the various photosynthetic systems.

For the family Cyperaceae, it is clear that the differences in the distribution of species are related to their photosynthetic status. However the results of the current study and other studies (Teeri and Stowe, 1980, Ueno et al., 1989, Ueno and Takeda, 1992) done in different localities have yielded more complex results than a simple relationship with environmental factors.

Geographical distribution

In the current study, it is clear that unlike the family Poaceae, where C_4 species show predominance in most of the areas of South Africa (Vogel et al., 1978), Cyperaceae species exhibit a predominance of C_3 species in most parts of the country. This agrees with what has been found in studies of Cyperaceae and Poaceae in Southern Florida (Teeri and Stowe 1976 and Teeri et al., 1980) where there were relatively more C_4 grasses (80%) than C_3 grasses as compared to 40% C_4 sedges. It is also evident, in the current study, that there is almost no area where there is exclusively C_4 or C_3 species, but species that are only restricted to the winter rainfall area are all C_3 species, whereas those that are restricted only to summer rainfall areas include both C_4 and C_3 species. In Vogel et al.'s (1978) study, however, areas with exclusively C_4 species where observed for grasses. As suggested by Sage et al. (1999), reduced Sedge representation, as compared to grasses, is probably due to the occurrence of sedges commonly on wet marshy soils where C_4 plants tend to be less abundant. However other factors, as explained below, also play a role in influencing this distribution.

Temperature

C_4 plants are known to posses a higher temperature optima (30 – 45°C) for net CO_2 exchange than C_3 plants whose temperature optima is about 10 – 25°C (Tieszen et al., 1979). This probably gives C_4 species some adaptive advantage in places with relatively high growing season temperatures.

For the Cyperaceae species in South Africa, this study has shown that percentage C_4 is significantly correlated ($R^2 = 0.3148$; $P = 0.0017$) with minimum temperature during the growing season. These results generally support the findings in studies of North American species for both C_4 grass and sedge representatives, which showed high correlation ($r^2 > 0.87$) with July average temperature and July minimum temperature (Stowe and Teeri, 1978, Teeri et al., 1980). Teeri et al. (1980) however found that the positive correlation was much weaker for Cyperaceae than it was for the grasses. The correlation of C_4% Sedges with minimum temperature in the current study, though significant, is quite low ($R = 0.5611$).

The low correlation between Minimum temperature and percentage C_4 in this study however, suggests that minimum temperatures may not be the only factor involved in the selection for C_4 species in certain environments. Teeri et al. (1980), for example, found that for Cyperaceae in North America, the abundance of C_4 species seemed to be modified by moisture availability. Many more factors may also be involved. The interpretation of the abundance of C_4 species of Cyperaceae therefore seems more complex than that of grasses. Differential distribution for C_4 and C_3 species of Cyperaceae in the current study is probably under the influence of such other additional environmental, as well as physiological, biochemical or anatomical factors.

Another factor that complicates our understanding of C_4 and C_3 species distribution is the existence of exceptions such as the C_4 species domination of wet warm habitats, while being absent from certain hot, dry locations (Sage, et al., 1999). This probably suggests further support for the existence of physiological, biochemical or anatomical attributes in certain species, which may overwhelm the effects of climatological factors and consequently also complicate their influence on the distribution of the different photosynthetic types. Further work along this line of research is therefore needed.

Minimum temperature for C_4 and C_3 transition

Earlier work (Vogel et al., 1978, Tieszen et al., 1979, Ueno and Takeda, 1992, Ehleringer et al., 1997) has suggested the existence of a transition temperature at which the percentage C_4 of species falls just below 50%. Transition temperature found in the current study for the species of Cyperaceae (i.e. minimum growing season temperature of 20.2°C) suggest that for Cyperaceae in South Africa there would generally need to be higher minimum temperatures than recorded for such a transition point to be attained. This transition temperature however can not be easily compared with that for grasses as in the case of grasses (Vogel et al., 1978, Ehleringer et al., 1997), the mean daily maximum temperatures were used for determining the transition points. The current study shows that only a few species of Cyperaceae were found at minimum growing season temperatures of less than 5°C (Fig 4). This is very similar to the results of Teeri and Stowe's (1976) study of C_4 species of Poaceae in North America, in which they found that that few or no C_4 species were found at minimum growing season temperatures below 8° C.

Altitude

For the Cyperaceae species of South Africa, altitude did not show any significant correlation with C_4 percentage. However a strong decline in the contribution of C_4 species to local floras and vegetation stands with increasing altitude has been reported (e.g. Tieszen et al. 1979, Sage et al., 1999). C_4 species have been observed to became less common with increasing altitude typically dropping out of the flora at 2000 to 3000 m (Tieszen et al., 1979, Sage et al., 1999). However correlation between altitude and %C_4 in these studies is probably only incidental, due to correlation with other major determinant factors such as temperature and water availability. As Sage et al. (1999) suggests, the pattern of correlation with precipitation also largely reflect temperature and light regimes with precipitation, N, and salinity playing secondary roles. In another study, Young and Young (1983) found that aridity modifies altitude trends with C_4 plants reaching higher altitudes on drier sites such as Mount Kenya. The effect of altitude therefore, could exist only when it is related to other factors such as temperature and water availability. Also in the case of the study by Tieszen et al. (1979), low altitudes along their transects were characterised by increasing water stress and high irrandiances as well as high temperatures; and C_4 species may have likely been selected in response to all these factors. This therefore supports the idea that altitude is not a major factor contributing to the selective distribution of C_3 and C_4 species, but is most likely a confounding factor. It is only strongly related to temperatures because of adiabatic cooling.

Mean annual precipitation

In the current study precipitation showed no correlation with C_4 percentage. This is unlike other studies for example Teeri et al. (1980), in which they found that the precipitation exhibited a positive correlation with C_4 percentage. However the area that had the highest precipitation (the South Eastern United States), also had the highest summer temperatures and moderately high evaporation rates. It would seem therefore that precipitation might only be a confounding factor, which appears to show high correlation, only because it is highly correlated with temperature. Therefore the real effect of precipitation could be tested in areas where the trends in temperature are incongruent with trends in precipitation or where trends in temperature are minimal whereas trends in precipitation tend to be quite high.

Tieszen et al. (1979) also found that the percentage of C_4 species remained equally high in both arid and less arid but still hot areas such as the lake Victoria. This further shows that precipitation amount, as such does not determine the distribution of C_4 and C_3 species. The most important factor seems to be the minimum temperature at the time of optimal water availability.

In Ueno and Takeda's (1992) study, a positive correlation was also found between C_4 % and annual precipitation according to them revealing a tendency for C_4 percentage to increase with increasing precipitation. However as mentioned by the same authors in Japan there is a high positive correlation ($r=0.785$, $p< 0.01$) between annual precipitation and annual mean temperature. The correlation between annual precipitation and annual mean temperature could just be incidental due to correlation with temperature (i.e. precipitation could only be a confounding factor). Any environmental variable that is associated with temperature is likely to show close correlation with percentage C_4.

Phylogeny

The presence of the C_4 syndrome has in previous studies shown some consistency especially within families and genera (Tieszen et al., 1979, Teeri et al., 1980). However a number of genera contain both C_4 and C_3 species.

In the current study, the distribution of C_3 and C_4 types of photosynthesis among the tribes and genera of Cyperaceae follows closely that of the recent taxonomies (Bruhl, 1995, Ueno and Takeda, 1992, Muasya et al, 1998). This study shows that Caricoideae is predominantly a C_3 subfamily with only *Rhyncospora rubra* here observed to be C_4, whereas the tribe Cypereae of the subfamiy Cyperoidea contains both C_3 and C_4 species (Table 3). The tribe Scirpeae contains predominantly C_3 species with only one species of *Schoenoplectus* being C_4. The results here supports similar results found by Teeri et al. (1980) and Ueno and Takeda (1992) for the tribe Cypereae and Scirpeae of the subfamily Cyperoideae, which was shown to contain entirely C_3 species. Further the current results shows some support for the their results that indicate a predominance of C_3 species for the subfamily Caricoideae.

The occurrence of similar photosynthetic types in species of the same tribes, occupying areas of different climatic types seems to suggest the influence of phylogenetic constraints in determining the photosynthetic status for particular genera and tribes. However the actual causal basis of this phenomenon is not fully understood (Sage et al., 1999). Further studies are required to gain more insight into the complex interactions between climatic, phylogenetic and other factors influencing the distribution of C_3 and C_4 species. This should involve fairly detailed case studies of a number of Cyperaceae species.

Another factor found to be more often limiting than either water or light in the humid tropics and during the wet season in the sub tropics is the availability of mineral nutrients (Saydon, 1991). Nitrogen and salinity for example have also been proposed as playing secondary roles in influencing C_4 and C_3 distribution (Sage et al., 1999). In another study, Jones (1988) suggested that the adaptive advantage of Papyrus, which thrives in the tropical swamps of Africa, could be found in the higher nitrogen use efficiency rather than in the water use efficiency.

Another point worth considering is the confinement of certain C_3 species exclusively to places where we would expect to find only C_4 species. *Fuirena ciliaris* for example is a C_3 species restricted to the summer rainfall areas. This species has been observed to contain a conspicuously large number of chloroplasts in the cells, yet it is considered to be C_3 species because it has C_3 like compensation concentrations (Ueno and Takeda, 1992) and shows $\delta^{13}C$ value typical of C_3 species (see appendix 1). This suggests possible existence of phylogenetic constraints. It is possible that such C_3 species found in Summer rainfall areas of south Africa where minimum growing season temperature are optimal for C_4 species, may have evolved in temperate type climate and then spread out to the subtropical type climate possibly due to possession of physiological or anatomical attributes that allow survival under sub-tropical type climates. They have however retained CO_2 compensation rates typical of C_3 species. Stowe and Tyree (1978) suggested that similarities in the micro-environmental attributes of disturbed sites could also override climatological differences. This can apparently result in such inconsistencies.

CONCLUSION

The results suggest that the relationship between the climatic factors and percentage of C_4 Cyperaceae species is similar to what has been found for the grasses (Vogel et al., 1978). However whereas in Vogel et al.'s (1978) study precipitation and temperature were the factors that were significantly correlated with percentage C_4 in grasses, this was not the case for the sedges in the current study. Only minimum growing season temperature is correlated to percentage C_4 in Cyperaceae. Altitude and Precipitation are not important factors in the determination of the distribution of C_3 and C_4 species. Any observed correlation between altitude, precipitation and $\%C_4$ would only be incidental, due to correlation with other major determinant factors such as temperature. The occurrence of some species of either C_3 or C_4 type in all types of rainfall regions with different growing season temperature regimes suggests a need for an examination of the physiology, anatomy and biochemistry of representative species, and other factors that may be contributing to their adaptiveness to a wide range of environmental conditions.

Case studies of a number of Cyperaceae species exhibiting these various distributional phenomena outlined above, related to photosynthetic type, are needed to gain insight into the real factors influencing their distribution. Further understanding of their physiology, biochemistry, and anatomy would shed more light on the interaction of these factors with environmental factors to influence the distribution of C_3 and C_4 species of Cyperaceae.

Study limitations

Difficulties were encountered in determining the environmental parameters as this was done by examination of climate maps and not by very accurate methods such as Geographical Positioning System so as to be able to pin point the exact temperatures for particular localities. However, the data obtained was accurate enough to give a general impression of the distributional phenomena for different photosynthetic type species of Cyperaceae. It was not possible to have a larger sample size because of the limited availability of species of Cyperaceae in the Bolus Herbarium and inadequate time to procure more specimens from other herbaria. However increasing the sample size is not expected to drastically change the general trend in the relationship between environmental factors and distribution of C_3 and C_4 species of Cyperaceae.

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APPENDIX

Appendix 1. Table of species studied with delta values, altitude, mean annual precipitation and temperature values for each of the species. MMAPT1 = minimum mean annual precipitation, MMAPT2 = maximum mean annual precipitation, AT1 = minimum altitude, AT2 = maximum altitude, MT1 = minimum growing season temperature), MT2 = maximum growing season temperature. Specimens with missing values are those for which distribution maps were not available from NBI.

GENUS & SPECIES/ STANDARD	delt13C	C3/C4 status	MMAPT1 (mm)	MMAPT2 (mm)	AT1 (m)	AT2 (m)	MT1 (^{0}C)	MT2 (^{0}C)
Chrysithrix juncifomis Nees	-23.33	C3	200	>1200	100	800	0	22
E. cernuus Levyns	-24.73	C3	200	>1200	200	400	4	22
Epischoenus complanatus Levyns	-26.21	C3	400	>1200	0	1250	2	20
E. dregeanus (Boekeler) Levyns	-24.45	C3	400	>1200	200	1750	2	16
E. lucidus (C.B. Clarke) Levyns	-25.20	C3	400	>1200	200	1250	2	20
E. villosus Levyns	-22.07	C3	1000	>1200	200	1250	0	20
Ficinia gydomontana Arnold	-21.20	C3	400	>1200	0	1500	0	22
F. angustifolia	-24.20	C3	200	1200	0	1750		
F. acuminata (Steudel) Nees	-25.40	C3	200	>1200	0	800		
Neesenbeckia punctoria (Vahl) Levyns	-26.01	C3	400	>1200	0	1250	2	22
Trianoptiles capensis (Steudel) Harvey	-28.02	C3	400	1000	200	1500	6	22
T. Solitaris (C.B. Clarke) Levyns	-25.82	C3	1000	1200	0	100	6	20
T. stipitata Levyns	-29.24	C3	200	>1200	200	1000	2	22
Tetralia compacta Levyns	-24.49	C3	200	1000	0	300	6	20
T. exilis Levyns	-25.94	C3	200	>1200	0	1250		
Lipocarpha chinensis (Obs.) Kern	-10.30	C4						
L. hemisphaerica (Roth?) Goetgebeur	-9.45	C4	400	600	1250	1500	10	30
L. nana (A.Rich) Cherm.	-10.34	C4	400	>1200	300	2000	10	34
L. rehmannii (Ridgley) Geotghebeur	-24.57	C3	400	1000	200	2000	12	32
Ascolepis capensis (Kunth) Ridgley	-10.31	C4	400	>1200	100	2500	<6	34

Appendix 1 continued

GENUS & SPECIES	**delt13C**	**C3/C4 status**	**MMAPT1 (mm)**	**MMAPT2 (mm)**	**AT1 (m)**	**AT2 (m)**	**MT1 (°C)**	**MT2 (°C)**
Cyperus amabilis Vahl.	-12.31	C4	400	>1200	100	1750	14	32
C. compressus L.	-9.85	C4	800	1200	0	1500	16	32
C. corymbosus Rottb	-10.33	C4						
C. fulgens CB Cl. var. fugens	-11.41	C4	200	>1200	600	1500	6	34
C. leptocladus Kunth	-27.37	C3	400	>1200	100	2000	12	30
C. natalensis Hoscht	-9.95	C4			0	1500	10	30
Pycreus flavescens (L.) Reichb	-10.93	C4	400	>1200	0	2000	12	32
P. polystachyos (Rottb.) Beau. var. laxiflorus	-10.43	C4						
P. pelophilus (Ridgley) C.B. Cl.	-10.93	C4	200	1200	200	1750	14	34
M. Keniensis (Kuenketh.) Hopper	-9.99	C4	400	>1200	600	2000	10	32
M. laxiflorus Turrill	-10.55	C4	400	1000	600	1750	16	32
M. vestitus (Hochst. ex Krauss) C.B. Cl.	-9.99	C4	600	1200	0	1750	16	32
Alinula paradoxa (Cherm.) Goetghebeur &Vorster	-25.08	C3	400	>1200	0	1250	16	32
Kyllinga elatior Kunth	-10.31	C4	600	1200	0	1000	12	30
K. nemoralis (J. R. & G. Forst.) Dandy	-9.53	C4	200	1200	0	600	18	32
K. pulchella Kunth	-9.99	C4	200	1200	600	1500	10	34
Fuirena ciliaris (L.) Roxb. var ciliaris	-26.63	C3	400	1200	0	1250	16	32
F. leptostachya Oliv. var nudiflora C.B. Cl.	-26.81	C3	400	1200	0	1500	16	32
F. abcordata O.L. Forbes	-26.03	C3						
F. pachyrrhiza Ridley var. pachyrrhiza	-25.04	C3	600	1000	200	1000	14	32
F. stricta Steud	-25.86	C3	200	>1200	400	2000	12	32
F. umbellata Rottb	-25.07	C3	200	>1200	0	>2500	6	34
Schoenoplectus confusus (N.E. Br.) K. Lye subsp. natalitus J. Browning	-24.11	C3	400	800	0	300	18	32
S. corymbosus (Roth. ex Roem & Schult.) J. Raynal var. brachyceras (A. Rich) K. Lye	-23.71	C3	400	1200	400	1750	<4	34
S. praelongatus (Poir.) J. Raynal	-23.99	C3	400	800	200	1500	14	34

Appendix 1 continued

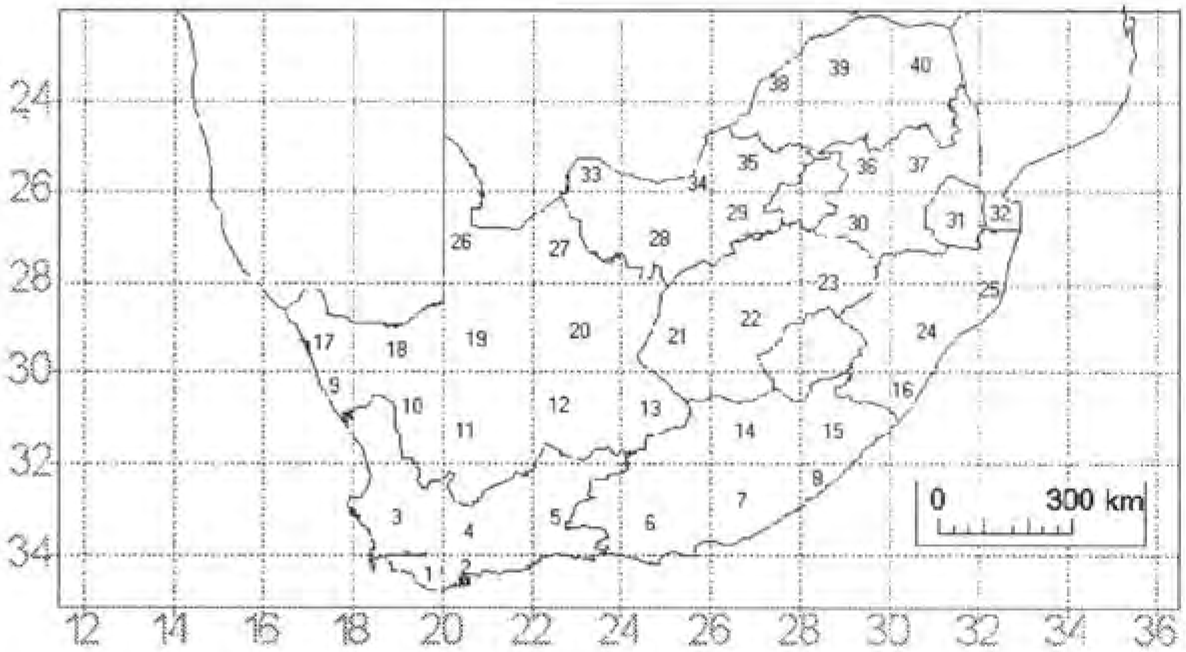
GENUS & SPECIES	**delt13C**	**C3/C4 status**	**MMAPT1 (mm)**	**MMAPT2 (mm)**	**AT1 (m)**	**AT2 (m)**	**MT1 (°C)**	**MT2 (°C)**
S. pulchellus (Kunth) J. Raynal	-10.59	C4	400	1000	600	2000	10	32
Eleocharis atropurpurea (Retz.) Presl	-26.90	C3	400	1000	0	1750	12	34
Fimbristylis longiculmis Steud.	-10.05	C4						
Rhyncospora rubra (Lour) Makino subsp. africana J. Raynal	-10.21	C4	600	>1200	0	300	>20	32
Tetraria natalensis (C.B.Cl.) Koyama check under Costularia natalensis	-25.80	C3	800	>1200	600	2000	10	32
Coleochloa setifera (Ridley) Gilly	-22.20	C3	600	>1200	400	2500	8	32
Scleria angusta Nees ex Kunth	-29.19	C3	600	>1200	0	800	16	30
S. greigiifolia (Ridley) C.B. Cl.	-25.98	C3	600	1000	0	600	16	32
S. rehmannii C.B. Cl.	-23.86	C3	800	>1200	600	2000	10	32
S. transvaalensis E.F. Franklin	-25.11	C3	800	>1200-	600	1750	12	30
Carex spicato-paniculata C.B. Cl. x C.zuluensis C.B. Cl.	-27.72	C3	1000	1200	1750	2000	10	24
C. subinflata Nelmes	-25.18	C3	400	>1200	1000	>2500	<6	28
Cyperus longus L. var. longus	-8.16	C4						
Cyperus longus L. var. lenuiflorus (Rottb.) Boeck	-10.90	C4	100	>1200	100	2000	-2	34
C. maginatus Thumb.	-26.27	C3	100	>1200	100	>2500	<2	34
Eleocharis limosa (Schrad.) Schult.	-26.17	C3	200	>1200	0	1750	0	32
Fucinia stolnifera Boeck	-25.31	C3	100	>1200	0	>2500	-2	32
Isolepis fluitans (L.) R. Br.	-25.71	C3	200	>1200	200	1750	-2	32
I. tenuissma Nees	-24.03	C3	200	>1200	0	1500	2	32
Cyperus laevigatus (L.) C.B. Clarke	-9.31	C4	<100	>1200	0	1750	-2	34
Mariscus congestus (Vahl.) C.B. Cl.	-9.56	C4	600	>1200	0	1500	<2	34
Pycreus nitidus (Lam.) J. Raynal (P. Lanceus)	-10.02	C4	200	>1200	0	2000	0	32
Schoenoxiphium sparteum (Wahlenb.) C.B. Cl.	-27.71	C3	200	1200	0	2000	-2	32

Appendix 2. Table of grid squares for the map of South Africa (Fig. 1) with their percentage C_4 and C_3 values, altitude, mean annual precipitation and temperature values. MAPPtm = maximum mean annual precipitation, AT1 = minimum altitude, AT2 = maximum altitude, MT1 = minimum growing season temperature), MT2 = maximum growing season temperature.

Grid square number	C_3 SPECIES.	C_4 SPECIES	Total SPECIES.	% C4 SPECIES.	% C3 SPECIES.	MT1	MT2	MAPPtm	AT1	AT2
1	13	3	16	18.75	81.25	4	18	1200	0	400
2	5	3	8	37.50	62.50	4	30	400	300	1500
3	19	3	22	13.64	86.36	2	20	1200	0	1500
4	7	2	9	22.22	77.78	-2	34	1000	200	1750
5	11	3	14	21.43	78.57	0	34	400	600	1500
6	8	3	11	27.27	72.73	-2	34	600	0	2000
7	9	5	14	35.71	64.29	2	30	600	0	1500
8	2	4	6	66.67	33.33	14		1000	0	800
9	0	1	1	100.00	0.00					
10	3	2	5	40.00	60.00	2	30	400	0	600
11	1	2	3	66.67	33.33					
12	1	2	3	66.67	33.33					
13	2	3	5	60.00	40.00	2	30	400	600	1750
14	5	2	7	28.57	71.43	8	30	600	800	2500
15	9	3	12	25.00	75.00	12	28	1000	400	2500
16	7	5	12	41.67	58.33	14	30	1000	0	1000
17	1	2	3	66.67	33.33					
18	0	1	1	100.00	0.00					
19	1	1	2	50.00	50.00					
20	4	4	8	50.00	50.00	2	32	400	800	1750
21	3	3	6	50.00	50.00	2	32	600	800	1500
22	6	6	12	50.00	50.00	12	30	800	1000	1750
23	8	5	13	38.46	61.54	5	30	1200	800	2550

Appendix 2 continued

Grid square number	C_3 SPECIES.	C_4 SPECIES	Total SPECIES.	% C4 SPECIES.	% C3 SPECIES.	MT1	MT2	MAPPtm	AT1	AT2
24	18	8	26	30.77	69.23	12	32	1200	0	1500
25	4	8	12	66.67	33.33	18	32	1200	0	300
26	0	0	0	0	0	–	–	–	–	–
27	1	1	2	50.00	50.00					
28	3	2	5	40.00	60.00	16	34	600	1000	1500
29	10	5	15	33.33	66.67	12	32	800	1250	1750
30	10	5	15	33.33	66.67	10	30	800	1500	2000
31	15	11	26	42.31	57.69	10	33	1200	200	2000
32	4	3	7	42.86	57.14	16	32	1000	0	200
33	0	0	0	0	0	–	–	–	–	–
34	0	1	1	100.00	0.00					
35	7	3	10	30.00	70.00	14	34	800	800	1750
36	9	4	13	30.77	69.23	12	30	800	800	1750
37	14	5	19	26.32	73.68	10	32	1200	200	2000
38	3	0	3	0.00	100.00					
39	9	3	12	25.00	75.00	14	34	600	600	1500
40	11	4	15	26.67	73.33	14	34	1200	200	800



Appendix 3: Map of South Africa showing the numbering for the grid squares as well as provincial boundaries.