What are southern Cape renosterveld geophytes storing in their underground storage organs?

Kirsten Packer

Supervisor: William Bond

Dissertation presented in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) in the Department of Botany, UCT, November 2011.

Abstract

Geophytes have been extremely successful in the Cape Floristic Region of South Africa, because they possess underground storage organs adapted to unpredictable climates. This study analysed the storage reserves of geophyte species from the southern Cape renosterveld, to determine if reserve concentrations varied with storage organ size and if different storage organ types (bulbs, corms, tubers and secondary thickening) store unique reserve concentrations. Plant species collected were analysed for water content (g/g fresh weight), nitrogen percentage (mass spectrometry), phosphorus percentage (dry-ashing extraction method) and starch content (*Megazyme* Total Starch Assay Kit, which follows an enzymatic procedure). Bulbs (mean = 30.89mm) and tubers (18.65mm) were found to have significantly larger widths than corms (11.13mm) and secondary thickening (7.00mm). Secondary thickening contained significantly less water than the other storage organ types (28.13%). Bulbs (83.81%) contained significantly more water than corms (68.33%). There were no observable differences in N, P and starch for the four storage organ types, although there was high variability. There were no substantial correlations between any of the reserves and storage organ width. The size variation is most likely due to annual versus perennial life cycle. Variability in water content is presumably due to microhabitat variation, and structures to prevent water loss. The average nutrient concentrations (nitrogen and phosphorous) were similar for all storage organ types possibly because all of the species are growing in a homogenous renosterveld environment. The results of this investigation were not entirely meaningful because they were a snapshot of a very dynamic system. Suggestions for further research include: considering time of year and age of geophytes when sampling, analysing local soils and assessing the variety of different carbohydrates and nutrients stored. Furthermore, the potential for phylogenetic signal, ecological similarity of species because they are phylogenetically related, could be explored.

Introduction

The 'geophytic' life form has been particularly successful in Mediterranean climate regions across the world, but none more so than in the Greater Cape Floristic Region of South Africa (Raunkaier 1934; Procheş *et al.* 2006).

The geophytic life form was first described by Raunkaier (1934) as a plant with a subterranean apical bud, a life-cycle that includes an annual dormant period and a fleshy underground organ (bulb, corm, tuber or rhizome). The geophyte was, therefore, suggested to be an adaptation to unpredictable or seasonal climates, such as the Mediterranean climate regime, largely due to the storage ability of the underground organ (Raunkaier 1934). Compounds synthesized by leaves during the growing season can be stored during the dry season and used for immediate growth at the start of the next growing season. Furthermore, during the 'dormant' period, the growth of new leaves and inflorescences may be initiated, again allowing for rapid development once the growing season arrives (Pate and Dixon 1982). Periods of drought may be overcome by storing water (Raunkaier 1934).

The geophytic diversity of the Greater Cape Floristic Region is unparalleled – 2096 species are found in the succulent karoo and fynbos biomes, accounting for 20% of the total flora, with just over 80% of these species being endemic (Procheş *et al.* 2005, Procheş *et al.* 2006). Interestingly, Procheş and colleagues (2006) noted in their comparisons of geophyte richness across the fynbos biome, that renosterveld plots contained considerably more geophyte species than fynbos plots at all the scales considered. The transition to a geophytic life form occurred independently in numerous plant groups within the Cape Floristic Region (CFR), and so it is thought that there must be some ecological advantage to this habit. Naturally, most studies undertaken regarding the geophytes of the CFR have questioned and investigated possible factors responsible for this diversity.

Arguably, the most spectacular characteristic of the CFR geophytes is their great floral diversity and, unsurprisingly, the role of pollination in the diversification of geophytes has been explored. Pollinator specificity is evident in a number of geophytic species, leading to unique and sometimes bizarre pollination systems (Procheş *et al.* 2006, Johnson 2010). However, besides the obvious candidates, pollination specificity is certainly not the sole driver of diversification. Herbivory has been tentatively suggested as a driver of diversification, because a number of geophyte storage organs contain highly toxic substances (Procheş *et al.* 2006). The mechanisms by which herbivory would cause diversification are yet to be investigated. On a global scale, geophyte species richness and success in Mediterranean climate regions may be due to the importance of fire in fragmenting landscapes and reducing competition from grasses or shrubs (Procheş *et al.* 2006). Both fynbos and renosterveld landscapes experience recurrent fires (Kraaij 2010). Fire may create special niches for geophytes; in fact many CFR species are adapted to flower or germinate directly after fire (Procheş *et al.* 2006). A key factor for diversification is likely to be the transition from simple/basal rhizomatous habits to more advanced storage organs such as bulbs and corms (Procheş *et al.* 2006). For example, there are ten species of the rhizomatous Agapanthaceae compared to 230 species in the sister taxon Amaryllidaceae, most of which are bulbous. Across the south-western Cape, 41% of species have corms, 32% are tuberous, 17% are bulbous and 10% have rhizomes (Procheş *et al.* 2006).

It is likely that edaphic and topographical heterogeneity of the CFR are two of the most important factors in the diversification of geophytes at the landscape scale (Procheş *et al.* 2006). But below the landscape scale this heterogeneity could be less important; considering the highest CFR geophyte diversity is found within the rather homogenous renosterveld landscape, as previously mentioned. Little is known about whether storage organ types (bulbs, corms, or tubers) are adapted to specific habitats; where different types of storage organs would follow different patterns of resource storage (Procheş *et al.* 2006). Also, underground storage organs could fulfil other basic functions besides water storage, such as the storage of mineral ions. Habitat specialisation may actually involve a combination of edaphic/topographical heterogeneity and storage organ type. It is possible that habitat specialisation could influence storage organ size (Procheş *et al.* 2006). Across the CFR, geophytic species are mostly photosynthetically active during the cooler and wetter winter months. It was hypothesized by Procheş and colleagues (2005) that the reliable and substantial winter rainfall of the south-western Cape has allowed the evolution of smaller storage organs, as opportunities for annual water replenishment are highly probable. In contrast, geophytes of the north-western Cape (succulent karoo), which receives very reliable but minimal rainfall, and the southern Cape, which receives less reliable rainfall, are thought to have larger storage organs to counteract the variability in rainfall. Patterns of storage organ size measured along a climatic gradient were found to be fairly consistent with this hypothesis (Procheş *et al.* 2005).

Although we are inclined to explore why the geophytic life form is so successful here in the CFR, most information gathered about the geophyte species relates to their taxonomic identifications, phylogenetic pictures and geographical patterns (Procheş *et al.* 2005). There seems to be a poor understanding of the underlying causes generating such richness and geographical patterns. Considering Raunkaier's (1934) first description of the geophyte relates its success to the underground storage organ, little work has been done in the CFR to quantify how storage organs and their ecological functions might adapt geophytes for survival, and ultimately lead to their success geographic patterns (Pate and Dixon 1982; Procheş *et al.* 2005, Al-Tardeh *et al.* 2008). The aim of this investigation was to attempt to answer questions relating to what geophytes are storing in underground storage organs (USOs), by analysing the storage reserves of a number of geophyte species from renosterveld vegetation of the southern Cape. Firstly, it was necessary to recognize if the geophyte species were storing more than just water, as first suggested by Raunkaier (1934), in terms of their possible edaphic adaptations. Considering Procheş and colleagues (2005) hypothesis, storage organ size may be an important ecological factor. Do water and other reserve concentrations vary with USO size? Also, if different types of storage organs are involved in habitat specialisation, it was necessary to question if different storage organ types store unique reserve concentrations.

Materials and Methods

Study Area

Sample collection for this study was conducted around the town of Napier (34°28'4" S and 19°54'10" E), which is situated in the southern Cape of South Africa, during the end of June (mid-winter) (Figure 1). According to Mucina and Rutherford (2006), two main vegetation types are found in the area: Elim Ferricrete Fynbos and Rûens Silcrete Renosterveld. Elim Ferricrete Fynbos is predominantly found on the Agulhas Plain and is characterised by asteraceous and low proteoid fynbos. Fragments of Rûens Silcrete Renosterveld are distributed across the undulating hills of the Overberg, found usually on silcrete outcrops or occasionally on quartz patches, and are characterised by short succulent species and the typical renosterbos (*Elytropappus rhinocerotis*).

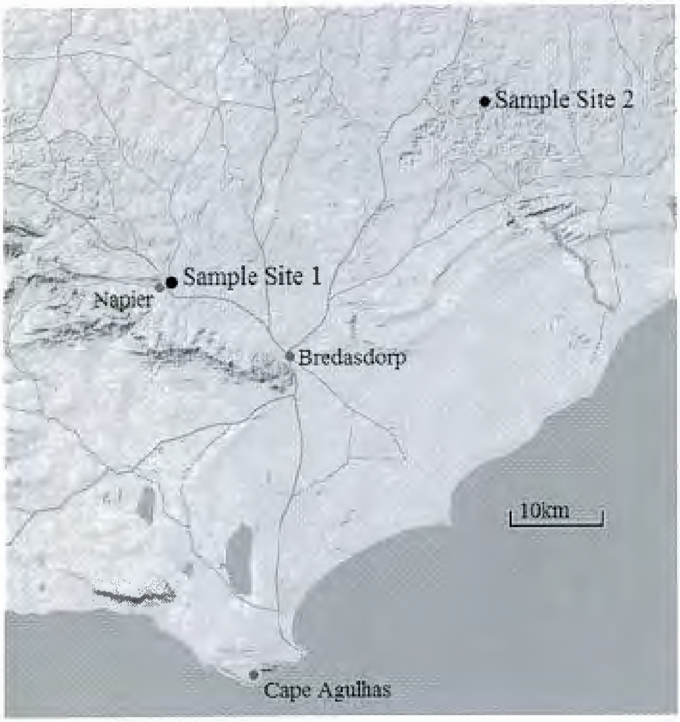

Figure 1: The location of the two sampling sites, one situated on the outskirts of Napier, and the other to the north-east of Napier. This map was constructed using *Google Maps*.

Although the classic Mediterranean climate prevails over the study area, rainfall is not necessarily concentrated during the winter months, as it is in the south-western Cape. The mean annual precipitation, of between 280 and 770mm, falls relatively evenly throughout the year, with a slight low from December to February, and is not as predictable as rainfall along the west coast (Mucina and Rutherford 2006). Temperatures range from a mean daily maximum of 26.9°C to a mean daily minimum of 6.0°C.

Geophyte samples were collected from fields directly surrounding the town of Napier (sample site 1), and also from a grazed field to the north-east of Napier (sample site 2). The two vegetation types mentioned previously have been heavily transformed into agricultural land and are, consequently, important conservation targets (Mucina and Rutherford 2006). It was personally observed that the fields directly surrounding Napier are heavily invaded by *Acacia saligna* and *Eucalyptus* trees. Also, only small fragments of Rûens Silcrete Renosterveld remain amongst a sea of wheat cultivation. Mucina and Rutherford (2006) unfortunately do not give any mention of the geophyte diversity of these two vegetation types.

Sampling Method

Thirty-seven plant species were collected at the two sampling sites – with three replicates of each species. The number of replicates was kept to a minimum due to the conservation status of the vegetation type. Each species was identified with the help of Cameron McMaster (a resident geophyte expert who runs *African Bulbs*[1], a small nursery that cultivates endemic geophytes).

Directly after collection, the plants were washed and dried to removes excess soil, the leaves and stem were removed, and each underground storage organ (USO) was longitudinally sectioned through the median axis. The widest cross-sectional diameter was measured to record the size of each USO. One half of each USO, to be used for the starch analysis, was frozen immediately in order for the sample to remain 'fresh' rather than dried. The other USO halves were weighed (fresh weight to 0.1 grams) and subsequently dried in an oven (70°C) for seven days, after which the dry-weight was recorded. Water content (% fresh mass) was calculated as (fresh weight – dry weight)/fresh weight x 100. The dried samples were ground using a *Wiley* ball-mill. It must be noted that before fresh weight was recorded, the outer layers of all corm sheaths were removed, but the innermost layers still surrounded the true USOs and were therefore incorporated into the dry sample weights.

Percentage nitrogen content (%N) was determined by mass spectrometry (dry samples were combusted in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit; in order to determine %N). The samples were run against two in-house standards (Lentil: $\delta^{15}N$ of 0.12 and New NASTD: $\delta^{15}N$ of 6.75). Percentage phosphorous content (%P) of the dry samples was determined by *Bemlab*[2] (an accredited analytical laboratory situated in Cape Town); using the dry-ashing extraction method.

Starch Analysis

Starch determination methods can be grouped into two categories: acid hydrolysis or enzymatic procedures. The enzymatic procedure is the preferred method because acid hydrolysis methods can be inaccurate. The acid used during hydrolysis may break down structural carbohydrates besides the non-structural starch that is being investigated, depending on the concentration of the acid, therefore leading to a larger percentage of starch than actually present in the specific plant material (Chow and Landhäusser 2004).

The *Megazyme* Total Starch Assay Kit[3], which follows the enzymatic procedure, was used to determine the percentage starch content of the fresh geophyte samples. In this method, starch is enzymatically broken down into a form of glucose, and the quantity of glucose is then determined by colourimetric reaction. Firstly, thermostable α-amylase hydrolyses starch into soluble maltodextrins, which are subsequently hydrolysed to D-glucose by amyloglucosidase. For the colourimetric reaction, D-glucose is oxidised to D-gluconate with the release of one molecule of hydrogen peroxide. Hydrogen peroxide reacts with peroxidise to form a quinoneimine dye that provides a colour for the spectrophotometer (Figure 2).

$$\text{1) starch} \xrightarrow{\text{thermostable } \alpha\text{-amylase}} \text{maltodextrins}$$

$$\text{2) maltodextrins} \xrightarrow{\text{amyloglucosidase}} \text{D-glucose}$$

$$\text{3) D-glucose} \longrightarrow \text{D-gluconate} + H_2O_2$$

$$\text{4) } H_2O_2 + \text{peroxidise} \longrightarrow \text{quinoneimine dye}$$

Figure 2: Schematic layout of the general principle behind the *Megazyme* Total Starch enzymatic assay.

The complete method used can be found on the *Megazyme* website[3]; the following are changes made to the method or specifics that need to be noted. The *Megazyme* method requires the plant material to milled to be able to pass a 0.5mm sieve. However, the USO material was unable to be milled due to it being fresh. Therefore, the fresh USO samples were ground in a mortar with liquid nitrogen until a consolidated paste was formed. It must be noted that all the corm sheaths and woody outer layers of tubers were removed before grinding. Also, the *Megazyme* method required 100mg of sample to be analysed but many of the USO halves would not have made sufficient material for the analysis. Therefore, for these species, the three replicate storage organ halves were combined when grinding.

Within the *Megazyme* Total Starch Assay, method C was carried out – determination of total starch content in samples containing resistant starch. This required the bulb samples to be dissolved in cold 2M KOH before the enzymatic hydrolysis. The samples were not centrifuged for 10 minutes at 3000rpm, but were rather passed through Wattman No.1 filter papers. After filtration, the samples were not diluted with water, and so the final solution volume (FV) for each sample was 10.4ml. For the colourimetric analysis, the blank contained sodium acetate buffer (pH3.8), while four standards contained 100μl of D-glucose. The following calculations were used to determine percentage starch on a dry weight basis:

$$Starch\ content\ (mg/mg) = \Delta A \text{ x } F \text{ x } \frac{FV}{0.1} \text{ x } \frac{1}{1000} \text{ x } W \text{ x } \frac{162}{180}$$

ΔA = absorbance of sample read against blank
F = 100 (μg of D-glucose)/ absorbance (for 100μg of D-glucose) – which converts absorbance to μg
FV = final volume of solution (10.4ml)
0.1 = volume of sample colourimetrically analysed
1/1000 = conversion from μg to mg
W = weight in milligrams of ground sample analysed
162/180 = adjustment from free D-glucose to anhydro D-glucose (that occurs in starch)

$$Starch\ (\%\ dry\ weight) = starch\ content \text{ x } \frac{FW}{DW} \text{ x } 100$$

FW = fresh weight (previously measured)
DW = dry weight (previously measured)

Graphical and Statistical Analyses

Scatterplot (XY) graphs were constructed in *Microsoft Excel (2007)* to visually evaluate if there was any correlation between reserve concentration (dependant variable) and storage organ size (independant variable). The longest cross-sectional width (mm) was used to indicate storage organ size however, due to extreme variation, the widths were log transformed. Moisture %, N%, P% and starch % were all plotted against storage organ size, each reserve differentiated into storage organ types.

Box and Whisker plots were constructed in *Statistica (version 10)* to display mean reserve concentrations (again Moisture %, N%, P% and starch %), as well as variation around the mean, for each storage organ type. A Box and Whisker plot was also constructed to display the mean and variation in storage organ widths; the widths were not log transformed here to indicate the extreme variability. It was stated by (Procheş *et al.* 2005) that the size of a rhizome cannot be quantified. Since only one species (3 replicates) of rhizome was collected, this storage organ type was excluded from the Box and Whisker plots for all variables, and also from the subsequent statistical analyses.

Statistical tests were performed in *Statistica (version 10)* to evaluate if the trends seen in the Box and Whisker plots were significant or not. A one-way ANOVA was performed on data that did conform to a normal distribution, which was only storage organ size (log transformed width, mm). If the one-way ANOVA confirmed that there was a significant difference between the mean sizes of storage organ types, then a post-hoc Tukey (HSD) test was performed to locate which storage organ types were different. A non-parametric Kruskal-Wallis (ANOVA by ranks) test was performed on data that did not conform to a normal distribution, which was Moisture %, N%, P% and starch %. Again, if the Kruskal-Wallis test confirmed significance, a Multiple Comparisons test was performed to locate which storage organ types were different.

An Analysis of Traits (AOT) was conducted in *Phylocom version 4.2* (Webb *et al.* 2008) to determine if the species collected showed phylogenetic signal. Phylogenetic signal is the tendency for closely related species to resemble each other more than they resemble species drawn at random from the phylogenetic tree (Losos 2008). Therefore, the AOT tests for node-level significance by comparing the mean values for all terminal taxa descended from a node against a randomization of 999 trait values across the tips of the phylogeny. In this analysis, water %, N%, P% and starch % were the traits considered. The phylogeny used for the AOT was constructed using the phylogenies from Kim *et al.* (2010) and Forest *et al.* (2007).

Results

The four types of underground organs analysed were: corms, bulbs, tubers and tap roots with secondary thickening. Corms, bulbs and tubers are all underground storage organs related to the geophytic life-form; whereas the plants displayed 'secondary thickening' of the tap root are not geophytes.

A significant difference in widths was found when comparing the four storage organ types (F=24.082, df=4, p<0.01). Bulbs and tubers were significantly larger than corms and secondary thickening (Figure 3). The large variability in bulb width, and specifically the very large maximum value was due to one species: *Drimia capensis* (average width = 109mm).

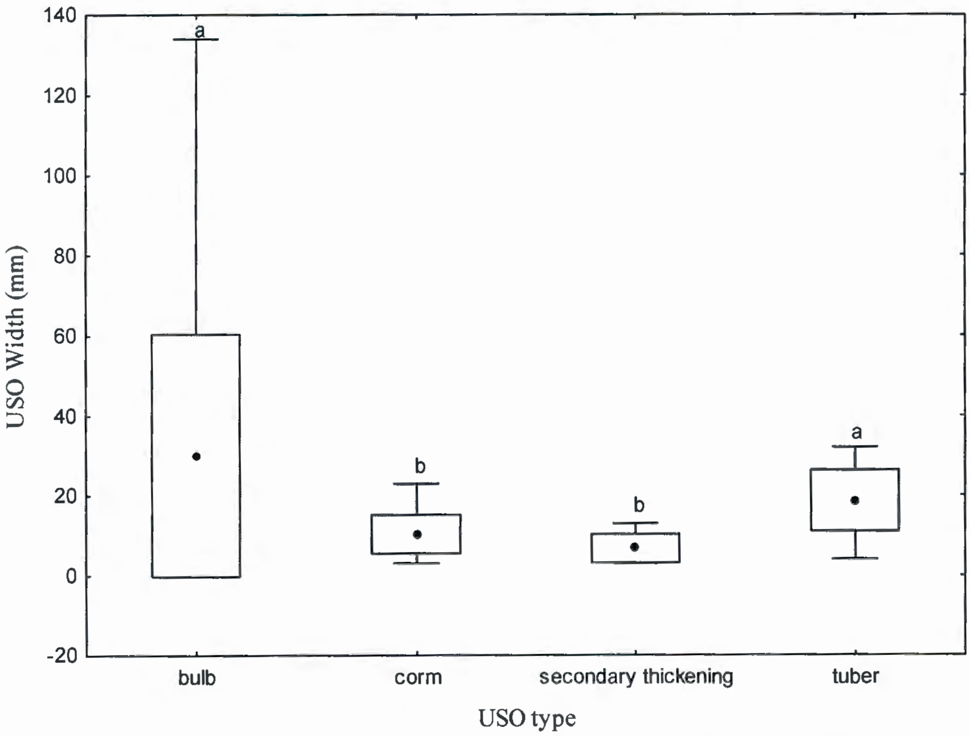

Figure 3: Illustrates the difference in storage organ width (mm) between the four storage organ types. The point represents mean width, the box represents standard deviation and the whiskers represent maximum and minimum values. Letter codes indicated above the whiskers show similarities based on a Tukey (HSD) test (df = 107, p<0.01).

A positive correlation was found (r = 0.633) when comparing water content against storage organ width, for all storage organ types combined (Figure 4A). When looking at each storage organ type separately, there was no correlation between water content and storage organ width for corms, bulbs and tubers. There was however, a positive correlation between the variables for secondary thickening (r = 0.85).

A significant difference in water content was found when comparing the four storage organ types (H=43.33, df = 3, $p<0.01$). Secondary thickening contained significantly less water than the other storage organ types. Bulbs contained significantly more water than corms. No conclusions could be drawn about the water content in tubers because the mean belonged to two populations (i.e. was similar to bulbs and corms). Again, it is important to notice the large variability in water content for all storage organ types (Figure 4B).

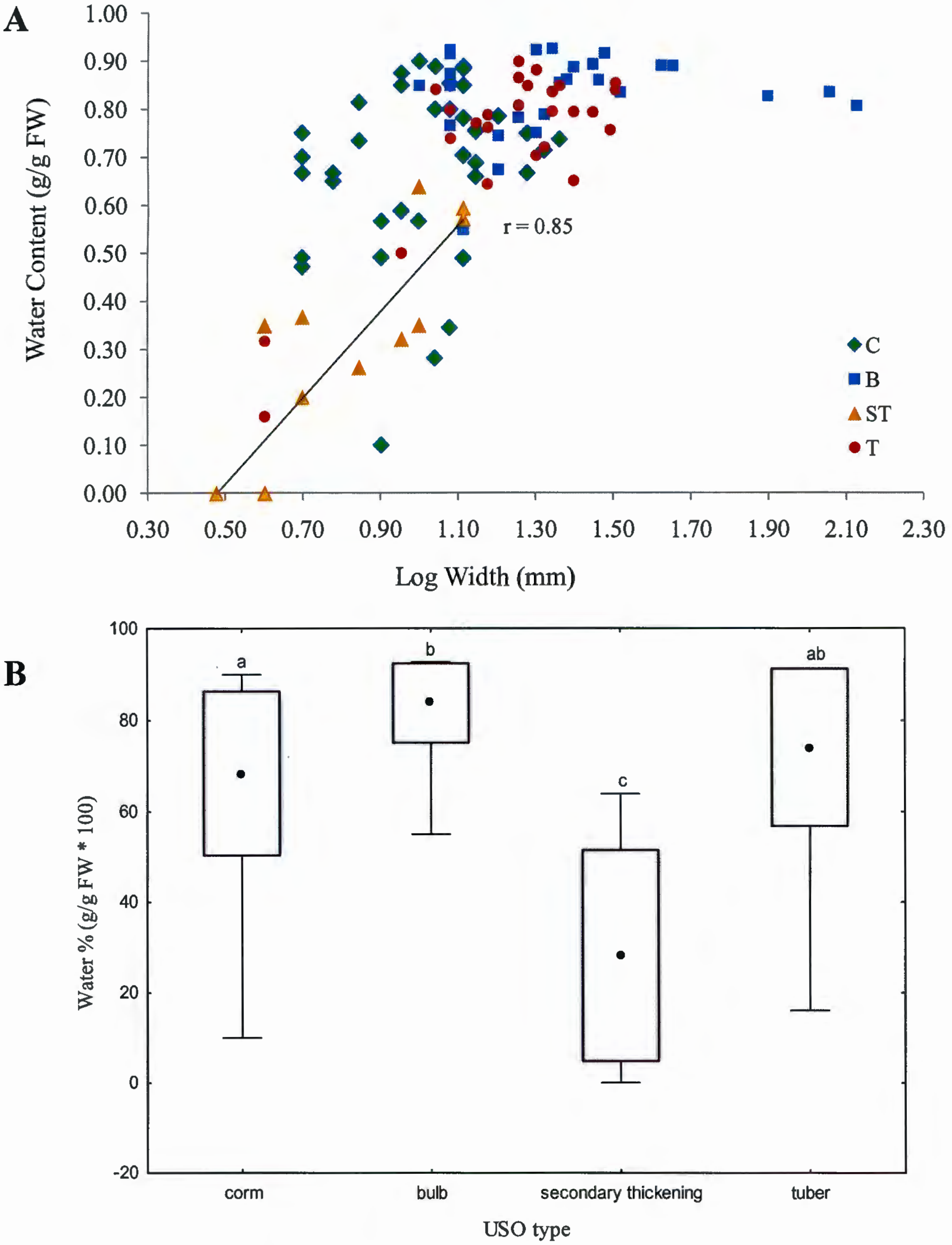

Figure 4: A) illustrates the relationship between storage organ width (log transformed) and water content (g/g Fresh Weight) for corms (C), bulbs (B), tap roots with secondary thickening (ST) and tubers (T). B) illustrates the difference in water % between the four storage organ types. The point represents mean water %, the box represents standard deviation and the whiskers represent maximum and minimum values. The letters represent the outcome of a Tukey (HSD) test.

There was no correlation between N% and storage organ width, for corms, bulbs and tubers; as well as for all storage organs combined. There was however, a positive correlation between the variables for secondary thickening (r=0.84) (Figure 5A).

A significant difference in N% was found when comparing the four storage organ types (H=15.40, df=3, $p<0.01$). However, this is only because corms had significantly less N% than secondary thickening and tubers. Upon visual evaluation, it seems as if the N% means for all storage organ types are similar (Figure 5B).

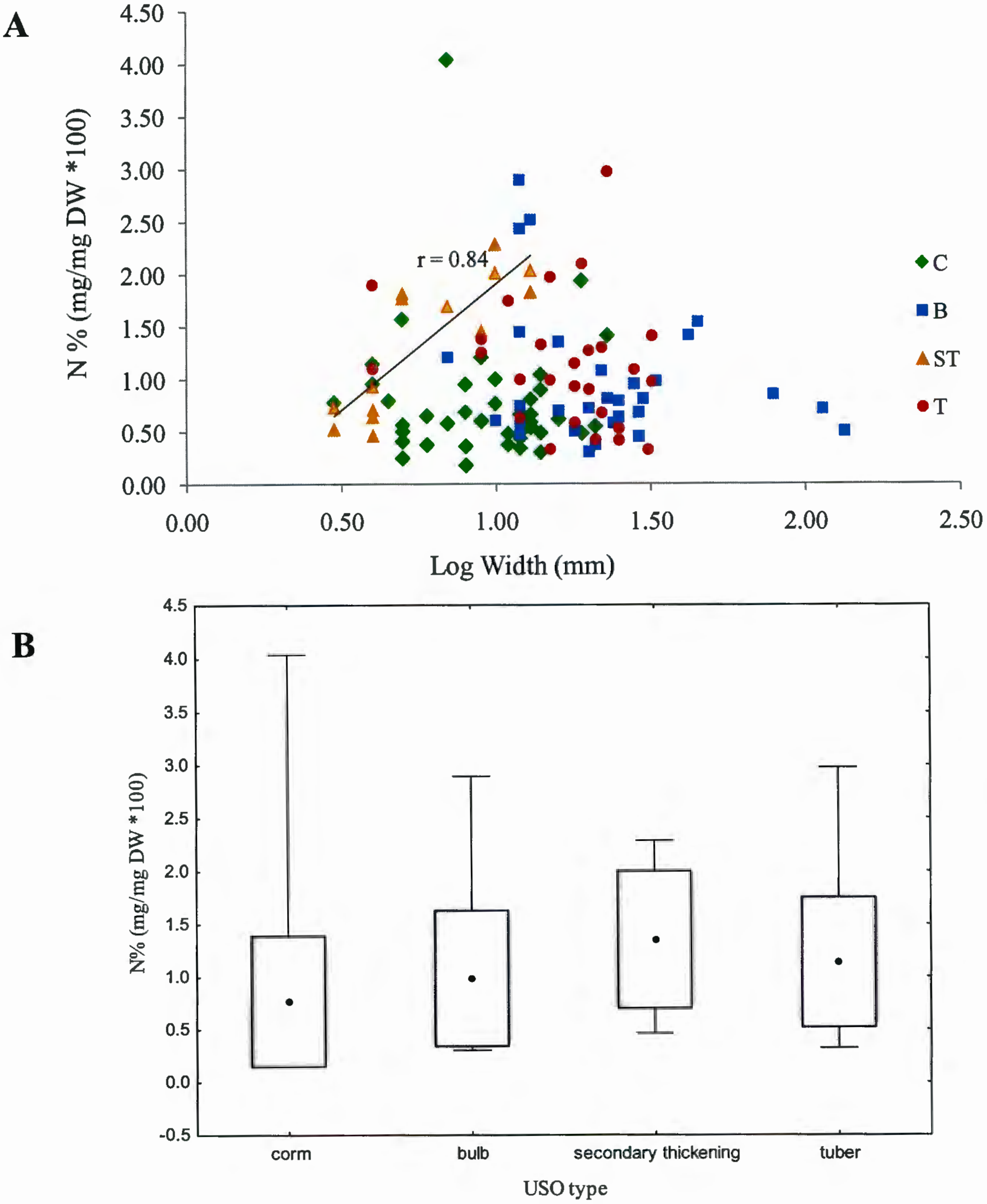

Figure 5: A) illustrates the relationship between storage organ width (log transformed) and N% (mg/mg DW *100) for corms (C), bulbs (B), tap roots with secondary thickening (ST) and tubers (T). B) illustrates the difference in N% between the four storage organ types. The point represents mean N%, the box represents standard deviation and the whiskers represent maximum and minimum values.

The P% of storage organs followed a similar trend to that of N%. There was no correlation between P% and storage organ width, for corms, bulbs and tubers; as well as for all storage organs combined. A positive correlation between the variables for secondary thickening was found (r=0.64) (Figure 6A). Furthermore, there were no significant differences between the mean P% of all four storage organ types (Figure 6B).

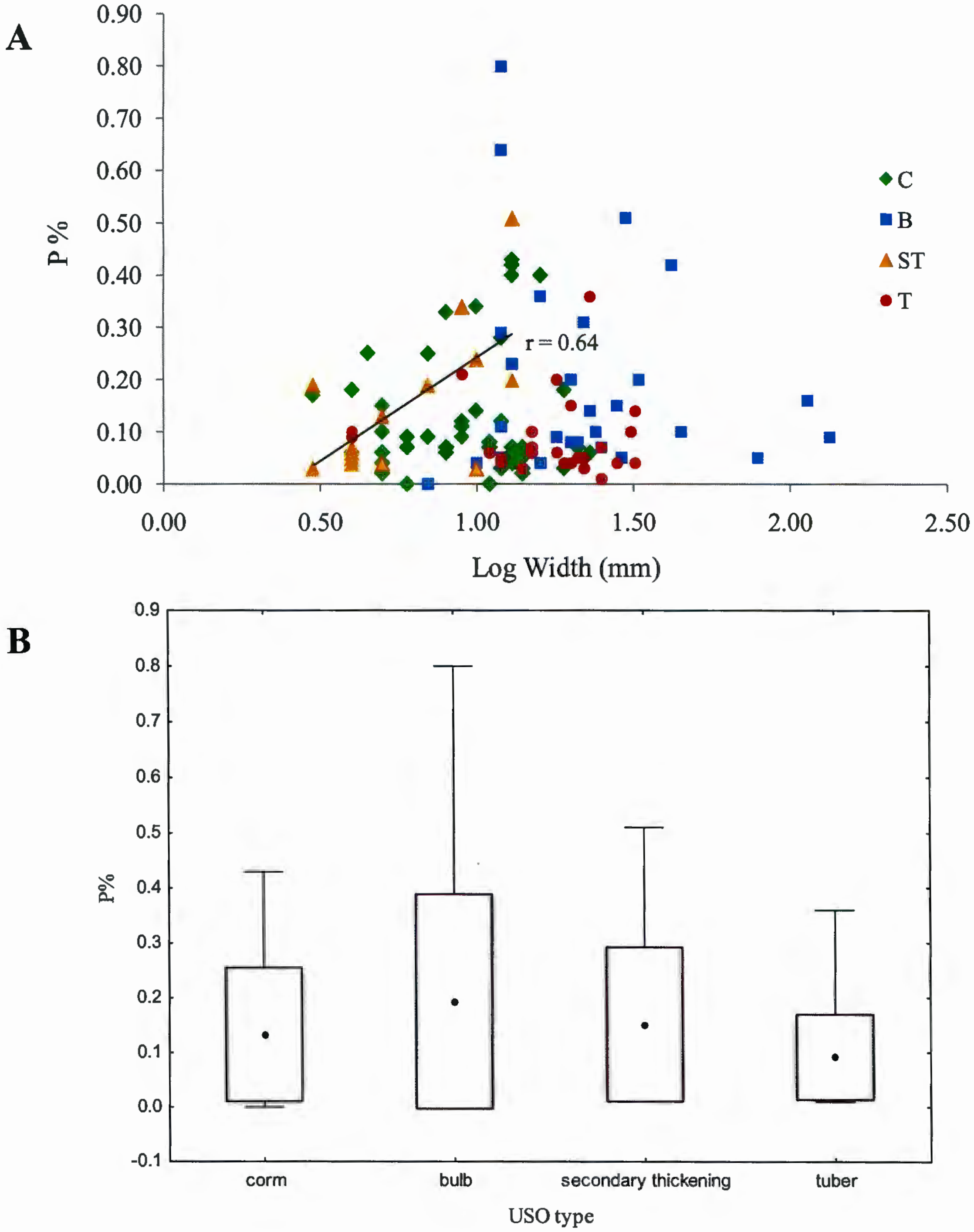

Figure 6: A) illustrates the relationship between storage organ width (log transformed) and P% for corms (C), bulbs (B), tap roots with secondary thickening (ST) and tubers (T). B) illustrates the difference in P% between the four storage organ types. The point represents mean P%, the box represents standard deviation and the whiskers represent maximum and minimum values.

As for N% and P%, there was no correlation between starch content and storage organ width overall and for the storage organ types, including secondary thickening (Figure 7A). Again, the starch content of all four storage organ types were not significantly different, but attention must be paid to the large variability in starch content seen in corms, bulbs and tubers (Figure 7B).

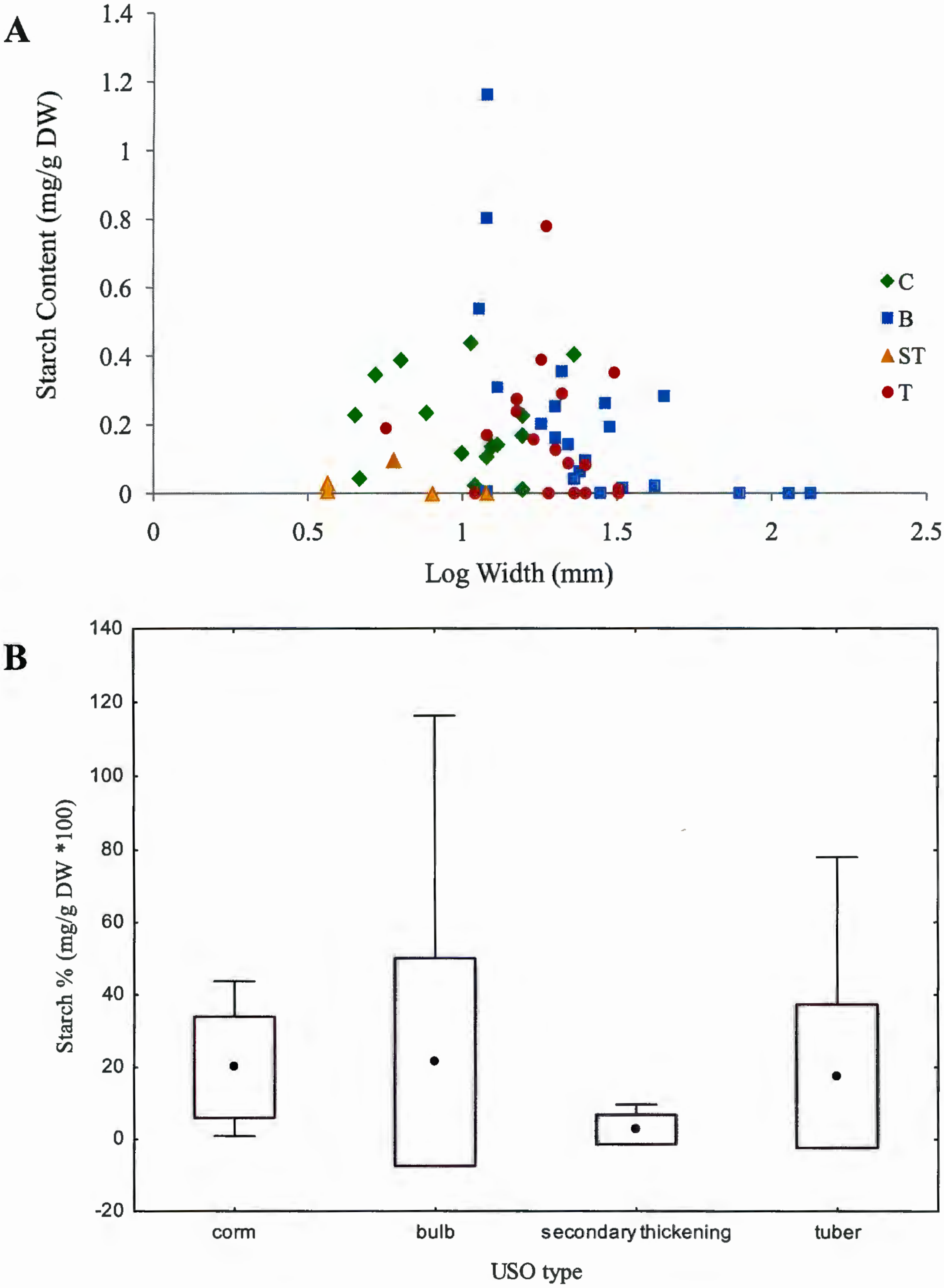

Figure 7: A) illustrates the relationship between storage organ width (log transformed) and starch content (mg/g DW) for corms (C), bulbs (B), tap roots with secondary thickening (ST) and tubers (T). B) illustrates the difference in starch % between the four storage organ types. The point represents mean starch %, the box represents standard deviation and the whiskers represent maximum and minimum values.

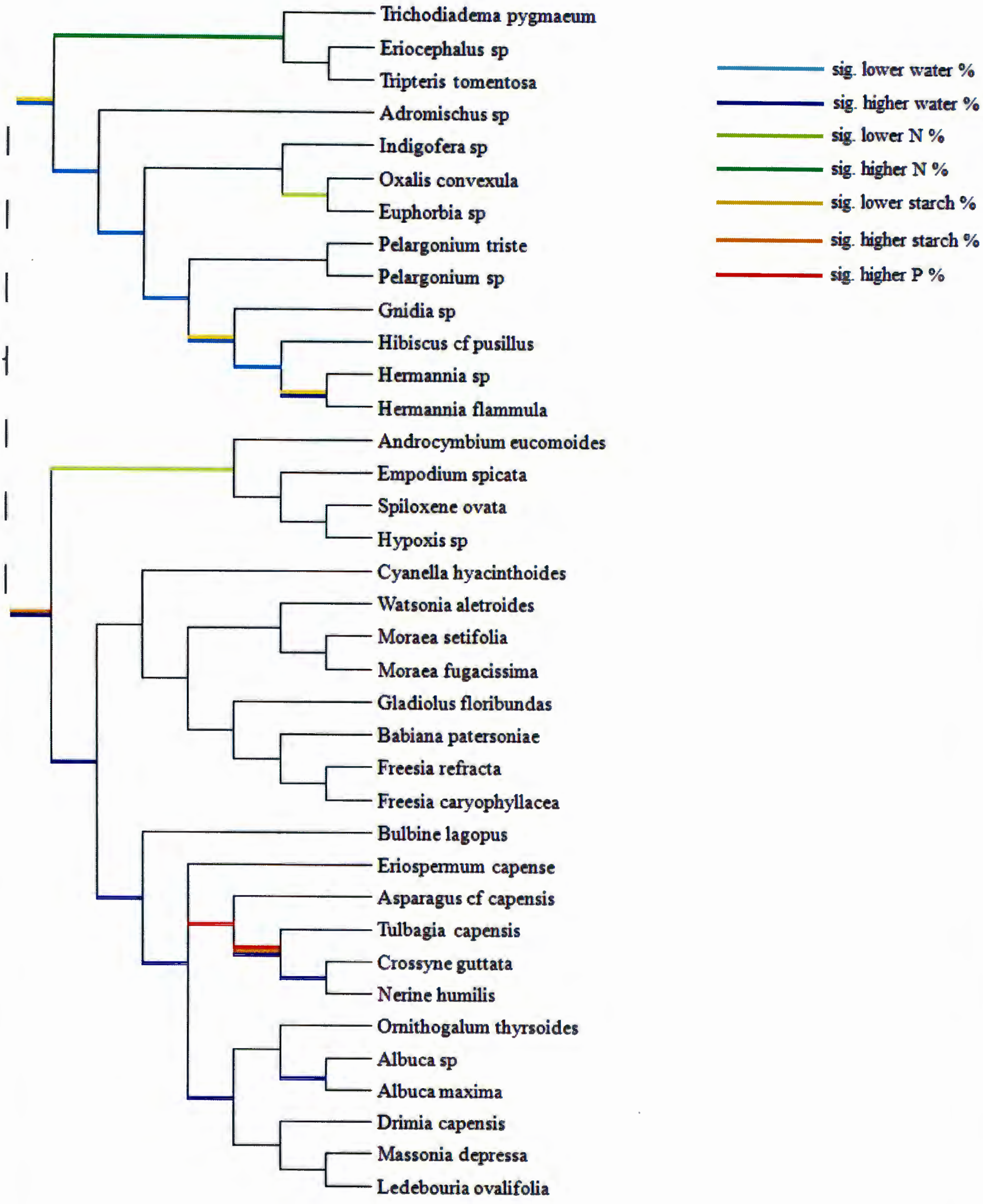

Figure 8: Phylogenetic tree of plant species investigated, with the significance results (at the 90% significance level) of the AOT (performed in *Phylocom 4.2*) mapped onto it. The key in the top right indicates which colours relate to the variables investigated: water %, N%, P%, starch %.

The AOT calculates the average of all the descended taxa from a node when analysing if this node is significant compared to the 'random' trait sample. As a result, nodes closer to the tips of the phylogeny, or nodes relating to a particular family, could possibly have greater importance. The water % trait seems to be significantly higher in a number of monocot nodes and significantly lower in a number of dicot nodes. The other traits do not give a large number of significant nodes.

Discussion

The significant differences in size between the different USO types can be partly explained by whether the storage organ has a perennial or annual life cycle. Generally, bulbs and tubers are perennial storage organs and so experience continuous growth over a number of years allowing for their size to be larger than corms (Procheş *et al.* 2006). Generally, corms are replaced annually and so a size limit is imposed. Because the secondary thickening of a tap root is not considered a true form of underground storage organ, it was not expected to be of significant size. Ruiters and McKenzie (1994) wisely state that it cannot be assumed that biomass will equal allocation, which holds true for this investigation – although storage organ width was considered, not dry mass.

A tentative explanation for why bulbs store more water than corms is that corms have a woody tunic which protects them from desiccation during summer, while bulbs have little protection – only older leaf bases/scales (Al Tardeh *et al.* 2008). A vital aspect for the storage organ is that cell turgor is maintained during the dry period; therefore, bulbs could store more water than corms to counteract their greater water loss (Al Tardeh *et al.* 2008). Water content in secondary thickening is significantly ? less than in true storage organs, because secondary thickening is not fleshy but woody and would not be composed of tissues with many vacuoles (Pate and Dixon 1982). Water may be a variable factor in storage organs because of microhabitat variation in soil moisture (Procheş *et al.* 2005). The depth of the storage organ in soil may increase this microhabitat variation. Ruiters and McKenzie (1994), when collecting geophytes for their study, noted that storage organs between 1.5 and 4 cm deep experienced high temperatures during summer and water-logging during winter.

Generally, the results describing mean nutrient and starch concentrations show no difference between the four storage organ types. The only feasible suggestion as to why the average nutrient concentrations (nitrogen and phosphorous) are similar is because all of the species were growing in the same homogenous renosterveld environment. Corms have a significantly different N% to secondary thickening and tubers most likely due to the large variation of N% in corms. This variation may be caused by one outlier – a replicate of *Freesia caryophyllaceae* with 4.04% N compared to the average of the other three replicates: 0.64%. Location in a similar environment cannot explain the insignificant difference in starch between the four storage organ types. Starch is synthesized in the plant and not gained from the environment.

Al Tardeh *et al.* (2008) state that geophyte reserve allocation patterns are a result of genotype and environmental conditions. Phylogenetic signal indicates that ecological similarity between species is because they are phylogenetically related (Losos 2008). One possible cause of phylogenetic signal is habitat specialisation; species prefer to remain in the environment to which they are best adapted. Phylogenetic relatedness, which takes into account time since divergence, is an important factor to consider in the AOT (Losos 2008). One would expect greater similarity in species that have more recently diverged from a common ancestor (Losos 2008). The phylogenetic tree in this investigation could be expanded by sampling more species to address phylogenetic relatedness. If there were a considerable number of nodes in Figure 8 that were either significantly higher or lower than the 'random' mean, it would imply that there is little trait/ecological similarity between species. Therefore, water % could be a trait that does not carry phylogenetic signal. However, if there were very few nodes in Figure 8 that showed significant differences, it would imply that there could be ecological similarity because of phylogenetic signal. The AOT performed in this investigation was very simplistic and so few conclusions can be drawn from it. But, considering the different storage organ types had similar reserves (N%, P% and especially starch %), as shown by the box and whisker plots, there is scope for investigation into how this might be caused by phylogenetic signal.

This investigation was restricted by theoretical and practical limitations, largely due to the lack of literature specifically regarding the ecology of CFR geophyte storage organs to guide questions and hypotheses for this study. Therefore, on evaluation of the results obtained, many suggestions and ideas for future research will be put forward. Firstly, I will discuss the limitations encountered in this study.

The first three limitations involve the sampling method. Firstly, the conservation restrictions that impose limits on the number of geophytes that are allowed to be removed from the southern Cape renosterveld vegetation hamper efforts to collect an accurate representative sample. Rather than aiming to collect more replicates for each species, additional species could be added to the sample size, and the age of the geophytes could be taken into account. It is possible that geophytes of different ages store different reserves, or different concentrations of the standard reserves. For example, Orthen (2000) discovered that fructan was always the predominate carbohydrate in developing bulbs of all of the CFR geophytes species sampled, even if it is not the major carbohydrate at maturity. Fructan and other carbohydrates will be discussed later on but, it was clear that the fructan to starch ratio depended entirely on bulb development stage. Also, the theory of 'critical mass' may be of importance. Here, the first flowering of a geophyte requires the storage organ to have reached a threshold mass, which must therefore contain the right amount of reserves to initiate flowering (Al Tardeh *et al.* 2008). For example, the Mediterranean geophyte *Urginea maritima* only flowers 6 years after germination (Al Tardeh *et al.* 2008).

The second important factor to consider when studying geophytes is the time of sampling. In other words reserve concentrations will differ across different periods of the year. Most authors investigating geophyte storage organ reserves collected their geophyte species soon after the end of the growing season – the seasonal die-back of leaves and initiation of dormancy (Pate and Dixon 1982; Ruiters 1995; Orthen 2001; Al Tardeh *et al.* 2008). This particular period was chosen because one is able to quantify the total nutrient content in the storage organ that would be available for reproduction and growth. At this period, the geophyte has most likely recycled resources from the leaves, but has not yet distributed any resources to inflorescence initiation (Al Tardeh *et al.* 2008). Although these authors have given their reasons for choosing this particular time period, any time period can be chosen as long as it relates to the ecological question being asked and is considered when interpreting the results. Unfortunately for this investigation, the geophytes were sampled in the middle of their growing period, making it difficult to interpret the results obtained.

The third factor that would be sensible to sample is the local soil characteristics. If the soils in which the geophytes were growing are analysed for moisture and nutrient concentrations, links could be made to the reserve concentrations in storage organs. Ecological questions such as 'to what extent are geophytes obtaining their water and nutrient contents from the soil?' can be asked. It would be interesting to know if uptake of nutrients by geophytes is correlated to winter rainfall, as rainfall allows for a 'flush' of nutrients to be made available (Ruiters and McKenzie 1994). Also, it would be interesting to note how geophytes may utilise nutrients made available by fire. Pate and Dixon (1982) reported that after fire, a specific Australian geophyte (*Drosera erythrorhiza*) showed enrichment of nitrogen and phosphorous in its tubers. None of the literature regarding geophyte storage organ reserves cited in this investigation mentioned or sampled local soil conditions. The limitations to this study were that age of geophytes and time of year for best sampling were not considered, as well as not quantifying the soil characteristics of the samples sites. These limitations made the results obtained tricky to interpret.

Due to the limitations encountered when trying to interpret the results of this investigation, many ideas for future research were noted. Firstly, the validity of the method used to quantify starch in this investigation was questioned and this lead to thoughts about future paths for starch analysis. The *Megazyme* Total Starch Assay Kit was designed to quantify starch in general food, cereal and animal feed products, and not specifically for natural plants. Consequently, throughout the methodology certain steps might not account for the resistance of the natural plant material. To give one specific example, Chow and Landhäusser (2004) examined the amount of enzyme and time required for complete hydrolysis of starch in woody plant material and found that both factors affect amount of starch digested. The amount of α-amylase and amyloglucosidase (enzymes), as well as the incubation time with these enzymes, were considerably greater than used in this investigation. Further, the *Megazyme* Total Starch Assay methodology did not include the production of a standard curve, which is surely necessary to evaluate whether the starch is digested in a linear manner, or if a plateau is reached after time.

A variety of different carbohydrates are actually found in underground storage organs, not exclusively starch. These include: soluble sugars, glucomannans and fructan (Al Tardeh *et al.* 2008; Ranwala and Miller 2008). Future investigations should take this into account, because it has been suggested that the different carbohydrates perform contrasting ecological roles. Starch is the carbon source most likely used exclusively for sprouting of leaves and inflorescences (Orthen 2001). Fructan however, may act as an osmoregulator, a drought and cold adaptation (Orthen 2001). Orthen (2001) explains that an accumulation of short-chain fructans and sucrose in the innermost leaf-bases of bulbs lowers the water potential of that section, compared to the outer leaf-bases/scales and the soil. This sets up a gradient for water to flow into the middle of the bulb. The hydrophilic nature of glucomannan implies that it may also have a water storage function (Ranwala and Miller 2008). To measure the different types of carbohydrates, most authors use a method whereby samples are extracted with 80% ethanol or hot water to obtain soluble sugars (fructans and glucomannans) after which the insoluble residues are used for the starch analysis (Chow and Landhäusser 2004; Ranwala and Miller 2008). It is suggested by Ranwala and Miller (2008) that extraction and analysis techniques should be validated for a true understanding of the diverse carbohydrate pool. Other minerals besides N and P should also be investigated; for example calcium may act as osmoregulator to withstand drought in dormant bulbs (Al Tardeh *et al.* 2008).

The most common description of the term 'geophyte' includes using underground storage organ reserves to initiate leaf growth at the start of a growing season. Thus, the obvious question to ask is 'does a storage organ contain enough resources for complete leafing to take place, or are there only enough reserves to initiate the leafing process?' Ruiters and McKenzie (1994) have made some attempts to determine seasonal allocation patterns to different plant parts, including leaves and inflorescences, for the species *Sparaxis grandiflora*. They state that allocation of resources to leaves during the early growing season (April) is important, where leaf biomass contained approximately 28% of the total macronutrients, 17% of the total micronutrients and 8% of the total non-structural starch (Ruiters and McKenzie 1994). Interestingly, allocation of resources to roots is just as important as it is to leaves. However, as these results suggest that only a percentage of the resources are allocated to leaves and roots, a substantial amount of the resources must remain in the parent corm.

As *Sparaxis grandiflora* is an annual, a new daughter corm develops during the growing season. Ruiters and McKenzie (1994) found that a considerable amount of resources are allocated to the daughter corm – from: transfer of parent corm resources, leaf photosynthate and nutrient withdrawal from senescing leaves and inflorescences. Furthermore, it appears that noticeably more macro- and micronutrients are allocated to the leaves and roots than starch or soluble carbohydrates, which remain in the parent or daughter corm (Ruiters and McKenzie 1994). The amount of reserves available and allocated to flowering is important. Allocation to an inflorescence is further complicated by whether a plant is hysteranthous (separated flowering then leafing) or synanthous (leafing and flowering concurrently), annual or perennial (Dafni 1981). The annual life cycle is more opportunistic, but more at risk from adverse environmental conditions, than the perennial life cycle, which has a 'storage fund' (more reserves than needed). Synanthy is more risky than hysteranthy, because the separation of phases in hysteranthy allows for intermittent storage of reserves (Dafni 1981).

In conclusion, the results of this investigation are not entirely meaningful because they are a snapshot of a very dynamic system. Geophyte underground storage organ reserves are not fixed; they are constantly being allocated to other plant parts such as leaves, roots and inflorescences. In addition, the concentration of reserves in the entire plant system is not fixed because of losses to reproduction and senescing leaves. The geophytes of the CFR should continue to be studied in order to begin to understand how the ecological functions of the 'storage organ' allow these remarkable plants to persist. Storage organs were most likely a major player in the diversification of the geophytes in the CFR.

References

Al Tardeh, S., Sawidis, T., Diannelidis, B.E., Delivopoulos, S. 2008. Water content and reserve allocation patterns within the bulb of the perennial geophyte red squill (Liliaceae) in relation to the Mediterranean climate. *Botany* **86**: 291 – 299.

Chow, P.S. and Landhäusser, S.M. 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology* **24**: 1129 – 1136.

Dafni, A., Cohen, D. and Noy-Mier, I. 1981. Life-cycle variation in geophytes. Annals of the *Missouri Botanical Garden* **68(4)**: 652 – 660.

Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning J.C., Procheş, S., van der Bank, M., Reeves, G., Hedderson, T., Savolainen, V. 2007. Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* **445**: 757 – 760.

Johnson, S.D. 2010. The pollination niche and its role in the diversification and maintainence of the southern African flora. *Philospohical Transactions of the Royal Society B* **365**: 499 – 516.

Kim, J.H., Kim, D.K., Forest, F. Fay, M.F., Chase, M.W. 2010. Molecular phylogenetics of Ruscaceae *sensu lato* and related families (Asparagales) based on plastid and nuclear DNA sequences. *Annals of Botany* **106**: 775 – 790.

Kraaij, T. 2010. Changing the fire management regime in the Renosterveld and lowland fynbos of the Bontebok National Park. *South African Journal of Botany* **76**: 550 – 557.

Losos, J.B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters* **11**: 995 – 1007.

Mucina, L. and Rutherford, M. 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute: Pretoria. pg 159, 160, 195 – 197.

Orthen, B. 2000. A survey of the polysaccharide reserves in geophytes native to the winter-rainfall region of South Africa. *South African Journal of Botany* **67**: 371–375.

Orthen, B. 2001. Sprouting of the fructan- and starch-storing geophyte *Lachenalia minima*: Effects on carbohydrate and water content within the bulbs. *Physiologia Plantarum* **113**: 308 – 314.

Pate, J.S. and Dixon, K.W. 1982. *Tuberous, cormous and bulbous plants*. University of Western Australia Press: Perth.

Procheş, S., Cowling, R.M., Goldblatt, P., Manning, J.C. and Snijman, D.A. 2006. An overview of the Cape geophytes. *Biological Journal of the Linnean Society* **87**: 27 – 43.

Procheş, S., Cowling, R.M. and du Preez, D.R. 2005. Patterns of geophytes diversity and storage organ size in the winter-rainfall region of southern Africa. *Diversity and Distributions* **11**: 101 – 109.

Ranwala, A.P. and Miller, W.B. 2008. Analysis of nonstructural carbohydrates in storage organs of 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection. *New Phytologist* **180**: 421 – 433.

Raunkiaer, C. 1934. *The life forms of plants and plant geography.* New York: Arno Press. pg 64 – 94.

Ruiters, C. 1995. Biomass and resource allocation patterns within the bulb of the perennial geophyte *Haemanthus pubescens* L. subsp. *pubescens* (Amaryllidaceae) in a periodic arid environment of lowland fynbos, South Africa. *Journal of Arid Environments* **31**: 311 – 323.

Ruiters, C. and McKenzie, B. 1994. Seasonal allocation and efficiency patterns of biomass and resources in the perennial geophyte *Sparaxis grandiflora* subspecies *fimbriata* (Iridaceae) in lowland coastal fynbos, South Africa. *Annals of Botany* **74**: 633 – 646.

Webb, C. O., Ackerly, D. D., and Kembel, S. W. 2008. Phylocom: software for the analysis of phylogenetic community structure and character evolution. *Bioinformatics* **24**: 2098 – 2100.

Websites:

1) http://www.africanbulbs.com/index.html
2) http://www.bemlab.co.za/index.php
3) http://www.megazyme.com/downloads/en/data/K-TSTA.pdf

Acknowledgements:

I would like to thank my supervisor William Bond for his advice in guiding the project and funding the project. Big thanks must go to Tony Verboom and Jeremy Midgley for helping me through quite a serious case of writers block. I am grateful to Odette Curtis and Rhonda and Cameron McMaster for helping me with my fieldwork and identifying my species. Thank you to Justin Nyanga for helping me get to grips with the lab work, and Brittany Arendse for helping me mash plants with liquid nitrogen. Lastly, I would like to thank the NRF for providing funding towards my Honours degree.