Longevity, population stage and size structures, morphology and reproduction of four long-lived grassland suffrutices

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DECLARATION

I declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science by coursework and research report in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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(Signature of candidate)

_____03_____day of

_____August_____2011_____

Abstract

Little is known about the longevity of grassland suffrutex plants and the relationship between longevity and plant morphological structures and seedling growth strategies. The aim of this study was to determine the longevity of four grassland suffrutex plant species, namely *Berkheya insignis, Callilepis laureola, Protea insignis* and *Tephrosia kraussiana*. Seed viability, seedling growth, morphology, habitat and population structure and demography were also assessed and related to plant age.

The rhizomes, seeds (if available) and canopies of *B. insignis*, C. *laureola, P. simplex* and *T. kraussiana* were removed from a site near Port Edward in the Pondoland region, KwaZulu-Natal, South Africa in April 2008. Seeds were available for *P. simplex* and *T. kraussiana* only. Seed germination and viability were tested in the field and laboratory using germination trials and tetrazolium tests. Greenhouse and field grown seedlings were used to monitor seedling growth and to record seedling morphology. The aerial and rhizome morphologies of adult plants excavated from the field were also recorded. The largest of these rhizomes were aged using radiocarbon dating. Attempts were made to develop morphological surrogates for plant age as no method currently exists. Plant density, demographies, number of inflorescences and various environmental variables of wild populations of *B. insignis*, C. *laureola, P. simplex* and *T. kraussiana* were sampled from the Red Desert Nature Reserve and the Umtamvuna Nature Reserve.

Species morphologies varied, however important similarities suggest convergent evolution. These included a single vertical main stem rhizome with multiple side branches, early rhizome development in seedlings, and high root: shoot ratios. Seed viability was high in the laboratory but low seedling emergence was observed in the field. Seedling growth was rapid for *T. kraussiana* and slow for *P. simplex*. The population stage structure for all four species comprised primarily adults with few or no juveniles. Wild population plant height and main stem diameter followed normal distributions. Number of stems, basal area, canopy area and the number of floral structures had distributions favouring the smaller size classes. Soil P, N, K and organic carbon were important soil nutrients in a PCA analysis of the habitats of the four species. Radiocarbon dating yielded the following ages: *B. insignis*: 49-51 years*, C. laureola*: 49-50 years*, P. simplex:* 49-51 years and *T. kraussiana:* 51 years. There was a significant relationship between rhizome mass and canopy area, basal area, height, number of stems and main stem diameter. Since rhizome mass had a positive relationship with age- a relationship between age and aerial structures is likely. Therefore, creating surrogates for age may be possible.

Overall, these species have moderate longevity, are poor seed producers with possibly slow population growth and are closely associated with soil nutrients. Therefore, these and other suffrutex species are particularly vulnerable to habitat destruction and climate change. The results of this study indicate that there needs to be a greater focus on below ground growth during ecological assessments in order to better understand the ecology of our diverse grassland biome plants.

Keywords: Demography, longevity, morphology, radiocarbon dating, rhizome, size structure, suffrutex, surrogates, viability.

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Nomenclature and abbreviations

Geoxylic suffrutices:	A plant growth form in African grasslands in which the plant develops a large perennial rootstock belowground and non-perennial canopy aboveground. Stems in the canopy are often burned and re-sprout after a fire. The stems of these underground trees can spread over a large area and yet may all be connected to a single underground rhizome. Their longevity is unknown, however, they have been suspected to be several decades old.
Rhizomatous:	An underground stem which produces shoots near its tips and roots from its dorsal surface.
Hemicryptophytic:	A perennial plant which produces over wintering buds at soil level. Buds are partially covered by litter cover.
Holocene:	The current Holocene interglacial epoch began approximately 12 000 years ago (equivalent to 10000 14C years). Human civilization dates entirely within the Holocene period.
AMS:	(Accelerator Mass Spectrometry) an accelerator-based mass Spectrometer is used to count all the C14 atoms, rather than just atoms which are decaying. AMS dates are more precise and less sample is required.
AAA:	(Acid-Alkaline-Acid) wash technique for extracting oils and other organic compounds from the sample. Samples are first immersed in an acid solution, rinsed with water and, then an alkaline and a final acid solution.

1. Introduction

Grasslands are one of the most threatened biomes worldwide. Only 1.8% of the Grassland biome in South Africa is currently formally conserved (National Grasslands Biodiversity Programme 2010). Industrialization, forestry and urbanization are serious threats to grasslands. Land cover information suggests that almost 30% of South Africa's Grassland Biome had been permanently transformed between 1988 and 2006 (Mucina *et al.* 2006b). These changes occur at rates which challenge the adaptive and evolutionary capability of grassland species.

Much of this transformation can be attributed to agriculture (crop cultivation). Many untransformed grassland areas in South Africa exist as fragmented patches between agricultural fields, some spanning only a few hectares (Mucina *et al.* 2006b). Higher rainfall plateaus are under particular threat from *Pinus* and *Eucalyptus* afforestation. These areas are often regions of high biodiversity and sometimes contain rare and endemic plant species. Anthropogenic habitat transformation also promotes the encroachment of alien vegetation into these grassland remnants (O'Connor and Bredenkamp 1997; Beater *et al.* 2008). Climate change models under a 2^{o}C temperature increase and 15% decrease in precipitation, predict a further reduction in the extent of the Grassland Biome (Mucina *et al.* 2006b). Under more ambitious predictions, the biome may be reduced by as much as 55% (Mucina et al. 2006), thus increasing pressure on grassland endemics.

At first glance, grasslands appear to be composed mainly of graminoids, however, upon closer inspection, the diversity of forb species becomes apparent. Forbs may represent as much as 75% of the alpha diversity in grasslands (Uys 2006). Forbs form an essential part of grasslands; however, until recently the ecological and conservation status of forbs have received less attention than the graminoids. Therefore, there needs to be a greater understanding of grassland forb biology in order for grassland management and conservation goals to be effective (Uys 2006).

Some Grassland suffrutescent forbs and shrubs have underground storage organs which are used for recovery or regeneration after disturbance events and seasonal deciduousness (Drechsler *et al.* 1999). Typical geoxylic suffrutices are plants with woody rhizomes which give rise to stems which do not persist throughout the year (Robbrecht 1979). Suffrutices have large underground woody rhizomes and usually imitate low shrubs aboveground (e.g. *Dichapetalum cymosum*) (Mucina *et al.* 2006). This plant form is thought to have evolved from ancestral climax trees which underwent dramatic evolutionary adaptations due to fluctuating environmental conditions, pushing them from massive, woody, mature arboreal structures to a subterranean form. Much remains to be discovered with regards to plants with this growth form. Field

ecological studies of suffrutex species have been recommended (Robbrecht 1979) as data on these species are infrequent and scattered.

Many of these species are suspected to be long-lived and form ancient components of grasslands. However, no information exists on their longevity and only surrogates for plant age have been used in the past. The recruitment patterns of these species are also little understood. Furthermore, the effects of harvesting of larger individuals from populations are unknown. The below ground storage organs of many of these species often contain chemicals which are highly sought after by muthi (medicinal plant) harvesters (Williams et al. 2007) for their remedial properties (e.g. *Dioscorea sylvatica* (Kunth) Ecklon; *Boophane disticha* (L.f.) Herbert.; and *Callilepis laureola* DC) and these activities may cause the species to be declining. The ecological impact of the loss of remnant grassland patches containing these potentially long-lived species will take a long time to become clear as species will continue to persist in these patches for a prolonged period after fragmentation.

Raimondo and Donaldson (2003) used radiocarbon dating techniques to determine plant age of the cycad species' *Encephalartos cycadifolius* and *Encephalartos villosus*. As far as we are aware, this was the first study to determine the age of South African grassland suffrutex plants (plants which were suspected to be long-lived but whose actual longevity was unknown) using radiocarbon dating techniques. Ageing and assessing the demography of plants provides vital information for calculating generation time, which is used as part of the IUCN Red List system for determining the conservation status of species (Keith 1998). Very few determinations of plant age have been done in South Africa. Addressing these gaps in knowledge will improve the effectiveness of conservation assessments and help with predictions of plant responses to current and future global changes. In addition, our basic lack of ecological knowledge of grassland forbs requires the collection of information on population structure, reproductive potential and habitats in order to better understand their persistence in these environments (Witkowski et al. 1997; Pfab and Witkowski 2000).

The aim of this study was to determine the age (longevity) of four grassland species using radiocarbon dating and relate age to aspects of their population demography, morphology, local habitat, growth and reproduction.

The broad approach used in this study was to determine the longevity (age) of individuals of *Tephrosia kraussiana* Meisn, *Berkheya insignis* (Harv.) Thell.*, Protea simplex* E.Phillips and *Callilepis laureola* DC. Population stage and size structure were also investigated for each species to assess population stability and trends in the field, as this information was also unknown and would be used to develop surrogates for age. Recruitment and germination studies

were also undertaken to provide more information on the reproductive potential of the four selected species. Information about the life history characteristics of these species may aid in their management and, hence, persistence in these threatened grasslands, where the tradeoffs between growth and reproduction versus longevity may increase their vulnerability to perturbations. The morphologies of aerial and subterranean parts of adult plants (excavated in the field) and seedlings (grown from seed collected from the field sites) were also described as this information was not previously recorded. Local habitat variables were also assessed. The following research questions were addressed for the species selected:

1. What is the longevity of suffrutex species and do the sizes of aboveground and belowground structures correlate with age?
2. Is the demography of field populations skewed to favour older (or larger) individuals because of low recruitment?
3. Can the age of existing field populations be estimated using surrogates?
4. What are the implications of population structure and suffrutex longevity for grassland management?

2. Literature review - The ecology of long-lived grassland plants

2.1 The components of Grasslands

The Grassland biome covers 29% of South Africa and has high species richness and biodiversity. The diversity of indigenous species in this biome is only surpassed by the Fynbos biome. Twenty-two percent of South Africa's endemic reptiles, one-third of South Africa's threatened butterfly species, the national bird, the Blue Crane, and the Critically Endangered Wattled Crane all occur in the biome (National Grasslands Biodiversity Programme 2008). It is floristically distinct and is the centre of diversity for many large genera (Nicholson and Hulett 1969)

Grasslands are an ancient and important biome with specially evolved plant growth forms. Previous theories attributing the development of grasslands to anthropogenic burning have now been discounted, as afromontane grasslands existed long before humans and cattle herding (O'Connor and Bredenkamp 1997). Grasslands are used extensively by humans. They usually occur in areas of relatively even topography. These are prime sites for grazing, agriculture and urban development (Nicholson and Hulett 1969). Such activities make them vulnerable to large scale disturbances, increasing the extinction risk for species which form an integral part of the biome.

Grasslands have a close relationship with fire (Knapp *et al.* 2004) as fire shapes species composition and dominance. The forb component of grasslands plays a pivotal role and has been suggested as a qualitative indicator of fire interactions along environmental gradients (Robinson *et al.* 1979). Forbs, which are geophytic and hemicryptophytic, are resilient to frequent fires (Uys *et al.* 2004), and often resprout after fire events as forbs hold their meristems below or at the soil surface. The exclusion of fire from grasslands for extended periods (±10 years) could reduce forb density (Uys *et al*. 2004); however, frequent fires favour sprouting forb geophytes over grasses, suggesting that individual forbs may have comparatively longer lifespans (Knapp *et al.* 2004). Many grassland forbs are suspected to be long-lived. Studies in the Eastern Cape have reported that changes in species composition with time after burning may depend more on species longevity and growth rates rather than changes in the environment (Robinson *et al.* 1979). The relative abundance of forbs also increases in grasslands from xeric to mesic habitats (Robinson *et al.* 1979). Other factors which decrease forb density and richness in grasslands include increased litter fall (accumulated in the absence of fire) and grazing (Overbeck and Pfadenhauer 2007). Although frost is usually a significant feature in grasslands, it plays a negligible role in coastal grassland vegetation units. These units have mean frost days varying

between zero and one. Typical (generally higher altitude) grasslands usually vary between 68 and 158 frost days per year (Mucina *et al.* 2006a).

Loss of forb species in grasslands increases the risk of invasive plant establishment (Martinez and Fuentes 1993). Therefore, forbs form an integral part of the dynamic processes which encompass grassland ecosystems. However, the effect of longevity of these plant forms on population dynamics and survival success remains to be adequately investigated.

2.2 Plant longevity

Published results regarding the full effect of fragmentation on grassland diversity is still unclear. The extinction debt created by the delayed response to habitat loss and fragmentation of the long-lived plants in this biome may be a possible explanation (Cousins 2009).

The ability to recover after disturbance is important when determining vulnerability and conservation status. Increased longevity allows populations to survive short term disturbance events which may drive short-lived species to extinction. However, when the population is faced with long term disturbance events (such as land transformation), the probability of recovery may be reduced, thus increasing extinction risk (Witkowski & Lamont 1997; Raimondo and Donaldson 2003). Some long-lived forbs and shrubs of high conservation value have a higher capacity to tolerate disturbance (such as livestock grazing and fires) as they have stored nutrients and energy. Morris *et al.* (2008) used matrix models to predict the long term effects of a changing climate on a species' lifespan. The population growth of shorter lived species was more influenced by climate driven demographic variability than for longer lived species. They suggest that life expectancy should be a more important consideration when predicting species vulnerability to extinction than taxonomic relations (Morris *et al.* 2008). The presence of a persistent adult stage in the models allowed long-lived species to tolerate small increases in annual variability of survival and reproduction.

For long-lived species, the number and size of populations may be deceptive indicators of the status of a species since large populations may consist mainly of aged individuals (Colling *et al.* 2002; Kohler *et al.* 2005). *Banksia goodii* R.Br. (an Australian rhizomatous shrub) populations have experienced a marked reduction due to land transformation (Witkowski and Lamont 1997; Drechsler *et al.* 1999). However, the conservation status of the species had not previously received significant attention as the long persistence of adult plants gave a false impression of immortality.

In South Africa, the age of some grassland suffrutex species have been inferred through observation, however, the actual age of individuals has yet to be investigated. Although growth

rings offer a simple, effective method of estimating the age of plants in temperate regions, individual rings are not always easily differentiated (Andersen and Krzywinski 2007), particularly in certain suffrutex species. Therefore, other means of establishing plant age must be employed for such species. Radiocarbon dating has been applied in these situations (Andersen and Krzywinski 2007).

2.2.1 The radio carbon dating technique

Three isotopes of carbon are present on earth (Protsch 1986). C^{12} and C^{13} are both stable forms of carbon, whereas C^{14} undergoes radioactive decay at a rate of approximately one percent for every 80 years. C^{14} is incorporated into plants along with carbon dioxide during photosynthesis. The mean lifespan of a C^{14} atom is roughly 8 000 years. It is believed that the specific radiocarbon activity of every organism is maintained so that it is in equilibrium with the atmosphere. When an organism dies, it stops acquiring additional C^{14}. At death C^{14} activity begins to decrease logarithmically through radioactive decay. Since the rate of decay is known, the time elapsed since the death of the organism can be determined (Protsch 1986).

C^{14} decays at a predictable rate with a half-life of approximately 5730 years with a 50 year margin of error. It decays by emitting a beta electron to form C^{12}. The ratio of C^{14} detected in the plant tissue to C^{14} currently in the atmosphere is used to date the material (Vogel 1969). The C^{14} isotope has been successfully used for dating woody tissue. It has also been used to confirm ages established from tree ring counts (Robertson *et al*. 2004, Andersen and Krzywinski 2007)

Samples pre-dating the Holocene can usually be dated with a precision of approximately 40 years. Samples from the Holocene can be dated to within 20 years. Nuclear weapons testing conducted during the 1950's elevated the atmospheric C^{14} levels until the Partial Test Ban Treaty in 1963 (Robertson *et al*. 2004). The level of ^{14}C has been declining slowly until the present (Andersen and Krzywinski 2007). This has allowed samples after 1955 to be dated to the nearest year (Robertson *et al*. 2004).

Secondary radiation (e.g. gamma rays from the sun) can affect the functioning of gas proportional counters. Therefore counter systems need to be insulated from secondary radiation to decrease noise (Theodórsson *et al*. 1992). Counters are insulated by encasing the counter in 10-15cm of lead or 20-30cm of iron. The best shielding conditions are achieved in laboratories which have counters in underground chambers (Povinec 1992) such as the QUADRU laboratory at the CSIR.

Radiocarbon dating can make a significant contribution to our understanding of a species for conservation purposes. The ages of long-lived grassland species are estimated in the field as

a more accurate measure of age does not currently exist. Determining the lifespan of individuals in a population of long-lived species may aid in more accurate estimations of the current status of a species.

2.3 Morphology and evolution of long-lived suffrutices

The origin of land plants has long been a subject of debate (Taylor 1988). However, geologists have held for a long time that herbaceous angiosperms are derived from more woody ancient forms, having been reduced and simplified along an evolutionary timescale to form herbaceous plants (Davy 1922; Sinnott and Bailey 1914). Thus the primitive arborescent form has been progressively reduced to lianas, shrubs and herbs. This change in plant form has been attributed to climatic cooling in temperate zones during the Tertiary period. Plants which were able to survive extreme conditions either underground or as seeds dominated in areas where extreme cold killed off plants with perennial aerial stems. Woody plants became more and more stunted until their hard woody stems retreated underground and their aerial parts only persisted for a single growing season. This led to the development of the suffrutescent growth form (Davy 1922).

Aridity may also have contributed to the development of the suffrutescent growth form (Davy 1922). In the South African Karoo and the Kalahari, arborescent plants are rare and replaced by suffrutices. Suffrutices can have annual stems arising from a perennial woody crown or from a perennial underground stem (Davy 1922). Fire may have also played a role in the development of suffrutex species. However, its contribution is less clear.

Increased carbon dioxide with climate change may influence root morphology and function in grasslands (Ferris and Taylor 1993). Species which use stored carbon to subsidize resprouting could resprout with greater vigor under higher concentrations of atmospheric carbon dioxide (Bond and Midgley 2001). Root morphology may, in turn, influence species abundance and nutrient cycling, thus affecting the structure of grassland communities in the future.

2.4 Reproduction and growth in long-lived plants

Species with life history characteristics which constrain their reproduction or recruitment may have a limited capacity to recover after a disturbance. These populations often have low rates of population growth, intrinsically increasing their vulnerability to extinction (Keith 1998). Different reproductive strategies employed by plants may stem from trade-offs between seed dispersal and seedling establishment (Parciak 2002). Larger seeds produced in small numbers are, in general, not dispersed as far as small seeds produced in large numbers. Environmental variation

may also contribute to influencing these seed dispersal patterns (Parciak 2002). For long-lived plants, regeneration by seeding would dominate in conditions of low environmental stress or under low competition (Garcia and Zamora 2003). Persistence by longevity or vegetative reproduction will take precedence under high levels of competition and abiotic stress (Garcia and Zamora 2003). Species with lower habitat specificity have longer-lived seeds and display lower rates of population extinction (Stöcklin and Fischer 1999).

Long-lived species and species with long seed bank persistence have been negatively correlated with high historical grassland connectivity and area (Lindborg 2007). Therefore long-lived species may be less influenced by the isolation of grassland patches than short lived species.

Herbaceous and suffrutescent plants dominate the landscape soon after fire. However, their dominance decreases as the time after fire increases. Some suffrutices are less likely to resprout and more likely to produce new seedlings after fire (Keeley *et al.* 1985) due to microsite availability for seedling establishment (Verkaar and Schenkeveld 1984). Reproduction and mortality in many plant populations depend on both individual age and size (Law 1983).

2.5 Demography and size structure of long-lived species

Plant-pollinator interactions are dependent on the size and spatial arrangement of populations as different pollinators show preferences to different population sizes and densities (Mustajarvi *et al.* 2001). Plant density and population size and demography, in turn, affect the genetic variability of a given population (Van Treuren *et al.* 1993).

Long-lived species tend to grow slowly, invest energy in roots and stems as juveniles and mature later than short lived species (Platt *et al.* 1988). Long-lived species also tend to form open canopy stands with frequent recruitment in open areas resulting in uneven stage structures (Platt *et al.* 1988).

The plant density of the threatened long-lived *Scorzonera humilis* (a perennial herb) was higher in regenerating populations compared to old populations (Colling *et al*. 2002). In sowing experiments, the higher frequency of older demographic classes was due to a lack of recruitment. The number of seedlings surviving were positively correlated with soil moisture and negatively correlated with productivity. Therefore the size and number of populations may not be good indicators of the status of a long-lived species since many of the individuals recorded in a census may have been older individuals which are just persisting in the landscape (Colling *et al*. 2002).This highlights the need for population-specific demographic studies. It also highlights the

need to properly investigate the morphology of a species' underground characteristics so that stage classes can be defined with greater accuracy.

Fire is also influential in population structure. Burning increases plant density and competition among the dominant C_4 grasses and decreases diversity and richness of subordinate grasses and forbs (Smith and Knapp 1999). Tree and shrub forms usually evolve fire resistance by increasing bark thickness or increasing individual height (Seymour and Huyser 2008). However, the meristematic tissues of suffrutex species are belowground and aerial shoots die back after a fire leaving little or no opportunity for bark thickening.

Harvesting, herbivory and the presence of invasive species (Klein and Steinger 2002; Williams and Crone 2006) also affect population structure and density (Van Hoang *et al.* 2008). Harvesters can influence a population either by harvesting of whole individuals or by harvesting reproductive parts, thereby reducing recruitment possibilities (Van Hoang *et al.* 2008), while herbivory and invasive species reduce forb density.

2.6 The local environment in grasslands

The influence of local environmental gradients may supersede that of disturbance in grasslands (Shackleton *et al.* 1994).

In discontinuous grasslands (grasslands with large gaps in the canopy), nutrients accumulate around resource islands which are centered on individual plants (Burke *et al.* 1998). These islands are strongly influenced by the individual's lifespan, growth form, root to shoot ratio and photosynthetic pathway. Nitrogen and phosphorus may be co-limiting biomass production in South African grasslands (Craine et al. 2008). In grasslands which do not have an overriding resource limitation, plant cover is usually relatively continuous and plant-soil interactions are governed by plant biomass production, litter quality and nutrient availability (Burke *et al.* 1998). Soil nitrogen, herbivory, fire and light are also important in ecosystem dynamics. There are strong feedbacks between plant community structure, diversity and soil attributes. In both dry and sub-humid grassland types, anthropogenic land transformation by changes in grazing, fire regime and alien plant introduction alters the pattern, quantity and quality of soil organic matter. Changes in soil nutrient supply in turn affect plant species composition, diversity and primary productivity (Burke *et al.* 1998).

Changes in a population's environment also influence the susceptibility of that patch of landscape to invading species (LeJeune and Seastedt 2001). This can, in turn, influence the population dynamics of long-lived species as once open spaces may become occupied by these ruderal species, thus reducing the available space for long-lived seedlings to establish.

3. Study species and study area

3.1 Species

Four suffrutex species were selected for this investigation (Table 3.1).

Table 3.1 Family, botanical name and growth form of the four grassland suffrutex species selected for investigation.

Family	Species	Growth form
Asteraceae	*Berkheya insignis* (Harv.) Thell.	Forb
Asteraceae	*Callilepis laureola* DC.	Forb
Proteaceae	*Protea simplex* E.Phillips	Dwarf shrub
Fabaceae	*Tephrosia kraussiana* Meisn.	Shrub

These four species (Table 3.1) were selected since they are all classified as grassland suffrutex species (low growing woody shrubs or perennials with a woody base). These species are predicted to be long-lived. During the field investigation in April 2008, the study area was surveyed for appropriate study species. Species which displayed characteristics such as: a) a stout rootstock and b) a traceable main root were chosen. *Berkheya insignis*, *Callilepis laureola*, *Tephrosia kraussiana* and *Protea simplex* were selected as they fit this profile (Table 3.1). Species, initially also short-listed, but which did not fit our requirements were *Eriosema kraussianum* Meisn. (Fabaceae); *Becium obovatum* (E.Mey. ex Benth.) N.E.Br. subsp. *obovatum* var. *obovatum* (Lamiaceae); *Eugenia capensis* (Eckl. & Zeyh.) Sond. subsp. *capensis* (Myrtaceae) and *Gerbera aurantiaca* Sch.Bip. (Asteraceae). Root diameters of these species were too narrow (i.e. less than 3cm). Therefore, too little tissue would have been available from the central root to meet the minimum 10g requirement for radiocarbon dating analysis. Furthermore, the roots of *E. kraussianum* formed complex networks of narrow roots extending over areas greater than $2m^2$. Thus, the oldest region of the plant could not be located. Table 3.2 outlines the characteristics of the selected species.

Table 3.2 Morphological and ecological traits of the four selected grassland suffrutex species, namely; *Tephrosia kraussiana, Berkheya insignis, Protea simplex* and *Callilepis laureola*.

Species	*Berkheya insignis* (Harv.) Thell	*Callilepis laureola DC.*	*Protea simplex* E.Phillips	*Tephrosia kraussiana* Meisn.
Representative photograph				
Growth form	herbaceous[2]	herbaceous[10]	dwarf shrub[5]	herbaceous[1]
Annual/Perennial	Perennial[10]	perennial[10]	perennial[1]	annual/perennial[1]
Leaf shape	numerous, sessile, linear or linear-lanceolate[2]	linear to elliptic, margins flat, entire[10]	oblong[5]	entire, narrow at base[1]
Leaf texture	glabrous or harshly pubescent, margins with flexible bristles, lower surfaces white felted[2]	glabrous/possess glandular hairs[10]	glabrous[5]	-
Leaf/Leaflet structure	Leaves are alternate or radical, but rarely opposite[10]	alternate, sessile[10]	-	imparipinnate or digitately (1)3-5-foliolate or pinnately trifoliolate[1]
Leaf dimensions (mm)	800X150[2]	-	-	-
Stipules	-	-	-	setaceous/broad and striate[1]
Stem height (mm)	500[2]	300-1000[10]	>100[5]	400-1000[1]
Stem diameter (mm)	2-3[2]	-	-	-
Stem texture	sparse soft hairs[2]	-	glabrous[5]	-
Stem structure	forms clumps, simple[2]	produced annualy[10]	numerous, unbranched[5]	-
Flower form	-	-	-	occur on long peduncles, bracts subulate/ovate/spatulate, calyx has a capanulate tube, petals vexillum, usually short with broad claw, velvety pubescent outside with obliquely oblong/obovate wings[1]
Flower structure	terminal, solitary[2]	flowers many, solitary, at end of peduncles[10]	solitary,terminal[12]	grow in racemes and are axillary/opposite leaves/terminal [1]
Flower head size (mm)	80[2]	-	30-60[5]	-
Flower sex	bisexual[10]	ray florets female with staminoides present, disc florets bisexual[10]	-	-
Flower colour	bright gold-yellow, orange-yellow[2]	ray florets white, disc florets purple, corolla white and tube shaped with 3 toothed lamina[10]	a pale red	purple
Stamen description	style terete, undivided/linear with obtuse branches[10]	anthers linear, tailed base with an apical appendage, style terete with obtuse braches with hairs[10]	-	monadelphous/diadelphous, anther uniform[1]

Ovary description	-	-	-	sessile, linear, many ovules, usually hairy, incurved style, terminal capitate stigma[1]
Receptacle	flat, honeycomb-shaped, fimbrilliferous[10]	-	flat, palea boat-shaped[11]	-
Fruit	-	-	-	pods, linear, compressed, 2 valved, sometimes septate[1]
Recorded habitat	submontane plateau grassland[3]	Coastal grassland*	grassland[4], isolated population in Drakensburg mist belt[5]	Coastal grassland*
Seeds	-	-	released 12 months after flowering[5]	oblong/subreniform, sometimes contain small strophiole, funicles short, arils greatly reduced/small/well developed[1]
Altitude (m.a.s.l.)	155-2100[10]	0-2150[10]	0-2000[11]	100-1220
Soil type	shallow, stony[3]	sandy loam*	sandstones*	sandy loam*
Distribution	From Limpopo to the Eastern Cape and inland to the North-west province	from Northern Province, Gauteng, Mpumalanga, Swaziland, KwaZulu-Natal and Eastern Cape[10]	from East London, through Natal, into Swaziland[5]	Eastern Cape and Kwa-Zulu Natal
Extent of distribution	widespread in Southern Africa[3]	endemic to Southern Africa[10]	widespread[5]	abundant, widespread in Africa[1]
Legislation to protect species	-	-	specially protected in the eastern cape[4]	-
Rainfall requirements (mm)	-	-	700-2000[5]	-
Root description	stout woody rootstock[2]	tuberous or subwoody[10]	subterranean rootstock[5]	-
Dieback	-	-	stems die down 1/2 years after flowering[5]	-
Special considerations	pyrophyte[3]	leaf and rhizome extracts used for medicinal purposes by native people[7], contains nephrotoxic atractylocide[9], causes liver necrosis often accompanied by renal tubular necrosis[7], however potency is reduced after long term storage of tubers[8]	flourishes under regular burning (3-4 year fire return interval)[5], stems wither and die after 2-5 years without fire[6]	prefer warmer regions[1]
Flowering season	September- November	August- November[10]	November-December[6]	September- November[10]
Fruiting season	-	-	November-December[6]	-
Population description	Loosely aggregated*	Dense or sparse isolated populations*	Sometimes in dense isolated stands, more often as scattered individuals[6]	Form dense to very dense stands*

[1](Germishuizen 2000); [2](Hilliard 1977); [3](Burrows and Willis 2005); [4](SANBI 2005); [5](Vogts 1982); [6](Rebelo 1995); [7](Popat *et al.* 2002); [8](Steenkamp *et al.* 2004); (Gates 1992)[9]; (Herman *et al.* 2000)[10]; (Rourke 2000)[11] ;(Pooley 2005)[12]; from this study*

Flowering for all species occurs during the summer rainfall season, however, peak flowering occurs in different months. *T. kraussiana* and *B. insignis* flower from September to November. *P. simplex* flowers from November to December while *C. laureola* flowers from August to November. This information is crucial to differentiate between mature flowering and non-flowering individuals during field collection. While all the selected species are classified as least concern under the IUCN Red List (Raimondo *et al.* 2009), *P. simplex* is specially protected in the Eastern Cape. Both *T. kraussiana* and *C. laureola* rhizomes are harvested for their remedial properties. Both are used to treat stomach ailments, however, *C. laureola* has greater efficacy as well as greater toxicity. Several deaths have been reported (Popat *et al.* 2002) as ingestion of the plant causes centrilobular liver necrosis, often accompanied with renal tubular necrosis. Flower displays for all species are colourful and conspicuous, thus aiding in identification of mature individuals in the field. All species are widespread. Two of the study species die-back during the dry season. We conducted fieldwork in summer to avoid problems with incorrect identification or under sampling due to die-back.

Typical geoxylic suffrutices are plants with woody rhizomes or a persistent woody stem base which give rise to stems which do not persist throughout the year (Heywood *et al.* 2007; Robbrecht 1979).

Incongruity and ambiguity exists in the terminology used to describe the underground stem of suffrutex species. Leistner (2000) describes the underground stem of the five species of *Callilepis* as tuberous or subwoody rootstocks, and SANBI (2008) describes it as a woody tuber. Leistner (2000) describes *T. kraussiana* as an undershrub; however an undershrub is vaguely described as a perennial with lower woody parts and herbaceous upper parts which die back annually (Heywood *et al.* 2007). *P. simplex* is described by Rebelo (1995) as having a woody rootstock. References describing the underground stem of *B. insignis* could not be found. A rhizome has been described as a perennial horizontally creeping underground stem which gives rise to seasonal aboveground shoots (Heywood *et al.* 2007b; Jackson 1928a; Rebelo 1995), while a tuber has been described as a perennial underground stem or root which is swollen with food reserves and may have buds or eyes (Jackson 1928c; Heywood *et al.* 2007a). However, a rootstock is also described as a partially underground stem base from which many stems arise after aerial parts of the plant have died, also referred to as a lignotuber (Rebelo 2005). Therefore the terms “lignotuber”, ‘tuber’, ‘rhizome’ and ‘rootstock’ all fit the description of the four study species to some degree. However, the term “tuber” also refers to a storage rhizome which is often distended to some degree (Dr. Stuart Sym 2010, pers. comm.), making it inappropriate for any of the study species.

For the purposes of this project the adult underground stems of mature plants of the study species will be referred to as woody rootstocks or rhizomes. Structures arising from these rootstocks will be referred to as branches or rootlets. The belowground structure of seedlings will be referred to as roots and rootlets.

3.2 The study area

3.2.1 Study site selection

The study area is located in the Pondoland region near Port Edward in KwaZulu-Natal, South Africa (Figure 3.1). Study sites lie between S 31º 04.079', E 30º 11.552' and S 31º 03.449', E 30º 10.547'.

Rhizomes were collected from two privately owned properties. *P. simplex*, *B. insignis* and *C. laureola* were excavated and removed from the Ipithi Retreat (an accommodation facility) on 14 April 2008. The owners of Ipithi Retreat demarcated an area of natural land on their property for development. Consequently, we were granted permission to remove individuals from this area before plants were lost during the perturbations of construction. We removed rhizomes of *T. kraussiana* from a patch of natural land in Clearwater Estate on 15 April 2008. The owner of this holiday destination (which lies adjacent to the Umtamvuna Nature Reserve) granted special permission for removal of the rhizomes. These unique circumstances allowed for the implementation of sampling methods, which would ordinarily be deemed destructive.

The Umtamvuna Nature Reserve (UNR; coordinates: 30.999227S 30.166146E, altitude: 386 m.a.s.l.) was selected for recording size structure, demography and environmental field data for *B. insignis*, *C. laureola* and *T. kraussiana.* We selected this reserve as it contained the study species and lay close to Ipithi Retreat and Clearwater Estate, thereby maximizing the likelihood that growth conditions were similar between the areas from which we removed rhizomes and measured field populations. Ipithi Retreat lies approximately 15km from UNR and Clearwater Estate lies adjacent to the reserve. *P. simplex* does not occur in the UNR. Therefore, size structure, demography and environmental field sampling for *P. simplex*, was undertaken in Red Desert Nature Reserve (lower Red Desert; coordinates: 31.0714S 30.1986E). This reserve was selected as it lies approximately 18km from Ipithi Retreat, again sufficiently close to minimize climatic and other environmental differences.

3.3 Study site descriptions

The region is classified as part of the Pondoland-Ugu Sandstone Coastal Sourveld (Molina *et al.* 2006a). This vegetation type supports species rich grasslands scattered with low shrubs and

small trees. Trees of the Proteaceae are locally common in some areas and geoxylic suffrutex growth forms occur in the sourveld (Molina *et al.* 2006a). Most threatened plant species in the Pondoland region have experienced population decline due to afforestation and land transformation from agriculture, overgrazing, high frequency fires, urban and rural development and exotic plant invasions (Hoare 2006). Almost half the natural vegetation of the Pondoland region has been lost to land transformation. More than 27% is entirely transformed and more than 20% is heavily degraded (Hoare 2006).

The study site for size structure measurements included three locations in the Umtamvuna Nature Reserve and one location at the Red Desert Nature Reserve. The Umtamvuna Nature Reserve (UNR) lies on the border between KwaZulu-Natal and the Eastern Cape (Abbott *et al.* 2000). The UNR lies within the Pondoland Centre, which is a region of high floristic endemism. The UNR is recognized as one of the most important centers of plant diversity and endemism in Africa. The reserve has an area of 3257ha. It encompasses the eastern side of the Umtamvuna River Gorge. Therefore, it forms a long narrow reserve, which follows a rough north-south profile (Abbott *et al.* 2000).

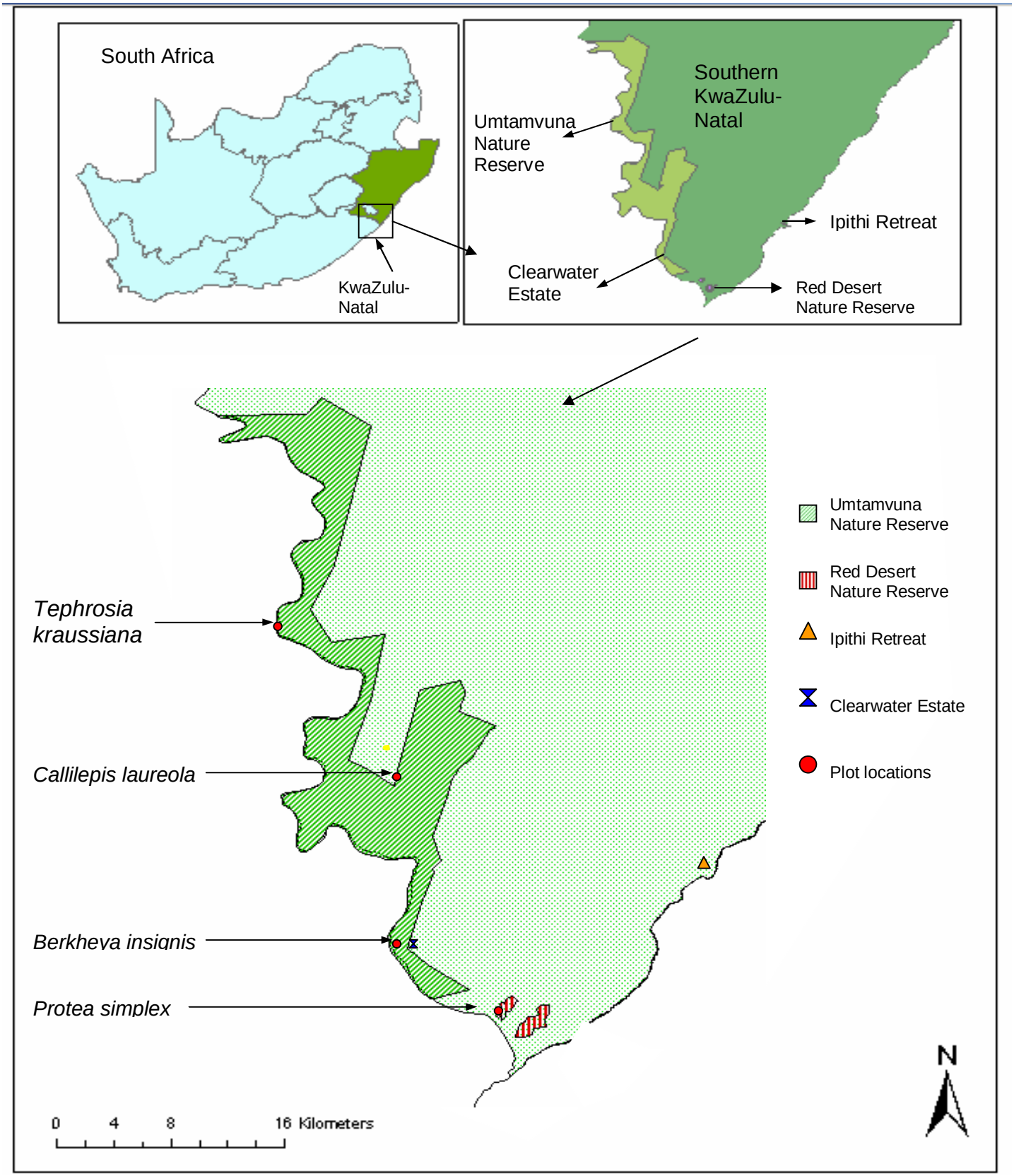


Figure 3.1 Map of a section of Pondoland in southern KwaZulu-Natal showing the study area. The two sites where rhizomes where excavated (Ipithi Retreat and Clearwater Estate) are represented by the triangle and hourglass respectively. The two reserves (Umtamvuna Nature Reserve and Red Desert Nature Reserve) from which size structure data was collected are also shown near the western edge of the province. The specific locations of plots for each species are indicated. *Tephrosia kraussiana, Callilepis laureola* and *Berkheya insignis* were sampled in Umtamvuna Nature Reserve and *Protea simplex* was sampled in the Red Desert Nature Reserve.

The Pondoland Centre contains some sandstone endemics, which are taxonomically isolated with no close relatives or with relatives on islands and continents of Gondwana origin (Abbott *et al.* 2000). Some of these paleoendemics are suspected to be relicts of a largely extinct tropical-subtropical Cretaceous-Tertiary forest.

The reserve is composed of grassland with isolated patches of forest. Forest patches are mainly found in riverine gorges, along south-west facing slopes, following the moisture gradient. Grasslands occur on the upper, drier slopes (Abbott *et al.* 2000). Cape Floral elements such as members of the Proteaceae occur on cool south-west facing slopes. Several streams, which flow towards the gorge, divide the grassland plateau of the reserve. Many of the woody endemics of the Pondoland Centre occur on the slopes of these ridges (Abbott *et al.* 2000).

Fire is important in the region (Abbott *et al.* 2000). Grasslands grow rapidly, facilitating a high fire frequency. However, the current management regime involves burning the grassland biennially in order to facilitate high species diversity. Umtamvuna Nature Reserve and the Red Desert Nature Reserve are separated into management burn blocks. The sites where populations of *T. kraussiana* and *P. simplex* were sampled were burnt in 2001, 2003, 2005, 2007 and July 2009. The block in which populations of *B. insignis* were sampled was burnt in the years 2000, 2001, 2002, 2004, 2005, 2007 and 2009. The site where *C. laureola* populations were sampled was burned in 2000, 2002, 2004, 2006 and 2008. Burns are conducted in early spring.

Most plant species occurring in the UNR are confined to the grasslands (Abbott *et al.* 2000). Non-graminoid herbs outnumber graminoids throughout the reserve, creating a thick groundcover. According to Acocks (1988), the area falls within the Pondoland Coastal Plateau Sourveld. This veld type is considered to be particularly well mixed with no single dominant species. Increasing human pressure has floristically degraded a large part of the grassland (Abbott *et al.* 2000).

Sandstones of the Msikaba Formation dominate the geology of the UNR. Rocks of this formation are chemically homogenous and have 70-96% quartz content and have yielded trace fossils. In some areas, sandstone deposits are only 500m thick. Soils overlying the sandstone are acidic, sandy, greatly leached and often shallow (Abbott *et al.* 2000).

The Red Desert Nature Reserve (RDNR) lies less than a kilometer south of UNR. It also falls within the Pondoland Centre of plant endemism (Abbott 2008b), and spans an area of 172 ha. It includes municipal land as well as two privately owned blocks of land donated to conservation by local farmers. It is an area of coastal grassland with two exposures of Berea red sand. Several rare archaeological artifacts have been found within the confines of the reserve. The reserve has open access to the public and a daily access route for local workers runs through

it. One theory for the origin of the desert-like landscape is overgrazing by cattle owned by Zulu tribes who occupied the region. However, the scenario favoured by Abbott (2008b) is that a game pathway slowly expanded due to heavy wind erosion. This coupled with severe drought would have removed top soil thus preventing the recovery of the vegetation in the reserve. The residents of Port Edward aim to make this reserve a world heritage site as it forms an important part of the Pondoland Centre of Plant Endemism. The Red Desert Nature Reserve is one of few remaining grassland areas in such close proximity to the coast. It harbours several endemic species in its forests and Black mangroves line the estuary. An undescribed endemic *Cussonia sp.* had been found in the reserve as recently as 2008 (Abbott 2008b).

The climate for the region, which encompasses both reserves, is subtropical with mild winters, warm summers and brief periods of high humidity (Abbott *et al.* 2000). Rainfall occurs in the summer months from September to April. Mean annual precipitation is 1075mm. Mean annual temperature is approximately 18.4^{o}C (Molina *et al.* 2006a) for the area in which both reserves occur.

Methods

4.1 Excavation and measurement of rhizomes for determining plant age

Rhizomes of each study species were collected using destructive sampling methods (i.e. the entire root structure was removed from the ground by carefully digging around the base of the plant by hand and with a spade and pickaxe). Rhizomes were removed carefully to preserve their structural integrity. It was assumed that bigger plants were most likely to be the oldest. Therefore, plants with the largest canopy were preferentially removed. For *C. laureola* and *B. insignis*, digging around the base of the central stem gave a better approximation of rhizome size. This aided in the selection of larger individuals.

Table 4.1 Numbers of individual plants sampled for rhizomes from each size class and each study species. Individuals were initially divided into size classes (large, medium and small) based on rhizome mass since rhizomes are older than aerial parts.

	Berkheya insignis		***Callilepis laureola***		***Protea simplex***		***Tephrosia kraussiana***	
Size classes	Mass per class (g)	Number of individuals sampled	Mass per class (g)	Number of individuals sampled	Mass per class (g)	Number of individuals sampled	Mass per class (g)	Number of individuals sampled
Large	>500	2	>200	8	>2000	4	>500	1
Medium	50-499	19	100-199	5	500-1999	9	50-499	11
Small	<50	2	<100	5	<500	2	<50	11
Total individuals collected		23		18		15		23

Originally, 20 individuals of each species were to be collected. Larger individuals of *B. insignis* and *C. laureola* were readily located and rhizomes were easier to handle and keep intact since these rhizomes form a corm-like system. However, rhizomes of *T. kraussiana* and *P. simplex* formed interconnected systems which proved more difficult to remove from the ground intact. Therefore, fewer individuals of these species were collected (Table 4.1).

Rhizomes were collected during April 14 to April 16 2008. Emphasis was placed on collecting the largest individuals of each species in the field. However, smaller plants were also collected to obtain a range of size classes for the purpose of comparison.

Canopy height and diameter of *P. simplex* plants were measured in the field, as extensive belowground branching caused difficulty in removing the rhizomes intact without damaging the canopy. Canopy height and diameter of *T. kraussiana, B. insignis and C. laureola* was measured later in the laboratory. For *P. simplex*, the numbers of stems arising from rhizomes were counted and mature seed cones were collected, if present. Aerial parts were separated from their

belowground parts at the base of the stem, to facilitate access to the plant rhizomes. The rhizomes were then excavated.

The remaining three species (*T. kraussiana, B. insignis and C. laureola*) were removed with the canopy and rhizomes still attached and intact whenever possible, since the rhizomes were removable without damaging the aerial structures. Excess soil was removed from rhizomes. Rhizomes and aerial parts were placed individually in labeled plastic bags to reduce water loss so that wet weight could be measured. These plants were used for investigating both plant age and plant morphology. Many of the *P. simplex* and *T. kraussiana* individuals bore seeds. These were collected in brown paper bags, labeled per individual plant, and stored in a cool dry room for seed germination and viability trials.

At the laboratory the length of the longest stem and stem diameter at the base of the thickest stem was measured for *T. kraussiana, B. insignis and C. laureola* using calipers. Basal diameter was measured at the region where stems connected to the rhizome. Two measurements were taken for basal diameter. The first measure was at the widest diameter and the second, at 90° from the first. The number of green stems arising from the rhizome was counted. Aerial parts were separated from the rhizomes. The aerial part of each individual was weighed using an electronic scale (BEL Engineering -model MARK 4100) with a 0.01g precision. The diameter of the main or thickest stem (if multiple stems arose from the rhizomes) was also measured.

4.2 Field population measurements for demography and size structure

Field measurements for stage and size structure of the four suffrutex species were conducted during late January 2009.

Size and stage structure measurements for *T. kraussiana*, *B. insignis* and *C. laureola* were obtained from the Umtamvuna Nature Reserve. Populations of *P. simplex* were measured in Red Desert Nature Reserve.

The two forms of sampling originally nominated to investigate population size and stage structure were: 1) the plotless point centre quarter (PCQ) method for species with sparse distributions, and 2) the plot method for species with densely distributed populations. The plot method was applied to all sampled populations as all species had dense population distributions. Approximately 300 individuals per plot was set as a target sample size. The size of plots depended on the degree of aggregation of the plants with smaller plots used for denser populations. Plot sizes were kept the same within species. Three populations were sampled for each species with one plot per population. Therefore, there were three plots per species, each

representing a different population. Where possible, populations from different topographic positions (e.g. upper, middle and lower slope) along the catena were selected.

Twenty by 20m plots were used for *P. simplex* and *B. insignis*, 20X5m plots for *T. kraussiana,* and 20X6m plots for *C. laureola*. All plants of the target species were recorded in each plot.

For demographic surveys, plants were categorized into four stage classes. These classes were juveniles; subadults (non-flowering plants); reproductive adults (flowering plants) and non-reproductive adults (non-flowering plants) which display evidence of past flowering events (Witkowski *et al.* 2001). Juveniles for *P. simplex* were defined as those individuals with a single stem (as opposed to the more mature multiple-stemmed habit). Juveniles of *P. simplex* were less than 15cm high and at least one meter away from sprawling adults, as regenerating adult (multi-) stems sometimes resemble juveniles. The soil was scraped carefully away from the base of the stem to make certain that the stem was not attached to a rhizome. Juveniles of *T. kraussiana*, *B. insignis* and *C. laureola* were first identified by their small stem diameter and canopy area. Soil around the basal stems of individuals suspected to be juveniles was again lightly scraped away to ascertain whether the stems were attached to a larger woody rhizome. If this was the case- then these were deemed regenerating adults (recovering from fire damage or other disturbances). If no rhizome was present, individuals were recorded as juveniles.

In each plot, the following information was obtained for each plant:

1) stage class
2) the number of flowers or flower bracts
3) seeds, if present, were collected
4) plant height
5) canopy dimensions (canopy area) based on diameters (two measurements were taken. The first diameter was taken at the widest part of the canopy and the second at 90° to the first)
6) stem basal diameter (two measurements were taken. Soil around the base of individuals was lightly cleared and all stems which appeared to be originating from the same rhizome were noted. The first diameter was taken at the widest part of the basal region and the second at 90° to the first)
7) number of live stems arising from the rhizome
8) main (or thickest, where multiple stems were present) stem diameter (at stem base - ±3cm above soil surface), using digital calipers (Toolquip and Allied digital caliper).

At the centre of each plot, the following were recorded: GPS position, slope angle, elevation and topographic position (position along slope). Soil samples (0-10cm depth) (Witkowski *et al.* 2001) were taken at the centre and at each quarter of the plot (n=5/plot).

Each plant was tagged with a unique number to prevent re-sampling. These tags will also be used for the long term monitoring of these individuals in future investigations (e.g. population viability analyses) conducted by the South African National Biodiversity Institute as the timeframe of this study was too short to incorporate such long term data.

Fieldwork for size structure, demography and environment data collection was originally planned for early December 2008 in order to coincide with the late flowering period of all four species. However, approval of permits was delayed; therefore fieldwork was delayed until late January 2009. It was expected that this period would coincide with fruit ripening and seed dispersal for *B. insignis* and *C. laureola* (species which were not in seed during the April 2008 fieldtrip- hence no seeds were collected). Unfortunately, possibly due to an irregular rainy season, seeds of these species had already dispersed when fieldwork commenced in January 2009.

4.3 Field germination trials

Due to low seed availability, germination, seed viability and growth trials were only carried out for *P. simplex* and *T. kraussiana.* Cones of *P. simplex* and pods of *T. kraussiana* were collected from mature individuals (16 individuals of *P. simplex* and 17 individuals of *T. kraussiana* sampled during April 2008). Seeds of *P. simplex* and *T. kraussiana* were planted in three 50X50cm quadrats (100seeds/quadrat) in the field in January 2009. Three sheets of welded mesh were used for quadrats. Each sheet was composed of 100 5X5cm blocks. Each sheet was secured adjacent to the plot sampled for size structure analysis. A seed was planted in each square. The number of emerged seedlings was recorded six months after planting in July 2009.

4.4 Seed size, viability and germination

Seeds were allowed to air dry for at least two weeks to ensure for post-dispersal drying and allow seed maturation processes to be completed (Weiersbye and Witkowski 2002). For each plant, the following information was recorded:

- number of cones for *P. simplex* only
- total number of seeds per plant
- individual seed mass per plant. Mass per seed was measured using a scale with a precision of 0.0001g (Precisa 92SM-202A). Seed mass was measured for each of 33

intact seeds per plant for *T. kraussiana.* For *P. simplex*, five cones were randomly selected from each plant and each of 20 seeds weighed per cone (100 seeds per plant).

Seeds were germinated during March 2009. *P. simplex* seeds were placed in Eppendorf vials with a layer of cotton wool at the base of the vial in order to trap moisture. *T. kraussiana* seeds were germinated in repli-dishes to facilitate rapid assessments of germination during seed checks. *T. kraussiana* seeds have a hard seed coat; therefore, seeds were immersed in freshly boiled water for 30 seconds (Ruppel 1967) before transfer into repli-dishes. This boiling treatment softened the seed coat and allowed for imbibition. Eight ml of distilled water was added to each vial. Vials were placed in natural light and temperature conditions.

Every three days, for six weeks, the vials were checked and germinated seeds were recorded. Germination was considered complete once the radicle exceeded 2mm.

Seeds which failed to germinate after 6 weeks were tested for viability using 2, 3, 5-triphenyl-tetrazolium chloride (TTZ) staining (Poulsen *et al.* 2006). Tetrazolium salt was dissolved in distilled water to make 100ml of solution. Seeds were soaked in distilled water for 12 to 24 hours at 20-25°C (Akagi *et al.* 2002). Seeds were bisected longitudinally and soaked in 1.0% TTZ solution for 24 hours at 20-25ºC. Viable seeds were recorded. Seeds with a red stained embryo were viable, whereas seeds with embryos, which remained unstained or darkened, were considered non-viable (Akagi *et al.* 2002). The presence of fungi on seeds was also recorded.

Fifty germinants each of *P. simplex* and *T. kraussiana* were planted and grown under green house conditions in soil collected from the field sites. Seedlings were planted at a depth of 1cm in polystyrene cups (Mbalo and Witkowski 1997). These cups were kept in the greenhouse at the University of the Witwatersrand under full sun, between 11-22°C and watered daily. Cups were monitored every two days until seedling emergence. The time to seedling emergence (shoots appear above soil surface) was recorded. Once seedlings emerged, the number of leaves, shoot/stem length and leaf lengths were recorded every four days for 30 days (Mbalo and Witkowski 1997). After 30 days, these measurements were recorded once a week for a six month period. A digital photo was taken of the aerial parts of each seedling. Variations in morphological features were also recorded. Measurements of seedlings were also used to record the morphological characteristics of juveniles of *P. simplex* and *T. kraussiana.*

4.5 Laboratory analysis of *in situ* soil samples

Soil samples obtained in the field were packaged and sent to the soil fertility and analytical services section of CEDARA at the Kwa-Zulu Natal Department of Agriculture and Environmental Affairs. Bulk density (mg/l), extractable phosphate (PO_4^{3-}, mg/l), K (mg/l), Ca (mg/l), and Mg

(mg/l), total Zn(mg/l), total Mn (mg/l), total Cu (mg/l), exchangeable acidity(cmol/l), total cations (cmol/l), acid saturation (%); pH (in KCl), organic carbon (%), total nitrogen and soil texture [(%),clay (%), fine silt (%), coarse silt and sand (%)] was analysed (Appendix 1).

4.6 Radiocarbon dating of rhizomes to determine plant age

Soil was removed from the rhizomes with a soft bristled brush and by thorough washing in tap water. The following measurements were recorded:

1) Wet mass of the rhizome.
2) The circumference of the rhizome was measured at the broadest part.
3) The diameter of the rhizome was taken at its thickest point and a second diameter was once again taken at 90 degrees to the first.
4) The depth of the main rhizome from the soil surface to its tip.
5) Rhizomes were air dried for four weeks. The dry mass of rhizomes was subsequently measured to a precision of 0.01g.

An original sample size of ten individuals per species (individuals with the largest rhizomes) was initially selected to be radiocarbon dated. Rhizome mass and rhizome diameter were considered as preliminary indicators of age. Rhizomes were cut in cross section and the amount of tissue available for carbon dating was estimated by quantifying the amount of sample that could be extracted from the central rings. It became clear after sectioning that only four individuals of *P. simplex*, and three for each of *T. kraussiana*, *B. insignis* and *C. laureola* were large enough for carbon dating (i.e. the central most rings would yield 10g after pretreatment). These rhizomes were then prepared for radiocarbon analysis. Soil was removed from the surface of rhizomes. Rhizomes were sliced along the horizontal plane. The surface of the horizontal axis was polished using an orbital sander in order to identify the central growth areas with greater clarity (Figure 4.1). Plant rhizomes are composed of a soft outer region and a harder central region called the heartwood. Both these areas are made up of several rings (not clearly visible in the study species) usually set down over successive growth years. The central-most rings were taken as the oldest for this investigation and the tissue from these central rings were excised for carbon dating. Eight to 10g of tissue was excised from the most central region using a wood borer and wood carving tools.

These samples were analysed at the Quaternary Dating Research Unit of the CSIR in Pretoria, South Africa (Vogel 1969). I undertook sample preparation and analysis under the guidance of Mr. Grant Hall and Dr. Stephan Woodborne. The radiocarbon dating procedure can

be divided into three phases: 1) The pre-treatment phase, 2) The purification and carbon dioxide isolation phase and 3) The isotope counting phase.

4.6.1) The pre-treatment phase

Samples were purified during a pre-treatment process to remove excess oils, tannins and protective waxes using the AAA (Acid Alkaline Acid) technique (Quarta *et al*. 2005). Samples were weighed before purification to a precision of 0.0001g. Each sample was placed in a glass beaker and approximately 150ml of 1% hydrochloric acid (HCl) was added. Beakers were kept in an oven overnight (24 hours) at 70ºC. Impurities were floating on the surface of samples when removed from the oven. Samples of *C. laureola* released a sharp pungent odor at this stage. The suspended solids in the beaker were allowed to settle for three hours. The unsettled suspended impurities were decanted along with as much 1% HCl as could be removed. Many oils were removed from *C. laureola* thus reducing the amount of sample. The samples remaining in the beaker were rinsed with distilled water by continual decanting and refilling until the sample reached pH 4 when in solution. Approximately 150ml of 1% sodium hydroxide (NaOH) was then added to each beaker and placed in an oven at 70ºC for 24 hours. NaOH removes the resins from plant tissues. Resins appeared to be abundant in *P. simplex*. Once again, the samples were left to settle for 3 hours and impurities, which did not settle, were decanted out. Samples were rinsed with distilled water until the sample reached pH 8 and until the wash-water was clear. 150ml HCl was once again added to samples and the process was repeated as before until samples reached pH 4. Excess water was decanted and the remaining solid sample was oven dried at 70ºC for 24 hours. The dry weight of samples was then determined at this point. A minimum mass of 3g (dry weight) was required for the next phase of the carbon dating procedure. Unfortunately, the remaining samples of *C. laureola* were insufficient for conventional radiocarbon dating. Consequently, these samples were sent to the Centrum voor Isotopen Onderzoek (Rijks*universitiet* Groningen, Nijenborgh, Netherlands) for radiocarbon dating using an alternate technique called Accelerated Mass Spectroscopy (AMS) (McNichol *et al.* 2001; Theodórsson 1991). This technique can cope with very small amounts of sample (i.e. samples less than three grams). AMS determines the concentration of ^{14}C by separating the isotope from other closely related molecules (i.e. ^{12}C) by accelerating ions to extremely high kinetic energies (Goodsite *et al.* 2001; Long *et al.* 1989). However, this is a more expensive technique (R5000/sample compared to R1000/sample for the standard procedure).

4.6.2) The purification and carbon dioxide isolation phase

Further impurities (i.e. sulphates, halogens and other volatiles) were removed using The Bench apparatus (Figure 4.2). This ensured that only carbon dioxide remained at the end of the purification processes before being read by the carbon 14 counters (John and TL/TR). Here samples were rinsed using nitrogen gas to remove volatile substances. Samples were then combusted by passing oxygen through the sample tube and, in this gaseous form, were shunted through three water traps to remove moisture. Samples were then passed through potassium permanganate solution in order to remove sulphates and sulphides. Thereafter samples were passed through coiled silver wire, which removed halogens, then cycled through a hot oven to remove further impurities. Thereafter gaseous samples were directed through two collecting tubes, which used liquid nitrogen to freeze the gas. Copper wires lining the inside of test tubes prevented the temperature from dropping low enough for oxygen to freeze (thus preventing a small explosion). The samples were then unfrozen and allowed to reform into gas. They were then passed through activated charcoal, which removed remaining impurities from the sample. Carbon dioxide gas remained after this process. The gas was then isolated in glass flasks. This procedure was run for approximately 16 hours at two eight hour sessions per sample.

4.6.3) The isotope counting phase

Samples were then taken to the gas proportional counters where the reader was allowed to run for a further 24 hours per sample. These readings were then interpreted using an existing model designed by technicians at the CSIR. Sufficient quantities were not obtained for some samples. Therefore, the process of adding gases from previously dated samples was used to compensate. The use of ‘adding gas’ increases the error margin inherent in carbon dating. However, the discrepancy created by the added gas was corrected for during calculations (Grant Hall, 2009 pers. comm.).

Subsequent to receiving radiocarbon dating results it was determined that many samples had two possible dates. Although growth rings were not visible with the naked eye, annual growth rings at a cellular level may have aided in obtaining an estimate of age so that we could decide which of the two possible dates were more accurate. Therefore, a stereo microscope (Olympus SZX16) was used to detect the presence or absence of growth rings by looking at cross-sections of rhizomes of *B. insignis, C. laureola, P. simplex* and *T. kraussiana*.

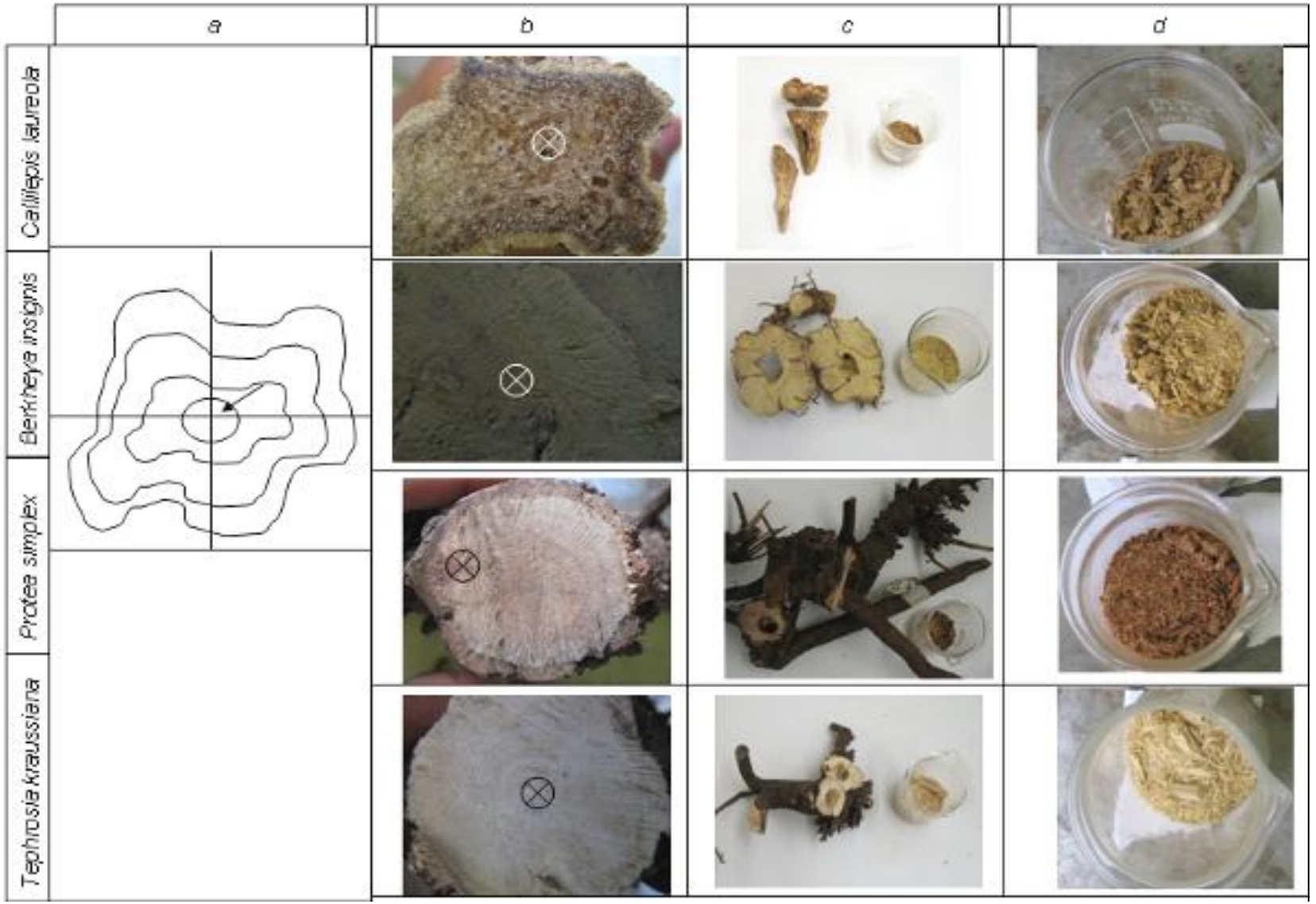
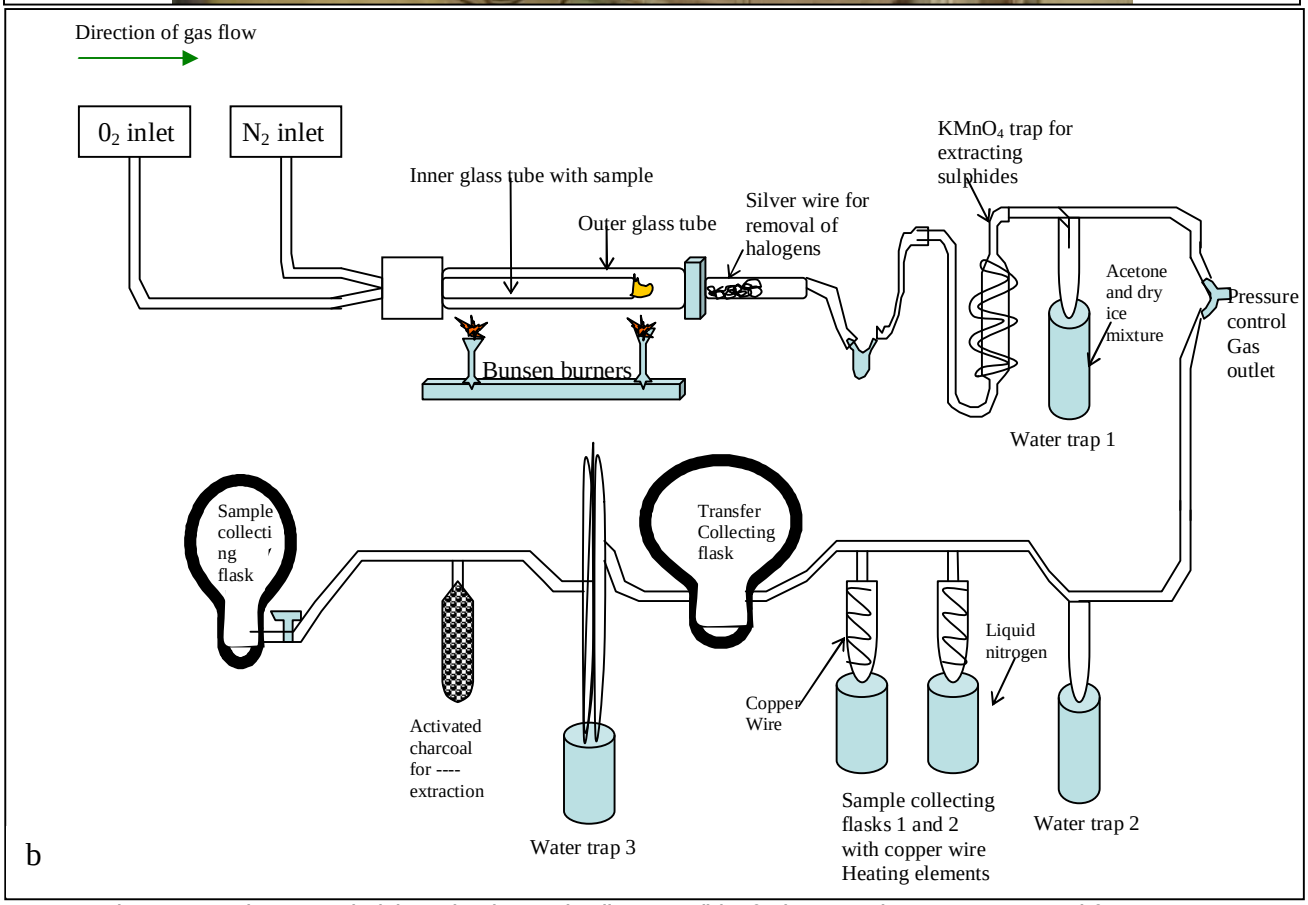


Figure 4.1 The process for the identification and excision of samples for radiocarbon dating first requires the identification of the oldest region. Rhizomes were cut in cross section. The most central rings of the rhizome were considered the oldest region (a) since this formed the heartwood (Stewart 1966) of the rhizomes. Once the central region was identified on the rhizome (b), the identified region was bored out down the central axis (c) and the wood sample collected for pretreatment (d).

a



b

Figure 4.2 Photograph (a) and schematic diagram (b) of The Bench apparatus used for the purification and isolation of Carbon dioxide for carbon 14 analysis.

4.7 Analysis

4.7.1 Morphology

Means and standard deviations of canopy area, basal area, number of stems/ plant, stem height and stem diameter of adults, sub adults and juveniles from field populations were calculated for each species. The mean and standard deviation of adult rhizome dry mass, depth, circumference, and diameter were also calculated for each species. Root to shoot ratios were calculated by dividing the mass of roots by the mass of shoots per plant as well as by dividing the length of roots by the length of shoots. The mean and standard deviation of *P. simplex* and *T. kraussiana* greenhouse grown seedlings main stem diameter, cotyledons cotyledon length, leaf length, number of leaves/leaflets, main root depth, root: shoot ratio (by length) and root: shoot ratio (by mass) was calculated.

4.7.2 Seed mass, viability and germination

Seeds and cones from *P. simplex*, plant 14 were omitted from seed mass, viability and germination analyses as the number of cones was unknown. Plants 2a and 2b were close to each other in the field; however these plants were treated as separate individuals throughout the analyses. Viability of a plant was calculated by adding the number of individuals which germinate to the number of individuals which were proven viable using TTZ testing.

Mean, standard deviation (SD), co-efficient of variation (CV) and range of adult plant rhizome mass, seed mass, percent germination, percent viability, total seed production/plant, cones/plant, cone mass and seeds/cone were calculated for *P. simplex* and *T. kraussiana* using Microsoft Excel 2003 (calculations for cones/plant, cone mass and seeds/cone were excluded for *T. kraussiana*). A one way ANOVA was used to compare seeds/cone, seed mass and cones/plant between individuals of *Protea simplex*. Seed mass of *P. simplex* and *T. kraussiana* were divided into classes and frequency graphs were constructed to determine the most frequently occurring seed masses. The percentage of viable and germinated seeds from each corresponding size class was also included in the graph so that the distribution patterns between seed mass, germination and viability could be established. Frequency graphs of the percentages of viable and non-viable, germinated and ungerminated seeds of *P. simplex* and *T. kraussiana* were also constructed in order to determine the seed mass range/s between which most seeds germinated or were viable. A t-test for unequal variances was used to test for a significant difference in seed mass between viable and non-viable seeds using STATISTICA 6.0.

The peak day for germination, germination lag and t_{50} germination (number of days for 50% of the seeds to germinate) were calculated according to Weiersbye and Witkowski (2002).

Peak value, mean days to germination and germination value were calculated according to Ranal and De Santana (2006). The mean, SD, CV and range of peak days, peak value, germination lag, t_{50} germination, mean days to germinate and the germination value were calculated for *P. simplex* and *T. kraussiana*. The cumulative percentage of the number of germinated seeds was plotted over the number of days of the trial to determine the pattern in the number of germinated seeds over time.

A multiple regression (all effects model using STATISTICA 6.0) was used to determine the relationship between percentage germination, percentage viability, seeds/cone, adult plant seed production/plant and cones/plant in *P. simplex* and the percentage germination, viability and total seed production/plant in *T. kraussiana* as a function of adult plant size variables (canopy area, basal area, stem diameter, root mass, height and number of stems). The backwards stepwise model was chosen as the best model fit for both species. Scatter plots with a simple linear regression fit were plotted for the significant relationships between canopy area, root mass, number of seeds/cone, seed production, cone mass, seed mass per plant, germination, seed mass and viability for *P. simplex*. The mean and SD of the percentage of *P. simplex* and *T. kraussiana* seedlings which emerged during field and laboratory germination trials were calculated and compared to determine the differences between the two methods.

4.7.3 Seedling growth (*Protea simplex and Tephrosia kraussiana*)

The dry mass allocations of six month old desiccated greenhouse grown seedlings of *P. simplex* and *T. kraussiana* were calculated by dividing the average total plant mass by average mass of roots, stems, leafs/leaflets and cotyledons. The mean and SD of seedling root to shoot ratios were calculated. Graphs of the cumulative growth of stem diameter, number of leaves, longest cotyledon length, height, longest leaf length and the number of stems (for *P. simplex* only) were constructed by calculating the average across individuals for each species over the study period. The number of leaflets were not counted after week 17 as leaflets became too numerous.

Scatter plots with regression fits were plotted to determine the relationship between the percentage dry mass of cotyledons whole seedling dry mass, whole seedling dry mass (excluding cotyledons), percentage root dry mass, percentage shoot dry mass, percentage leaf dry mass and seed mass to ascertain whether or not a relationship existed between parent plant investment in seedlings via cotyledons and seedling structures. Simple regression analyses were used to find significant relationships between seed mass (as an indicator of parent plant investment via seed) and *P. simplex* and *T. kraussiana* seedling dry mass allocations to roots,

shoots, leaves, cotyledons and the final growth values for stem diameter, number of leaves, longest cotyledon length, height, longest leaf length and the number of stems (for *P. simplex* only). Multiple regression analyses were used to determine the relationship between whole seedling dry mass, percentage root dry mass, percentage shoot dry mass, percentage leaf dry mass and parent plant size variables (canopy area, basal area, stem diameter, root mass, height and the number of stems). A backwards stepwise model was used to determine the best model fit for significant relationships.

The percentage of germinants surviving in the field and greenhouse trials was compared. The mean, SD and CV of stem diameter, the number of leaves, cotyledon length, height, leaf length and the number of stems of greenhouse and field grown seedlings were calculated. An independent two sample t-test was used to test for a significant difference between the stem diameter, the number of leaves, cotyledon length, height, leaf length and the number of stems between field and greenhouse grown seedlings.

4.7.4 Size structure, demography and plant density

The plant density of each plot and the overall plant density (plants/ha) were calculated for *B. insignis, C. laureola, P. simplex* and *T. kraussiana* so that plant density could be compared across species. A Kolmogorov-Smirnov test was used for a pair-wise comparison of height, canopy area, basal area, number of stems and main stem diameter between plots to determine congruency between plots and which plots were significantly different from each other within a species. Size classes were developed for height, canopy area, basal area, number of stems, main stem diameter and the number of floral structures/plant and frequency distributions were constructed for each plot separately and for an average of the three plots for each species. The frequency distributions of juveniles, sub-adults, non-reproducing adults and reproducing adults were constructed in order to ascertain the demographic allocation of each stage in the population.

The number of inflorescences, height, canopy area, basal area, number of stems and main stem diameter between juvenile, adult and sub adult stage classes between field populations were compared using a one way ANOVA and Fishers Least significant difference (LSD).

4.7.5 Habitat and environmental characteristics

The mean and SD of soil density, exchangeable phosphorus, acidity, available potassium, calcium, magnesium, zinc, manganese and copper, total cations, acid saturation, pH, organic carbon, altitude, slope, percentage clay, fine silt and coarse silt and sand, and total nitrogen were

calculated across plots for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. These variables were compared across species using a one way ANOVA and Fishers Least significant difference (LSD). A Correspondence analysis was conducted and it was found that a principal components analysis was the best model. The PCA (CANOCO for windows 4.5) was used to determine which habitat variables were the most influential across the species.

4.7.6 Plant longevity

Plant age (AD 2008) represented the age of plants in the year 2008. Scatter plots were constructed for plant age along the ascending and descending slopes, rhizome mass and circumference for each sample for *B. insignis, C. laureola, P. simplex* and *T. kraussiana* so that any correlations between these variables could be identified. Data was analysed across species first as the sample sizes of carbon dated plants were small. Simple linear regressions (STATISTICA 6.0) were used to determine the relationship between i) age along the ascending slope, ii) age along the descending slope of carbon dated plants and iii) percentage modern carbon and: rhizome mass, rhizome circumference, height, canopy area, basal area, number of stems, and main stem diameter. Scatter plots with linear regression fits of relevant significant relationships were constructed. Species were then analysed separately to determine whether any significant relationships were lost when species were analysed as a single data set. Therefore, the relationship between i) the age (AD 2008), ii) the percentage modern carbon (AD 2008) of carbon dated plants and rhizome mass, rhizome circumference, height, canopy area, basal area, number of stems, and main stem diameter was analysed separately for each species using simple linear regressions. Linear regressions were also used to analyse the relationship between rhizome mass and height, canopy area, basal area, number of stems and main stem diameter in order to find the relationship between belowground and aboveground plant structures. If any structures had a significant relationship with both age and rhizome mass, then these would be used to construct age surrogates.

The ages of field populations were estimated by comparing the structural variables of the carbon dated population with known ages with the structural variables of field populations. The mean and SD of height, canopy area, basal area, number of stems and main stem diameter were calculated and compared across plots using a one way ANOVA and Fishers Least significant difference (LSD).

5. Results

5.1 Morphology

5.1.1 Aboveground morphology and general characteristics

B. insignis, *C. laureola*, *T. kraussiana* and *P. simplex* display a range of sizes, stem textures and morphologies even though all occupy areas of similar environmental conditions and all display a suffrutex growth form (Table 5.1). All species have multiple aboveground stems. Stems are generally low growing (not more than ~60cm high) for all species. Flowers of all four species are colourful and greater than 8mm in diameter. *T. kraussiana* appears to invest more in numerous smaller flowers compared to the few larger flowers produced by *B. insignis*, *C. laureola* and *P. simplex.*

Table 5.1 General characteristics of *Berkheya insignis*, *Callilepis laureola*, *Tephrosia kraussiana* and *Protea simplex* plants sampled during April 2008. Where measurements or counts are recorded, the mean and standard deviations are shown.

General morphology and description	*Berkheya insignis*	*Callilepis laureola*	*Protea simplex*	*Tephrosia kraussiana*
Leaf/Leaflet structure	Leaves are alternate or radical, but rarely opposite[10]	Alternate, sessile[10]	Leaves alternate, margins entire	Imparipinnate or digitately (1)3-5-foliolate or pinnately trifoliolate[1]
Leaf length x width (mm)	800X150[2]	150X20	100X30	17X5
Adult canopy area (m^2)	0.092±0.096	0.032±0.036	0.51±0.61	0.075±0.076
Sub-adult canopy area (m^2)	0.013±0.016	0.014±0.015	0.08±0.12	0.009±0.005
Juvenile canopy area (m^2)	0.025±0.047	-	-	0.004±0.005
Adult basal area (cm^2)	55±67	87±148	1383±2913	49±0.9
Sub-adult basal area (cm^2)	4±4	17±3	200±547	3±7
Juvenile basal area (cm^2)	24±47	-	-	0.7±2
Number of stems/plant	5±3	6±4	42±21	28±23
Adult stem height (cm)	55±1	43±8	55±15	56±12
Sub-adult stem height (cm)	47±1	28±6	33±10	35±7
Juvenile stem height (cm)	42±1	-	-	29±7
Adult stem diameter (mm)	4.7±2.2	1.7±0.3	9.03±2.8	2.9±1.5
Stem texture	Sparse soft hairs[2]	Glabrous	Glabrous[5]	Woody with soft hairs
Stem structure	Forms clumps, simple[2]	Produced annualy[10], multiple stems arising from rhizome	Numerous, unbranched[5]	Sometimes one main stem arising from rootstock with multiple branching 2° stems, often multiple stems arising from rootstock which appear separate above soil surface
Flower structure	Terminal, solitary[2]	Flowers many, solitary, at end of peduncles[10]	Terminal, solitary, multiple flowers per plant	Grow in racemes and are axillary/opposite leaves/terminal [1]
Flower head diameter range (mm)	8-10	45-50	30-60[5]	10-12
Flower colour	Bright gold-yellow, orange-yellow[2]	Ray florets white, disc florets purple, corolla white and tube shaped with 3 toothed lamina[10]	Creamy white	Pinkish-purple

[1](Germishuizen 2000); [2](Hilliard 1977); [3](Burrows and Willis 2005); [4](SANBI 2005); [5](Vogts 1982); [6](Rebelo 1995); [7](Popat *et al.* 2002); [8](Steenkamp *et al.* 2004); [9](Gates 1992); [10](Herman *et al.* 2000); [11](Rourke 2000)

5.1.2 Rhizome morphology

Rhizomes of *Berkheya insignis*, *Callilepis laureola*, *Tephrosia kraussiana* and *Protea simplex* are highly variable in size, shape, texture, smell and branching structure between species (Table 5.2). However suffrutescent rhizomes were composed of a thickened central region (except *P. simplex* which was composed of multiple thickened branches) with storage potential. The belowground architecture of individual rhizomes showed no signs of connectivity to other individual plants; hence these species are not clonal. Although *P. simplex* forms complex rhizome networks, these networks were confined to discrete individuals. Therefore individuals displayed no evidence of vegetative reproduction. Cross-sections of rhizomes of *P. simplex*, *C. laureola*, *B. insignis* and *T. kraussiana* revealed that they did not form defined growth rings. The internal structure and texture of rhizomes was also highly variable. The junction between the rhizome and aerial shoots was cryptic for all species. The points of attachment were only established once rhizomes were excavated and carefully examined.

The rhizomes of *B. insignis* (Figure 5.1) were composed of multiple tendril-like rootlets attached to the main rootstock. Rootlets were 10-20cm long, often branching off further into smaller rootlets. Rootlets occasionally ended in a single fleshy fusiform root (tapering at both ends). The main rootstock was irregular and shapes varied between individuals. Rhizomes of *B. insignis* were light and not dense. Rhizomes of *C. laureola* were carrot-shaped with a wide diameter at the top tapering off gradually into a narrower tip. *C. laureola* rhizomes were dense and woody. Side branches were few, if any. Larger rhizomes were less carrot-shaped and ends were less tapered. Evidence of decay was present in larger rhizomes creating even greater irregularity in the shape. Rhizomes of *P. simplex* were large, dense and heavy with numerous thick branches with connections to other branches on the same plant (Figure 5.1). The main rootstock and its branches were covered by a rough, bark-like outer layer. Branches penetrated deep into the soil profile (>1m) and the main rootstock was often removed before the deepest point could be unearthed. Therefore, the network of rhizomes of *P. simplex* is both wide and deep. Rhizomes of *T. kraussiana* resembled a denser woody structure from the outside (lighter and less dense than *P. simplex*). However, the internal structure of rhizomes was more fibrous. Side branches were more profuse in larger individuals. Branches grew laterally as opposed to deeper into the soil profile. The internal structure and texture of *C. laureola* and *B. insignis* rhizomes were less woody and may be due to the fact that they contain chemicals in greater abundance than *T. kraussiana* and *P. simplex* (Figure 5.2). The more tuber-like anatomy of *C. laureola* and *B. insignis* for storage of defensive chemicals and their sticky, aromatic characteristics could help decrease palatability of the rhizomes, thus increasing the longevity of plants. *T. kraussiana* also possessed milder chemical defenses while *P. simplex* had less abundant chemical defenses (volatiles burned for a very brief period during the sample purification for radiocarbon dating).

Table 5.2 Rhizome morphology measurements (mean and standard deviation) and descriptions (from rhizomes excavated in April 2008) for *Berkheya insignis*, *Callilepis laureola*, *Tephrosia kraussiana* and *Protea simplex*.

Rhizome morphology				
Graphical Representation of rhizome (scale: 1 block=1cm)				*Tep* 
Sample size (no. of plants)	23	23	15	18
Rhizome shape	Main rhizome highly irregular in shape. Generally a large fist shaped mass	Composed of a main corm like structure. Bullet-shaped with a tapering endpoint. Larger rhizomes more irregularly shaped with evidence of tissue necrosis.	Composed of a network of multiple, interconnected, branch-like roots. Rhizome network can span a diameter of >1m^2 in medium-sized adults. Thicker rhizomes taper down into soil profile (>1m)	A single main rhizome extending downward. Main root tapering to reduced diameter.
Adult rhizome mass (g)	94.3±115.8	173±178.7	1449.6±1005.9	172.5±117.5
Adult rhizome depth (cm)	7.7±2.9	10.6±3	>8.9	10.7±4.7
Adult rhizome circumference (cm)	14.9±6.6	19.1±8.4	48.1±22.1	13.5±5.7
Adult rhizome diameter (cm)	3.4±1.5	4.9±2.1	15.7±7.7	3.7±1.5
Rhizome colour	Pale brown scales interspersed white grooves between scales. Secondary branches light brown	Pale grey to brown	Dark to reddish brown	Light orange-brown
Rhizome surface texture	Striations running parallel to direction of root growth. Root hairs present on secondary branches only.	Striations vertical to direction of root growth. Protruding scale-like, appearance. Individual scales smooth.	Rough bark-like texture. Have nodular protrusions.	Striations running parallel to direction of root growth, no root hairs present. Surface glabrous and scaly
Branching from main rhizome (single/ multiple)	Multiple and numerous.	Branching rare, more often only main rhizome present	No central axis, however multiple branching of woody rootstock present.	Multiple sparse branches from main rhizome
Secondary branches (vertical, lateral angular)	Secondary branching mostly lateral. Secondary branches thin adventitious-like structures. Fusiform roots present on several secondary roots. Fusiform root swellings fleshy but firm.	Branching, if present, downward into soil surface. Secondary branches identical in texture to main rhizome, however, thinner and more gradually tapered	Branching off individual main rhizomes very sparse, sometimes only one branching and directional.	Secondary branching lateral, at multiple angles
Rhizome smell	No distinct smell	Sharp pungent, sour	No distinct smell	Slightly nutty
Rhizome internal tissue (density, texture, colour)	Composed of tiny beehive shaped cells forming a firm tissue structure. Texture similar to pastry, flaky. White. Growth rings present.	Thick, soft, waxy texture. Resembles the appearance of bone marrow with light beige circling rings interspersed with dark brown patches.	Very dense, compact, woody tissue. Pale reddish-brown. Growth rings present and easily differentiated.	Composed of densely packed fibrous structure, very stringy, pale cream coloured tissue. Growth rings present.

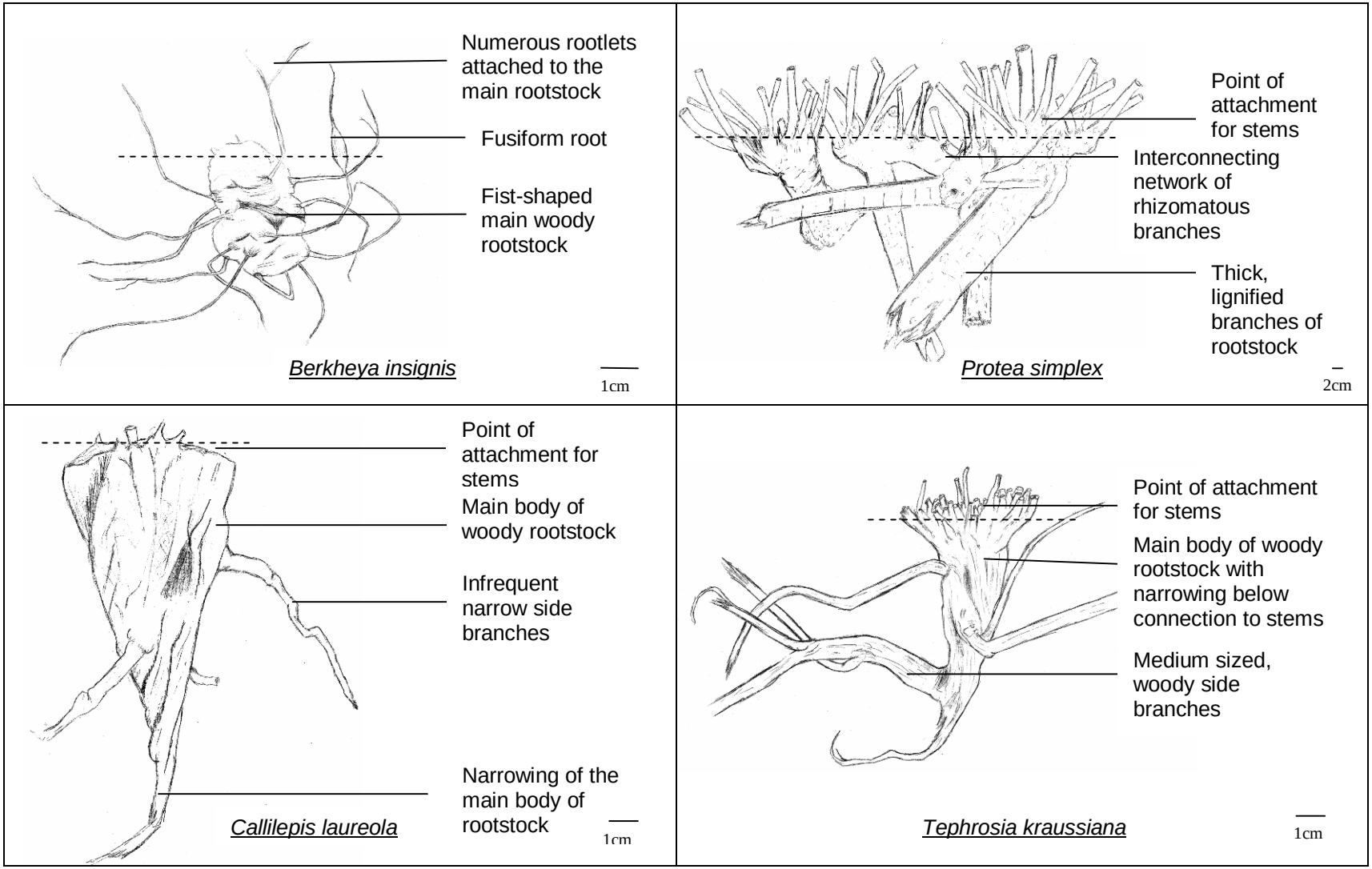


Figure 5.1 Root system morphologies of *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Points of attachment to aerial stems are highlighted. Sketches based on woody rootstocks collected from Ipithi Retreat and Clearwater Estate in April 2008. The dotted line indicates the ground surface level.

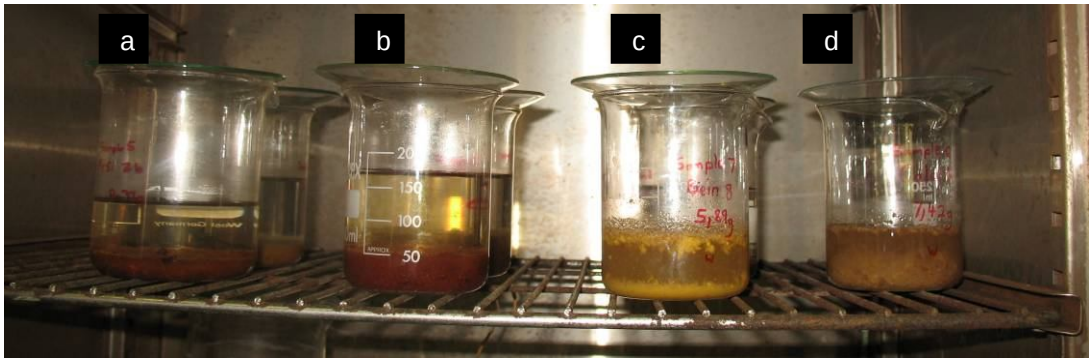


Figure 5.2. Acid-Alkaline-Acid (AAA) treated samples of (from left to right) a) *Protea simplex,* b) *Tephrosia kraussiana,* c) *Berkheya insignis* and d) *Callilepis laureola* showing the amount of volatile chemicals in suspension.

5.1.3 Seedling morphology (*Tephrosia kraussiana* and *Protea simplex*)

The morphology of seedlings at 5 months closely resembled that of adults (Table 5.3 and Figure 5.3). The leaf structure of *T. kraussiana* was particularly representative of the adult form at an early age. However, the stems of seedlings were fleshier and smoother compared to the deeply veined, woodier stems of adults. Leaves of *P. simplex* were smaller, softer and less coarse than those of adult plants. Stems were also fleshier. *P. simplex* already showed signs of a multiple stem habit at this age. Cotyledons of *T. kraussiana* were not as thick as those of *P. simplex* and persisted for only 1.5-4.5 months. The seedlings of *P. simplex* still retained their cotyledons after five months. While most were still plump and green, some showed signs of reddening and desiccation. Both species also displayed early signs of a thickened area where the stem and roots joined. This could be representative of early rootstock development.

T. kraussiana seedling roots were already showing signs of lignification at five months (Table 5.3 and Figure 5.4). Rootlets were fibrous and numerous. Seedlings of *T. kraussiana* had longer roots (main root depth: 110±35mm) compared to shorter stems (height: 49±22mm). Root to shoot ratios indicated that although the mass ratio of roots was greater (5.1±2), the ratio in length was only 1.5. This indicated that these seedlings may invest more energy in making roots denser and thicker rather than longer (i.e. may be due to lignification). The roots of *P. simplex* seedlings were composed of a single main root with several fleshy rootlets (Table 5.3). Rootlets displayed typical proteoid characteristics with the presence of cluster roots. *P. simplex* seedlings did not root as deep as *T. kraussiana* with a main root depth of only 63±35mm. Although the difference between root to shoot ratios were not as great as those for *T. kraussiana*, the mass ratio of roots was also higher in *P. simplex* than the ratio in length.

Table 5.3 Morphological measurements and descriptions of shoots and roots of five month old greenhouse grown *Tephrosia kraussiana* and *Protea simplex* seedlings (mean and standard deviation).

Seedling morphology (5 months)	*Tephrosia kraussiana*	*Protea simplex*
Photograph of seedlings		
Sample size	48	28
Height (mm)	49±22	10.7±6.3
Main stem description	Single	Mostly single, however, sometimes multiple stems present after 3 months (2-3)
Main stem diameter (mm)	1.9±0.5	1.56±0.47
Description of cotyledons	Cotyledons slightly thicker than secondary leaves, non-aristate	Cotyledons thick and succulent, obtuse
Length of cotyledons (mm)	8.9±0.4	11.3±2.3
Cotyledon senescence	Cotyledons turn yellow before senescence, persist for 1.5-4.5 months	Cotyledons desiccate and turn red before senescence, persist for longer than 6 months
Leaf length (mm)	17.1±11.5	27.2±6.3
Number of leaves/leaflets	21±13	13±5
Main root description	Lignification evident, smaller seedlings display single tap root- like structure	Single main root with numerous branches/rootlets. Lignification at base of stem and becoming fleshier as it tapers down to the tips
Main root depth (mm)	110±35	63±35
Rootlet description	Numerous tough- fibrous rootlets	Numerous fleshy rootlets with cluster roots
Root: shoot ratio (by length)	1.5±1:1	1.3±0.7:1
Root: shoot ratio (by mass)	5.1±2.4:1	2.9±2:1

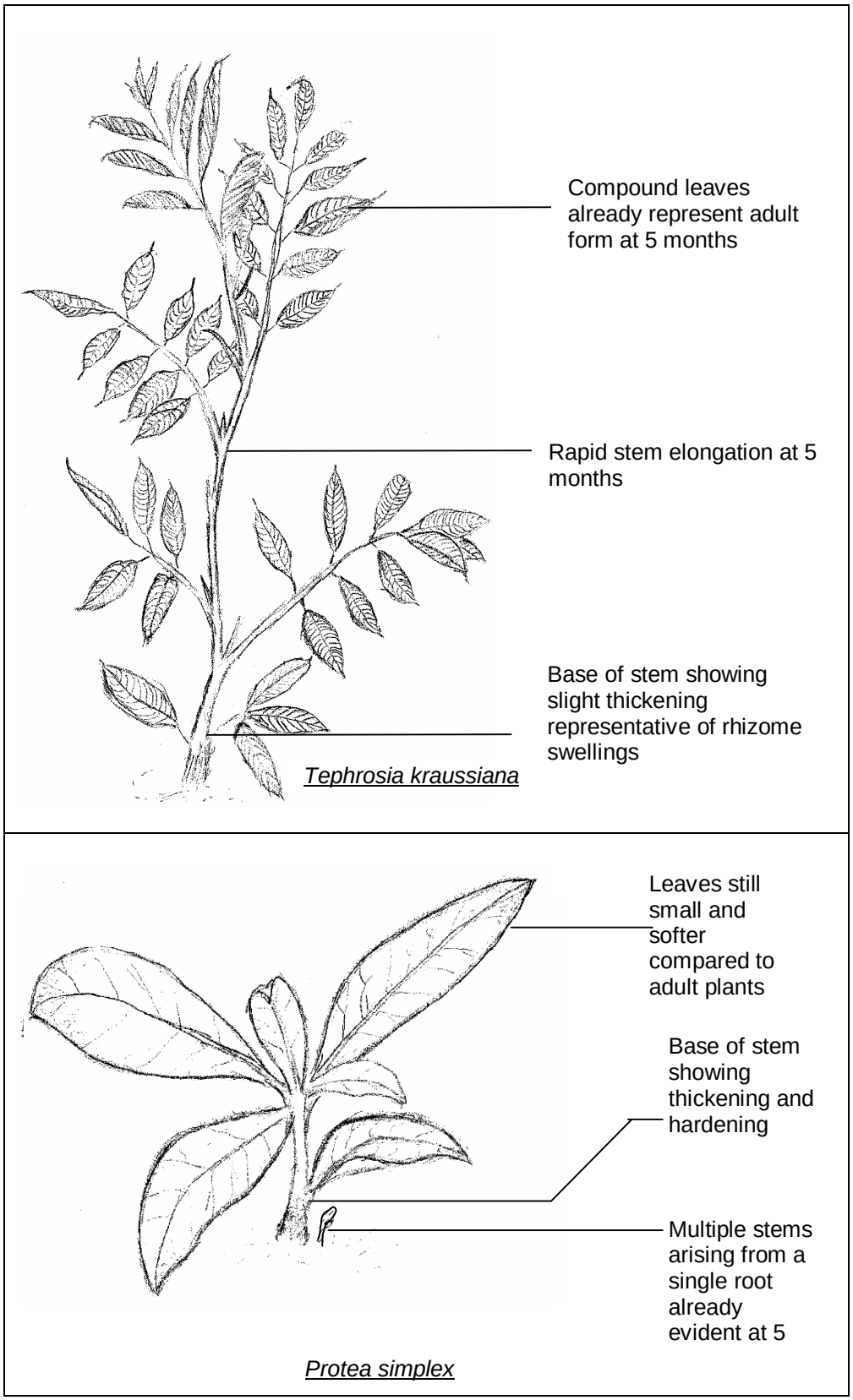


Figure 5.3 Seedling shoot morphologies of *P. simplex* and *T. kraussiana*. Sketches based on 5 months old seedlings grown in the greenhouse.

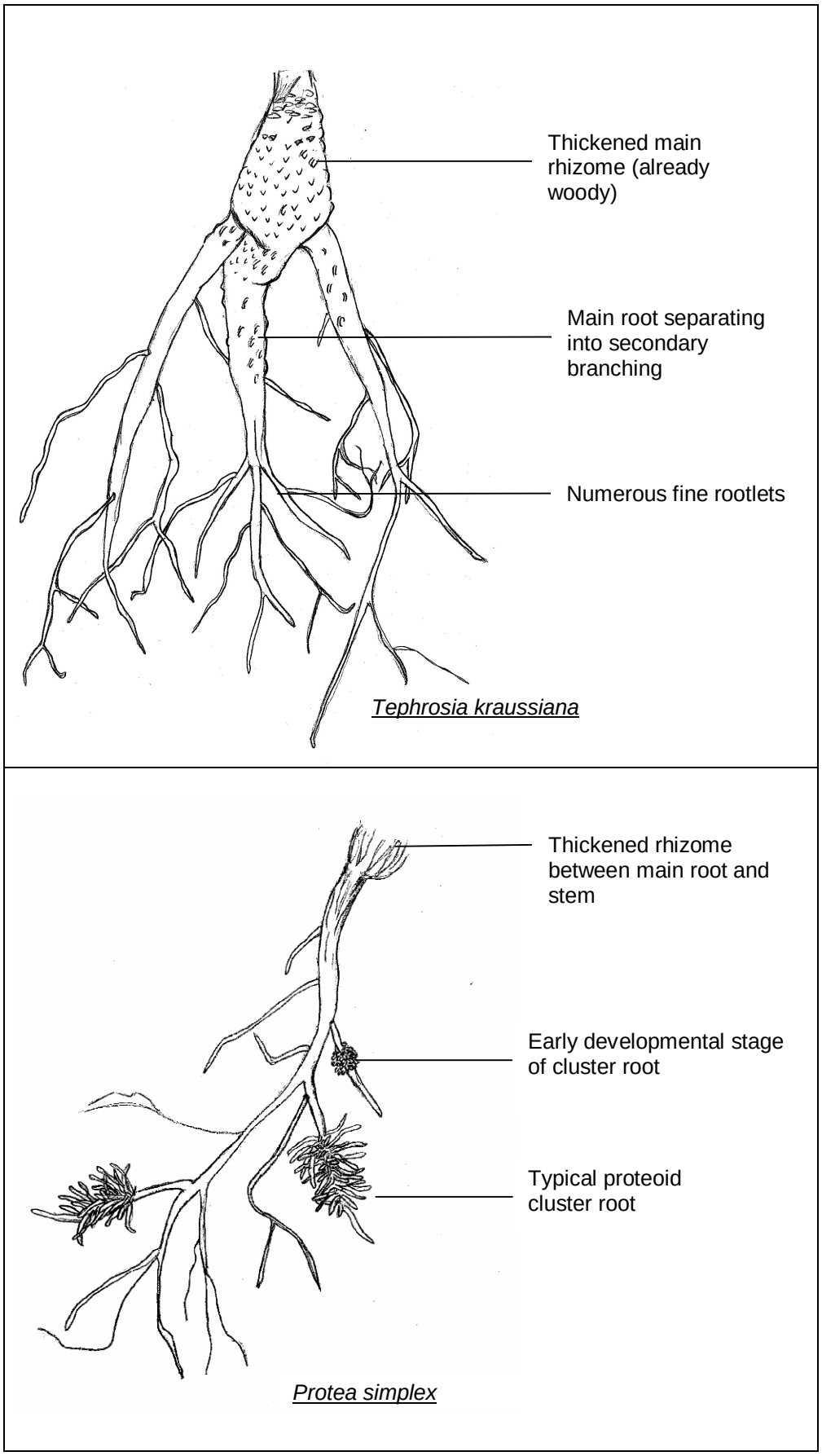


Figure 5.4 Seedling root morphologies of *P. simplex* and *T. kraussiana*. Sketches based on 6 months old seedlings grown in the greenhouse.

5.2 Seed mass, viability and germination

5.2.1 Seed viability and germination and the relationship with seed mass: *Protea simplex*

Seed mass/seed for *P. simplex* varied considerably between plants, ranging from 6.5 to 26.4 mg/seed with a mean and SD of 15.5±5.3 mg/seed (Table 5.4). Plants 3 and 5 had the lowest mean seed mass, and percentage viability and germination. However, plant 3 had the heaviest rhizome (3216g) and the highest number of seeds per cone. Plant 4 had the highest mean seed mass; comparatively low seed viability of only 19%, and relatively low total seed production as it only had four cones. Plants 1, 2a, 2b, 15 and 12 were among the plants with the highest seed viability. Rhizomes of plants 2a and 2b were among those with the greatest mass (2909 and 3036g, respectively). However, rhizomes of plants 12 and 15 weighed only 581 and 900g. The percentages of seed germination and viability amongst individuals of *P. simplex* were low to moderate compared to other *Protea* species (Van Staden 1966; Brown and Van Staden 1971; Brown and Van Staden 1973; Van Staden and Gilliland 1977) at 21.8±15.5% and 32.7±18.8%, respectively. Seed production ranged from 140 to 2814 seeds/plant and had a relatively high CV (84.2%). In contrast cone mass had a low CV (26.8%); however the number of cones per plant was highly variable, ranging from 2 to 37 (CV=81.8%) (Table 5.4). The number of seeds/cone, seed mass and cone mass was significantly different between individual plants of *P. simplex* ($p < 0.01$) (Table 5.5).

The highest frequency of seeds was recorded between 10.21-11.90mg/seed (Figure 5.5). This was also the smallest size class in which germination was evident and had the lowest germination. However, viability was evident in seeds with mass 6.81-8.50mg/seed. The smaller size classes (11.9mg/seed) had higher frequencies and fewer viable and germinating seeds than the larger size classes (11.91-32.30mg/seed), suggesting that many sterile empty seeds were produced. Both seed germination and viability followed a normal distribution and peaked at 18.71-20.40mg/seed. The percentage of viable and germinated seeds increased with increasing seed mass for *P. simplex* (Figure 5.6). The viable seed in the 0-1.7mg/seed class was only a single seed. Therefore, seeds greater than 10.21-11.90mg/seed were considered viable. Optimum germination was achieved in seeds with mass between 27.21-28.90mg and 30.61-32.30mg. Optimum viability was achieved in seeds with mass between 30.61-32.30mg. There was a significant difference between the seed mass of viable and non-viable seeds for individuals of *P. simplex* (Table 5.6) and overall for the species. Overall (across all individuals) 67.08% of all seeds sampled were viable, and 23.91% germinated. While germination was low compared to other *Protea* species, viability was relatively high (Van Staden 1966; Brown and

Van Staden 1971; Brown and Van Staden 1973; Van Staden and Gilliland 1977). Whilst seed predation is a common feature in Proteaceae, seeds displaying signs of predation were excluded from germination trials and, therefore, cannot account for the low numbers of viable seed that germinated.

The mean germination peak value (and range) of *P. simplex* was relatively low (0.7±0.5) (range 0-1.5) (Table 5.7), suggesting a slow rate of germination overall. Plant 10 had the highest peak value (1.47), and then plant 2a (1.23). Peak day and germination lag values highlighted the substantial delay in germination of *P. simplex* seeds after imbibition.

Table 5.4. Means, standard deviation and counts of seeds, cones, germination and viability for individuals of *Protea simplex* (n=15 plants, n=1360 seeds).

Plant number	n (seeds)	Adult rhizome mass (g)	Seed mass (mg)	Germination (%)	Viable seeds (%)	Total seed production/ plant	Cones/ plant	Cone mass (g) n=5	Seeds/ cone
1	100	670	19.9±4.1	26	56	827	10	2.0±0.3	82±23
2a*	100	2909	19.8±4.7	41	49	646	7	2.5±0.5	92±9
2b	100	3036	18.9±5.1	34	47	2814	37	2.3±0.9	76±13
3*	100	3216	8.1±1.4	0	0	1249	12	2.8±0.7	104±17
4	80	1047	20.6±5.8	9	19	275	4	2.3±0.4	69±3
5	40	719	10.3±1.8	0	3	140	2	1.3±0.1	70±1
6	80	1973	14.4±3.5	28	33	301	4	1.7±0.3	75±4
7	100	1633	16.7±3.4	34	40	1954	23	2.3±0.3	85±21
8*	100	2107	12.1±3.6	8	26	371	5	1.5±0.5	74±6
9	100	778	15.3±3.3	3	18	818	11	2.1±0.7	74±13
10	100	674	14.8±2.8	32	43	1033	14	2.5±0.6	74±20
11	100	277	14.6±4.5	32	39	397	5	1.5±0.4	79±4
12	60	581	14.3±4.6	45	53	304	3	2.6±0.4	101±4
13	100	2301	12.2±5.7	8	11	1103	14	1.8±0.3	79±16
15	100	900	17.0±3.1	27	54	1157	17	2.2±0.3	68±20
Mean+SD		1521±998	15.5±5.3	21.8±15.5	32.7±18.8	852±717	11±9	2.1±0.6	80±28
CV		65.6	34.2	71.1	57.5	84.2	81.8	28.6	35.0
Range		277-3216	6.5-26.4	0-45	0-56	140-2814	2-37	1.0-3.5	48-121

* Rhizomes which were radiocarbon dated

Table 5.5. One way ANOVA comparing seeds/cone, seed mass and cones/plant between individuals of *Protea simplex*. (n=15 plants, n=1360 seeds, p=0.01)

	Adjusted r^2	df	F	p
Seeds/cone	0.45	1,14	10.20	< 0.001
Seed mass (g)	0.41	1,14	66.56	< 0.001
Cone mass (g)	0.31	1,14	3.16	0.0012

Plant 8 had the longest germination lag (24 days), the largest peak day value (33 days) and the biggest value for t_{50} germination (33 days), suggesting that plant 8 seeds experienced a greater lag phase. Plant 8 also had the highest value for mean days (31.5 days) suggesting that seedlings of this plant were more likely to be different ages as there were long intervals between the germination of successive seeds. Plant 12 had the highest uniformity (19 days) and a small

germination lag (9 days). The overall germination value was 15.5±11.4 (CV = 73.4%) for *P. simplex*, suggesting high variability between individuals. Plant 10 had the highest germination value (35.9). The cumulative germination curve for *P. simplex* (Figure 5.7) followed a typical sigmoidal curve. Germination went through a lag phase, followed by a gradual increase in the number of germinants, and then leveled-off.

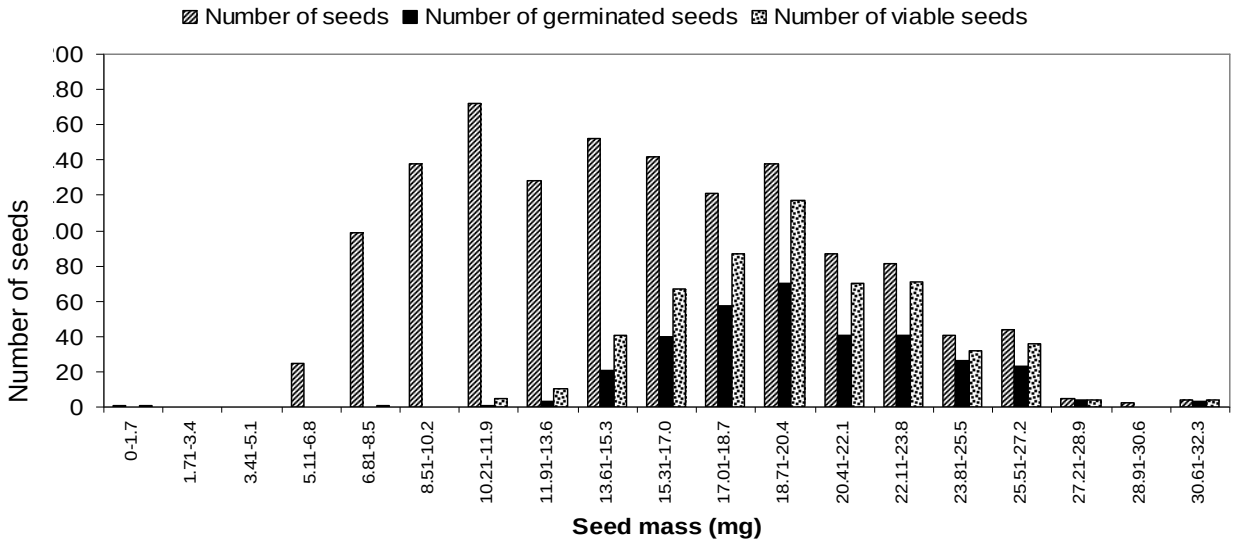


Figure 5.5. Seed size distributions showing the relationship between seed mass and a) the number of germinated and b) viable seeds per mass class for *Protea simplex.*

Table 5.6. Statistical comparison of the seed mass of viable and non-viable seeds per plant (and across plants) for *Protea simplex* using t-tests.

Plant no.	Viable seeds		Non-viable seeds		t	DF	p
	Mean	SD	Mean	SD			
1	0.0222	±3.3025E-06	0.0164	±1.8000E-05	8.0398	47	<0.0001
2a	0.0223	±8.0131E-06	0.0158	±1.8925E-05	8.2828	59	<0.0001
2b	0.0220	±8.0135E-06	0.0154	±2.3585E-05	8.1003	72	<0.0001
3	0	0	0.0081	±0.0014	-	-	-
4	0.0259	±1.6865E-05	0.0177	±1.8424E-05	8.4508	61	<0.0001
5	0.0148	±0.0	0.0102	±0.0017	17.0109	0	-
6	0.0173	±3.2731E-06	0.0118	±5.3240E-06	12.0276	77	<0.0001
7	0.0189	±3.8788E-06	0.0143	±9.2701E-06	8.8581	79	<0.0001
8	0.0159	±9.1310E-06	0.0104	±5.6838E-06	8.9951	47	<0.0001
9	0.0205	±4.1359E-06	0.0141	±4.2748E-06	12.2823	27	<0.0001
10	0.0163	±1.7210E-06	0.0130	±9.2230E-06	6.7782	57	<0.0001
11	0.0190	±9.5935E-06	0.0116	±4.7887E-06	13.3280	67	<0.0001
12	0.0181	±2.5257E-06	0.0096	±2.8124E-06	20.1970	54	<0.0001
13	0.0233	±2.7046E-06	0.0101	±9.8005E-06	24.6878	39	<0.0001
15	0.0179	±8.3412E-06	0.0158	±8.4002E-06	3.6651	92	0.0004
Mean across plants	0.0198	±1.3378E-06	0.0125	±1.7041E-06	34.1319	1258	2.8374E-181

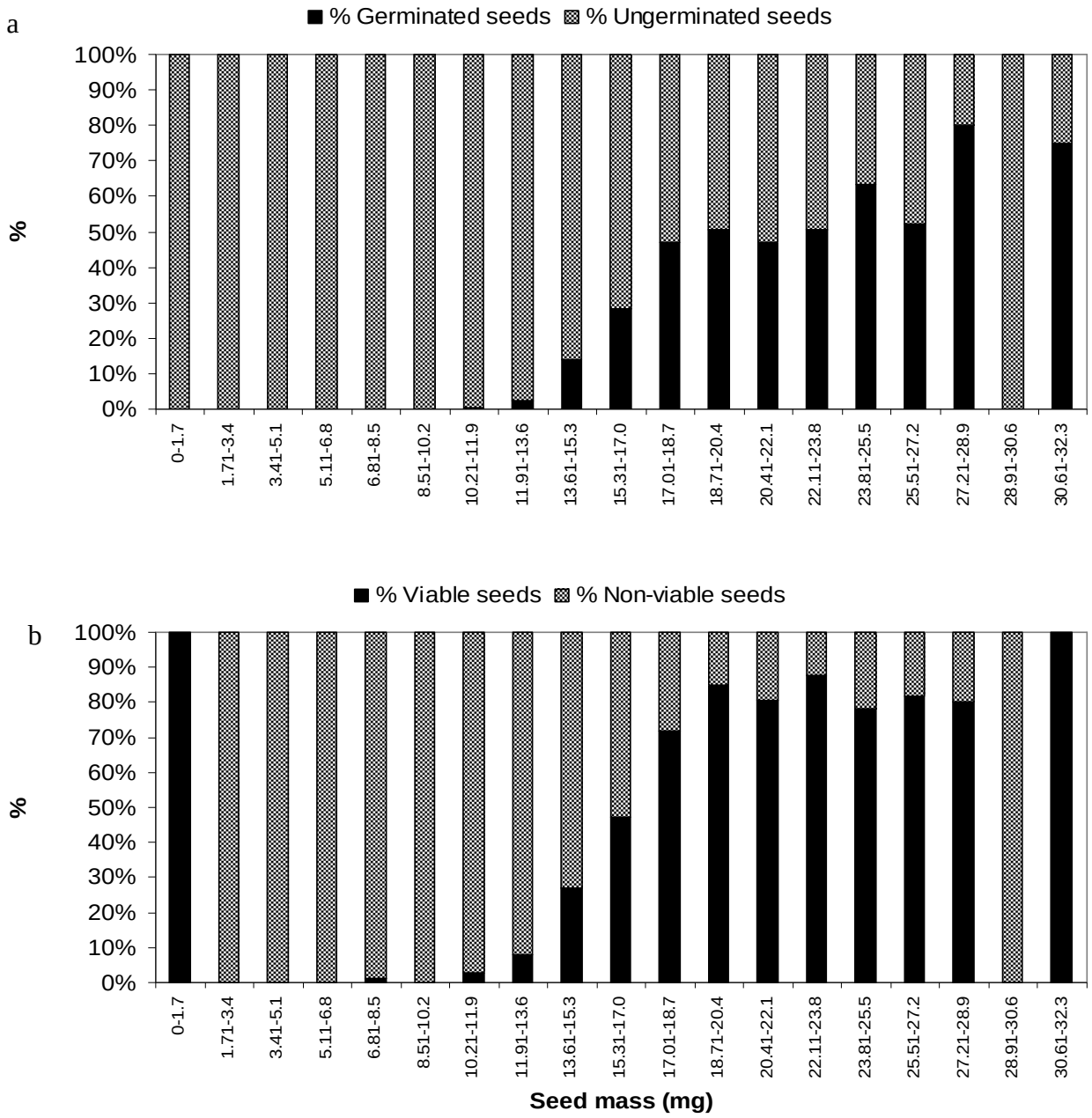


Figure 5.6. Seed size distributions showing the relationship between seed mass and a) the percentage of germinated and b) viable seeds per mass class for *Protea simplex.*

Table 5.7. Germination indices [Peak day; the time taken to the day when the most number of seeds germinated, germination lag; the number of days before germination commenced, t_{50} germination; number of days for 50% of the seeds to germinate, mean days and germination value (Ranal and De Santana 2006)] were calculated for *Protea simplex* plants that produced seeds (n=15).

Plant	n (seeds)	Peak day (days)	Peak value	Germination lag (days)	t50 germination (days)	Mean days to germination (days)	Germination value
1	100	24	0.78	12	24	23.7	18.5
2a*	100	24	1.23	12	24	24.9	30.6
2b	100	18	1.04	12	21	21.8	22.7
3*	100	0	0	0	0	0	0
4	80	30	0.10	21	30	31.3	3.1
5	40	0	0	0	0	0	0
6	80	27	0.58	15	30	28.9	16.8
7	100	18	1	3	24	24.9	24.9
8*	100	33	0.21	24	33	31.5	6.6
9	100	18	0.09	18	24	24.0	2.2
10	100	24	1.47	15	24	24.4	35.9
11	100	24	1.08	15	24	22.0	23.8
12	60	15	1	9	18	19.0	19.0
13	100	27	0.36	15	27	26.5	9.5
15	100	15	0.96	12	18	19.8	19.0
Mean ± SD	90.7±18.3	19.8±9.6	0.7±0.5	12.2±6.9	21.4±9.6	21.5±9.5	15.5±11.4
CV	20.2	48.5	75.2	56.9	44.9	44.0	73.4
Range	40-100	0-33.0	0-1.5	0-24.0	0-33.0	0-31.5	0-35.9

* Rhizomes which were radiocarbon dated

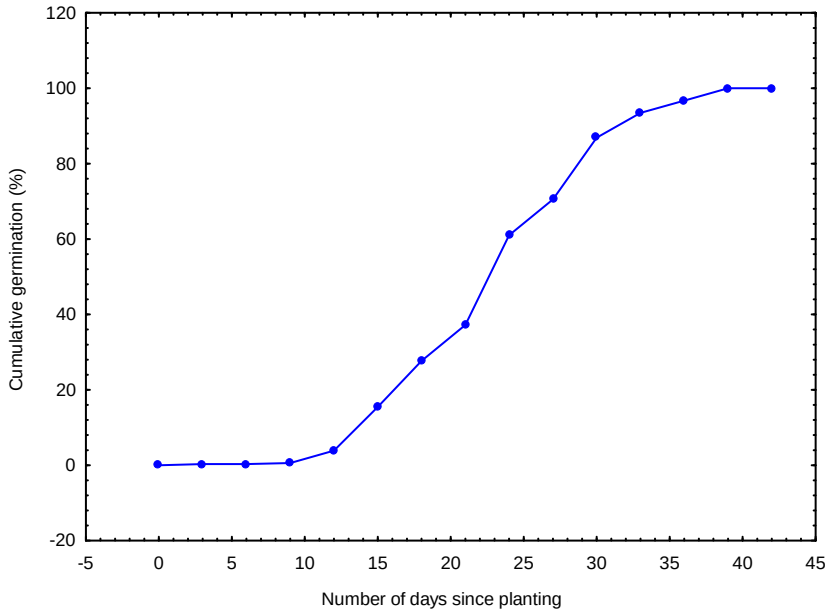


Figure 5.7. Cumulative germination for *Protea simplex* (all plants combined) n=15.

5.2.2. Parent plant investment: the relationship between parent plant structures, seed mass, viability and germination in *Protea simplex*

There was a linear relationship between parent plant canopy area and root mass [r^2= 0.727, p<0.001 (Figure 5.8)]. Hence canopy area provides a good above-ground measure of plant size in *P. simplex*. There was a linear relationship between canopy area and seed production (r^2=0.569, p<0.001). The relationship between cone mass and the number of seeds/cone also showed a positive relationship (r^2= 0.417; p<0.001). Seed mass and viability also had a positive linear relationship (r^2 =0.450, p=0.006). However, there was no relationship between germination and seed mass (r^2 =0.237, p=0.054). Only seed production/plant and cones/plant had a significant relationship with any of the plant structural variables tested (root mass, height, canopy area, stem basal area, number of stems and main stem diameter) in the multiple regression models (Table 5.8). Only canopy area (CA) remained as a significant predictor of seed production/plant (SP) in the backwards stepwise model (SP=39.932 + 0.192CA, p<0.001, r^2=0.56). Root mass (RM) and canopy area (CA) remained as predictors of the number of cones/plant (CP) in the backwards stepwise model (CP=1.969-0.007RM + 0.005CA, p<0.001, r^2=0.63).

Table 5.8. The relationship (multiple regression analyses) between adult plant seed production/plant and cones/plant as a function of plant size variables (canopy area, basal area, stem diameter, root mass, height and number of stems) in *Protea simplex*. Only variables included in the model are shown.

Model	Dependent variable	Predictor variable	Best fit model	Adjusted r^2	df	F	p
Linear regression	Seed production/plant (SP)	Canopy area (CA)		0.54	1,13	17.15	0.001
Backwards stepwise	Seed production/plant (SP)	Canopy area (CA)	SP=39.932 + 0.192CA	0.56	6,8	3.99	0.038
Linear regression	Cones/plant (CP)	Root mass (RM)		0.16	1,13	2.57	0.133
Linear regression	Cones/plant (CP)	Canopy area (CA)	CP=1.969-0.007RM + 0.005CA	0.50	1,13	15.15	0.002
Backwards stepwise	Cones/plant (CP)	Root mass (RM) Canopy area (CA)		0.63	6,8	5.03	0.020

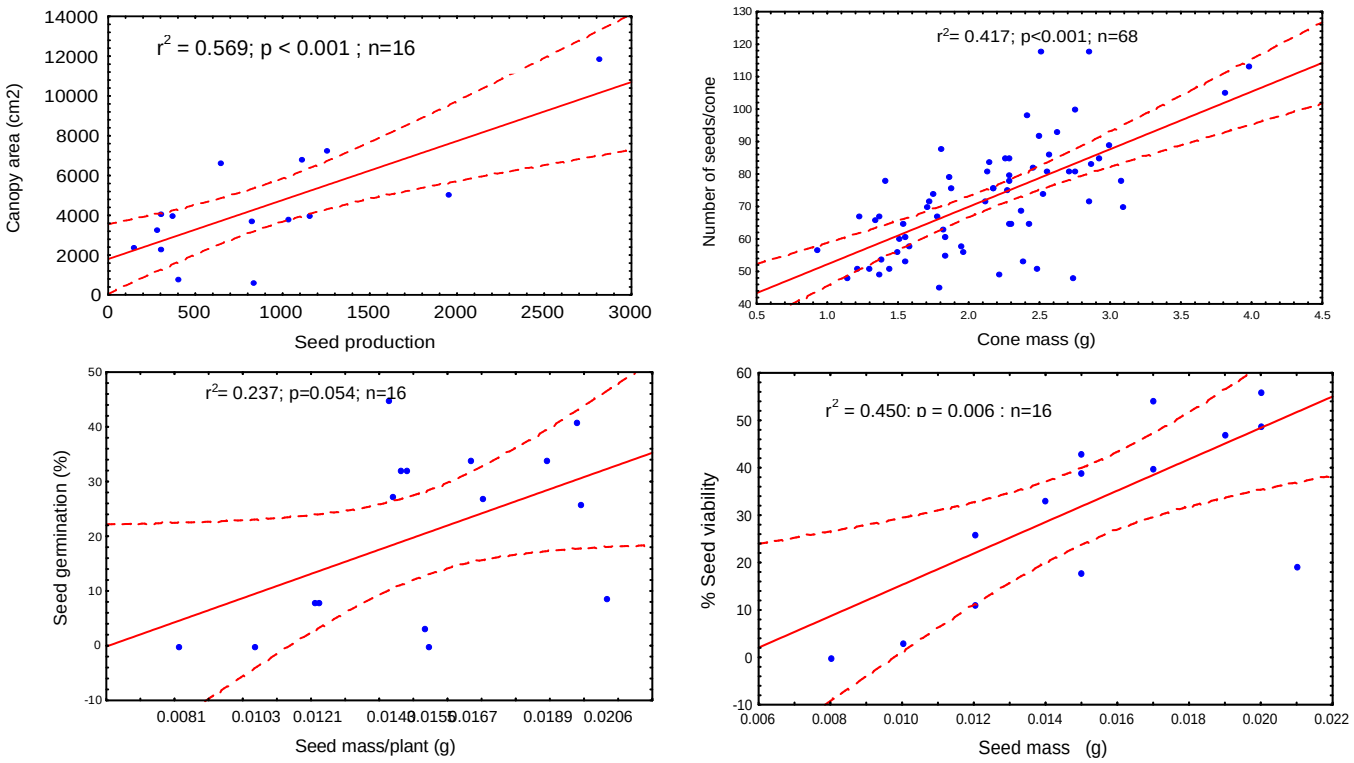


Figure 5.8. Relationships between canopy area, root mass, number of seeds/cone, seed production, cone mass, seed mass per plant, germination, seed mass and viability of *Protea simplex* using linear regressions. Broken lines represent 95% confidence limits.

5.2.3 Seed viability and germination in relation to seed mass: *Tephrosia kraussiana*

Seed mass per seed did not vary greatly for *T. kraussiana,* ranging from 4.5 to 7.7mg/seed with a mean and SD of 5.9±0.4 mg/seed (Table 5.9). The seed masses of plants 10, 11, 9 and 5 were the most significantly different from the rest of the individuals (Table 5.9). Plants with a higher mean seed mass (i.e. plants 4, 6 and 7) had higher seed viability. Plants with a low mean seed mass (i.e. plants 10 and 11) also had 100% seed viability. Plant 5 had the lowest seed viability but not the lowest mean and SD for seed mass (5.4±0.6mg). Plants with the same mean seed mass did not have the same seed viability and germination percentages, but overall there were few differences between plants because seed mass variation is very low in this species (CV= 6.8%).

Overall 98.7% of all *T. kraussiana* seeds sampled were viable. The number of viable and germinated seeds followed a normal distribution (Figure 5.9), together with the frequency of seeds in each size class, suggesting that seed mass does not influence seed germination and viability. The most frequent seed mass was between 5.51-6.0mg/seed. The percentage of viable seeds peaked between 8.51-9.00mg/seed (Figure 5.10), however, the sample size for this size class was small. Viability decreased marginally between 5.01-6.65mg/seed. The seed which was

recorded as viable between 2.01-2.50mg/seed is considered an outlier as it was based on a single seed. Only plant number 5 had more than one non-viable seed. Seed mass was not significantly different between viable and non-viable seeds for *T. kraussiana* (Table 5.10); however, seeds with mass less than 3.01mg/seed are likely to be non-viable (Figure 5.10).

The germination indices for *T. kraussiana* (Table 5.11) confirmed a rapid germination rate with a mean peak value of 6.1±2.1, a germination lag of 3.0±0 days, t_{50} germination of 3.9±1.4 days and mean days to germinate of only 4.2±0.7 days. The seeds all germinated around the same time with a short lag phase. Therefore, the seedlings were all of a similar age. This pattern is reflected in the cumulative germination curve (Figure 5.11). The curve indicated a period of rapid germination followed by a phase where germination slowed and then stopped altogether. Plant 14 had both the lowest peak (3) and germination values (12.6). Plants 10 and 18 had relatively high germination values (27.4, 25.8).

Table 5.9. Seed mass, viability and germination and total seed production (means and standard deviations) for individuals of *Tephrosia kraussiana* (n=17 plants, n=546 seeds). Superscripts with the same letter are not significantly different from each other (LSD, $p<0.05$) using Fishers Least significant difference (LSD) test after a one way ANOVA.

Plant number	Root mass (g)	Seed mass (mg)	Germinated (%)	Viable seeds (%)	Total seed production/plant
1	120	5.9±0.6 ^{bcde}	100	100	342
2	253	5.8±9.5 ^{cde}	100	100	684
3	170	5.9±0.7 ^{bcde}	100	100	453
4	200	6.1±0.5 ^{abc}	100	100	50
5	200	5.4±0.6 ^{f}	75.8	87.9	132
6	160	6.0±0.7 ^{abcd}	100	100	246
7	18	6.0±0.6 ^{bcd}	100	100	47
8	160	5.7±0.5 ^{de}	97	100	180
9	48	6.4±1.3 ^{a}	97	100	166
10*	253	5.0±0.5 ^{g}	94	100	462
11	126	5.0±0.5 ^{g}	97	100	436
12*	435	6.2±0.6 ^{ab}	94	100	487
13	200	6.2±0.8 ^{ab}	97	97	313
14	22	5.9±1 ^{abcd}	93.3	100	284
16	232	5.7±0.7 ^{ef}	100	100	583
17	45	5.9±0.6 ^{bcde}	97	97	200
18*	400	5.6±0.7 ^{ef}	100	100	723
Mean + SD	178.9±117.9	5.9±0.4	96.6±5.9	98.9±3.0	341±207
CV	65.9	6.8	6.1	3.0	60.7
Range	18-400	4.5-7.7	75.8-100	87.9-100	47-723

* Rhizomes which were radiocarbon dated

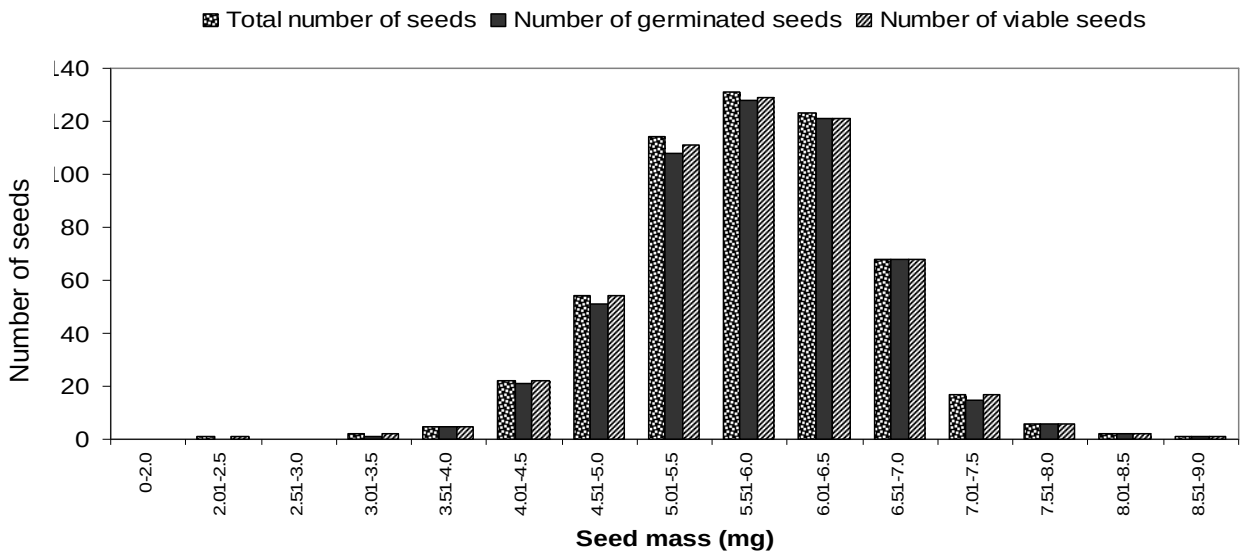


Figure 5.9. Seed size distributions showing the relationship between seed mass and a) the number of germinated and b) viable seeds per mass class for *Tephrosia kraussiana.*

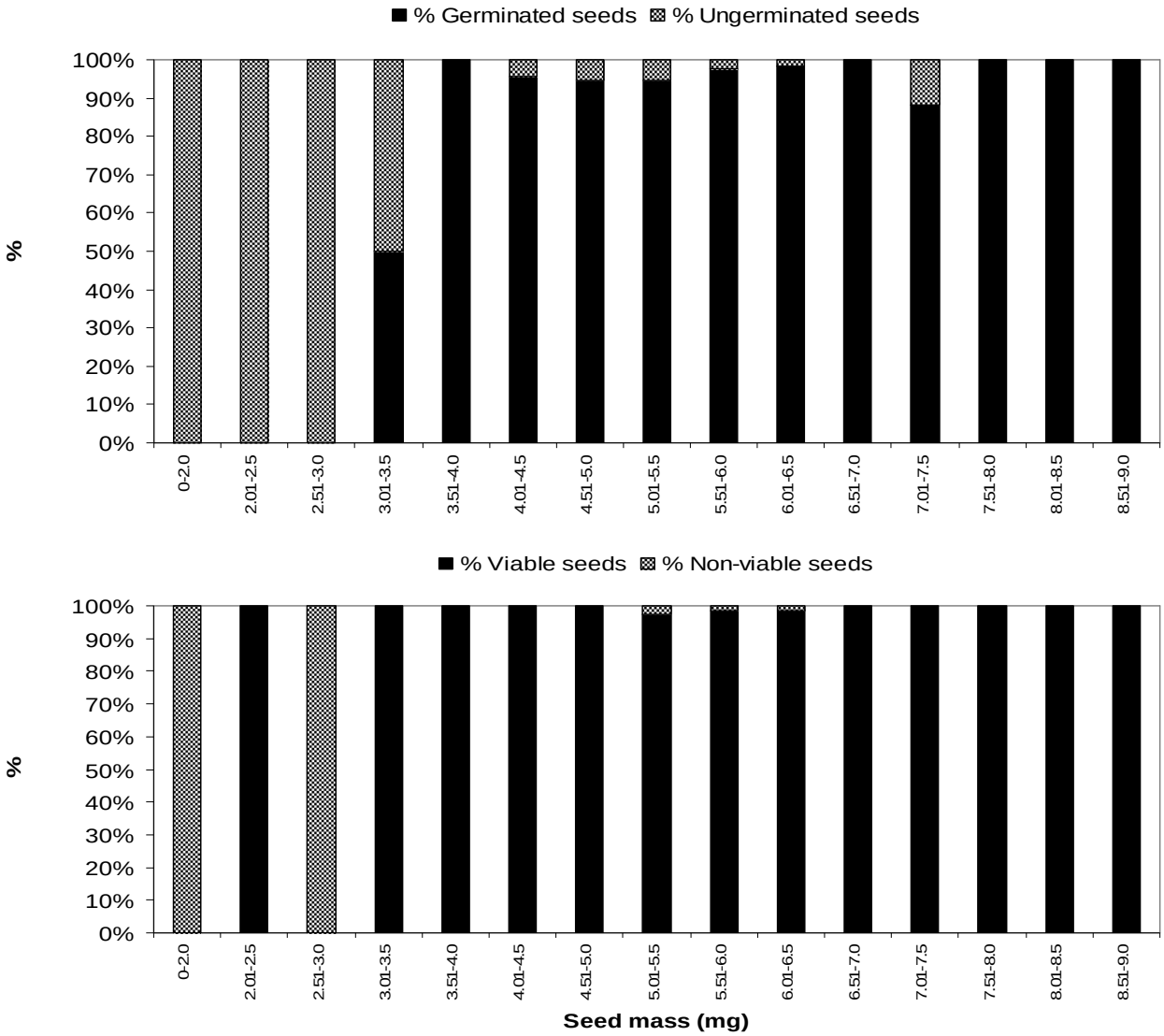


Figure 5.10. Seed size distributions showing the relationship between seed mass and a) the percentage of germinated and b) viable seeds per mass class for *Tephrosia kraussiana.*

Table 5.10. T-test (two sample with unequal variance) comparing the overall seed mass of viable (n= 541) and non-viable (n= 5) seeds across plants for *Tephrosia kraussiana*. (p>0.01)

Plant number	Viable seeds		Non-viable seeds		t	df	p
	Mean	SD	Mean	SD			
Mean across plants	6.0	±0.01	5.6	±1.73E-04	1,50	10	0.16

Table 5.11. Germination indices [Peak day; the time taken to the day when the most number of seeds germinated, germination lag; the number of days before germination commenced, t_{50} germination; number of days for 50% of the seeds to germinate, mean days and germination value (Ranal and De Santana 2006)] were calculated for *Tephrosia kraussiana* plants that produced seeds (n=17).

Plant	Peak day (days)	Peak value (days)	Germination lag (days)	t_{50} germination (days)	Mean days to germination (days)	Germination value
1	3	8.33	3	3	3.5	29.2
2	3	9	3	3	3.3	29.7
3	3	6	3	3	4.2	25.2
4	3	5	3	3	4.8	24
5	6	3.7	3	6	5.1	18.9
6	3	10	3	3	3.5	35
7	3 & 6**	5	3	3	4.5	22.5
8	3	4.7	3	6	4.8	22.6
9	3 & 6**	4.7	3	6	4.7	22.1
10*	3	8.3	3	3	3.3	27.4
11	6	4.5	3	6	4.9	22.1
12*	3 & 6**	3.7	3	6	5.5	20.4
13	3	8.7	3	3	3.4	29.6
14	3	3	3	3	4.2	12.6
16	3	5.7	3	3	4.4	25.1
17	3	7.7	3	3	3.6	27.7
18*	3	6.3	3	3	4.1	25.8
Mean ± SD	3.6±1.1	6.1±2.1	3.0±0	3.9±1.4	4.2±0.7	24.7±5.1
CV	30.6	34.4	0	35.9	16.7	20.6
Range	3-6	3-10		3-6	3.3-5.5	12.6-35

* Plants which were radiocarbon dated

** Peak value for germination was on both day 3 and 6

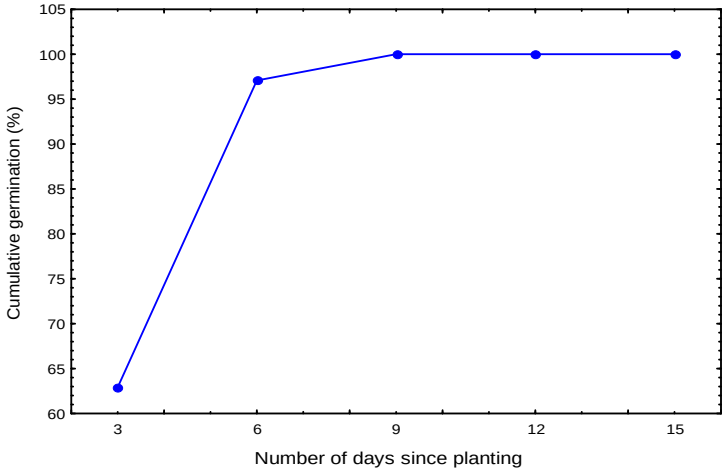


Figure 5.11. Cumulative germination curve for *Tephrosia kraussiana* (all plants combined).

5.2.4. Parent plant investment: relationship of parent plant structures with seed mass, viability and germination in *Tephrosia kraussiana*

There was a weak, non-significant relationship between seed mass and both percentage viability and germination (r^2=0.1518, p=0.1222 and r^2=0.0868, p=0.2511, respectively). There was a positive relationship between seed viability and seed germination (r^2=0.78, p<0.001) and between root mass and canopy area (r^2=0.53, p=0.001). However, root mass and canopy area did not have any significant relationships with any of the tested germination or viability variables (all other values for seed mass, % germinated, total seed production/plant, canopy area and root mass were p>0.05, r^2 <1.0). Only seed production/plant had a significant relationship with any of the size structure variables (canopy area, basal area, stem diameter, root mass, height, root depth and number of stems) tested in the multiple regression models (Table 5.12). Root mass, height, canopy area and the number of stems were significant predictors of seed production/plant in the backwards stepwise model (SP=-527.642 -1.885RM + 12.079H + 0.334CA + 12.224NS, p<0.001, r^2=0.83).

Table 5.12. *Tephrosia kraussiana* seed production/plant as a function of parent plant size variables (canopy area, basal area, stem diameter, root mass, height, root depth and number of stems) (n=17).

Model	Dependent variable	Predictor variable	Best fit model	Adjusted r^2	df	F	p
Linear regression	Seed production/plant (SP)	Root mass (RM)		0.39	1,15	11.17	0.004
Linear regression	Seed production/plant (SP)	Height (H)		0.11	1,15	2.94	0.107
Linear regression	Seed production/plant (SP)	Canopy area (CA)		0.24	1,15	6.09	0.026
Linear regression	Seed production/plant (SP)	Number of stems (NS)		0.67	1,15	33.92	<0.001
Backwards stepwise	Seed production/plant (SP)	Root mass (RM) Height (H) Canopy area (CA) Number of stems (NS)	SP=-527.642 - 1.885RM + 12.079H + 0.334CA + 12.224NS	0.83	4,12	20.12	<0.001

5.2.5 Comparison between field and laboratory germination of *Protea simplex* and *Tephrosia kraussiana*

Germination for *P. simplex* was based on two seed plots, 1 and 3, six months after planting as plot 2 could not be located. The emergence of seedlings in the field was more than four times lower than laboratory germination trials (Table 5.13).

For *T. kraussiana*, the study site had been burned 24 hours (27/07/2009) before measurements were taken and all three plots were burnt. This may have influenced results as

plants may have emerged but were completely burned and, therefore, not counted. Plants which were measured were scorched and desiccated but the remaining skeletons remained intact. No seedlings were found in seed grids for plot 2. The emergence of seedlings in the field was 20 times lower than laboratory germination trials. Emergence of seedlings in the field was lower for *T. kraussiana* than for *P. simplex*. However, *T. kraussiana* emergence was higher than *P. simplex* in laboratory trials.

Table 5.13. Comparison between the emergence of *Protea simplex* and *Tephrosia kraussiana* seedlings in field plots six months after sowing and laboratory trials.

Species	Plot	% Emergence/plot	Field trial % Emergence (mean±SD)	Laboratory trial % Emergence (mean±SD)
Protea simplex	1	8	5±4.2 (n=300)	21.8±15.5 (n=1360)
	2	Plot missing*		
	3	2		
Tephrosia kraussiana	1	1	4.7±7.2 (n=300)	96.6±5.9 (n=546)
	2	0		
	3	13		

* The markers for this plot could not be found during the survey of seedling emergence.

5.3 Seedling growth (*Protea simplex and Tephrosia kraussiana*)

5.3.1 Dry mass allocation and growth rates of six month old *Protea simplex* seedlings

P. simplex seedlings allocated the greatest mass (39.6±17%) to leaf production (Figure 5.13), then roots (29.2±14%) and shoots (11.5±5%). Cotyledons had a high percentage allocation for six month old plants (19.8±26%). All cotyledons persisted (and were green) on plants at six months with the exception of one individual on which the cotyledons had desiccated. Mean root to shoot ratios indicated that the dry mass of roots were 2.9 times heavier than shoots (Table 5.14), suggesting a high investment in a woody rootstock at this early stage. Seedling roots also showed early signs of lignification at the stem-root interface at six months old (Figure 5.12).

Figure 5.12. Early signs of lignification of the woody rootstock of *Protea simplex* seedlings at six months old.

Cumulative growth for the stem diameter and height of *P. simplex* seedlings (Figure 5.14) followed a gradual increase in length during weeks one and two, followed by a brief period where growth remained stable. A second phase of gradual increase then followed. Cumulative growth

of the number of leaves and longest leaf length increased gradually in length and number, then the curve began to rise at a quicker rate. The number of stems increased gradually over 26 weeks. The length of the longest cotyledon remained relatively constant throughout the trial. Cotyledons remained on 93% of seedlings at the end of the trial. The increase in slope angle for height, number of leaves and stem diameter all occurred after week 7. No change in greenhouse conditions was noted during this period. Week 7 was during early May 2009 and, therefore, the beginning of the colder, less favourable season for optimum growth. The exclusion of any known external favourable conditions suggests the possibility of an internal catalyst for growth. Proteoid roots may have been fully developed for more effective nutrient uptake at this stage.

The remaining cotyledon dry mass (a possible indicator of the quality of parent investment) had significant logarithmic relationships with the dry mass of the whole seedling ($r=-0.7717$, $p<0.0001$), dry mass of the whole seedling excluding cotyledons ($r=-0.7772$, $p<0.0001$), and root dry mass allocation ($r=-0.6563$, $p<0.001$). Shoot dry mass allocation had a polynomial fit with cotyledon dry mass ($r^2=-0.5581$, $p=0.002$) and there was a significant linear relationship between cotyledon mass and leaf mass ($r^2=0.7447$, $p<0.0001$). There was a logarithmic relationship between seed mass and cotyledon dry mass ($r^2=-0.1369$, $p=0.4871$), with plants A, B, C and D as outliers (Figure 5.15). Plants A, B and C were plants which had poor root and no shoot development. Plant D had poor root development and strong shoot development. Plant E was the only individual on which cotyledon senescence occurred. Cotyledon mass decreased over time as reserves were utilized by the seedlings. These results only provide what remained, not what was utilized nor the original cotyledon mass, however, the latter would be represented by seed mass. Overall, plants which grew more rapidly and invested in early rhizome formation utilized greater cotyledon mass, grew shoots and had greater overall seedling mass.

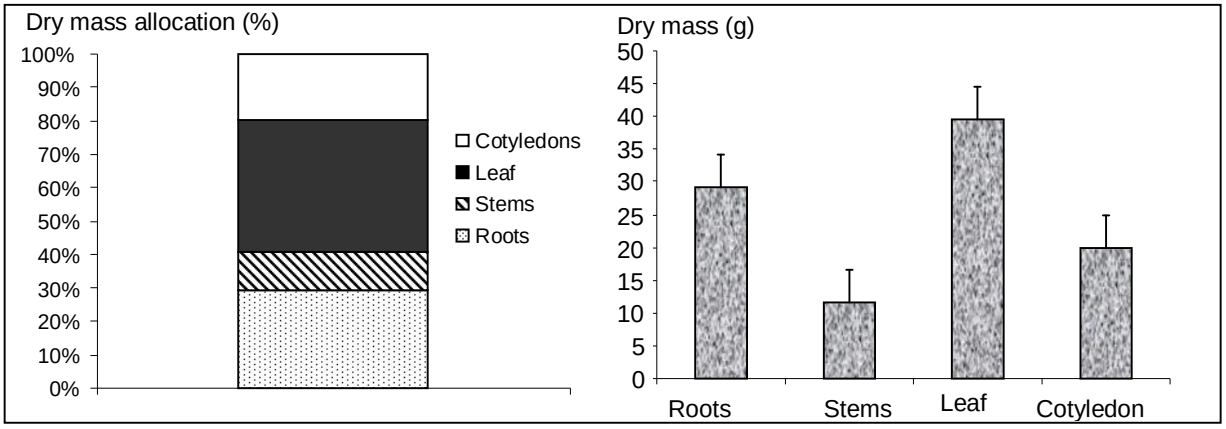


Figure 5.13. Dry mass allocation (%) and dry mass (g) of cotyledons, stems, leaves and roots for six month old (greenhouse grown) seedlings of *Protea simplex* (n=28).

Table 5.14. The percentage of roots and shoots of total seedling mass and the root: shoot ratio for *P. simplex* (n=28).

% Root mass	% Shoot mass	Root: Shoot ratio
29.2±13.9	11.5±4.6	2.9±2.0

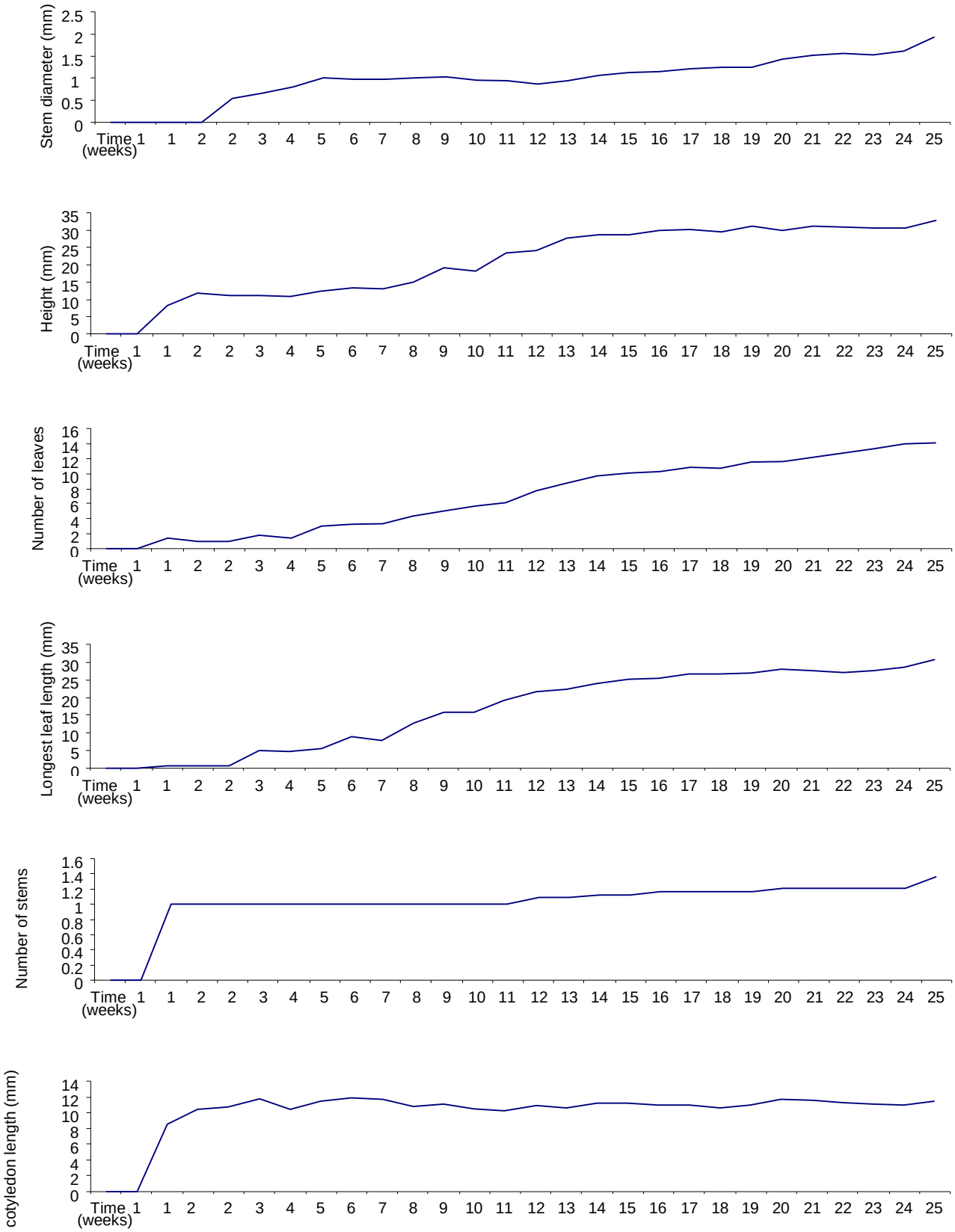


Figure 5.14. Cumulative growth of stem diameter, number of leaves, longest cotyledon length, plant height, longest leaf length and the number of stems of *Protea simplex* seedlings over 6 months. Results of seedling trials were recorded twice a week for the first four weeks (n=28).

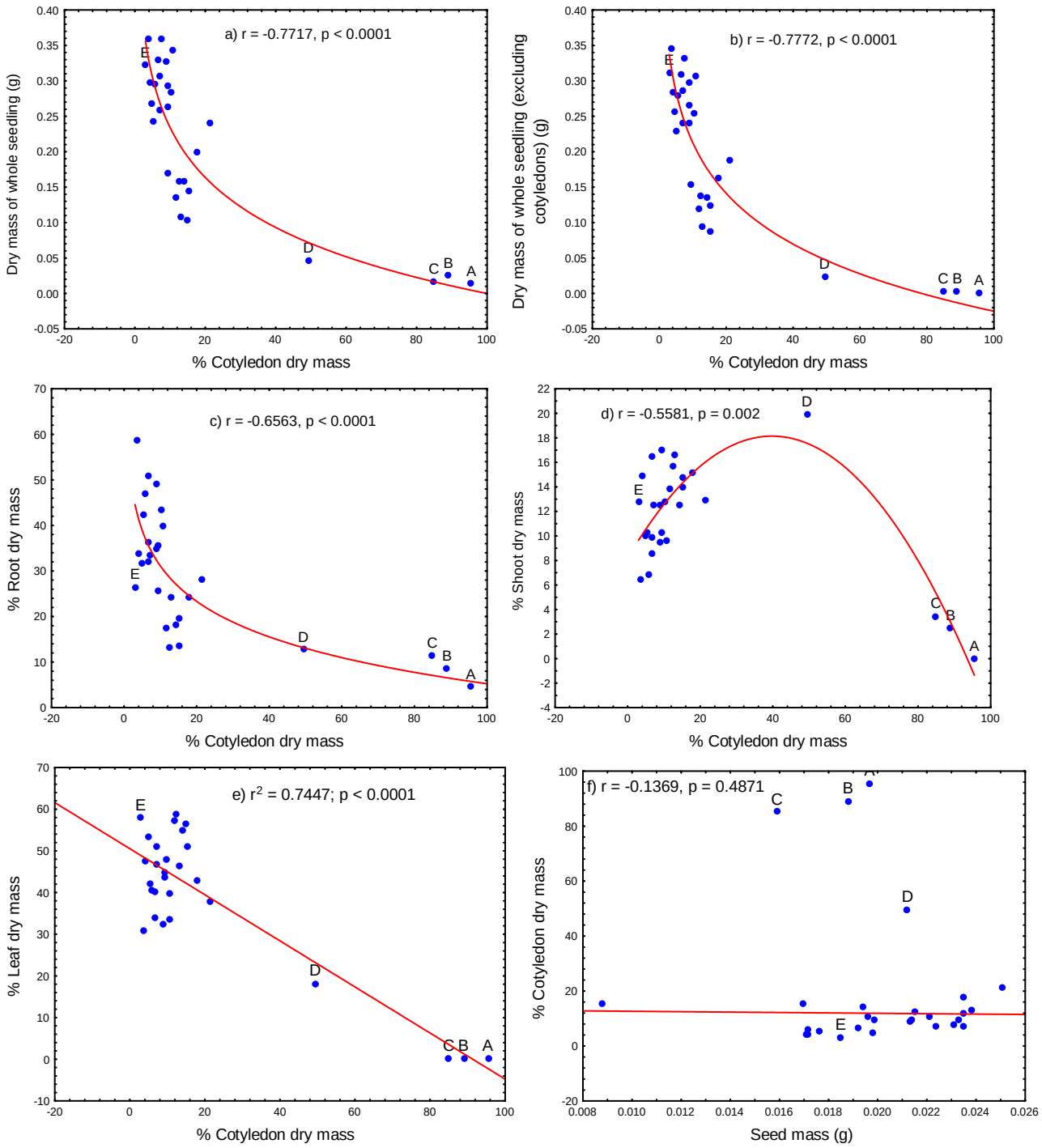


Figure 5.15. The relationship between the percentage dry mass of cotyledons of *P. simplex* seedling and a) whole seedling dry mass, b) whole seedling dry mass (excluding cotyledons), c) % root dry mass, d) % shoot dry mass, e) % leaf dry mass and f) seed mass (n=28, p<0.01). A, B, C, D and E represent plants which were of particular interest.

5.3.2 Parent plant investment in seedlings of *Protea simplex*

Seed mass had a significant relationship with the percentage allocated to shoot dry mass (p=0.018) after the outlier 0.0088g/seed was removed (Table 5.15). Seed mass and shoot dry

mass shared a weak positive relationship (r^2=0.2117, p=0.018) (Figure 5.16). Final stem diameter, shoot dry mass, longest cotyledon length, height, longest leaf length, number of stems, dry mass of root, leaves and cotyledon were not significantly related to seed mass (p>0.05).

There was also a relationship between parent plant height (r^2=0.399, p=0.040) and rhizome mass (r^2=0.441, p=0.030) and seedling shoot mass. However, parent plant rhizome mass remained as the stronger predictor of seedling shoot mass in the multiple regression model (r^2=0.443, p=0.030) (Table 5.16).

Table 5.15. The relationship between seed mass and seedling dry mass allocations to roots, shoots, leaves, cotyledons and the final growth values for stem diameter, number of leaves, longest cotyledon length, height, longest leaf length and the number of stems for *P. simplex* using linear regressions (n=23 seed mass categories, p=0.05).

	r^2	df	p
Stem diameter (mm)	0.0313	1,22	0.4194
Number of leaves (mm)	0.0343	1,22	0.3973
Longest cotyledon length (mm)	0.0893	1,22	0.1661
Height (mm)	0.0617	1,22	0.2533
Longest leaf length (mm)	0.0756	1,22	0.2043
Number of stems (mm)	0.0142	1,22	0.5880
% Root dry mass	-0.0500	1,22	0.9904
% Shoot dry mass	0.2117	1,22	0.0180
% Leaf dry mass	0.0148	1,22	0.2648
% Cotyledon dry mass	0.0179	1,22	0.2533

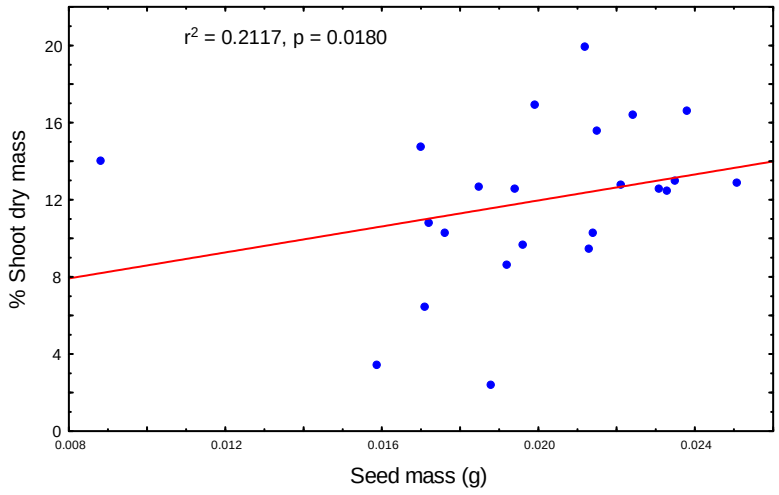


Figure 5.16. The relationship between seed mass and % allocation to shoot dry mass for seedlings of *P. simplex* (the outlier 0.0088g/seed is shown) (n=23, p<0.05).

Table 5.16. The relationship (multiple regression analyses) between seedling shoot dry mass (SDM) as a function of parent plant size variables (canopy area, basal area, stem diameter, root mass, height and the number of stems) in *P. simplex*.

Model	Dependent variable	Predictor variable	Best fit model	Adjusted r^2	df	F	p
Linear regression	Shoot dry mass	Root mass (RM)		0.441	1,7	7.353	0.030
Linear regression	Shoot dry mass	Height (H)		0.399	1,7	6.313	0.040
Backwards stepwise	Shoot dry mass	Root mass (RM)	SDM=9.673 + 0.002RM	0.443	1,7	7.350	0.030

5.3.3 Dry mass allocation and growth rates of six month old *T. kraussiana* seedlings

T. kraussiana seedlings invested the greatest mass in the development of roots (Figure 5.18) (45.4%), followed by leaves (42.5%). Shoot mass was allocated 11.4% and cotyledon mass 0.7%. However, this was not unexpected as cotyledon senescence occurred earlier than that of *P. simplex* (at about 3 months) and only two individuals remained with green cotyledons still attached to the plant at the end of six months. The ratio of roots was 5.1 times higher than shoots (Table 5.17) in seedlings of *T. kraussiana*, placing greater emphasis on early energy investment in the woody roots. The roots of *T. kraussiana* also showed signs of early lignification (Figure 5.17). The junction between roots and shoots was hard and rough. There were significant inverse relationships between seedling root dry mass and shoot (r^2=0.3368, p<0.0001) and leaf (r^2=0.1882, p=0.0021) dry mass (Figure 5.19). There was also an inverse relationship between cotyledon dry mass and leaf dry mass (r^2=0.7447, p<0.0001). Therefore, faster growing plants utilized and depleted resources from the cotyledons quicker than slower growing plants.

The cumulative growth curves of stem diameter and height (Figure 5.20) followed a pattern of short, rapid initial growth, followed by a phase in which the two variables remained relatively constant. However, after week 12, the curve for both variables began increasing rapidly once again. Week 12 also coincided with the early winter months and less favourable light and temperature conditions. Therefore, once again, this change could be attributed to the development of sufficient root mass to facilitate rapid growth. The number of leaflets had exponential growth throughout the trial while the length of cotyledons and leaflets remained constant.

Figure 5.17. Early signs of lignification of the junction between roots and shoots of *Tephrosia kraussiana* seedlings at six months old.

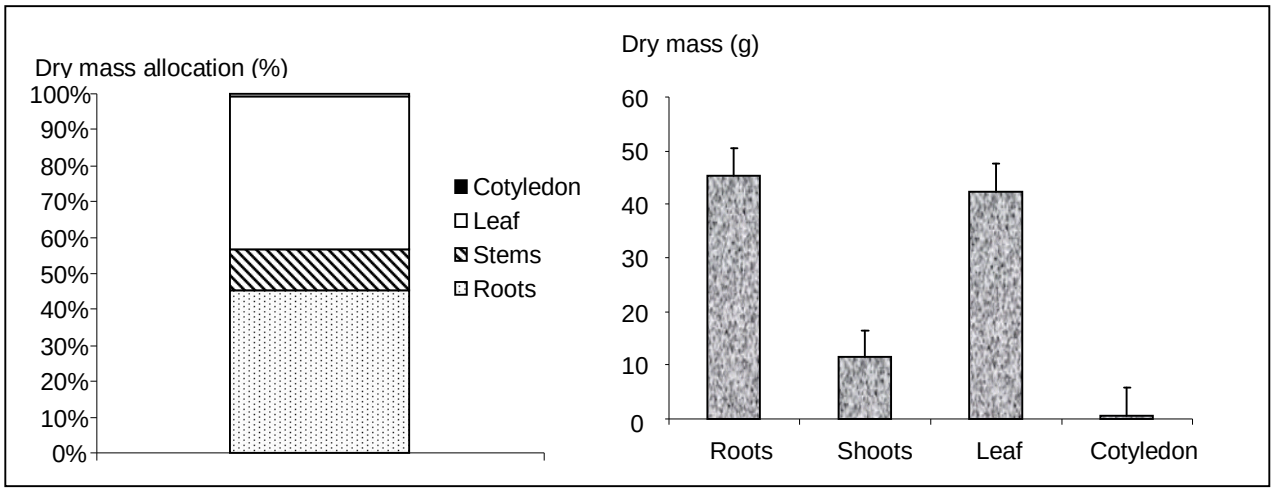


Figure 5.18. Dry mass allocation (%) and dry mass (g) of cotyledons, stems, leaves and roots for six month old (greenhouse grown) seedlings of *Tephrosia kraussiana* (n=48).

Table 5.17. The percentage of roots and shoots of total seedling mass and the root: shoot ratio for *T. kraussiana* (n=48).

% Root mass	% Shoot mass	Root: Shoot ratio
45.4±9.0	11.5±7.5	5.1±2.4

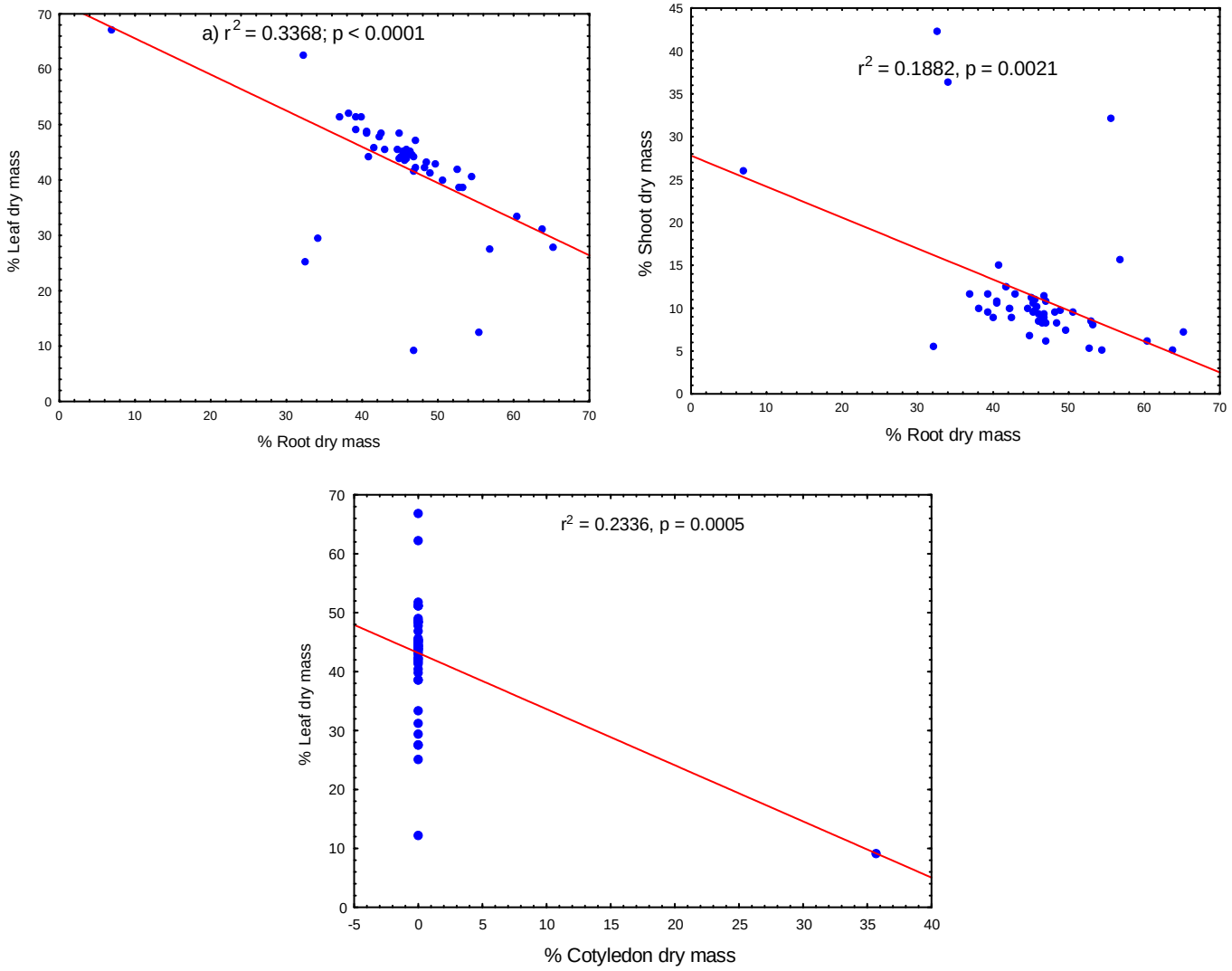


Figure 5.19. Significant relationships between seedling allocations to roots, shoots, leaflets and cotyledons for *T. kraussiana* (n=48, p<0.001).

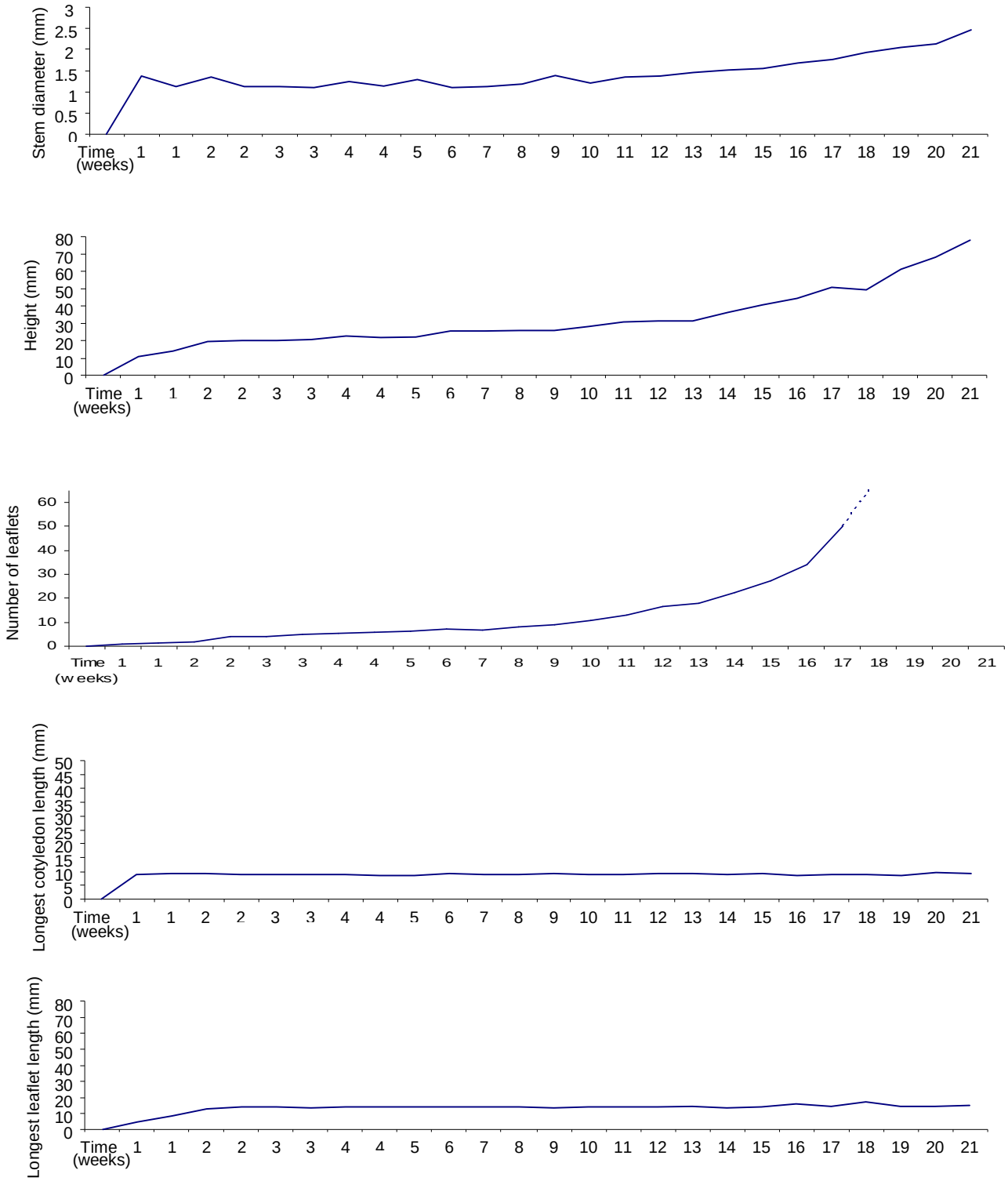


Figure 5.20. Cumulative growth of *Tephrosia kraussiana* seedlings over 6 months. Results of seedling trials were recorded twice a week for the first four weeks (n=48).

5.3.4 Parent plant investment in seedlings of *Tephrosia kraussiana*

There were no significant relationships between seed mass and the final growth of seedling stem diameter, number of leaflets, longest cotyledon length, height, and the dry mass of roots, shoots, leaflets and cotyledons for *T. kraussiana* (Table 5.18). However, there was a significant relationship between seedling leaflet dry mass and parent plant height (r^2=0.2651, p=0.0413) and seedling root dry mass and parent plant main stem diameter (r^2=0.2651, p=0.0413) (Figure 5.21). The dry mass allocation of seedling roots increased with increasing main stem diameter of the parent plant whereas there was an indirect relationship with parent plant height and seedling leaflet dry mass. There was also a significant relationship between the percentage of germinated seeds/parent plant and the dry mass of cotyledons (r^2=0.8731, p<0.0001) and leaflets (r^2=0.4173, p=0.0069) in corresponding seedlings.

Table 5.18. The relationship between seedling dry mass and final growth values for *T. kraussiana* using linear regressions. (n=25, p=0.01)

	r^2	p
Stem diameter (mm)	0.049208	0.347239
Number of leaflets	0.036010	0.422942
Longest cotyledon length (mm)	0.061070	0.293527
Height (mm)	0.011225	0.656641
% Root dry mass	0.001343	0.878109
% Shoot dry mass	0.046465	0.361386
% Leaflet dry mass	0.005853	0.748533
% Cotyledon dry mass	0.061070	0.293527

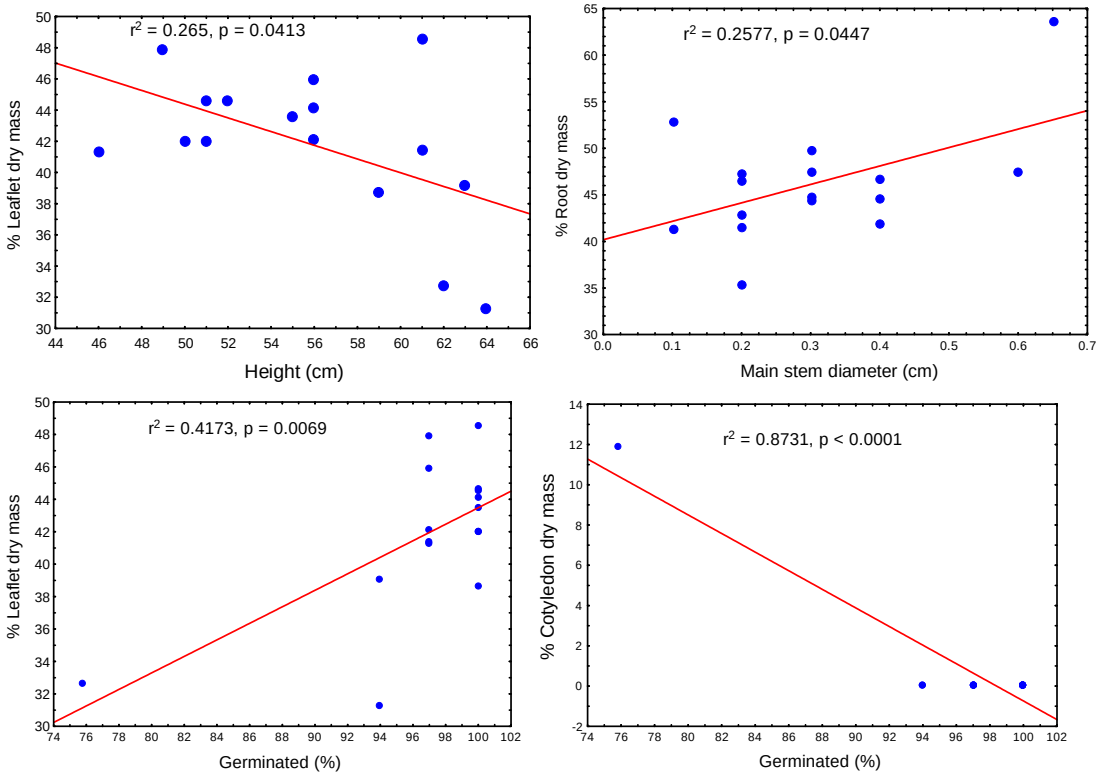


Figure 5.21. Significant relationships between parent plant size (height, canopy area, number of stems, stem diameter, basal area) and viability (% viability and germination) and seedling dry mass allocations for roots, shoots, leaflets and cotyledons) (n=16, p<0.05)

5.3.5 Comparison between 6 month old germinants of field and greenhouse trials: *Protea simplex* and *Tephrosia kraussiana*

Ninety-eight percent of *T. kraussiana* germinants planted in the greenhouse emerged, and 96% survived through to the end of the six month trial. Only 60% of greenhouse grown *P. simplex* germinants emerged and only 56% survived till the end of the trial. Some of the plants which emerged but did not survive dried out during a malfunction in the greenhouse sprinkler system during day 21 of the trial. The percentage of *P. simplex* and *T. kraussiana* germinants surviving in the field after six months was only 5±4.2%.

Mean seedling stem diameter, leaf number, cotyledon length, leaf length and the number of stems was higher in greenhouse grown compared to field grown seedlings of *P. simplex* (Table 5.19). Only mean height of field germinants was higher than greenhouse grown germinants. There was a significant difference between stem diameter (t= -0.578, p<0.0001), cotyledon length (t= -2.804, p=0.0082) and height (t= 3.332, p=0.0020) between field and greenhouse grown germinants (Table 5.20).

Greenhouse grown seedlings of *T. kraussiana* had consistently higher mean values than field grown seedlings for stem diameter, number of leaflets, height and leaflet length (Table 5.21 and 5.23).

Table 5.19. Mean, SD and CV of stem diameter, leaf number, cotyledon length, height leaf length and the number of stems of field (n=10) and laboratory grown (n=28) seedlings of *P. simplex* after 6 months.

		Stem diameter (mm)	Number of leaves	Cotyledon length (mm)	Height (mm)	Leaf length (mm)	Number of stems
Field	Mean	0.89	10.40	9.63	48.40	27.95	1.00
	SD	0.08	2.88	1.55	16.32	7.51	0
	CV	8.88	27.65	16.13	33.71	26.88	0
Greenhouse	Mean	1.93	14.11	11.50	32.91	30.60	1.25
	SD	0.56	5.61	1.87	11.12	4.89	0.70
	CV	29.10	39.74	16.30	33.77	15.99	56.04

Table 5.20. A comparison between stem diameter, leaf number, height and leaf length of field (n=10) and laboratory grown (n=28) seedlings of *P. simplex* after 6 months using independent t-test (p<0.05). Certain plants did not grow all structures resulting in variation in df.

	t	df	p
Stem diameter	-5.788	1,33	<0.0001
Number of leaves	-1.987	1,36	0.0545
Cotyledon length	-2.804	1,35	0.0082
Height	3.332	1,36	0.0020
Leaf length	-1.236	1,33	0.2251
Number of stems	-1.119	1,36	1.0000

Table 5.21. Mean, SD and CV of stem diameter, leaflet number, cotyledon length, height, leaflet length and the number of stems of field (n=14) and laboratory grown (n=48) seedlings of *T. kraussiana* after 6 months.

		Stem diameter (mm)	Number of leaflets	Height (mm)	Leaflet length (mm)
Field	Mean	0.66	17.00	29.78	10.17
	SD	0.19	15.31	9.37	2.64
	CV	29.09	90.04	31.46	25.98
Greenhouse	Mean	2.46	44.33	78.00	14.82
	SD	0.72	14.05	42.55	3.51
	CV	29.38	31.68	54.55	23.66

Table 5.22. A comparison between stem diameter, leaflet number, height and leaflet length of field (n=14) and laboratory grown (n=48) seedlings of *T. kraussiana* after 6 months using independent t-tests ($p<0.05$).

	t	df	p
Stem diameter	-9.154	1,60	<0.0001
Number of leaflets	-6.280	1,60	<0.0001
Height	-4.188	1,60	<0.0001
Leaflet length	-4.590	1,60	<0.0001

5.4. Size structure, demography and plant density of *Berkheya insignis*, *Callilepis laureola*, *Protea simplex* and *Tephrosia kraussiana*

The variation in plant densities of *B. insignis*, *C. laureola* and *T. kraussiana* was relatively high (CV= 48, 59, 78) (Table 5.23); compared to *P. simplex* (CV=15). *T. kraussiana* (with its compact corm-like rhizome structure with few secondary branches) (Figure 5.22) had the highest mean plant density (14067 plants/ha), then *C. laureola* (6194 plants/ha). *C. laureola* also had a compact corm like structure with multiple thin side branches. *P. simplex* had the lowest mean density (2292 plants/ha). This species had a complex and widespread underground rhizome system. Plot two for *T. kraussiana* had an especially high plant density (26 000 plants/ha) (Table 5.23). More compact rhizome morphologies may allow for the occupation of a greater number of plants in any given area as the distance between adjacent plants is reduced, whereas a widespread rhizome network may increase the distance between individuals, thus increasing and decreasing plant densities, respectively.

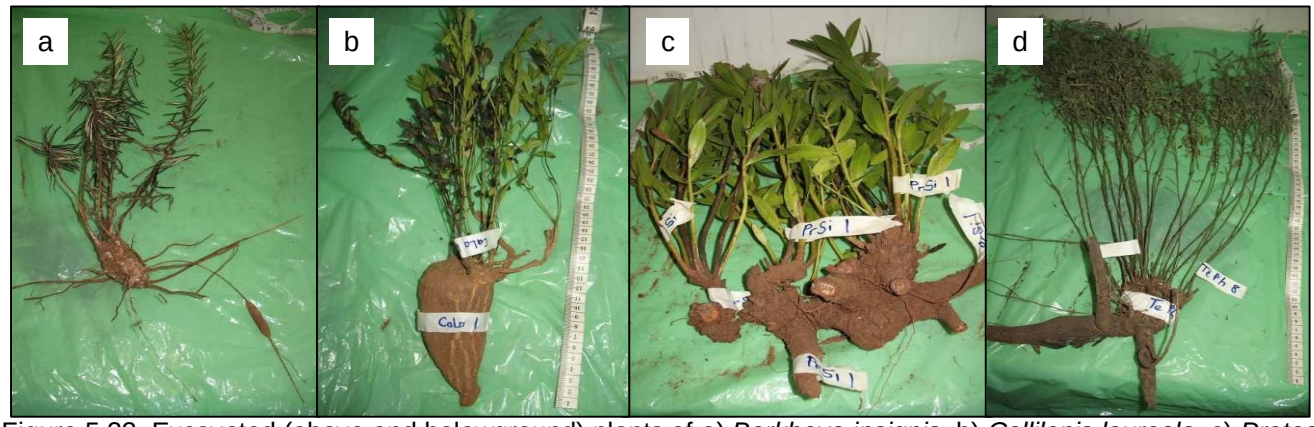


Figure 5.22. Excavated (above and belowground) plants of a) *Berkheya insignis*, b) *Callilepis laureola*, c) *Protea simplex* and d) *Tephrosia kraussiana*

Plots two and three of *B. insignis* (Table 5.24) were not significantly different from each other for any of the tested size structure variables (p=0.54). Plots two and three had the greatest likelihood of being drawn from the same population for *C. laureola* with three out of the five variables (canopy area: p=0.299, basal area: p=0.363 and number of stems: 0.53) showing no significant difference between each plot. Only canopy area (p=0.80) and basal area (p=0.78) between plots two and three were not significantly different. *T. kraussiana* had the highest congruency between the distributions of size structure measurements with only height (p=0.008) and main stem diameter (p=0.03) significantly different between plots 1 & 2 and 2&3, respectively. Plots one and three were the most similar. Across species, height and main stem diameter were the most significantly different. These differences may have been due to soil nutrient availability.

Height (Figure 5.23) had a bell shaped distribution for all species, with low numbers of very short and very tall plants and a higher number of medium sized plants. Height for *C. laureola* was skewed to the right. Main stem diameter for all species (Figure 5.24) followed a normal distribution. The number of stems/plant (Figure 5.25) for all four species followed an inverse J-shaped distribution. Stem basal area (Figure 5.26) and canopy area (Figure 5.27) had more individuals in the smaller size classes. The frequency of individuals then decreased with an increase in size class. The frequency distributions for the number of floral structures (Figure 5.28) varied across species. *C. laureola* had a distribution which was skewed to the left. Most plants had a few floral structures. *T. kraussiana* had high frequencies in the lowest and highest size classes but low frequencies in the intermediate size classes. No floral structures were found on individuals of *B. insignis* during the field investigation.

All four species had similar stage class frequencies (Figure 5.29). The frequency of individuals was higher in the mature stage classes. *B. insignis* and *T. kraussiana* had very few juveniles and few sub-adults. There were more adults in *B. insignis* and *T. kraussiana* ; with more reproductive than non-reproductive adults for *T. kraussiana* (differentiation could not be made between reproductive and non-reproductive adults for *B. insignis* as no flowers or flower bracts

were present on plants at the time of sampling). No juveniles were found for *C. laureola* and *P. simplex*. Reproductive adults were the most frequent for *C. laureola* and *P. simplex*. There was a significant difference between the main stem diameter of juveniles and sub adults of *B. insignis*; however, there was no significant difference between the number of inflorescences, height, canopy area, basal area and number of stems (Table 5.25). *B. insignis* adults were significantly different from juveniles and sub adults for the number of inflorescences, height, basal area, number of stems and main stem diameter. Adults and sub adults of *C. laureola* were significantly different from each other for all structures measured. For *P. simplex*, only height, canopy area and basal area were significantly different between adults and sub adults. Five of the six structures (number of flowers/bracts, height, canopy area, number of stems and main stem diameter) were not significantly different between juveniles and sub adults of *T. kraussiana*. Adults were significantly different from juveniles and sub adults for all structures. Basal area was significantly different between the three stages.

Table 5.23. Mean and coefficient of variation of plant density for each species. Density for each plot (sampled during January 2008) and overall plant density is shown.

Species	Plot	Plant density/plot	Overall Plant	
Berkheya insignis	1	2875		
	2	3250	2408	48
	3	1100		
Callilepis laureola	1	8500		
	2	8083	6194	59
	3	2000		
Protea simplex	1	2625		
	2	2325	2292	15
	3	1925		
Tephrosia kraussiana	1	11700		
	2	26000	14067	78
	3	4500		

Table 5.24. Pair-wise comparison of size structure variables between plots of *B. insignis*, *C. laureola*, *P. simplex* and *T. kraussiana* using the Kolmogorov- Smirnov test. D and p values are shown. P values in bold are significantly different ($p < 0.05$).

Species	**Plot**	**Height (cm)**		**Canopy area (cm^2)**		**Basal area (cm^2)**		**Number of stems**		**Main stem diameter (mm)**	
		D	***p***	**D**	***p***	**D**	***p***	**D**	***p***	**D**	***p***
Berkheya insignis	1 & 2	0.38	**<0.001**	0.09	0.75	0.09	0.67	0.16	0.07	0.34	**<0.001**
	2 & 3	0.14	0.540	0.15	0.44	0.22	0.08	0.09	0.96	0.17	0.250
	1 &3	0.40	**<0.001**	0.13	0.63	0.26	**0.02**	0.21	0.11	0.22	0.090
Callilepis laureola	1 & 2	0.23	**0.01**	0.31	**0.001**	0.35	**0.001**	0.19	**0.04**	0.1	0.73
	2 & 3	0.59	**0.001**	0.22	0.299	0.2	0.363	0.18	0.53	0.3	**0.04**
	1 & 3	0.48	**0.001**	0.34	**0.018**	0.28	0.075	0.28	0.08	0.3	**0.03**
Protea simplex	1 & 2	0.12	0.461	0.16	**0.02**	0.12	**0.31**	0.4	**0.001**	0.23	**0.011**
	2 & 3	0.74	**0.001**	0.07	0.80	0.08	0.78	0.35	**0.001**	0.7	**0.001**
	1 & 3	0.77	**0.001**	0.15	**0.04**	0.17	**0.05**	0.53	**0.001**	0.58	**0.001**
Tephrosia kraussiana	1 & 2	0.19	**0.008**	0.09	0.427	0.145	0.06	0.11	0.33	0.12	0.19
	2 & 3	0.11	0.743	0.73	0.001	0.16	0.24	0.11	0.69	0.22	**0.03**
	1 & 3	0.22	0.067	0.66	0.001	0.121	0.68	0.2	0.12	0.16	0.37

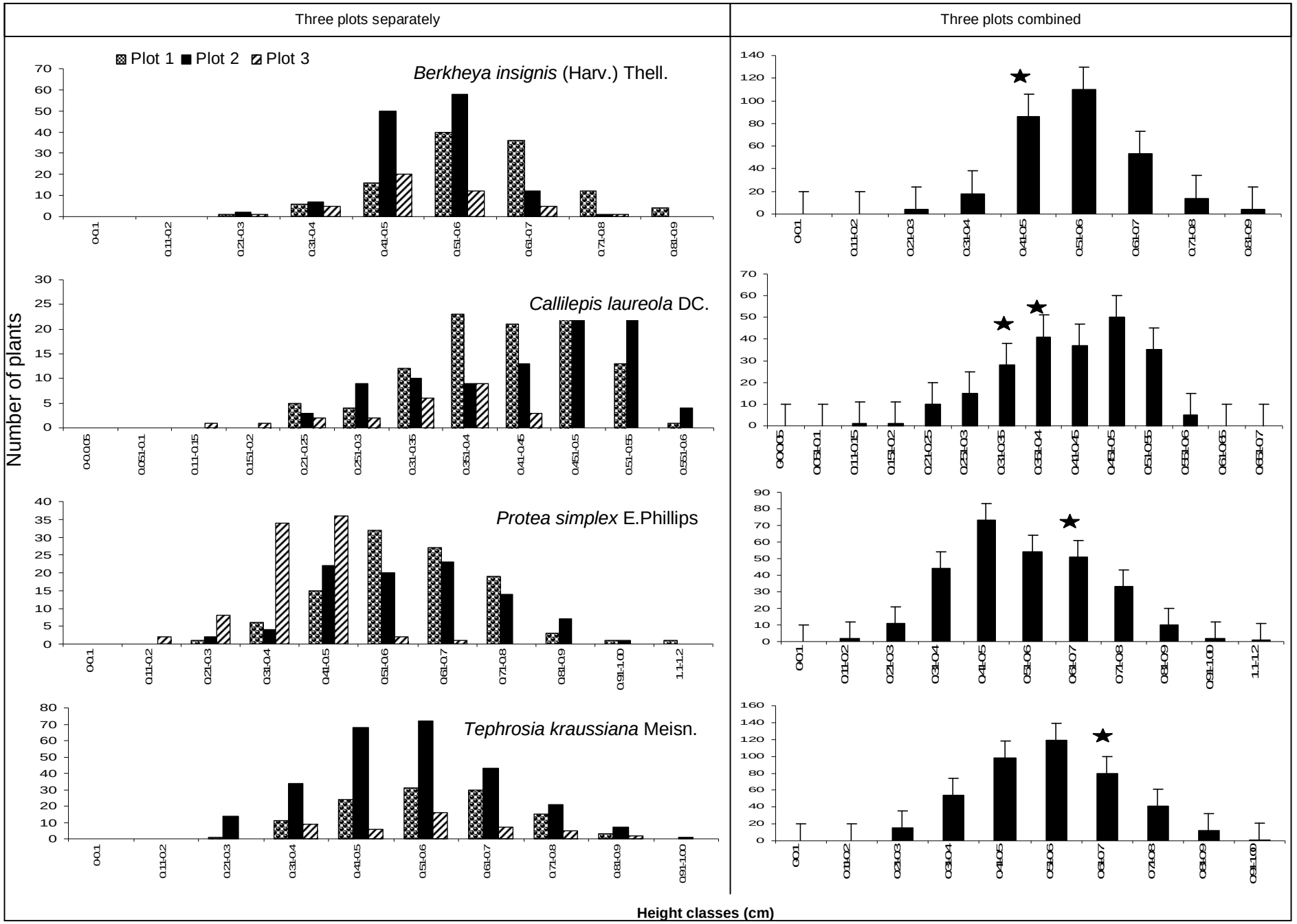


Figure 5.23. Distribution of the number of plants in each class showing height for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE). ★ Indicates where the sizes of radiocarbon dated plants fall.

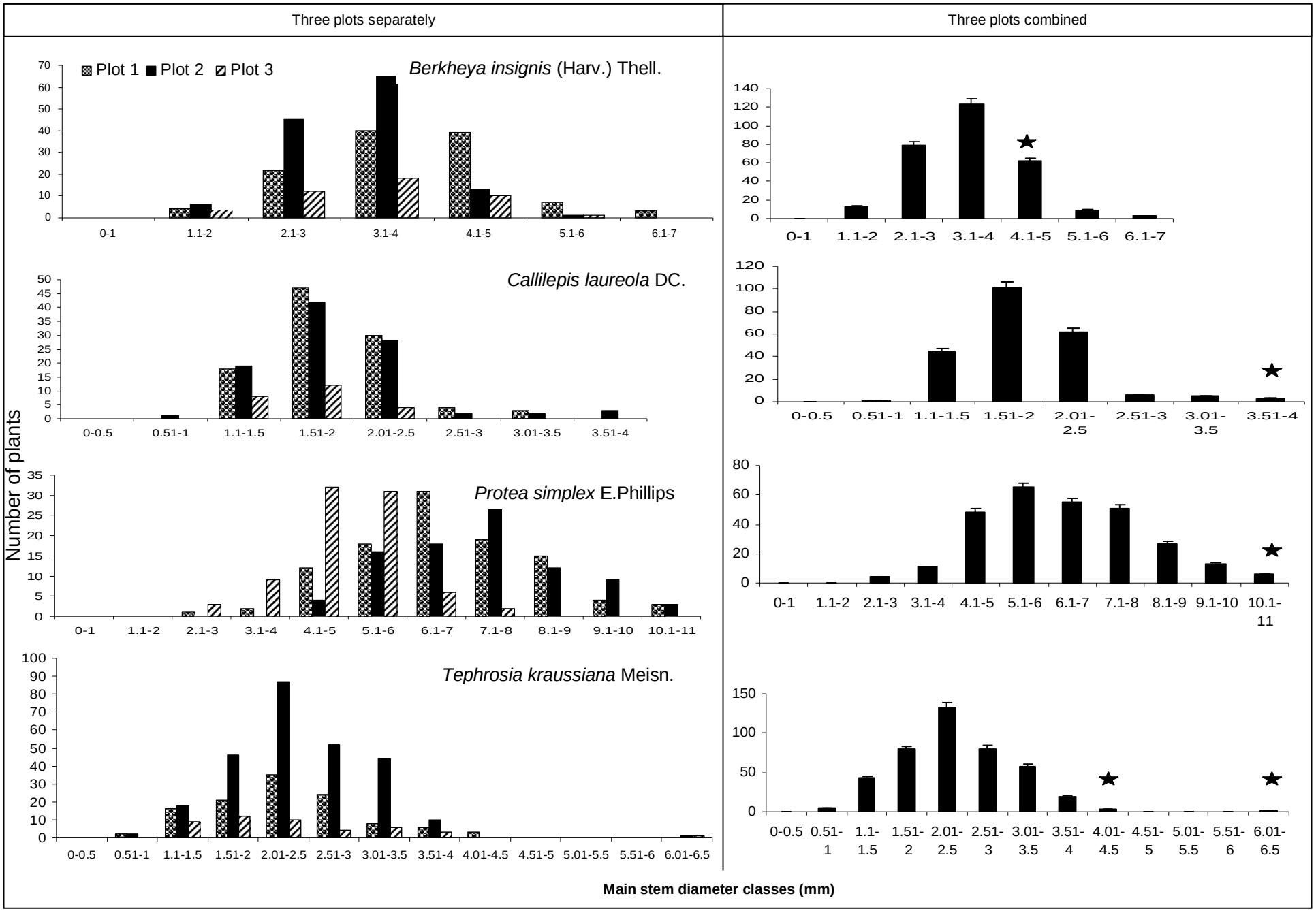


Figure 5.24. Distribution of the number of plants in each class for main stem diameter for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE). ★ Indicates where the sizes of radiocarbon dated plants fall.

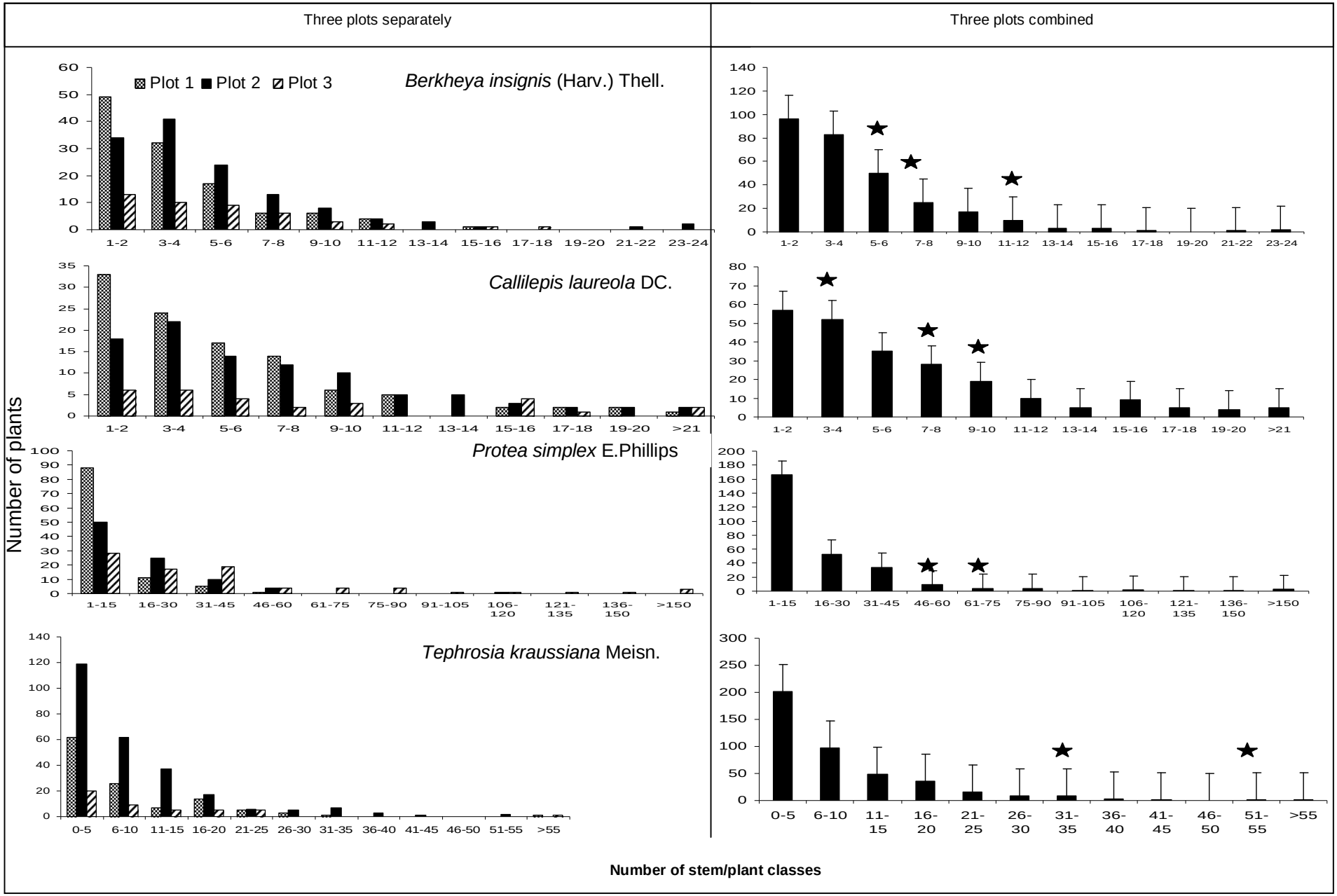


Figure 5.25. Distribution of the number of plants in each class for the number of stems for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots (mean ± SE). ★ Indicates where the sizes of radiocarbon dated plants fall.

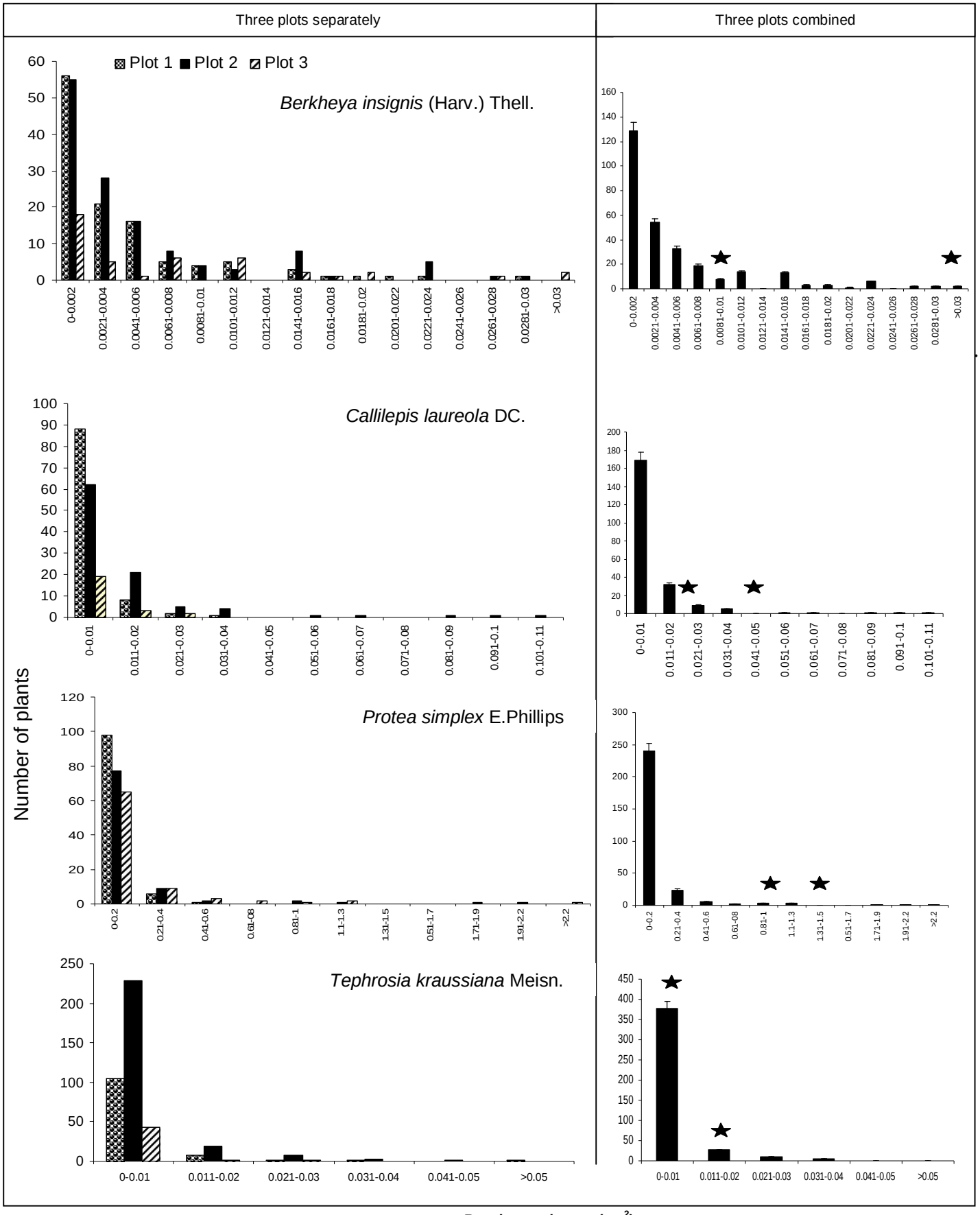


Figure 5.26. Distribution of the number of plants in each class for basal area for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions are constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE). ★ Indicates where the sizes of radiocarbon dated plants fall.

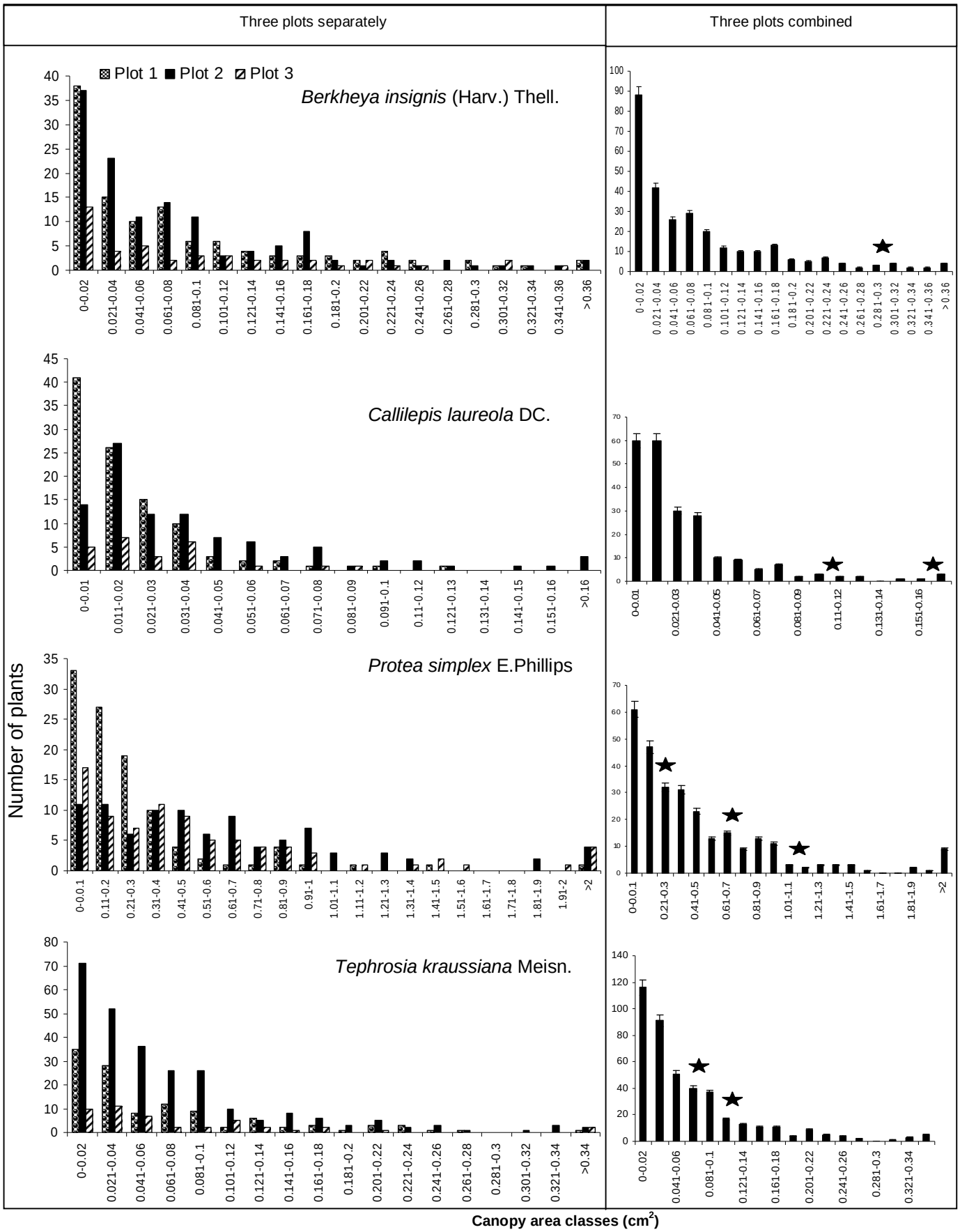


Figure 5.27. Distribution of the number of plants in each class for canopy area for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE).
★ Indicates where the sizes of radiocarbon dated plants fall.

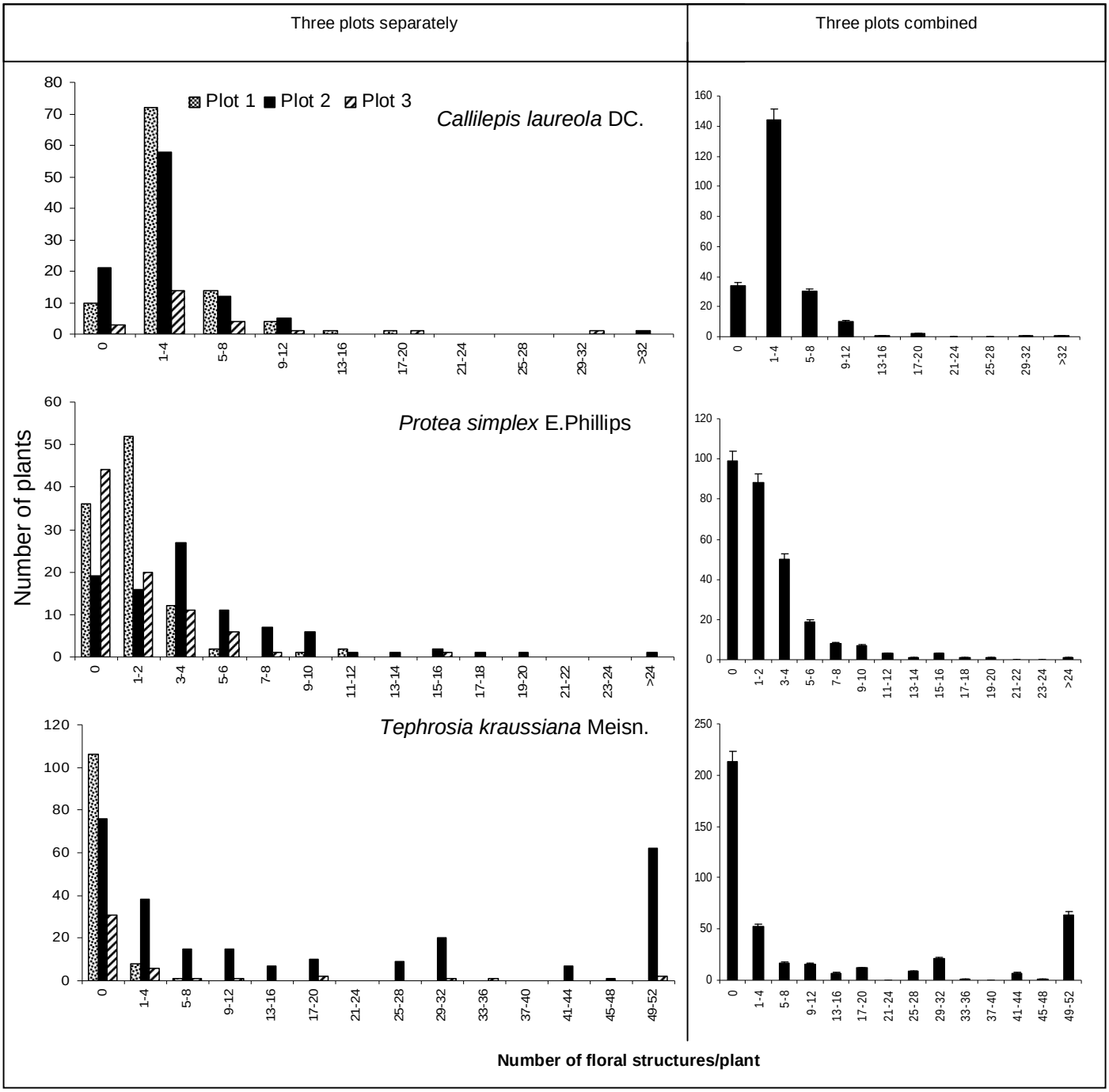


Figure 5.28. Distribution of the number of plants with the given number of floral structures in each class for *C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE). No floral structures were present on *B. insignis* plants.

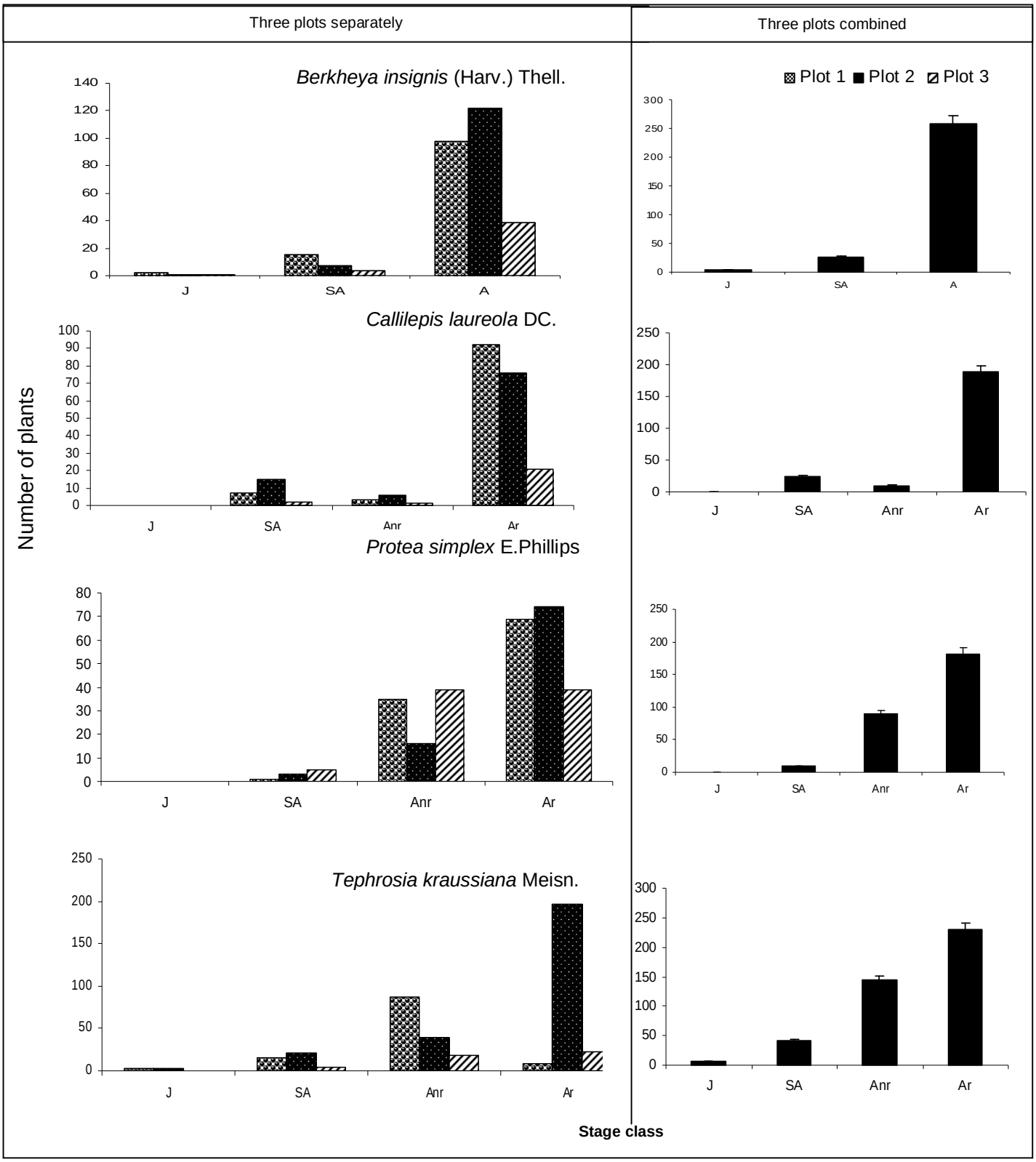


Figure 5.29. Distribution of the number of plants in each stage class for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Distributions were constructed for the 3 field plots separately and then for all 3 plots combined (mean ± SE). J: juvenile, SA: sub-adult, A: Adult, Anr: Adult (not reproducing), Ar: Adult (reproducing).

Table 5.25. Comparison of the number of inflorescences, height, canopy area, basal area, number of stems and main stem diameter between juvenile, adult and sub adult stage classes for field populations [one way ANOVA and Fishers Least significant difference (LSD)]. Means with the same superscript letter are not significantly different from each other. Means and standard deviations shown. Juveniles of *C. laureola* and *P.*

Species	Stage	Juvenile	Sub adult	Adult
Berkheya insignis	Number of inflorescences	0	0	0
	Height (cm)	42 ± 15^{b}	47 ± 7^{b}	55 ± 10^{a}
	Canopy area (cm^2)	248 ± 474^{ab}	130 ± 160^{b}	923 ± 957^{a}
	Basal area (cm^2)	23 ± 47^{b}	4 ± 4^{b}	54 ± 67^{a}
	Number of stems	1.5 ± 1.0^{b}	1.8 ± 0.8^{b}	4.8 ± 3.6^{a}
	Main stem diameter (mm)	2.1 ± 7.0^{b}	2.6 ± 0.7^{b}	3.5 ± 0.8^{a}
Callilepis laureola	Number of inflorescences	-	0^{b}	3.41 ± 4.97^{a}
	Height (cm)	-	28 ± 6^{b}	$43 \pm {}^{a}$
	Canopy area (cm^2)	-	138 ± 147^{b}	316 ± 361^{a}
	Basal area (cm^2)	-	17 ± 30^{b}	87 ± 147^{a}
	Number of stems	-	3.0 ± 2.2^{b}	7.1 ± 6.6^{a}
	Main stem diameter (mm)	-	1.4 ± 0.4^{b}	1.9 ± 0.4^{a}
Protea simplex	Number of inflorescences	-	0^{a}	2.58 ± 4.04^{a}
	Height (cm)	-	3 ± 10^{b}	55 ± 15^{a}
	Canopy area (cm^2)	-	880 ± 117^{b}	6482 ± 5934^{a}
	Basal area (cm^2)	-	200 ± 547^{b}	1383 ± 2913^{a}
	Number of stems	-	3.7 ± 4.6^{a}	20.9 ± 28.8^{a}
	Main stem diameter (mm)	-	4.3 ± 1.3^{a}	8.1 ± 28.6^{a}
	Number live flowers	-	0^{a}	0.32 ± 0.27^{a}
	Number of cones	-	0^{a}	1.36 ± 0.93^{a}
Tephrosia kraussiana	Number of flower bracts	0^{b}	0^{b}	13.77 ± 19.21^{a}
	Height (cm)	31 ± 8^{b}	36 ± 7^{b}	57 ± 12^{a}
	Canopy area (cm^2)	50 ± 54^{b}	81 ± 52^{b}	754 ± 777^{a}
	Basal area (cm^2)	1 ± 3^{c}	3 ± 7^{b}	49 ± 99^{a}
	Number of stems	1.5 ± 1.2^{b}	2.0 ± 1.3^{b}	10.0 ± 9.4^{a}
	Main stem diameter (mm)	1.0 ± 0.3^{b}	1.6 ± 0.5^{b}	2.5 ± 0.7^{a}

5.5. Habitat and environmental characteristics of *Berkheya insignis*, *Callilepis laureola*, *Protea simplex* and *Tephrosia kraussiana*

Soil from *B. insignis* plots had high levels of essential elements such as manganese (2.33 ± 0.58 mg/L) and total nitrogen (0.3 ± 0.0 %) (Table 5.26) suggesting the potential for higher biomass production for either growth or storage. However, both canopy and rhizome structures for this species was small compared to *P. simplex* which occurred in nutrient poor soil. Soil for this low altitude, shallow slope species was acidic (pH 4.76 ± 0.23) and contained low levels of total nitrogen (0.1 ± 0.0 %) and phosphorus (2.00 ± 0.00 mg/L). Soil texture was sandy with high coarse silt and sand composition thus promoting a lack of soil nutrient retention. *C. laureola* had the highest values for exchangeable phosphorus (13.33 ± 3.05

mg/L). However, the soil also had relatively high concentrations of zinc (1.77 ± 0.96 mg/L) which is a heavier metal suggesting evidence of nutrient leaching. Fishers LSD indicated that manganese was the only element with no significant difference across all four species.

Axis 1 of the principle components analysis (PCA) (Table 5.27) explained 84% of variation in the data. Axis 2 explained only 13%. In the PCA triplot (Figure 5.30) variables which were collinear or which were strongly correlated were removed. Zinc and acid saturation were strongly correlated with exchangeable acidity. Total nitrogen and total cations were strongly correlated with potassium. Manganese was positively correlated with potassium. *T. kraussiana* appeared to be the species most influenced by habitat characteristics. Potassium appeared to have the greatest influence on *T. kraussiana. B. insignis* and *P. simplex* were not as greatly influenced by the habitat variables. However, they were closely associated with copper and clay. *C. laureola* was influenced by exchangeable acidity and acid saturation by a small degree. Overall, the essential soil nutrients: potassium, total nitrogen, organic carbon, and phosphorus were the most important variables in the PCA.

Table 5.26. Soil physical and chemical composition and environmental characteristics (Mean ± SD) for *Berkheya insignis*, *Callilepis laureola*, *Protea simplex* and *Tephrosia kraussiana*. Means with the same superscript are not significantly different from each other (Fisher's least-significant-difference test (LSD), p<0.05, n=3).

	Berkheya insignis	*Callilepis laureola*	*Protea simplex*	*Tephrosia kraussiana*
Soil density (g/mL)	0.88 ± 0.03^{c}	1.07 ± 0.02^{b}	1.13 ± 0.04^{b}	1.30 ± 0.04^{a}
Exchangeable P (mg/L)	2.67 ± 0.58^{c}	13.33 ± 3.05^{a}	2.00 ± 0.00^{c}	8.00 ± 4.35^{b}
Available K (mg/L)	59.7 ± 7.0^{b}	66.7 ± 12.5^{b}	76.0 ± 30.3^{ab}	113.7 ± 25.7^{a}
Available Ca mg/L)	696.3 ± 153.5^{a}	334.3 ± 55.1^{b}	279.0 ± 85.0^{b}	580.6 ± 120.8^{a}
Available Mg (mg/L)	344.0 ± 38.5^{a}	141.3 ± 26.2^{bc}	109.7 ± 14.5^{c}	208.0 ± 60.7^{b}
Exchangeable acidity (cmol/L)	0.097 ± 0.031^{b}	1.397 ± 0.176^{a}	0.093 ± 0.051^{b}	0.383 ± 0259^{b}
Total cations (cmol/L)	6.45 ± 1.05^{a}	4.39 ± 0.26^{b}	2.58 ± 0.55^{c}	5.29 ± 0.89^{ab}
Acid saturation (%)	1.33 ± 0.58^{b}	32.33 ± 5.68^{a}	4.00 ± 2.65^{b}	7.67 ± 6.43^{b}
pH (KCl)	4.66 ± 0.08^{ab}	4.04 ± 0.03^{c}	4.76 ± 0.23^{a}	4.45 ± 0.21^{b}
Available Zn (mg/L)	1.97 ± 0.74^{a}	1.77 ± 0.96^{ab}	0.53 ± 0.25^{b}	1.57 ± 0.55^{ab}
Available Mn (mg/L)	2.33 ± 0.58^{a}	2.33 ± 0.59^{a}	1.67 ± 1.15^{a}	2.00 ± 1.00^{a}
Available Cu (mg/L)	3.77 ± 0.42^{a}	0.63 ± 0.25^{b}	0.30 ± 0.10^{b}	0.53 ± 0.06^{b}
MIR org. C (%)	3.70 ± 0.18^{b}	5.23 ± 0.35^{a}	1.43 ± 0.67^{c}	5.77 ± 1.07^{a}
Altitude (m)	379 ± 8^{a}	363 ± 17^{a}	84 ± 3^{c}	171 ± 5^{b}
Topographic position	Midslope - upper midslope	lower slope - hilltop	Midslope - hilltop	upper slope - hilltop
Slope angle	3.69 ± 1.67^{b}	3.25 ± 1.76^{b}	0.92 ± 0.85^{a}	2.54 ± 1.04^{ab}
Clay [% (<0.002 mm)]	51.3 ± 2.1^{a}	16.3 ± 1.5^{b}	9.0 ± 2.0^{c}	19.3 ± 4.2^{b}
Fine Silt [% (0.02 - 0.002 mm)]	13.7 ± 2.1^{a}	6.3 ± 0.6^{b}	1.7 ± 0.6^{c}	4.7 ± 0.6^{b}
Coarse Silt & Sand [% (0.02 - 2 mm)]	35.0 ± 1.0^{c}	77.3 ± 1.5^{b}	89.3 ± 2.1^{a}	76.0 ± 4.7^{b}
Texture Class	Clay	Sandy loam	Sandy	Sandy loam
Total N (%)	0..25 ± 0.0^{b}	0.21 ± 0.0^{c}	0.08 ± 0.0^{d}	0.31 ± 0.0^{a}

Table 5.27. Summary of axis values for principle components analysis.

Axes	1	2	3	4
Eigenvalues	0.841	0.132	0.022	0.005
Species-environment correlations	0	0	0	0
Cumulative percentage variance				
of species data	84.1	97.3	99.5	100
of species-environment relation	84.1	97.3	99.5	100

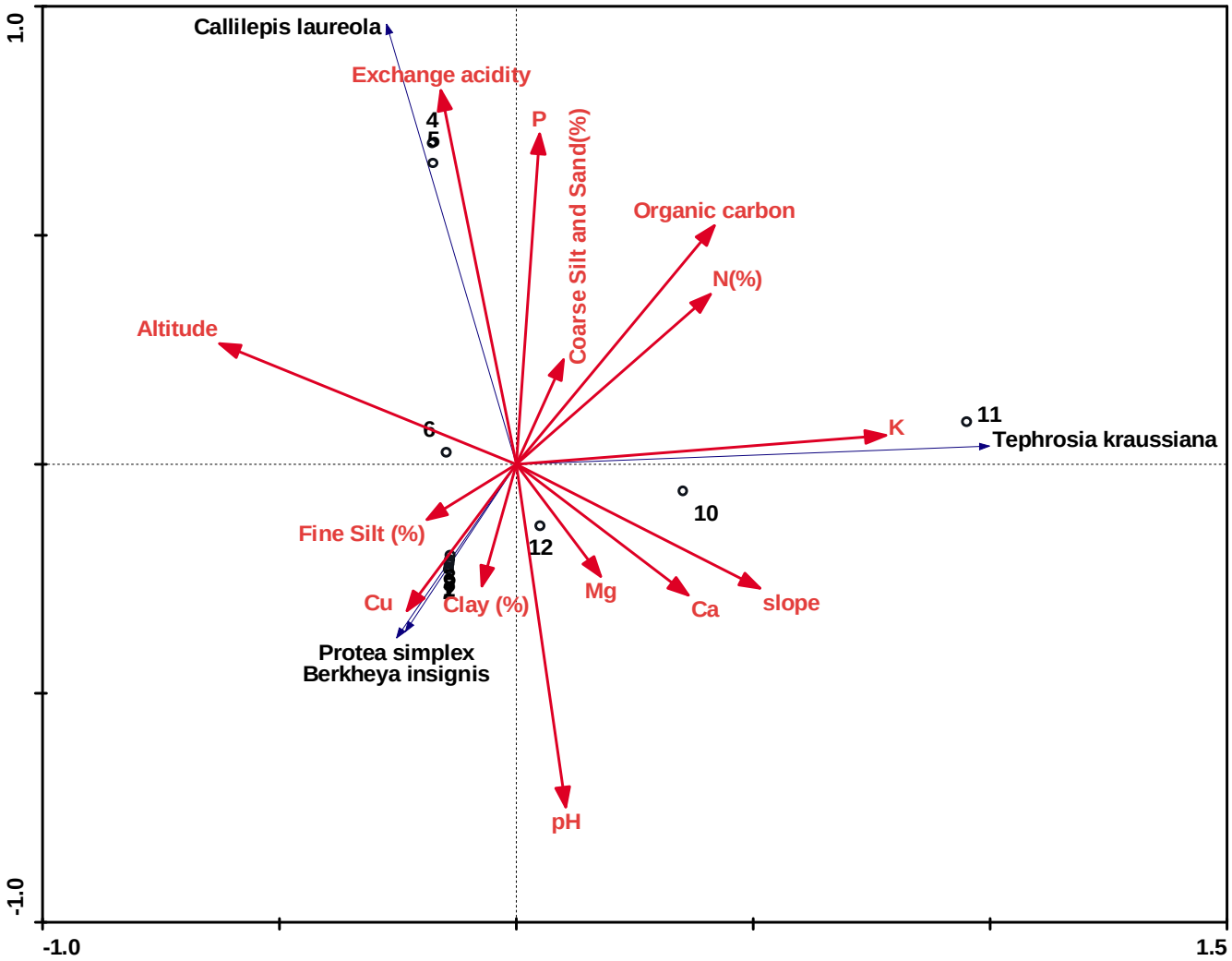


Figure 5.30. Triplot of the relationship between habitat variables, the four study species and the plots sampled using principle components analysis (PCA). Circles represent plots, narrow arrows represent species and bold arrows represent habitat variables.

5.6 Plant longevity: age and its relationship with plant structures

5.6.1 Longevity of *B. insignis*, *C. laureola*, *P. simplex* and *T. kraussiana*

All individuals which were radiocarbon dated had percentages of ^{14}C which fell within the modern carbon period (i.e. % modern carbon>100), therefore, the Bomb Carbon Curve (Figure 5.31) was used to determine the ages of these plants. However, since the concentration of ^{14}C on the Bomb Carbon curve increased rapidly during the 1950's and began a gradual decline after the nuclear test ban, ^{14}C concentrations usually coincide with two or sometimes three possible dates on both the ascending and descending slopes of the curve. Dates along the ascending slope of the curve are often reported close to the year 1950 as the C^{14} concentration increased swiftly during this period. Therefore the distinction between years is more difficult to determine as concentrations were increasing too rapidly. Therefore, both dates have been reported and analysed here as both results may be valid. However the legitimacy of results on the ascending and descending slopes is discussed where applicable. These results will also be an underestimate of plant age as the central ring (Figure 5.32) of the rhizome yielded insufficient material for carbon dating and material had to be extracted from adjacent (younger) rings as well.

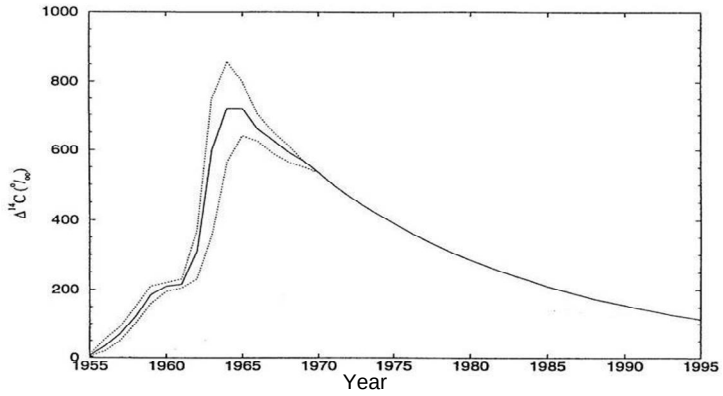


Figure 5.31. The bomb carbon curve used to determine the age of the radiocarbon dated plants.

According to the ascending slope of the bomb carbon curve, *T. kraussiana* rhizomes were the oldest (AD 1957 ± 1 yr; i.e. AD2008 minus AD1957=51 years old) (Table 5.28). However, according to the descending slope these individuals were only between 13 and 16 years old. *P. simplex* plants were between 45 and 49 years old on the ascending slope and between 16 and 29 years on the descending slope. *C. laureola* rhizomes were between 49 and 50 years old on the ascending slope and 12 and 21 years on the descending slope. *B. insignis* plants were between 49 and 51 years old on the ascending slope and 10 years on the descending slope.

a.Berkheya insignis	
b.Callilepis laureola	
c.Protea simplex	
d.Tephrosia kraussiana	

Figure 5.32. Cross-sections of a) *Berkheya insignis*, b) *Callilepis laureola*, c) *Protea simplex* and d) *Tephrosia kraussiana* rhizomes. One block on the tape in the photograph represents 1cm.

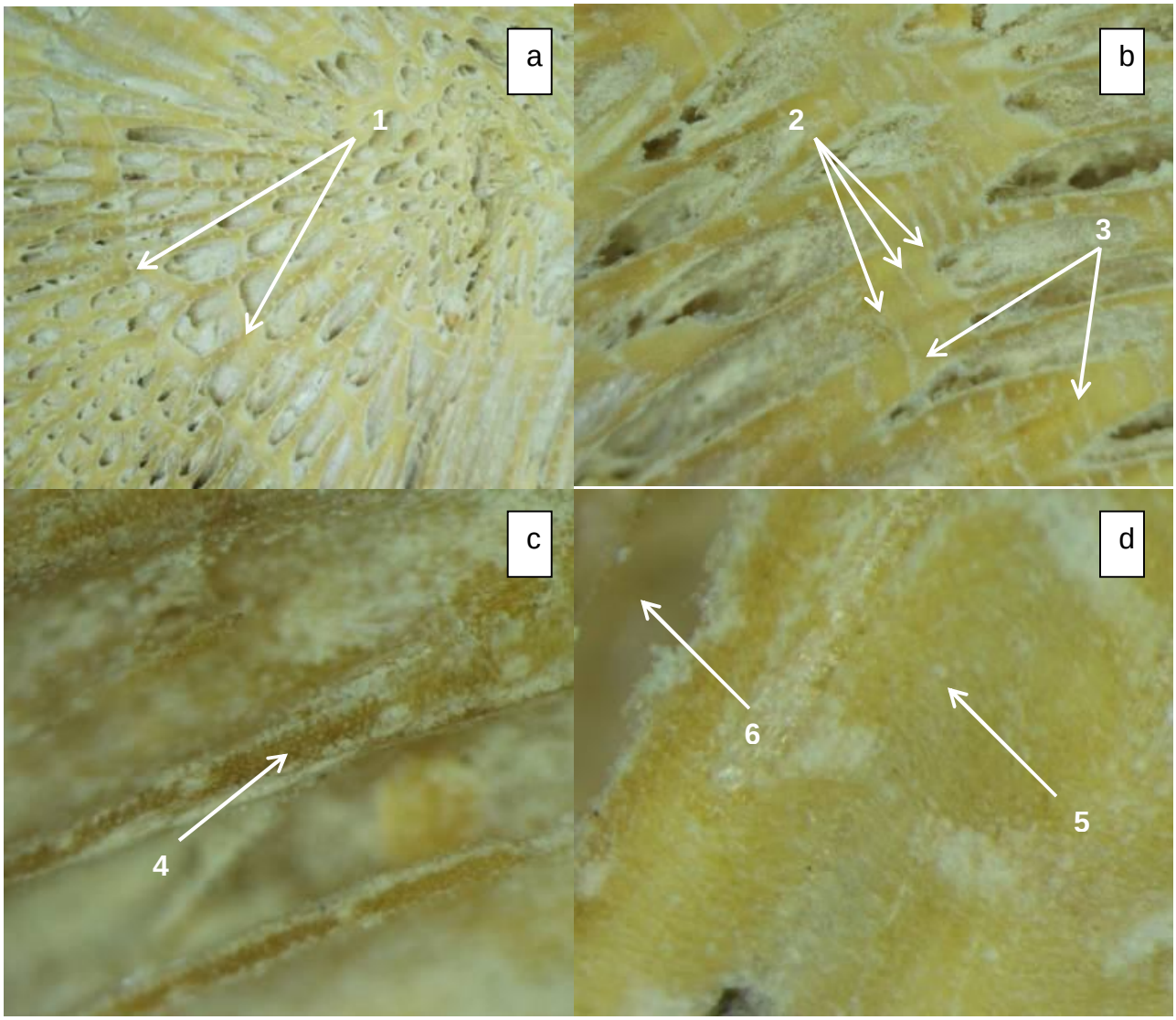


Figure 5.33. Cross-section of *Berkheya insignis* rhizomes using a stereomicroscope under a) low magnification (7x) with rays visible (1), b) medium magnification (50x) with light bands of parenchyma (2) and rays (3) visible, c) high power magnification (100X) of rays (4) and d) high magnification of a ray (100X) with very small vessels in fiber tissue (5) visible. Smaller vessels are sometimes indicative of climate pressures. Plants in drier areas often have smaller vessels for water conduction (Barajas-Morales 1985). *B. insignis* rhizomes are composed of hard and soft tissues which form grooves (6) after desiccation. The lack of annual ring structure is evident.

The dates on the descending slope are generally considered a better approximation of age as the more gradual slope allows for a greater differentiation between concentrations of the ^{14}C isotope (Dr. Stephan Woodborne 2010, pers. comm.). Since older ages are often considered spurious, opting for these younger ages would be a more conservative approach for the study. However, older ages could not be discounted.

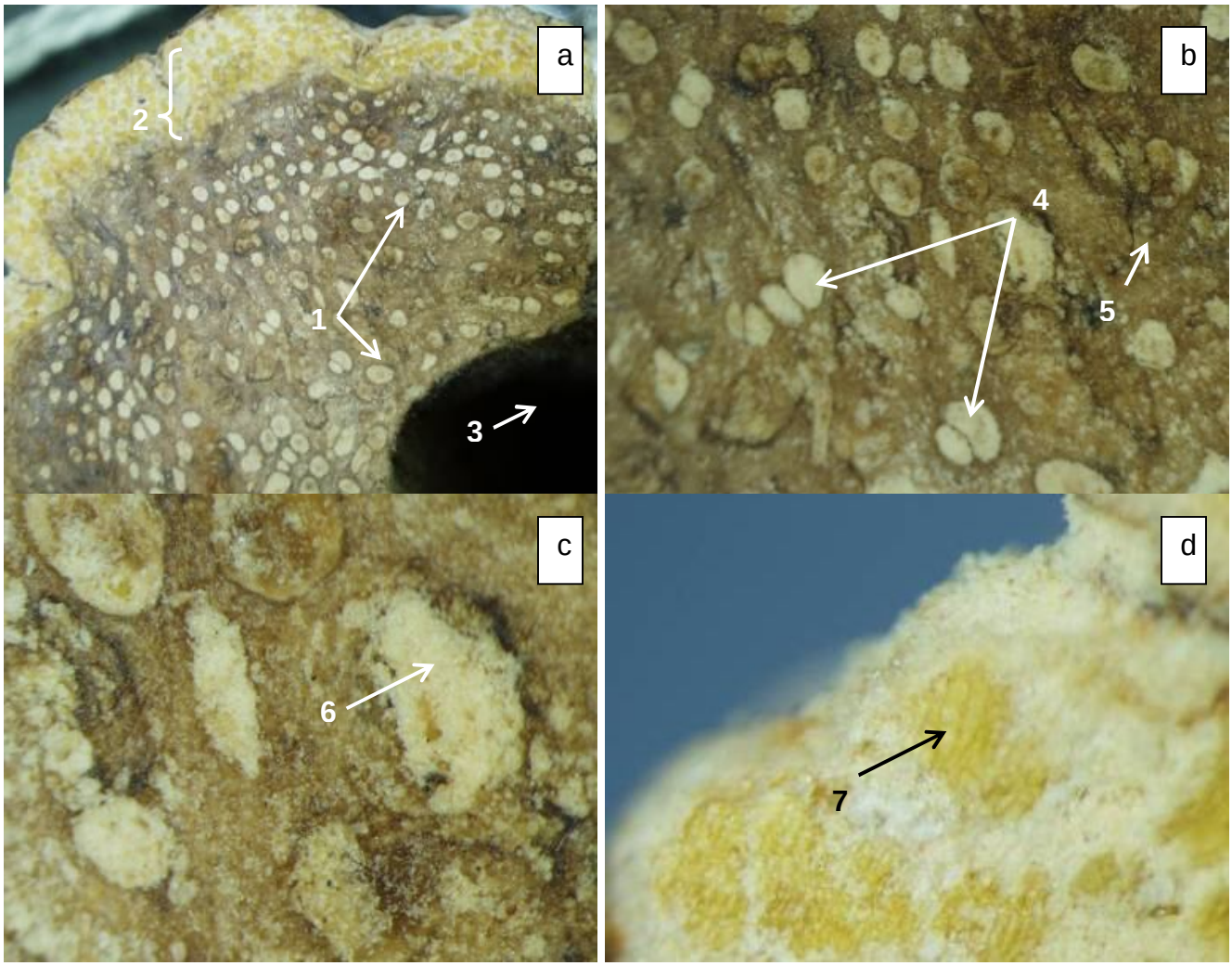


Figure 5.34. Cross-section of *Callilepis laureola* rhizomes using a stereomicroscope under a) low power (7x) with numerous starch bodies (1) visible throughout the rhizome. The yellow, relatively thick bark or cortex layer is also clearly visible (2). A hollow center (3) represents the area from which tissues were excised for radiocarbon dating. Under medium power (b) (50X) the close association of starch bodies (4) and the lack of any obvious rays (5) is evident. Starch bodies under d) high magnification (100x) are shown in (c) with the granular texture of the starch within these bodies visible (6). The apparent presence of parenchyma between starch in the cortex is also visible (7). The lack of annual ring structure is evident.

Therefore, interpretation of the results was not a straightforward process. Microscopic analyses of the rhizomes (for annual growth rings) revealed a lack of annual ring structure even at a cellular level (Figures 5.33, 5.34, 5.35 and 5.36). Due to the 'perplexity' surrounding the precision of each set of ages, analyses were carried out on ages along both the ascending and descending slopes. While not representative of age, percentage modern carbon was also analysed as it represented the concentration of carbon 14 in the atmosphere.

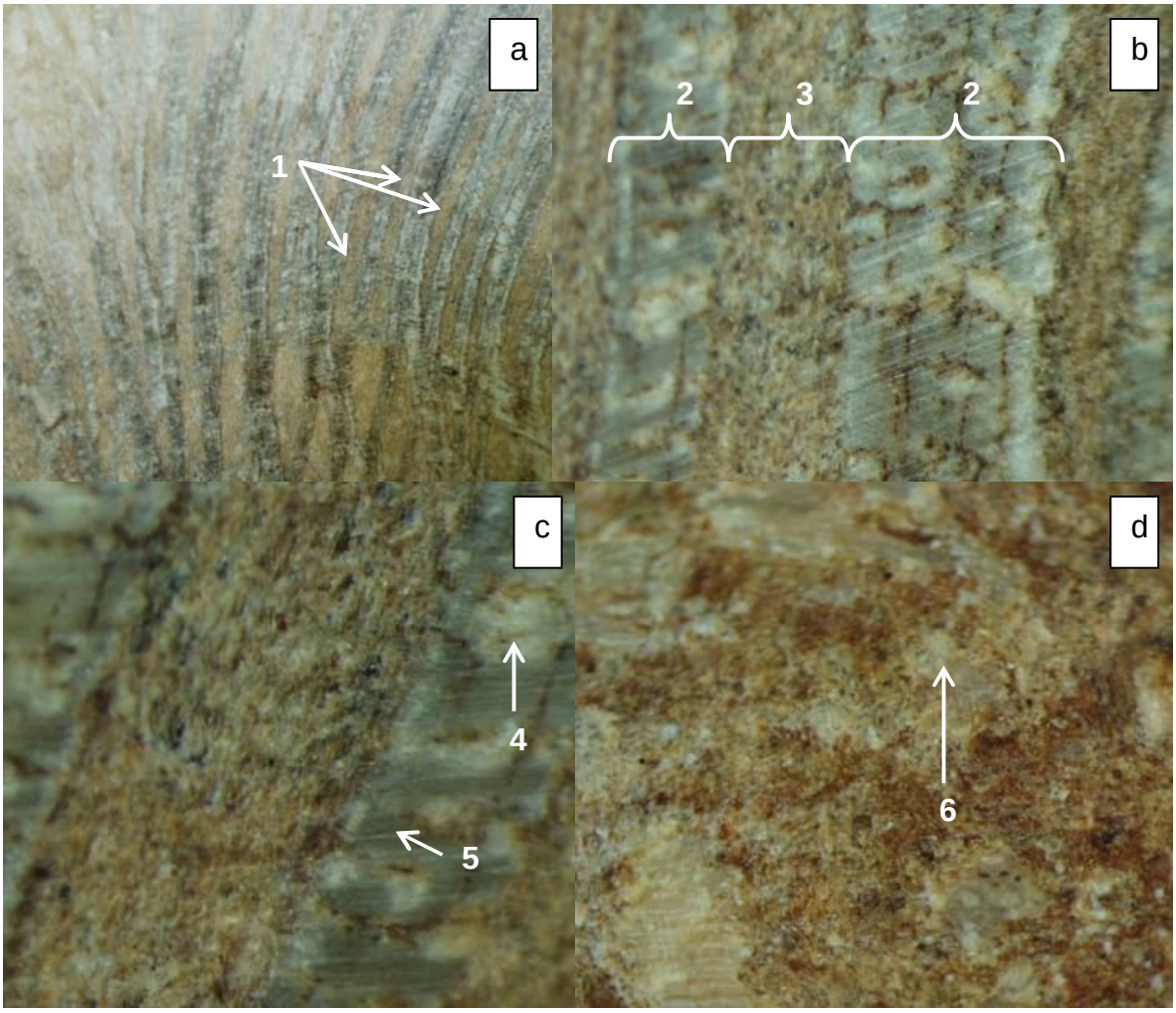


Figure 5.35. Cross-section of *Protea simplex* rhizomes using a stereomicroscope under a) low (7x) magnification showing the arrangement of large, thick rays (1); under b) medium magnification (50x) with bands of rays (3) and vessels embedded in fibrous tissue (2) clearly visible. (c) Under high magnification (100x) light colored vessels (4) become visible in between darker fibers (5) which form ground tissue. d) Vessels are also visible (6) in the bark tissue under high magnification. The lack of annual ring structure is evident.

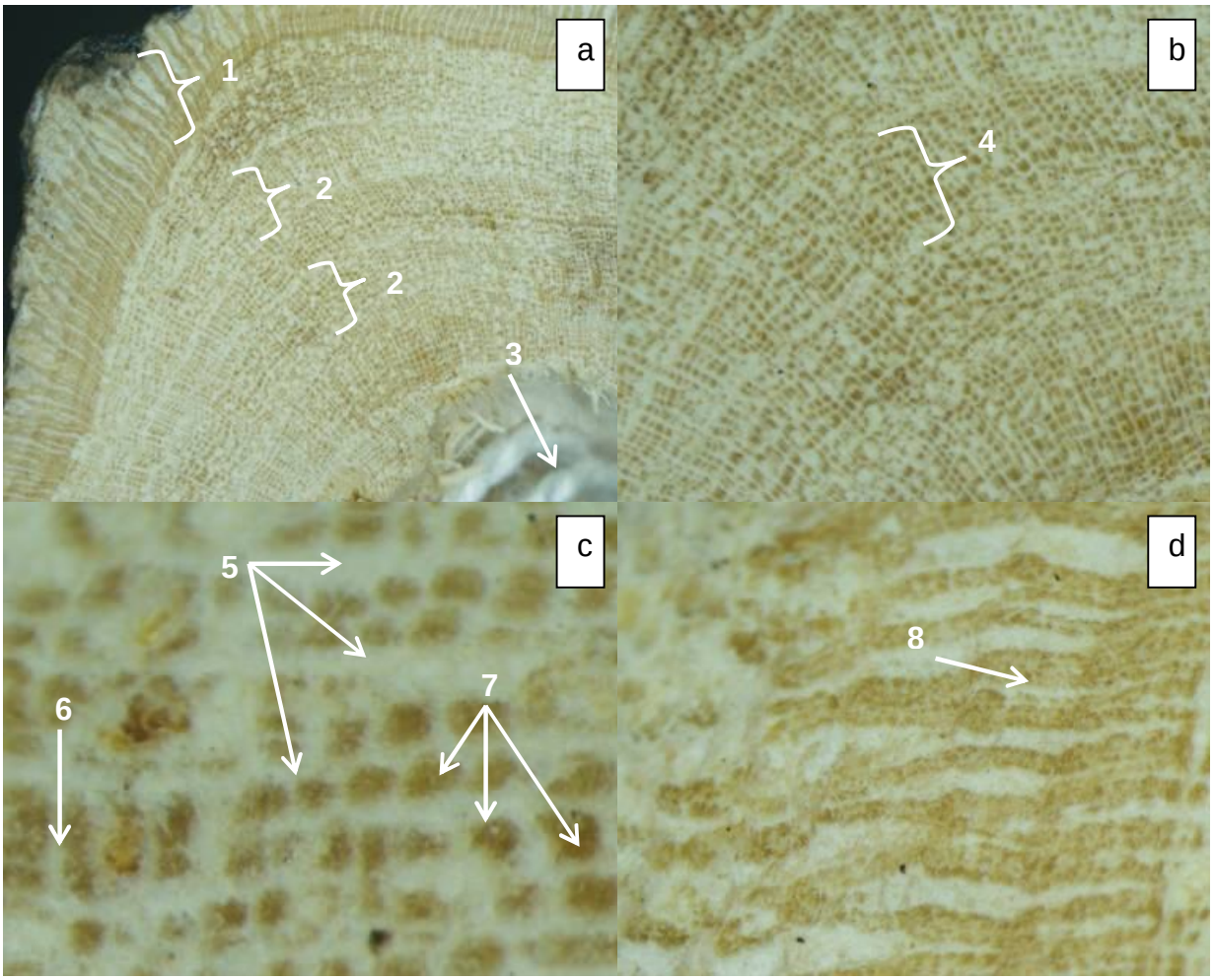


Figure 5.36. Cross-section of *Tephrosia kraussiana* rhizomes using a stereomicroscope under a) low magnification (7x) with the outer cortex (1) visible. Bands of growth rings (2) are also visible. However, these growth rings are unlikely to be annual rings as the numbers of rings are fewer than the youngest age obtained from radiocarbon dating. The hollow centre (3) from which tissue was excised for radiocarbon dating is also visible. Under b) medium magnification (50x) the non-annual bands (4) are more clearly visible. c) Light-coloured, horizontal banded parenchyma (5) is visible under high magnification (100x). Light vertical bands represent rays (6). Dark patches of fibres and small vessels (7) are also visible. d) Under high magnification (100x), the presence secondary parenchyma (8) was evident.

Table 5.28. The age AD (as per the ascending and descending slope of the bomb carbon curve), laboratory number, percentage modern carbon, $\delta_{13}C$ and the age of radiocarbon dated plants in the year they were excavated (i.e. AD 2008).

Laboratory #	Plant number	% Modern carbon	Age AD	$\delta_{13}C$	Plant Age (at AD 2008)
Pta-9809	*P. simplex* 8	116.30 ± 0.37	AD 1959 or AD 1992 ± 1 yr	-26.16‰	49 or 16
Pta-9808	*P. simplex* 2a	125.00 ± 0.59	AD 1959, 1962 or AD 1981 ± 1 yr	-27.15‰	49, 46 or 27
Pta-9806	*P. simplex* 2b	120.62 ± 0.67	AD 1961 or AD 1984 ± 1 yr	-27.78‰	47 or 24
Pta-9802	*P. simplex* 3	129.99 ± 0.62	AD 1963 or AD 1979 ± 1 yr	-27.82‰	45 or 29
Pta-9805	*B. insignis* 8	104.70 ± 0.59	AD 1957 ± 1 yr	-29.18‰	51
Pta-9811	*B. insignis* 10	109.16 ± 0.71	AD 1958 or AD 1998 ± 1 yr	-29.66‰	50 or 10
Pta-9803	*B. insignis* 4	105.16 ± 0.58	AD 1959 ± 1 yr	-28.96‰	49
Pta-9807	*T. kraussiana* 10	113.96 ± 1.43	AD 1957 or AD 1992 ± 1 yr	-28.78‰	51 or 16
Pta-9810	*T. kraussiana* 12	114.20 ± 0.66	AD 1957 or AD 1993 ± 1 yr	-29.36‰	51 or 15
Pta-9804	*T. kraussiana* 18	112.67 ± 0.49	AD 1958 or AD 1995 ± 1 yr	-29.38‰	50 or 13
GrA-46079	*C. laureola* 15	118.13 ± 0.47	AD 1958/9 or AD 1987 ± 1yr	-30.57‰	50, 49 or 21
GrA-46078	*C. laureola* 1	113.50 ± 0.47	AD 1958 or AD 1993 ± 1yr	-30.80‰	50 or 15
GrA-46077	*C. laureola* 14	111.72 ± 0.45	AD 1958 or AD 1996 ± 1 yr	-29.87‰	50 or 12

5.6.2 The relationship between longevity and the sizes of rhizomes and aerial structures

Given the low samples sizes due to the cost of carbon dating, the results from following regressions should be considered preliminary and explorative. There were no significant relationships between rhizome mass or circumference with plant age according to the ascending or descending slopes for *P. simplex, T. kraussiana, B. insignis* and *C. laureola* (Figure 5.37). However, while not statistically significant, there may be an allometric relationship between rhizome mass and plant age along the descending slope (r^2=0.84, p=0.28) in *P. simplex*. There may also be an allometric relationship between rhizome mass and age along the descending slope (r^2=0.89, p=0.15) in *C. laureola*. There was also a comparatively weaker relationship between rhizome mass and age on the ascending slope (r^2=0.55, p=0.32) in *B. insignis*. No relationships were apparent in *T. kraussiana*. There also appeared to be positive relationships between rhizome circumference and age in *B. insignis* (r^2=0.34, p=0.6), *C. laureola* (r^2=0.45, p=0.53) and *P. simplex* (r^2=0.75, p=0.13)

Given the low number of samples (due to costs and lack of sufficient sample mass), the results were also analysed by pooling across species, despite the inherent problems in doing so. There was no significant relationship between the age of carbon dated plants (all species combined) according to the descending slope (AD 2008) and the following plant measurements: rhizome mass (r^2< 0.001, p= 0.84), rhizome circumference (r^2=0.01, p= 0.75), height (r^2< 0.001, p= 0.88), canopy area (r^2< 0.001, p= 0.83), basal area (r^2< 0.001, p= 0.96), number of stems

(r^2= 0.04, p= 0.51) and main stem diameter (r^2< 0.001, p= 0.99) (Table 5.29). However, there was a significant relationship between the percentage of modern carbon in rhizomes and the following: rhizome mass (r^2= 0.69, p< 0.001), rhizome circumference (r^2= 0.66, p< 0.001), canopy area (r^2= 0.53, p< 0.001), basal area (r^2= 0.52, p= 0.01), number of stems (r^2= 0.31, p= 0.05) and main stem diameter (r^2= 0.58, p< 0.001). There was no relationship between the percentage of modern carbon and height (r^2= 0.19, p= 0.14). Rhizome mass, rhizome circumference, canopy area, basal area, number of stems and main stem diameter had a positive relationship with the percentage of modern carbon (Figure 5.38). Rhizome mass and circumference shared the most significant relationships with percentage modern carbon. There were no relationships between the age along the descending slope and all the size structure variables (all p>0.05) (Table 5.29). There was a significant relationship between age on the ascending slope and rhizome mass (r^2=0.83, p<0.001), rhizome circumference (r^2=0.85, p<0.001), canopy area (r^2=0.67, p<0.001,), basal area (r^2=0.58, p<0.001,) and main stem diameter (r^2=0.65, p<0.001). However, since all these relationships were inverse and thus contrary to our assumption that older plants have larger sizes (irrespective of the structure), and coupled with the prevailing notion that ages along this slope are generally unreliable and should be treated with caution, these relationships are tentative at best. Different combinations of ages from ascending and descending slopes for each species were also analysed, however, there were still no significant relationships. While none of the age combinations had a convincing relationship with any of the plant structures, the positive relationship between the size of plant structures and the percentage of modern carbon suggests that there is an allometric relationship between the percentage of sequestered carbon 14 and size. However, more data is necessary.

Only the basal area of *C. laureola* had a significant relationship with age along the descending slope (r^2= 0.999, p= 0.01) and percentage modern carbon (r^2= 0.998, p= 0.03) when the relationship between structure size and age was tested in each species (Table 5.30). The height and number of stems of *T. kraussiana* had significant relationships with the ascending slope (r^2= 0.99, p= 0.05) and percentage modern carbon (r^2= 0.99, p= 0.05) and age along the descending slope (r^2= 0.996, p= 0.04), respectively. The main stem diameter of *B. insignis* had a significant relationship with age along the descending slope (r^2= 0.99, p= 0.03). . However, all these relationships- except the relationship between height and age along the ascending slope and % modern carbon- were negative.

For all species combined, there was a significant positive relationship between rhizome mass and the sizes of all aerial plant structures: height (r^2= 0.4114, p= 0.0181), canopy area (r^2= 0.8792, p< 0.0001), basal area (r^2= 0.8606, p< 0.0001), number of stems (r^2= 0.3632, p= 0.0293) and main stem diameter (r^2= 0.9280, p< 0.0001) (Figure 5.39). Furthermore, rhizome mass had a significant relationship with % modern carbon, which is related to age. Therefore, plants with higher % modern carbon may be larger and older. There were also allometric relationships between age and rhizome mass when *P. simplex, C. laureola* and *T. kraussiana* were analysed separately. Therefore, the links in these relationships suggest a positive relationship between plant age, rhizome mass, height, canopy and basal area, number of stems and main stem diameter. Unfortunately, these relationships were not sufficiently convincing to establish surrogates for age based on the sizes of plant aerial structures.

Although the data gathered was insufficient to make a final decision on whether the ages along the ascending or descending slope of the bomb carbon curve more precisely reflected the age of plants, an informed and logical deduction is presented based on the information at hand. A Palaeobotanist familiar with the growth patterns in rhizomes of older plants suggested that the higher density and greater woodiness of the plant tissue samples in this study indicated that plants were older than ages corresponding to both the ascending and descending axes of the bomb carbon curve (Dr. Marion Bamford 2010, pers. comm.). Furthermore, for *B. insignis*, plants 8 and 4 had a single date along the ascending slope of the bomb carbon curve (i.e. AD 1957 and 1959), and the sizes of these two plants were similar to plant 10 (AD 1958 or 1998). It is unlikely that these similar sized plants would have a 40 year difference in age. Therefore, the ages along the ascending slope of the bomb carbon curve may be more precise. The ascending slope may also be more precise for *T. kraussiana* as it is unlikely that plant 10 was older than plant 12 since the rhizome mass for plant 12 was 1.7 times greater than plant 10.

Furthermore, a sample of a large *Dioscorea* was also dated along with the samples used in this study. This species was found to be 46 or 31 years old according to the radiocarbon dating technique. However, subsequent analysis of individuals of the same species using scars on stems and counting rings [both methods endorsed by an expert, Dr. Paul Wilkin (D. Raimondo 2010, pers. comm.)] estimated the age of the carbon dated plant to be >300 years old. Hence, for this study, the older ages were considered as more accurate and even these ages may be a gross underestimate of the true ages of these plants.

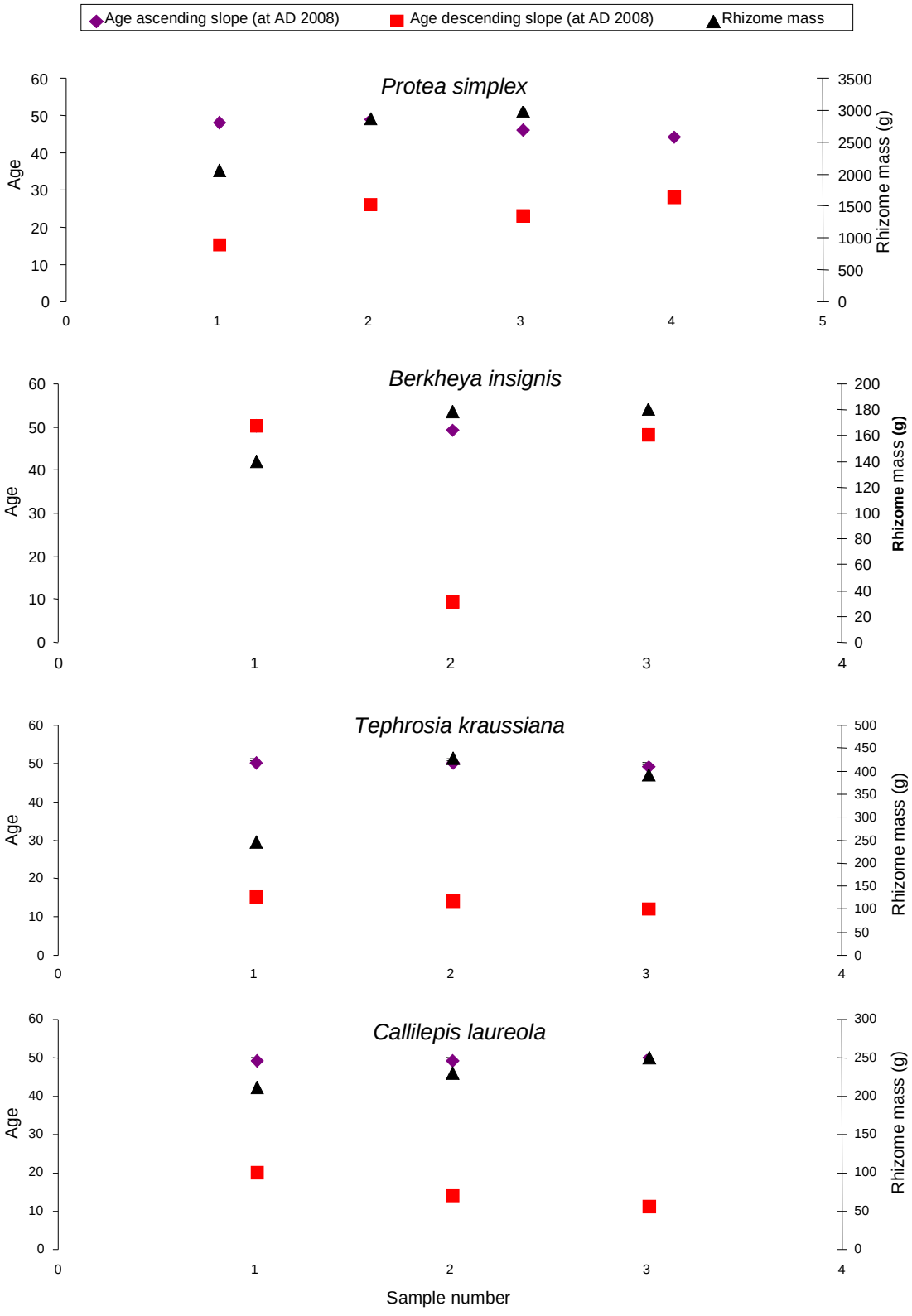


Figure 5.37. Comparison between rhizome mass (right axis) and the age of each radiocarbon dated plant (for *P.simplex, B. insignis, T. kraussiana* and *C. laureola*) at AD 2008 according to the ascending and descending slopes (left axis) of the bomb carbon curve ($p<0.05$).

Table 5.29. The relationship (linear regression analysis) between i) the Age along the ascending slope and ii) descending slope of carbon dated plants and percentage modern carbon and rhizome mass, rhizome circumference, height, canopy area, basal area, number of stems, and main stem diameter (p<0.05)

		Rhizome mass	Rhizome circumference	Height	Canopy area	Basal area	Number of stems	Main stem diameter
i) Age ascending slope (AD 2008)	r^2	0.83	0.85	0.17	0.67	0.58	0.22	0.65
	df	1,11	1,11	1,11	1,11	1,11	1,11	1,11
	F	53.28	61.34	2.30	22.39	15.18	3.15	20.86
	p	<0.001	<0.001	0.16	<0.001	<0.001	0.10	<0.001
ii) Age descending slope (AD 2008)	r^2	<0.001	0.01	<0.001	<0.001	<0.001	0.04	<0.001
	df	1,11	1,11	1,11	1,11	1,11	1,11	1,11
	F	0.04	0.11	0.03	0.05	<0.001	0.46	<0.001
	p	0.84	0.75	0.88	0.83	0.96	0.51	0.99
iii) % Modern carbon	r^2	0.69	0.66	0.19	0.53	0.52	0.31	0.58
	df	1,11	1,11	1,11	1,11	1,11	1,11	1,11
	F	24.77	21.45	2.58	12.35	11.90	4.97	15.36
	p	<0.001	<0.001	0.14	<0.001	0.01	0.05	<0.001

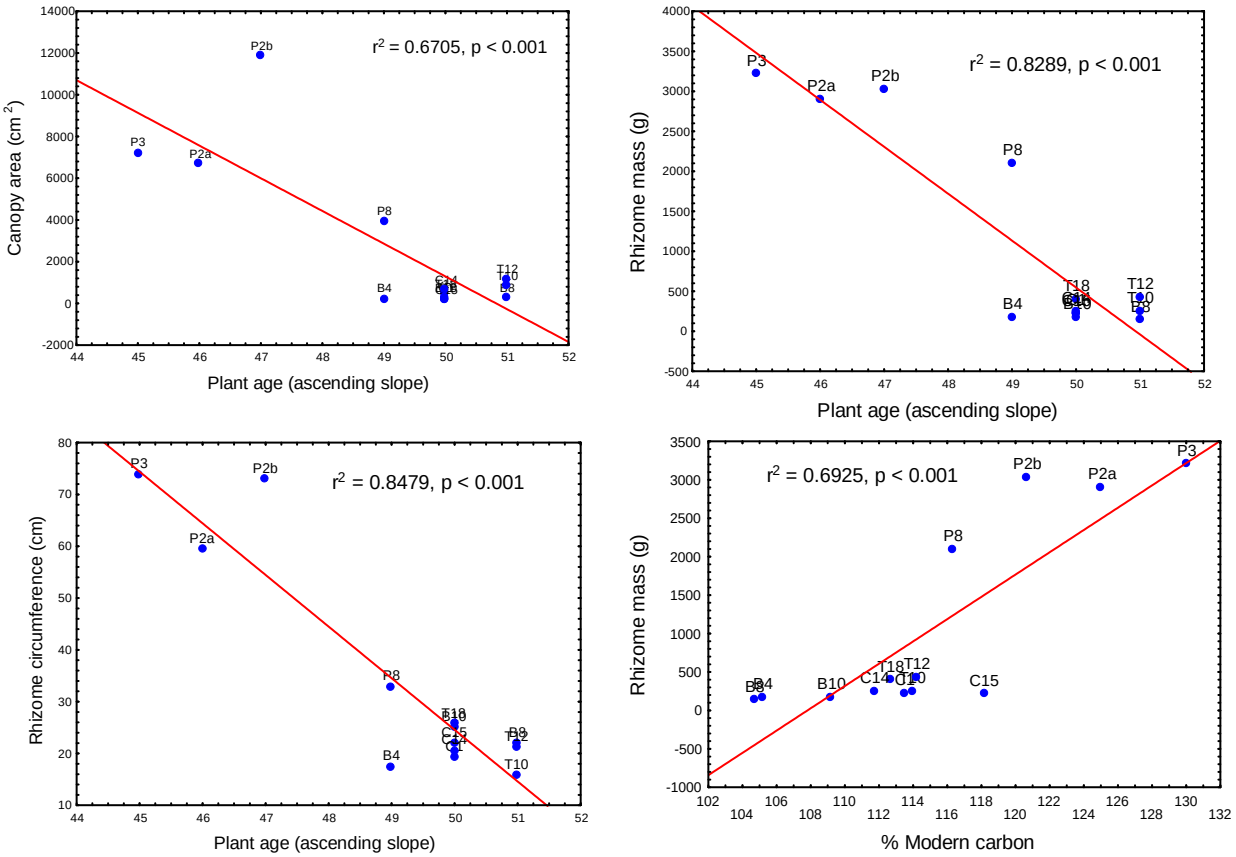


Figure 5.38. Significant linear regression relationships between the percentage of modern carbon and rhizome mass and the relationships between plant age (ascending slope) and rhizome mass, circumference and canopy area (p<0.05).

Table 5.30. The relationship (using simple linear regressions) between i) the age (at AD 2008), ii) the percentage modern carbon (AD 2008) of carbon dated plants and rhizome mass, rhizome circumference, height, canopy area, basal area, number of stems, and main stem diameter for each species ($p<0.05$).

	Ascending slope				Descending slope				% Modern carbon			
	r^2	df	F	p	r^2	df	F	p	r^2	df	F	p
B. insignis												
Rhizome mass	0.77	1,2	3.41	0.32	0.26	1,2	0.36	0.66	0.31	1,2	0.45	0.62
Rhizome circumference	0.34	1,2	0.51	0.60	0.62	1,2	1.63	0.42	0.57	1,2	1.33	0.45
Height	0.96	1,2	27.00	0.12	0.05	1,2	0.06	0.85	0.08	1,2	0.09	0.82
Canopy area	0.52	1,2	1.08	0.49	0.52	1,2	1.10	0.48	0.57	1,2	1.35	0.45
Basal area	0.87	1,2	6.43	0.24	0.17	1,2	0.20	0.73	0.20	1,2	0.26	0.70
Number of stems	0.36	1,2	0.55	0.59	0.60	1,2	1.52	0.43	0.55	1,2	1.24	0.47
Main stem diameter	>0.001	1,2	>0.001	1.00	0.99	1,2	533.33	0.03	0.99	1,2	112.75	0.06
C. laureola												
Rhizome mass	0.86	1,1	6.40	0.24	0.95	1,2	17.74	0.15	0.92	1,2	10.89	0.19
Rhizome circumference	0.59	1,1	1.45	0.44	0.45	1,2	0.82	0.53	0.51	1,2	1.04	0.49
Height	0.28	1,1	0.39	0.64	0.16	1,2	0.20	0.73	0.21	1,2	0.27	0.70
Canopy area	0.58	1,1	1.41	0.45	0.72	1,2	2.58	0.35	0.66	1,2	1.98	0.39
Basal area	0.98	1,1	58.39	0.08	0.999	1,2	5720.33	0.01	0.998	1,2	434.66	0.03
Number of stems	0.86	1,1	6.26	0.24	0.75	1,2	3.00	0.33	0.80	1,2	4.02	0.29
Main stem diameter	0.96	1,1	27.00	0.12	0.89	1,2	8.33	0.21	0.93	1,2	12.82	0.17
P. simplex												
Rhizome mass	0.85	1,2	11.09	0.08	0.90	1,2	17.28	0.05	0.70	1,2	4.62	0.16
Rhizome circumference	0.70	1,2	4.58	0.17	0.75	1,2	6.16	0.13	0.54	1,2	2.33	0.27
Height	>0.001	1,2	0.00	0.97	0.01	1,2	0.02	0.90	0.05	1,2	0.10	0.78
Canopy area	0.12	1,2	0.27	0.66	0.19	1,2	0.47	0.56	0.03	1,2	0.06	0.83
Basal area	0.34	1,2	1.03	0.42	0.46	1,2	1.71	0.32	0.19	1,2	0.46	0.57
Number of stems	0.21	1,2	0.52	0.55	0.13	1,2	0.30	0.64	0.36	1,2	1.12	0.40
Main stem diameter	0.06	1,2	0.14	0.75	0.01	1,2	0.03	0.88	0.19	1,2	0.47	0.56
T. kraussiana												
Rhizome mass	0.11	1,1	0.13	0.78	0.39	1,2	0.64	0.57	0.04	1,2	0.04	0.88
Rhizome circumference	0.70	1,1	2.30	0.37	0.94	1,2	15.47	0.16	0.56	1,2	1.25	0.46
Height	0.99	1,1	176.33	0.05	0.84	1,2	5.33	0.26	0.99	1,2	196.65	0.05
Canopy area	0.77	1,1	3.30	0.32	0.45	1,2	0.81	0.53	0.88	1,2	7.20	0.23
Basal area	0.21	1,1	0.27	0.69	0.02	1,2	0.02	0.91	0.34	1,2	0.53	0.60
Number of stems	0.93	1,1	13.37	0.17	0.996	1,2	225.33	0.04	0.84	1,2	5.20	0.26
Main stem diameter	0.25	1,1	0.33	0.67	0.04	1,2	0.04	0.88	0.39	1,2	0.63	0.57

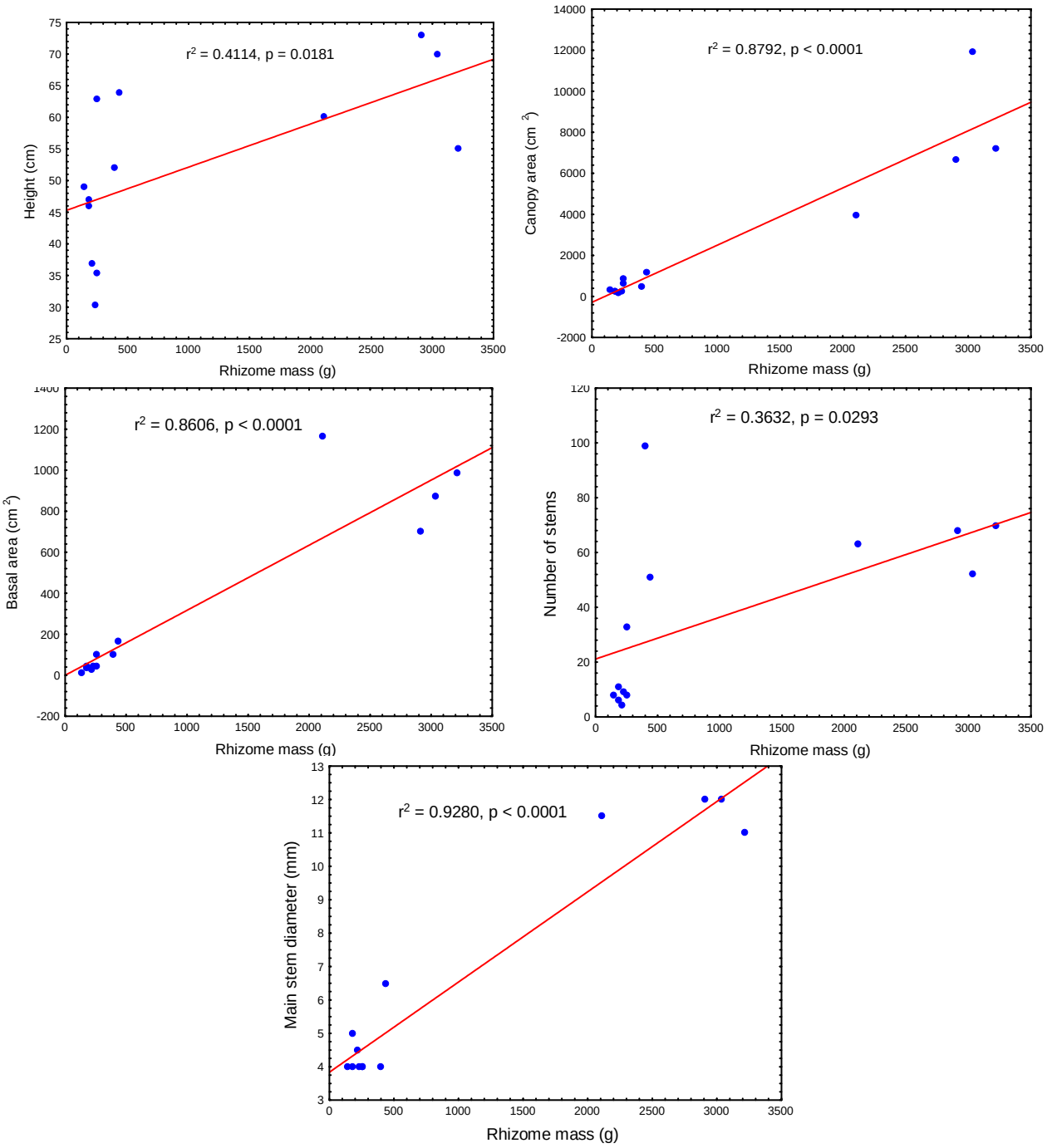


Figure 5.39. Significant relationships (linear regressions) between rhizome mass and height, canopy area, basal area, number of stems and main stem diameter (n=12, p<0.05).

5.6.3 Estimation of the age of field populations

Although a precise measure of the age of wild populations was not possible, an estimation of the ages was attempted by comparing the sizes of wild plant canopy structures with radiocarbon dated canopy structures and then relating these sizes with age (Table 5.31).

Field populations of *B. insignis* had mean heights and canopy areas which were significantly larger than the mean measurements of plants which were radiocarbon dated (height: 43.2 ± 7.5cm, canopy area: 242 ± 547 cm^2, main stem diameter: 4.6 ± 2.2 mm) (Table 5.31). Field populations of *C. laureola* had smaller main stem diameters compared to radiocarbon dated plants, however, the means for height, canopy area, basal area and the number of stems was larger in the field populations suggesting that these plants may be older than the ages obtained for radiocarbon dated plants in this study.

Table 5.31. Comparisons (mean ± standard deviation) between the field populations (sampled from Umtamvuna and Red Desert nature reserves: 2009) and the carbon dated population (sampled from Ipithi Retreat: 2008) [one way ANOVA and Fishers Least significant difference (LSD)]. Means with the same superscript are not significantly different from each other (LSD, $p<0.05$).

	Field populations			Carbon dated Population
Plot	1	2	3	
Berkheya insignis	(n=115)	(n=130)	(n=44)	(n=23)
Height (cm)	59.5 ± 11.5^{a}	51.3 ± 7.7^{b}	50.0 ± 9.9^{b}	43.2 ± 7.5^{c}
Canopy area (cm^2)	827 ± 943^{a}	813 ± 936^{a}	968 ± 949^{a}	242 ± 547^{b}
Basal area (cm^2)	40 ± 53^{bc}	50 ± 63^{b}	75 ± 91^{a}	19 ± 35^{c}
Number of stems	3.8 ± 2.9^{b}	4.9 ± 3.8^{a}	5.1 ± 3.8^{a}	5.0 ± 3.1^{ab}
Main stem diameter (mm)	3.8 ± 1.0^{c}	3.2 ± 0.7^{b}	3.4 ± 0.8^{b}	4.6 ± 2.2^{a}
Callilepis laureola	(n=102)	(n=97)	(n=24)	(n=23)
Height (cm)	41.6 ± 7.7^{a}	43.7 ± 9.1^{a}	33.3 ± 7.8^{b}	28.9 ± 9.1^{b}
Canopy area (cm^2)	190 ± 201^{b}	414 ± 450^{a}	275 ± 206^{ab}	168 ± 142^{b}
Basal area (cm^2)	44 ± 75^{b}	120 ± 190^{a}	65 ± 72^{ab}	22 ± 19^{b}
Number of stems	5.3 ± 5.7^{b}	7.4 ± 6.1^{a}	9.5 ± 9.1^{a}	6.4 ± 4.3^{ab}
Main stem diameter (mm)	1.9 ± 0.4^{b}	1.9 ± 0.5^{b}	1.7 ± 0.3^{b}	3.7 ± 1.9^{a}
Protea simplex	(n=105)	(n=93)	(n=83)	(n=16)
Height (cm)	60.7 ± 13.1^{a}	59.9 ± 13.5^{a}	39.3 ± 8.1^{c}	47.5 ± 11.2^{b}
Canopy area (cm^2)	2679 ± 3174^{b}	6574 ± 6471^{a}	5882 ± 7547^{a}	3769 ± 1853^{ab}
Basal area (cm^2)	556 ± 917^{b}	1737 ± 3152^{a}	1904 ± 3837^{a}	739 ± 457^{ab}
Number of stems	8.8 ± 9.3^{c}	17.4 ± 16.0^{b}	38.2 ± 43.3^{a}	39.2 ± 22.1^{a}
Main stem diameter (mm)	6.7 ± 1.6^{a}	7.2 ± 1.4^{a}	10.6 ± 52.0^{a}	8.7 ± 2.8^{a}
Tephrosia kraussiana	(n=114)	(n=260)	(n=45)	(n=18)
Height (cm)	57.1 ± 12.5^{a}	52.9 ± 13.8^{b}	53.6 ± 14.6^{ab}	54.9 ± 6.4^{ab}
Canopy area (cm^2)	665 ± 841^{a}	673 ± 714^{a}	743 ± 819^{a}	475 ± 300^{a}
Basal area (cm^2)	45 ± 145^{a}	45 ± 70^{a}	30 ± 42^{a}	51 ± 42^{a}
Number of stems	8.2 ± 9.2^{b}	9.2 ± 9.1^{b}	10.4 ± 9.8^{b}	28.3 ± 23.8^{a}
Main stem diameter (mm)	2.3 ± 0.7^{b}	2.5 ± 0.7^{b}	2.3 ± 0.9^{b}	2.9 ± 1.5^{a}

Field populations of *P. simplex* were not significantly different from radiocarbon dated plants for any aerial structures except height. Field populations of *T. kraussiana* had fewer stems, smaller main stem diameters and smaller basal areas than radiocarbon dated plants. However, the mean canopy area of *T. kraussiana* field populations was larger than radiocarbon dated plants.

Therefore, individuals of *B. insignis* and *C. laureola* in the field may be greater than 50-51 years old as the canopy structures were larger than those of radiocarbon dated plants. Individuals of *T. kraussiana* in the field may be less than 51 years old as overall the aerial structures were smaller than radiocarbon dated plants. *P. simplex* plants from plots 2 and 3 had structures which were larger than radiocarbon dated plants and, hence, may be older than 49 years, whereas individuals from plot 1 may be younger. Overall the individuals in field populations may be older than the plants which were radiocarbon dated in this study.

6. Discussion

6.1 Morphology and adaptations of suffrutices

Overall *B. insignis*, *C. laureola*, *P. simplex* and *T. kraussiana* had various rhizome shapes, sizes and internal structures, leaf and stem shapes, sizes and textures. Most of these characteristics followed the convention associated with the genus to which the species belonged. However, several fundamental patterns emerged with both the belowground and aboveground morphological structures. All four species had completely submerged rootstocks which enable plants to quickly produce organs after dormancy or physical damage to the plant (Suzuki and Stuefer 1999). The individuals of all four species were below ±60cm in height. All species produced multiple herbaceous stems from the rhizome. This would be advantageous to suffrutex plants in fire prone grasslands since herbaceous stems require a smaller energy investment than woody stems and immediately after a fire, shorter post fire recruiting species undergo rapid growth in height at a rate faster than that of taller species, so that they capture enough light for growth and reproduction before taller species can attain their maximum height and shade out the suffrutex species (Falster and Westoby 2005).

Even though the rhizomes of each species varied in shape and size, in general, the main rhizome was perpendicular to the soil surface with a few or numerous roots growing parallel to and just below the surface. While the main rhizome extended deep into the soil profile to anchor the plant, the roots are free to extend laterally for nutrient acquisition (Pregitzer *et al.* 1993). Seedlings of both *P. simplex* and *T. kraussiana* started developing a woody rhizome at an early age (within 6 months). This emphasizes the importance of this adaptation to these suffrutex plant species.

The network formed by *P. simplex* rhizomes could be an adaptation to maximize the area over which roots extend [e.g. similar to other Proteaceae such as *Banksia goodii* (Witkowski and Lamont 1997)] thereby increasing access to nutrients since they grow in sandy nutrient-poor coastal soils (Brooker *et al.* 1999). *B. insignis* and *C. laureola* grow in soils with greater nutrient availability (section 6.4) and may, therefore, possess more compact rhizomes with less extensive root systems (Pregitzer *et al.* 1993).

All four species had multiple shoots which could be an adaptation against herbivory since the likelihood of the entire canopy being lost is reduced. All four species also produce large, colourful inflorescences and flowers. Producing attractive flowers is a strategy used by grassland plants to ensure pollination since smaller, less conspicuous flowers held on shorter peduncles

may not be clearly visible to pollinators above the grassland canopy (Lortie and Aarssen 1999) whereas conspicuous flowers are more likely to be seen (Lovell 1912).

6.2 Viability, germination and seed mass of suffrutex plants

Seed mass is important in the viability and germination of *P. simplex*. Seeds of *P. simplex* are relatively lighter than seeds of some other non-resprouting *Protea* species (i.e. *P. simplex*: ~0.016g, *P. compacta*: ~ 0.16g, *P. obtusifolia*: ~ 0.03g) (Esler *et al.* 1989), which may facilitate more effective wind dispersal (Augspurger and Franson 1987). The inflorescences of *P. simplex* were also comparatively small (compared to other *Protea* species), which may reduce the visibility of flowers to pollinators (Lortie and Aarssen 1999) thus reducing viability. However, overall seed viability (67.08%) was relatively high for a resprouter (Rebelo and Rourke 1986). Furthermore, 67.08% may be an underestimate of seed viability in *P. simplex* in the field, as Proteaceae seeds lose their viability with increasing storage time in the laboratory (Davey and van Staden 1977) and seeds were stored for 12 months before sowing. Although seeds were stored under appropriate dry storage conditions, cell organelles and membranes begin to disintegrate during storage, leaving limited energy available for embryos to germinate (Davey and van Staden 1977). This may explain the lower overall germination in laboratory trials (23.9%). However, field germination of *P. simplex* seeds was also low (5±4.2%). Germination may have been more successful in the greenhouse as conditions (abundance water supply and lack of competition) are more conducive to growth (Van Straten *et al.* 2000). However, generally low germination in both trials may suggest low germination in the species. This may also hold true for the high viability, low field germination and low juvenile recruitment in field populations of *T. kraussiana*.

Field populations of *P. simplex* were situated in a reserve with open access to the public and rural workers used a pathway through the reserve daily. Evidence of flower cutting and grazing was noted during the field investigation. These disturbances may reduce cone and seed production and the percentage of viable seed in a plant (Witkowski *et al.* 1994), thereby, resulting in low recruitment and slow population growth in the Red Desert Nature Reserve, thus making these populations more vulnerable.

Seeds weighing between 13.61-28.90mg/seed were the most viable. The abundance of light, sterile seeds supports the idea that *P. simplex*, like other members of the Proteaceae invests greater energy in storage for resprouting instead of seed production (Enright and Goldblum 1999). The production of sterile seeds may also be a strategy against predators.

Parthenocarpy (production of seedless fruits) is a strategy used by plants to attract dispersers and reduce predation of viable seeds by insects and birds, thereby maximizing the plant's reproductive success (Ramos-Ordonez *et al.* 2010; Wright 1994; Fuentez and Schupp 1998). The significantly lower seed mass of non-viable seeds suggests that the energy investment in these seeds is lower and these seeds are expendable.

P. simplex had a long germination lag (12.2±6.9 days) and long intervals between the germination of successive seeds under favourable laboratory conditions. These lag periods are likely to be amplified in the field. Seeds of *P. simplex* may germinate long after neighbouring grassland species (e.g. *T. kraussiana*) establish, thus reducing the availability of nutrients and light to the establishing *P. simplex* plants (Berntson and Wayne 2000). However, having a long lag phase before germination could also be an advantage in these fire prone coastal grasslands. The exposed tender shoots of seedlings of other species emerging soon after a fire may also be more vulnerable to grazers and seedling predators (Bryant *et al.* 1983). Germination between individual *P. simplex* seedlings was staggered (mean days to germination= 21.5), suggesting that germination will be staggered over time and seedlings will be of different ages. This could also be advantageous if the early seedlings die from unfavourable conditions and are replaced by others that may face better conditions (Hyatt and Evans 1998). Staggering germination also reduces competition between each set of seedlings (Kaplan 1980; Hyatt and Evans 1998).

Although there was no relationship between root mass and the number of cones produced, there was a relationship between root mass and the number of seeds per cone, suggesting that root mass indirectly influences inflorescences. Therefore, the stored energy provided by larger rhizomes may provide for the production of a greater volume of seed (Schmid *et al.* 1995).

Seed mass was also important in the germination and viability of *T. kraussiana*; however, not to the same degree as in *P. simplex*. The high seed viability across all mass categories above 0.00301g/seed may be due to the low variability in mass between sampled seeds. High variability in seed mass within a species is a more evolutionarily stable strategy (Geritz 1994). Therefore, while *T. kraussiana* seeds have greater viability, the higher variability in seed mass in *P. simplex* may be a more stable evolutionary strategy. The seeds of *T. kraussiana* occurred in an area with relatively fertile soil (compared to the soil in which *P. simplex* occurs). Higher soil fertility increases the probability that competing species will also establish easily (Williamson and Fitter 1996). However, by producing seeds of a similar mass, *T. kraussiana*, increases juvenile mortality (Geritz 1994), which may explain the low number of juveniles in field populations even though viability was high. The populations tended to form dense population stands. In a dense stand,

new seedlings are prevented from establishing (White and Harper 1970) as they become shaded by larger plants, reducing the amount of energy reaching their roots (Schmitt and Wulff 1993), thereby reducing the ability of these roots to absorb nitrogen (Lemaire 2001). These plants are less likely to survive, thus reducing the number of juveniles recruited into adulthood in the population. A short germination lag and a rapid germination rate (Table 5.11) would also ensure that *T. kraussiana* germinants establish quickly when microsites become available (Anderson and Winterton 1996). However, this strategy could also be disadvantageous as all seedlings will be of a similar age, thus increasing intra-specific competition (Hyatt and Evans 1998). Furthermore, if a fire had to spread through the population (as was the case during the survey of field germination trials), many of the seedlings will be lost and there will be no subsequent set of germinating seeds to replace them.

The viability and germination of *T. kraussiana* plants 10, 12 and 18 (plants which were carbon dated) were not significantly different from other parent plants of *T. kraussiana* for germination and viability. This lack of difference could be because carbon dating required the selection of larger individuals and, therefore, the size difference may have been insufficient for variations in germination and viability to be detected. Alternatively, size may not be influential in germination and viability.

The lower percentages of germinating *T. kraussiana* seeds in the field compared to greenhouse trials may have been further exacerbated by the fire, which burned field populations a day before surveying; therefore, many seedlings could have been lost. Even seedlings which were counted were desiccated, thus reducing the magnitude of main stem diameter and height. Therefore, the percentage of emerged seedlings in the field may be a slight underestimate of the initial number and an overestimate of the “final” number. Germination of seedlings in the greenhouse was also augmented by boiling of the seeds (Ruppel 1967), whereas seeds planted in the field germinated naturally. However, overall, the marked differences between the field and laboratory trials suggest that the percentage of new seedlings entering the population each year is low. Furthermore, the germination and emergence of these seedlings was probably improved by clearing away competing vegetation surrounding the area in order to install the grids for plots. Therefore the actual germination and emergence of both species may be even lower. With climate change, the optimal climate which supports the species may shift (Loarie *et al.* 2009); however, the species may be unable to produce new seedlings quickly enough to track these climatic shifts (Loarie *et al.* 2009). Every species has its own germination niche (i.e. the specific conditions required for germination to occur successfully) in which different environmental

conditions are required (Liu *et al.* 2011). Therefore, the effects of climate change on seed germination requirements (such as dormancy breaking) are unknown, as are the implications of these changes on species composition in a plant community (Liu *et al*. 2011).

Furthermore, suffrutex species often occur in grasslands which are used as grazing land by local communities. Long-lived forb species with low reproductive output and infrequent recruitment could be driven to local extinction by overgrazing as defoliation and trampling (non-graminoid plants are poorly adapted to tramping and grazing) increases adult and seedling mortality and consumption of reproductive parts leads to a further reduction in the seed bank and seedling recruitment (O'Çonnor *et al.* 2011, in press).

6.3 Growth strategies in suffrutescent seedlings

Both *P. simplex* and *T. kraussiana* seedlings showed signs of early rhizome development. At six months rhizomes were already thickened and lignifying. Development of rhizomes at this early stage requires a significant energy investment in below ground structures (Schwilk and Ackerly 2005). This is highlighted by the higher ratio of roots to shoots for both species, an occurrence commonly found in seedlings grown in nutrient poor soils (Gedroc *et al.* 1996) (like that of *P. simplex*) as seedlings adjust their root to shoot ratios according to the nutrients available (Gedroc *et al.* 1996).

Early investment in belowground structures may be advantageous in the fire- prone coastal grasslands in which the study species occur (Bond and van Wilgen 1996a). After a fire (such as the managed block burn which burnt through all three seedling plots of *T. kraussiana* at six months), plants may be able to regenerate from the rhizome from as early as six months, thereby giving them a competitive advantage over neighbouring grassland species as aerial structures grow soon after a fire by resprouting using energy stored in the rhizome (Suzuki and Stuefer 1999) as opposed to beginning the germination process from seed.

P. simplex seedlings had relatively slower growth rates compared to *T. kraussiana*. This is expected as *Proteas*, in general, have relatively slow growth rates (Witkowski 1991). The higher root to shoot ratio in *T. kraussiana* compared to *P. simplex* was unexpected as leguminous species generally invest less in belowground biomass (Power *et al.* 2010). *P. simplex* displayed the typical tradeoff of resprouting species by investing more resources in below ground growth, resulting in a slower canopy growth rate (Bond and Midgley 2001) of seedling aerial structures such as stem height. Slow growth rates may also be because the species grows in nutrient poor soils and, therefore, requires the assistance of proteoid roots for efficient nutrient uptake

(Lambers *et al.* 2006; Neumann and Martinoia 2002). Proteoid roots are costly to manufacture (Lambers *et al.* 2006); therefore, investing in cluster root development slows the initial rate of aerial growth.

Nutrient-poor soils may also be the reason for high mass allocation to cotyledons and their persistence on seedlings for greater than 6 months. In members of the Proteaceae, cotyledons play a vital nutritional role in the early establishment of seedlings (Milberg and Lamont 1997). Nutrients such as N, P, K and Cu are translocated from the cotyledons to the seedling. Species with larger seeds may be more reliant on nutrients supplied by the cotyledons since larger seeds may be an adaptation to increase successful establishment in infertile soils (Milberg and Lamont 1997). This may have been why the seedlings of the larger seeded *P. simplex* retained cotyledons for a long time. Parent plants may invest sufficient energy in cotyledons to facilitate the initial growth of seedlings until proteoid roots form- since the soil is low in important macro nutrients (N and P) (Lambers *et al.* 2006).

T. kraussiana seedlings grew rapidly in terms of both height and leaflet growth. While resprouter seedlings have to make a tradeoff between allocating resources to structural defence against herbivory and growth, investing energy in defence reduces the growth of vegetative structures as seedlings have to grow fast enough to outcompete other plants but still maintain the physiological adaptations required for survival in a particular environment (Herms and Mattson 1992). Therefore, *T. kraussiana* may tradeoff investing resources in storage for rapid growth. Rapid growth may have been facilitated by the dark- nutrient-rich (e.g. high in nitrogen and phosphorus) soils in which the species grows (Brooker *et al.* 1999). The high density of adult populations suggests that seedlings may have to compete with taller adult plants for light and space (Bond and van Wilgen 1996b; Falstor and Westoby 2005). Therefore, a rapid increase in height and leaflet number may ensure that seedlings grow out from beneath the shading canopy to expose leaves to light so that the plant can begin photosynthesizing and storing energy in the rhizome before the next fire event (Bond and van Wilgen 2006a). Cotyledons may fall off soon after seedling emergence as the nutrients available in the soil negates the need for the persistence of cotyledons after six months. Greenhouse grown seedlings were larger then field grown seedlings of *P. simplex* and *T. kraussiana* (except height for *P. simplex*). Therefore, the actual growth rates of seedlings are likely to be slower compared to greenhouse trials.

Overall, the two species used growth strategies which were different from each other and which may be dictated by their resource allocation, soil nutrient composition and population

densities. Growth trials need to be done with other suffrutex species in order to better understand different patterns of seedling growth among suffrutices.

6.4 Suffrutex size structure and demography in coastal grassland habitats

All four species had few or no juveniles in the wild population. Low recruitment is common in resprouters which occur in fire prone habitats (Bond and Midgley 2001). Juvenile mortality is higher in resprouters than non- resprouters as the growth of juveniles are stunted by the cost of producing rhizomes for fire survival (Bond and van Wilgen 1996a). Furthermore, those seedlings which do survive may be outcompeted (White and Harper 1970). Low numbers of juveniles is daunting as it implies slow population growth which, coupled with rapid habitat reduction could leave populations vulnerable to disturbance. However, low numbers of juveniles may also have been recorded because the plot sampling method was used. Seed density declines with increasing distance from the parent population (Nathan and Muller-Landau 2000; Clarke *et al.* 1999).

Therefore, seedlings which may have established outside the range of the main population (and the plot) due to the high competition for space and resources would have been excluded from the plot. Therefore, a PCQ (Point-center quarter) method may have been better suited to finding juveniles which dispersed and established outside the main population (Mueller-Dombois and Ellenberg 2002). However, no juveniles were spotted outside the main population during preliminary surveys of the area, thus supporting the evidence that populations were composed primarily of adult plants.

The relative sizes of plant organs (leaf, roots, stems) are influenced by the environmental constraints and resource sharing among individuals in different stage classes in the population (Lemaire 2001). These individual interactions determine the emerging properties for the functioning of the whole population. Asymmetric distributions of plant structure (such as that found in this study) are observed more often than normal distributions (Lemaire 2001). Individuals in plot three of *P. simplex* had particularly low values for height (Figure 5.23), which may be attributed to a reduction in surface area to minimize stem breakage by coastal winds. There is also a maximum height to which herbaceous stems can grow before it collapses under its own weight (Niklas 1995), which may explain the relatively similar heights of adults in all four species.

A larger proportion of plants in the sampled populations had fewer stems for *B. insignis, C. laureola, P. simplex* and *T. kraussiana*. Investing energy in developing and maintaining a few healthy shoots may be a strategy for conserving energy as shoots are non-perennial. Producing

high numbers of shoots which only last a year or two would be a waste of stored resources. The high frequency of fewer stems contributed to higher frequencies of smaller basal and canopy areas. Therefore, small canopy areas were not necessarily attached to younger plants. Plants which had large rhizomes often had only two or three shoots. The high frequency of plants with few floral structures emphasized the fact that these plants invest more energy in storage (Bond and Midgley 2001) than in seed production, thus contributing to the slow population growth of these species. Resprouting species may have low recruitment; however, they are more resistant to disturbance events and can tolerate long periods without recruitment due to their persistence (Bond and Midgley 2001). They are also more likely to preserve the genetic diversity of the population as they have long generation times (Bond and Midgley 2001). However, an increase in adult mortality due to habitat destruction is a threat, which is compounded by poor recruitment in resprouters, reducing the ability of these species to establish in new areas (Bond and Midgley 2001).

The plant density of *B. insignis*, *C. laureola*, *P. simplex* and *T. kraussiana* differed significantly. *T. kraussiana* had the highest plant density (14067 plants/ha). The high concentrations of important essential elements such as nitrogen, phosphorus and organic carbon in the soils in which it occurs may facilitate dense growth (Boyer 1982). High nitrogen concentrations in *T. kraussiana* soils could be due to nitrogen fixing by the legume itself. Therefore, having a short distance dispersal strategy may be advantageous to the species since it ensures that seeds germinate in a nitrogen-rich site.

C. laureola had the second highest plant density. The soil in which *C. laureola* occurred had high concentrations of phosphorus. Acid saturation was also high. Therefore, higher concentrations of phosphorus may support a higher concentration of individuals. However, since nitrogen and phosphorus function as co-limiters, (i.e. biomass production is limited by the ratio of N and P concentrations in the plant) (Craine *et al.* 2008), the lower levels of nitrogen restrain the species from having population densities as high as *T. kraussiana*. *B. insignis* soils had lower concentrations of N and P, which may have also resulted in a lower plant density. However, relatively high concentrations of magnesium, calcium and manganese indicated relatively nutrient-rich soil. *P. simplex* soils were nutrient-poor. These soils had the lowest levels of N and P and low concentrations of Mg and Ca. This may be the reason for low densities of *P. simplex* populations.

Overall it seemed that plant density and the sizes of aerial structures varied according to environmental conditions. However, all species appeared to have certain constraints and trends

which could be attributed to having a suffrutescent growth form. All species were below 60cm in aerial height, suggesting an adaptive constraint. Basal area reflected rhizome extent but not its size. There appeared to be low investment in seed production and a higher investment in storage. There appeared to be few juveniles in populations and, consequently slow population growth (Drechsler *et al.* 1999).The evidence from this study suggests that existing populations are persisting in the grassland and not reproducing at a rate which will enable them cope with the effects of climate change (Thomas *et al.* 2004) and habitat transformation.

6.5 Longevity of grassland suffrutices

Radiocarbon dating results were inconclusive. Ages may have been underestimated since younger tissues were included in the samples. Furthermore, these underestimates may have contributed to poor regression fits and correlations during analyses. However, while plant ages may have been younger than anticipated, overall ages along the ascending slope (*B. insignis*: 49-51 years, *C. laureola*: 49-50 years, *P. simplex*: 45-49 years and *T. kraussiana*: 51 years) were still remarkable when plant size is taken into consideration. The oldest *B. insignis* plant (51 years) was just 47 cm high, was composed of eight stems with a main stem diameter of four millimeters and had a canopy and basal area of just 286 cm^2 and 11 cm^2 respectively. The oldest (according to the descending slope) *P. simplex* plant (29 years old) was comparatively larger at 55 cm high, with 70 stems and a minimum stem diameter of 11 mm and canopy and basal areas of 7240cm^2 and 988cm^2 respectively. Long-lived plants which have ages in the region of hundreds of years are generally large trees or clonal plants (Hofgaard 1993; Mitton and Grant 1980; Dickinson *et al.* 1991). Therefore, these plants are relatively old for non-clonal, suffrutescent plants. These individuals were diminutive compared to similar aged plants of other growth forms (Hofgaard 1993; Mitton and Grant 1980; Dickinson *et al.* 1991). The results of radiocarbon dating highlighted the mismatch between the perception of age and plant size for these suffrutex plants. It is essential that the growth form and belowground habit of plants are known as the importance of investigating the belowground component of forbs in grasslands is slowly gaining credence (Mommer *et al.* 2010). The sizes and extents of these rhizomes must be quantified as long-lived rhizomes have important long term ecological contributions within a grassland community. Suffrutex plants may also be useful in assessing the age of veld since the last fire event (derived from the age of the resprouting canopy) since other species in the grassland community are likely to be annuals, geophytes or clonal grasses which tend to be short-lived (Kelly 1989) and may be

more difficult to date (Pornon and Escaravage 1999). A rough idea of age can be estimated from the circumference and mass of the plant rhizome.

However, given that rhizome mass has a relationship (albeit non-significant) with plant age, and rhizome mass has significant relationships with the size of plant aerial structures, it follows that aerial structures may have a relationship with age. Hence, surrogates for age using aerial plant structures may be achievable. However, substantially more work needs to be done to gain a better idea of the relationship between the aerial structure and plant age. A greater number of replicates with a wider range of sizes coupled with better dating techniques would be required.

During the course of this study a few important points were learned and may be useful to future investigations of rhizomes using radiocarbon dating:

- The CSIR laboratory in South Africa has limited resources and no longer does commercial radiocarbon dating. Samples will therefore have to be sent overseas, where the cost of AMS and gas proportional counting techniques are similar. Therefore, it is recommended that the AMS technique be used since a smaller amount of sample is required (McNichol *et al.* 2001) (therefore decreasing the sample size constraints on species selection).
- Furthermore, AMS yields more precise results as less material is required; decreasing the likelihood of having to excise material from younger tissue regions.
- During field collection- larger rhizomes/tubers must be collected as the oldest regions must yield at least four grams of material (even for AMS).
- Plants which show signs of decomposition must be excluded as it is likely that the decomposition extends into the central tissue.
- Even larger plants must be collected for species which have high concentrations of chemical defenses (e.g. *C. laureola*) as a lot of these chemicals are extracted during the AAA purification phase and are also burned off as volatiles during combustion, therefore, reducing the amount of sample available for radiocarbon dating.
- The basal area of the plant can be used as a rough guide to the extent of the rhizome, however, since it does not always correlate with size- this method may not always be accurate and would need to be assessed per species studied.
- The whole rhizome must be removed so that masses can be compared with greater accuracy
- Rhizomes should be dated across a wider range of sizes for greater comparability.

- After a fire the rhizome nutrient reserve of a plant may be decreased by as much as 50% and may take 2 years before pre-burn reserve levels are regained (Bond and Midgley 2001). Therefore, the time since the last burn must be considered when measuring the sizes of rhizomes.

Overall, suffrutex species and the importance of the rhizomes have received little attention previously. In conclusion, the results of this study suggest that suffrutex species are long-lived and longevity may exceed 50 years. The sizes of aboveground structures do not correlate with the sizes of belowground structures and may, therefore, lead to spurious estimations of the demography of populations. There appears to be a greater number of persisting adult plants and very low recruitment in wild populations. Surrogates for age using plant aerial structures would be very useful in gaining a more accurate estimation of individual age. However, further investigation with larger sample sizes needs to be conducted before these surrogates can be formulated. Long-lived species have low temporal variability in population structure (Garcia 2003). Higher mortality and low recruitment may reduce population growth but the population will continue persisting in the landscape. However, a continued increase in the death of adults would drive the population to extinction. These species have an extremely slow capacity for recovery after a disturbance (such as harvesting for the *umuthi* trade) (Garcia 2003).

7. Conclusion: Longevity, population structure, viability, habitat, growth and the implications for suffrutex species survival and management

B. insignis, C. laureola, P. simplex and *T. kraussiana* are relatively long-lived species with slow population growth and poor re-seeding capability. Although only four suffrutex species were investigated, they are from three different families, yet similar overall patterns still emerged. This suggests the possibility that these trends may be common to other suffrutex species.

Management strategies for these species (and possibly all grassland suffrutex species) need to take cognisance of the following:

- Suffrutex species die-back during the dry season; however, large underground rootstocks remain and sprout non-robust shoots during the growing season. Therefore, these species are in danger of being overlooked in grasslands

- Species are likely to do well in both small isolated patches as well as in large reserves since populations appear to form dense stands; therefore, large areas for establishment are not essential.
- For small reserve patches with long-lived suffrutex plants such as the Red Desert Nature Reserve, restoration is unfeasible as recruitment is slow and conservation of these grassland remnants should take precedence.
- The focus must be on preserving larger individuals since these plants are likely to be older. These individuals have a more significant ecological contribution to the grassland community. They have a greater capacity for seed production and, therefore, a greater contribution to the population of the species.
- Suffrutex species with *umuthi* uses in reserves with open access (such as the Red Desert Nature Reserve) need to be specially protected as the larger rhizomes, which are important for reproduction, are a target for harvesters.
- Periods of more frequent burning (i.e. biennially or every three years) may encourage seedling growth in slow growing populations since fire reduces both interspecies and intraspecies competition for light and canopy space. Since seedlings displayed early rhizome development and high root to shoot ratios, burning every two years also gives seedlings and adults an opportunity to store energy in their rhizomes so that they can resprout quicker than competing species after the fire.
- Conservation efforts at a species level must also consider the conservation of the local environment as local conditions are important for predicting the size structure of plants in a population.

Slow population growth and poor recruitment and the fact that these species appear to be closely linked with soil nutrient composition makes them particularly vulnerable to habitat destruction, flower picking, cattle grazing, excavating of larger plants for the *umuthi* trade and the effects of rapid climate change. Therefore, habitat conservation for the preservation of these long-lived adult plants is vital.

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Appendix 1. Procedure for soil analysis at CEDARA

Soil samples were air dried, and then air was forced over them in drying trays (Manson and Roberts 2001). Dried soil was crushed on a soil crusher, and then passed through a 1mm sieve. Particles coarser than 1mm were removed. Bulk density was measured on a volume rather than mass basis. The mass of 10mL of sample was measured to obtain a sample density. To measure pH (KCl), 25ml of 1M KCL solution was added to 10ml of soil. This solution was stirred. The suspension was allowed to stand for 30 minutes. pH was measured using a gel-filled combination glass electrode while stirring. Extractable calcium, magnesium and acidity was measured by adding 25ml of 1M KCl to 2.5 ml of soil. The solution was then stirred for 10 minutes. Extracts were removed with filter paper. 5ml of filtrate was diluted with 20ml of 0.0356M $SrCl_2$. Ca and Mg values were determined using atomic absorption (Manson and Roberts 2001). Ten milliliters of filtrate was diluted with 10 mL of de-ionised water mixed with 2-4 drops of phenolphthalein, and titrated with 0.005M NaOH to determine extractable acidity. Extractable phosphorus, potassium, zinc and manganese were determined using an Ambic-2 extracting solution with a concentrated ammonia solution. Twenty-five ml of the solution was added to 2.5ml of soil. The solution was stirred for 10 minutes. Extracts were filtered. A modification of the Murphy and Riley (1962) molybdenum blue procedure was used to determine phosphorus. Potassium was determined by diluting 5ml of filtrate with 20ml of de-ionised water for use in atomic absorption. Atomic absorption was used to determine zinc and manganese using the remaining undiluted filtrate. Acid saturation was calculated as a function of Ca, Mg and K values. Organic carbon and clay content was estimated using near-infrared reflectance. Total nitrogen was analysed according to the Automated Dumas dry combustion method using a thermal conductivity cell (Manson and Roberts 2001). Suspended clay and fine silt were determined after dispersion and sedimentation (Manson and Roberts 2001). Sand was determined by sieving. Twenty grams of soil (<2 mm) was treated with hydrogen peroxide to oxidise organic matter. De-ionised water was added to the sample and left overnight. A clear supernatant formed and was siphoned off. De-ionized water was added again and the sample was stirred and left overnight. Clear supernatant was siphoned off again. NaOH and sodium hexametaphosphate were used as dispersing agents. They were added to the sample and stirred on Hamilton Beach stirrers. The suspension was made up to 1 liter in a measuring cylinder and the clay (<0.002 mm) and fine silt (0.002-0.02 mm) fractions measured with a pipette after sedimentation. Fine silt and clay was measured after four to five minutes at 100 mm. Clay was measured after five to six hours at 75 mm. Sand fractions include very fine sand (0.05 - 0.10 mm), fine sand (0.10 - 0.25 mm), medium sand (0.25 - 0.50 mm) and coarse sand (0.50 - 2.0 mm) which are determined by sieving. Coarse silt (0.02-0.05 mm) is estimated by calculating the difference. Textural classes were determined using a textural triangle (Manson and Roberts 2001).