The ecology and nitrogen-fixing ability of selected *Aspalathus* spp. in fynbos ecosystems.

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Contents.

Abstract.

Aspects of the ecology of selected species of *Aspalathus* spp. (Fabaceae) were investigated to elucidate the role that this genus may play in the nitrogen economy and ecology of fynbos ecosystems. From the literature it is argued that fire plays a pivotal role in these ecosystems as decomposition is very slow. Decomposition returns nutrients trapped in the vegetation back to the soil. However, nitrogen is lost via volatilisation during fires and as deposition of nitrogen in precipitation is low, symbiotic and non-symbiotic nitrogen-fixation may be of importance in replacing lost nitrogen.

The influence of soil total nitrogen and phosphorus on the occurrence of dense stands of *Aspalathus* plants in vegetation of different ages was investigated. Soil of young vegetation containing stands of *Aspalathus* plants had the same or higher concentration of phosphorus as nearby older vegetation which lacked stands of *Aspalathus*. Total nitrogen however showed no trends.

Nodulation of 15 *Aspalathus* spp. at 11 study sites showed varying amounts of nodulation although all these species nodulated well under pot culture. Nodulation of three *Aspalathus* spp. at three seasonal study sites was followed over the course of one year. *A. carnosa* showed peak nodulation in the late winter months but *A. retroflexa* and *A. abietina* displayed no similar trends. Across the three seasonal sites, which had very similar soil, nodulation was positively correlated with soil total phosphorus and negatively correlated with soil total nitrogen in a multiple regression analysis. The ratio of soil total nitrogen to phosphorus was negatively correlated with nodulation. The opposite correlations for soil total nitrogen and phosphorus were found across all 11 study sites. Differences in soil types and organic content may be responsible for this apparent contradiction. Other soil factors such as pH, temperature and moisture were not correlated with nodulation. Total organic content, however, was negatively correlated with nodulation across the geographic survey study sites.

The Nitrogen fixation in 13 species of *Aspalathus* at 10 study sites was determined using the $\delta^{15}N$ natural abundance technique for determining nitrogen fixation. Instead of using nearby non-fixing species as reference plants, plants of the same species were used. These were grown on their native soil and supplied with oxygen to prevent

nodulation. There appeared to be some trend between nitrogen fixation determined in this way and nodulation in the *Aspalathus* spp. at the various study sites. This method shows promise for future ecological studies as it removes the problem of finding a suitable reference plant.

Various vegetational aspects of *Aspalathus* spp. and Fabaceae were investigated. The Fabaceae where found to be associated with young vegetation as there was a great reduction in the cover of this family when it was compared with nearby older vegetation. This fact was further investigated in the growth and senescence of three *Aspalathus* species over the course of one year. Two of these species, *A. retroflexa* and *A. abietina*, appeared to be very early successional species which die out 1-3 years post-fire while the third, *A. carnosa* appeared to be prominent in later succession. Circumstantial evidence suggested that summer drought may be an important immediate cause of death and senescence in all three species. The dense stands of *Aspalathus* were also found to affect alpha species diversity. This appeared to be a spatial rather than an overtopping effect which has been observed in previously Proteaceae.

An investigation into the effect of heat exposure in stimulating germination in 13 species of *Aspalathus* and three other Fabaceae species showed temperature between 80 °C and 100 °C to be most effective. Temperatures above 120 °C were lethal in most cases. The thickness of the seed coat relative to the embryo was shown as important in determining the most effective heat exposure for germination.

The ecology of *Aspalathus* spp. and their role in the fynbos is discussed in the light of the results obtained in these studies.

Chapter 1

Introduction.

Fire plays an important role in cycling nutrients in fynbos ecosystems (Stock & Allsopp 1992). These fires may lead to nitrogen, a scarce element in these environments, being lost via volatilisation. The role of nitrogen-fixing organisms in these environments is therefore of interest, as the replacement of lost nitrogen depends on atmospheric inputs, which are very low in this case, and symbiotic and non-symbiotic nitrogen fixation. It has been suggested that *Aspalathus* spp. may be of particular importance in replacing lost nitrogen because these plants form large dense stands in fynbos environments after fires. It is hypothesised that they have a competitive advantage over other plants because they are able to fix nitrogen and therefore do not have to compete for the limited supplies present in the soil aftert fire. The argument continues by suggesting that as the vegetation ages, changes in the nutrient status of the soil, specifically in phosphorus, cause the plants to loose this advantage and rapidly give way to other species (Rundel 1983).

The aim of this thesis was to investigate aspects of the ecology of *Aspalathus* spp. which may help to elucidate the role they play in the nitrogen economy of fynbos. This included the ability of *Aspalathus* spp. to nodulate and fix nitrogen and the influence of other environmental factors. Other interesting aspects of the ecology of these species are also investigated. For instance, are *Aspalathus* species generally less common in the later stages of succession and, is there a discernible trend in the nutrient status of the soils of vegetation containing stands of *Aspalathus* plants and older vegetation where these are absent ? Very dense stands of *Aspalathus* plants may also have an effect on community structure and diversity. In dense stands of *Aspalathus*, the plant may grow fast, occupying space and out-competing slower growing species thus leading to a lower species diversity in the community. The seed biology of these plants is also investigated in order to determine the role of fire in the germination of *Aspalathus* seeds. It may be hypothesized, for example, that like other legume species, *Aspalathus* seed dormancy is broken by fire. The heat from more intense fires should penetrate the soil to a greater depth thus causing larger numbers of seeds to germinate. Dense stands of *Aspalathus* species would thus be associated with hotter fires (Auld & O'Connell 1991).

This thesis poses to answer some of these questions by reviewing the literature and proceeding to selected aspects of the ecology of nitrogen fixation of these species. In the literature review (Chapter 2), the importance and cycling of nitrogen and phosphorus to Mediterranean-type ecosystems, and the role of fire in cycling them, is highlighted. The replacement of nitrogen lost by fire via atmospheric input and nitrogen fixation is also discussed. A discussion of the different techniques used to measure nitrogen fixation is followed by a presentation of the literature detailing the ecological position of legumes in pyric succession in Mediterranean-type ecosystems, the emphasis being on *Aspalathus* spp. in fynbos.

The twelve study sites used for this study are described in Chapter 3, which includes details of their location, soils and vegetation, and of the climate of the area. In the following chapter (Chapter 4), the nodulation of different *Aspalathus* spp. is investigated. This includes a survey of nodulation found in different species at a variety of geographical localities as well as the nodulation found over the course of a year. The role of soil nutrient status in determining whether stands of *Aspalathus* plants are associated with younger vegetation is investigated as well as the influence of various soil factors on *Aspalathus* nodulation. Nitrogen fixation in different *Aspalathus* spp. was determined (Chapter 5) using the stable isotopes of nitrogen ^{14}N and ^{15}N, in the so-called $\delta^{15}N$ natural abundance method. The rationale behind this method is discussed in detail in the literature survey (Chapter 2). The following chapter (Chapter 6) investigates various vegetational aspects. These include the occurrence of legumes in vegetation of different ages in fynbos ecosystems, the growth and senescence of three species of *Aspalathus* over a year and the effect of dense stands of *Aspalathus* on community alpha diversity. The effect of exposure to dry heat on *Aspalathus* seed germination is also investigated (Chapter 7). These data are related to the embryo to seed coat mass ratio. The importance of heat stimulated germination for *Aspalathus* and fynbos ecology is discussed with reference to legumes from other Mediterranean regions. The thesis concludes (Chapter 8) with a general discussion of the information obtained during this study and provides scope for further research concerning *Aspalathus* spp..

Chapter 2

Literature review.

1. Introduction.

In this chapter areas of the literature which have bearing on the research in later chapters of this thesis are discussed. These are: the cycling of nitrogen and phosphorus, including decomposition and the role of fire in nutrient release and nutrient loss in fynbos and other Mediterranean-type ecosystems; gains in nutrients to these ecosystems, including atmospheric deposition; the gains from nitrogen fixation of which a detailed account is provided and a brief discussion of the importance of *Aspalathus* as a major legume genus in the Cape Floral Region. The chapter concludes with a general discussion of fynbos legumes and fire ecology.

2. Nutrient cycling in Mediterranean-type ecosystems with specific reference to nitrogen and phosphorus.

2.1. The importance of nitrogen and phosphorus in ecosystem functioning.

Both nitrogen and phosphorus are noted as nutrients important to the functioning of heathlands as well as Mediterranean-type ecosystems (Groves 1983). As plant nutrients, these elements also appears to have a high level of interaction between them (Groves 1983). However, it should be noted that phosphorus is cycled in a local sedimentary fashion whereas nitrogen is cycled globally in a process which includes a gaseous phase.

Phosphorus and nitrogen are the elements in shortest supply in heathlands and Mediterranean-type ecosystems (Groves 1983). The generally low total and available phosphorus content of fynbos soils is related to the low total phosphorus

concentration of the parent material. For instance, the aeolian derived soils of the west coast fynbos (Mitchell *et al.* 1984), as well as the soils of mountain fynbos are all derived from Table Mountain Sandstone which is low in total phosphorus (Witkowski & Mitchell 1987). Granite derived soils in mountainous areas tend to have somewhat higher levels of total phosphorus (Witkowski & Mitchell 1987). The limestone derived soils of the south coast are rich in total phosphorus but nonetheless have a low available phosphorus content because of the insolubility of $CaPO_4$ which predominates in these soils (Witkowski & Mitchell 1987).

There are no wide-scale studies of the nitrogen content of fynbos soils, although from the limited data available they are generally regarded as low in nitrogen. This is probably owing to the impoverished nutrient status of the parent material. The accretion of nitrogen in an ecosystem, however, is related to the biological inputs because of nitrogen's more global cycle. Low (1983) found soil total nitrogen for mountain fynbos and coastal fynbos to be low when compared to other Mediterranean-type shrublands. Low total nitrogen of coastal fynbos soil was also noted by Stock and Lewis (1986a). In these ecosystems the largest reserve of both nitrogen and phosphorus is in the soil and not in the phytomass (Low 1983; Stock & Lewis 1986a).

2.2. Litter production and decomposition.

The rate of litter production and its decomposition is obviously important to the nutrient cycling of any ecosystem and, although it may vary greatly, the rate of litter production in Mediterranean-type shrublands is far less than that of tropical forests and savannas. The highest values recorded for Mediterranean-type shrublands are similar to those obtained for deciduous forests (Read & Mitchell 1983), while for litter production generally, fynbos ecosystems tend to be on the lower end of the scale (Stock & Allsopp 1992).

Not only is the quantity of litter produced important in the cycling of nutrients in an ecosystem but so is the quality. Sclerophyllous vegetation of Mediterranean-type shrublands produces litter which is slow to decompose. The high carbon to nitrogen ratio of this litter results in slow decomposition rates (Read & Mitchell 1983) while components such as lignin, holocellulose and phenolics also affect the decomposition rate. Schlesinger and Hasey (1981), for example, found no decrease from the initial lignin content of *Ceanothus megacarpus* after a year of decomposition. Their study also suggested that a high phosphorus to carbon ratio

may be of importance in determining decomposition rates in sclerophyllous vegetation. This has been confirmed for *Protea repens* decomposition in fynbos (Mitchell & Coley 1987).

The already low nutrient content of sclerophyllous vegetation is lowered further by efficient withdrawal of nutrients before abscission. In California, *Ceanothus megacarpus*, a sclerophyllous evergreen, was able to remove 61 % of the nitrogen and 75 % of the phosphorus from its leaves whereas *Salvia mellifera*, a deciduous shrub, removed 22 % of the nitrogen while the phosphorus concentration increased by 49 % (Schlesinger & Hasey 1981). *Banksia ornata* in South Australia recycled 90 % of its phosphorus and 30 % of its nitrogen before abscission (Specht 1981). Various fynbos species have also been shown to be efficient at relocating nitrogen and phosphorus. *Leucospermum parile*, for instance, transported 41 % of the nitrogen and 25 - 50 % of the phosphorus from its leaves before abscission (Mitchell *et al.* 1986). Values of 3 - 25 % nitrogen and 33 - 66 % phosphorus were recorded for *P. repens* (Mitchell & Coley 1987) and of 70 % nitrogen in *Thamnocortus punctatus* culms (Stock *et al.* 1987).

High phenolic content in leaves affects their decomposition rates. Phenolic compounds are relatively inactive in healthy tissue but as senescence takes place they form complexes with proteins and become more condensed. This renders the substrate less favourable to bacterial and fungal decomposers (Read & Mitchell 1983). Schlesinger and Hasey (1981) note that the rapid decrease in soluble phenolics seen in the litter of *Ceanothus megacarpus* and especially *Salvia mellifera*, which they examined in the Californian chaparral, may represent the formation of complexes with protein. When the litter is analysed these are isolated with the lignin component which would explain the increase in lignin over time during decomposition that has been observed in some studies.

Thus high lignin and phenolic concentrations, together with low nitrogen and phosphorus concentrations and an unfavourable climate (the warm season is dry), lead to low decomposition rates in sclerophyllous, Mediterranean-type shrublands.

Schlesinger and Hasey (1981) calculated a mean turnover time of 2.5 years for the less lignified litter of *Salvia mellifera* and 4.6 years for the sclerophyllous shrub, *Ceanothus megacarpus*. Similar values have been found in other heathlands and Mediterranean-type shrublands, namely three years in *Quercus ilex* woodlands in the south of France (Lossaint 1973) and 3.8 years for coastal scrub in New South Wales

(Maggs & Pearson 1977). Mean turnover times appear to be much longer in fynbos with 20.4 years and 14.9 years having been calculated for *Protea repens* litter at Pella (coastal fynbos) and Swartboskloof (mountain fynbos), respectively (Mitchell & Coley 1987). Similar values have been recorded for other fynbos species, for example 14.4 years for *Leucospermum parile* and 11.4 years for *Thamnocortus punctatus* culms at Pella (Mitchell *et al.* 1986).

The nitrogen and phosphorus in litter is not released quickly. In the first year after litterfall, Schlesinger and Hasey (1981) actually found an increase in the amount of nitrogen and phosphorus in the litter of *Ceanothus megacarpus*. Turnover times for these elements were therefore not calculated. The increases were probably due to absorption from the environment and accumulation by the colonising microfauna. Specht (1981) found that *Banksia ornata* leaf litter in South Australia showed no change in nitrogen or phosphorus content after two years while Maggs and Pearson (1977) calculated a four year turnover for nitrogen and a two year turnover for phosphorus in New South Wales scrub. No significant mobilisation of nitrogen or phosphorus took place over the first 18 months of decomposition of *Leucospermum parile* litter at Pella and the turnover rates were calculated at 5.4 years for nitrogen, 124.7 years for phosphorus and 19.0 years for lignin (Mitchell *et al.* 1986). An increase in the phosphorus concentration of *Protea repens* litter was observed at Pella while at Swartboskloof the concentration of phosphorus in the same species remained constant; no turnover rates were thus calculated for phosphorus (Mitchell & Coley 1987).

These results probably reflect the unfavourable carbon to nitrogen and carbon to phosphorus ratios, as well as the high lignin content of sclerophyllous shrubland litter. This has an apparently detrimental effect on the growth of microorganisms capable of decomposition and nutrient mineralisation, for instance the carbon to nitrogen ratios of *Ceanothus megacarpus* only dropped from 80:1 to 64:1 in the first year of decomposition (Schlesinger & Hasey 1981). Net mineralisation probably only occurs at a ratio of 30:1 (Lutz & Chandler 1946 In: Schlesinger & Hasey 1981). This ratio could be as high as 230:1 for phosphorus (Lousier & Parkinson 1978).

With the restricted litter production, slow decomposition rates and little resulting mineralisation of nitrogen and phosphorus in Mediterranean-type shrublands, especially fynbos, it appears that fire probably plays the major role in returning

nutrients from the above-ground phytomass and litter to the soil and is thus an essential process within these ecosystems.

2.3. The role of fire in nutrient cycling in Mediterranean-type ecosystems.

The return of nutrients to the soil by means of fire is not an easily understood process. Nutrients which are not lost from the vegetation by volatilisation during the fire (see section 2.4) are deposited as ash. The amount released and deposited depends on the nature of the fire which may vary greatly in terms of heat, duration and degree to which the vegetation is burnt. This depends on the prevailing climatic conditions during the fire and the nature of the vegetation (Raison 1979). Ash is initially deposited on the surface of the soil but the lower levels of the soil are rapidly affected as nutrients are readily leached from the ash (Raison 1979; Stock & Lewis 1986a). The amount of nutrients released by fire varies greatly depending on the nature of the fire. Debano and Conrad (1978) found that 75 % of the nitrogen and 92 % of the phosphorus were released from the phytomass in chaparral. Various mountain fynbos communities showed similar levels with 53 - 100 % of the nitrogen and 50 - 100 % of the phosphorus released from the litter and phytomass (Van Wilgen & Le Maitre 1981).

The amount of nitrogen in the phytomass returned to the soil is likely to vary as nitrogen is readily volatilised. Increases in levels of soil ammonia have been observed in burnt areas as opposed to unburnt areas. Christensen & Muller (1975) reported immediate increases in ammonia levels in *Adenostoma* chaparral which remained high over the months following the fire. This was related to the high ammonia content of the ash. Nitrate concentrations were, however, similar for burnt and unburnt areas immediately after fire and increased over time as conditions for nitrification of the ammonia deposited in the ash improved (Christensen 1973). Similar increases in ammonia followed by nitrate have since been found in other studies, for example in sandplain fynbos (Stock & Lewis 1986a) and in *Eucalyptus regnans* forest in south-eastern Australia (Weston & Attiwell 1990). In a study by Stock and Lewis (1986a) the ammonia flush was of short duration while the nitrate increase was observed for over nine months before dropping to pre-fire levels. Ammonia release has been related to fire intensity. Weston and Attiwell (1990), for instance, found the highest levels of ammonia in a slash-fire plot which experienced the highest temperature during the fire. In experiments, this increased ammonia release has also been associated with higher temperatures e.g. chaparral soil samples

(Dunn & Debano 1977) as well as undisturbed soil and litter samples (Debano *et al.* 1979). The amount of ammonia produced was moderated, in both cases, by soil moisture content.

Total nitrogen in soils, on the other hand, has shown conflicting results. Some studies have reported increases (Christensen & Muller 1975; Debano & Conrad 1978; Rundel 1983; Stock & Lewis 1986a). Others have reported a loss of soil total nitrogen (St John & Rundel 1976) and a loss at the soil surface (Christensen & Muller 1975; Dunn & Debano 1977). Dunn and Debano (1977) and Debano *et al.* (1979) showed that a decrease in total nitrogen occurred in soil samples as well as soil and litter samples when heated above 200 °C. These conflicting results in field studies probably reflect differences in the intensity of the fire and the nature of the vegetation which would lead to differences in the amount of ash deposited.

Most of the phosphorus present in the phytomass and soil is likely to be present after the fire because phosphorus, unlike nitrogen, is not easily volatilised (see section 3). Debano and Conrad (1978), for example, could account for all the phosphorus present in the litter and phytomass as being present in the ash or charred remains of the plants after a chaparral fire. There may thus be an increase in soil total phosphorus after fire (Christensen & Muller 1975; Debano & Conrad 1978). Water soluble phosphorus has also been reported to increase after fire (Christensen & Muller 1975; St John & Rundel 1976). Brown and Mitchell (1986) found no increase in total phosphorus after a fire in the coastal fynbos at Pella. They did find a flush of resin extractable phosphorus, however, which lasted for four months following the fire. Here again, conflicting results probably reflect the nature of the fire and vegetation.

3. Losses of nitrogen and phosphorus due to fire.

As already mentioned, fire is not only the major agent mineralising nutrients from the phytomass and litter back to the soil, it can also be a major source of loss. The loss may be due to volatilisation, wind-blown ash or increased losses in run-off.

The amount of nutrients lost depends on the nature of the fire and vegetation. Using controlled fires over soil slabs, Debano *et al.* (1979) investigated the amount of nitrogen lost from the upper layers of the soil and the litter. They found that the rate

of loss increased linearly with fire intensity. In an intense fire, which may reach 700 °C at the litter surface, up to 90 % of the nitrogen may be lost from the litter and 50 % from the upper 20 mm of the soil. Van Wyk *et al.* (1992) obtained similar results for fynbos biomass where 80 % of the nitrogen was lost at temperatures of 400-500 °C. Debano and Conrad (1978) were also able to relate nitrogen loss to the amount of organic matter consumed by fire. Little nitrogen was lost when under 25 % of the organic matter was destroyed; with the destruction of 70 % of the organic matter, 44 % of the nitrogen was lost.

A 10 % loss of total nitrogen in the soil and litter was recorded under field conditions in chaparral (Debano & Conrad 1978). Few other studies have recorded losses under field conditions in Mediterranean-type ecosystems. Results from *Calluna* heathlands, however, suggest that it can be substantial with losses of 67-76 % (Allen 1964), 95 % (Chapman 1967) and 70 % (Lloyd 1971) having been recorded.

Loss of phosphorus due to fire could be far less than that of nitrogen. Debano and Conrad (1978) could account for all but 2 % of the total phosphorus in the combined litter, upper soil and phytomass in chaparral after fire. They suggested that 100 % of the phosphorus is returned from the biomass to the soil. Rundel (1983) could also find no loss of phosphorus in *Adenostoma* chaparral. Little further field evidence is available from Mediterranean-type ecosystems. Experimental results by van Wyk *et al.* (1992) have shown high (73 %) volatilisation of phosphorus from fynbos biomass at temperatures of 400 - 500 °C. Other experimental studies in *Calluna* heathlands in Britain have shown varying losses from the standing phytomass: 0.6 - 3.5 % (Allen 1964), 26 % (Chapman 1967) and 25 % and 39 % (Lloyd 1971). The loss of phosphorus, if it does take place in the field, may be due to wind blown ash (Rundel 1983).

Not only can fire cause loss of nitrogen and phosphorus via volatilisation and wind blown particles, it may also increase loss due to run-off. Increased losses of nitrogen and phosphorus have been observed in chaparral between burnt and unburnt areas. Most of this was in the form of plant litter (15 kg $N.ha^{-1}.y^{-1}$ and 3.37 kg $P.ha^{-1}.y^{-1}$). In the run-off water, however, only trace amounts of nitrogen and phosphorus were found (Debano & Conrad 1976 In: Debano & Conrad 1978). After a fire in Swartboskloof on the other hand, marginal increases in nitrates and phosphates were observed in spates (Van Wyk *et al.* 1992).

4. Input of nitrogen and phosphorus into the ecosystem.

4.1. Atmospheric deposition.

Nutrients lost from an ecosystem owing to the action of fire may be replaced via precipitation (wet and dry). The nitrogen concentration of rainfall for various heathland areas of Britain has been reported as 8.7 - 19.0 kg $N.ha^{-1}.y^{-1}$ (Allen *et al.* 1968) and 6.89 kg $N.ha^{-1}.y^{-1}$ (Gore 1968). These high nitrogen inputs may well be due to the heavy industries in Europe (Gore 1968).

Atmospheric inputs of nitrogen in the chaparral were found to be much lower at 1.5 kg $N.ha.y^{-1}$ although deposition rates nearer to large industrial cities may be higher (Schlesinger *et al.* 1982). Stock and Lewis (1986b) measured an input of 1.99 kg $N.ha^{-1}.y^{-1}$ for a coastal fynbos ecosystem at Pella. The sources of the nitrogen measured at Pella was thought to be oceanic in wet precipitation from north-westerly winds with little industrial input of dry sources of deposition (Stock & Lewis 1986b). These small inputs from precipitation are insufficient to replace the nitrogen lost due to the effect of fire in the south-western Cape or in California. In the chaparral ecosystems of southern California atmospheric inputs of 70 years would be necessary to replace the calculated loss of nitrogen from a single fire, which is similar to the values calculated by Stock and Lewis (1986b) for fynbos.

Brown *et al.* (1984) measured an input of 0.19 kg $P.ha^{-1}.y^{-1}$ for a coastal fynbos site at Pella. This is similar to the 0.2 -1.0 kg $P.ha^{-1}.y^{-1}$ (Allen *et al.* 1968) and 0.27 kg $P.ha^{-1}.y^{-1}$ (Gore 1968) measured for various heathlands in Britain. Again, oceanic and not industrial sources were thought to be the major source of the phosphorus deposited by rain at Pella. The annual input of phosphorus at Pella was calculated as 4.5 % of the mean available phosphorus content in the surface 10 cm of the soil (4.2 kg $P.ha^{-1}$) and may therefore be a major input into this ecosystem (Brown *et al.* 1984).

4.2. Nitrogen fixation.

4.2.1. Non-symbiotic nitrogen fixation in Mediterranean-type ecosystems.

Nitrogen fixation by free-living bacteria has not been extensively studied in Mediterranean-type ecosystems. Debano and Dunn (1982) report gains of 1.0 kg $N.ha^{-1}.y^{-1}$ in chaparral in California and they noted that fixation could be increased by adding a carbon source or by maintaining moisture conditions near field capacity. Poth (1982), for example, noted that the most favourable conditions for non-symbiotic nitrogen fixation started with the onset of winter rains when there is sufficient carbon due to the litterfall of summer, moisture levels are raised and oxygen partial pressures are low because of the soil microflora's rapid respiration. Similar conditions may exist in the fynbos at the onset of winter. Under these favourable conditions the bacteria present in chaparral soils appear to be capable of rapid reproduction.

Little is known about non-symbiotic nitrogen fixation in South Africa in general, and in the fynbos biome in particular. Two studies measured nitrogenase activity in soil samples from the savannah of the northern Transvaal but most of the activity could be attributed legume-*Rhizobium* associations (Grobbelaar & Rosch 1981; Zietsman *et al.* 1981). No other studies appear to have investigated rates of fixation but possible nitrogen-fixing bacteria have been discovered in various soils. Becking (1959) found *Azotobacter* and *Beijerinckia* in the soil of a Tokai arboretum and *Azotobacter* alone under a *Pinus taeda* plantation near Humansdorp. Neither of these species was in natural fynbos or forest, and Martin (1966) could not isolate *Azotobacter* in soils with a pH below 6 in fynbos near Grahamstowm. Van Reenen *et al.* (1992), on the other hand have recorded "surprisingly high" levels (0.5 - 18.4 millions /g dry soil) of apparent nitrogen-fixing bacteria in rhizophere and non-rhizophere samples of three soil types after a fire at Swartboskloof. These micro-organisms were often at the same or higher levels than the non-specific aerobic bacteria which were present. These were tentatively identified as being from the genus *Azotobacter*. Greatly increased levels of nitrogen fixation have been recorded in burnt as opposed to unburnt areas in *Pinus taeda* forests in South Carolina (Jorgensen & Wells 1971). This suggests that fire may be beneficial to the growth of free-living, nitrogen-fixing bacteria. Van Reenen *et al.* (1992) suggest that the levels of these bacteria in the fynbos at Swartboskloof may enhance the rates of replacement of nitrogen lost in this ecosystem. However, the actual amount of nitrogen fixed was not quantified.

4.2.2. Symbiotic fixation of nitrogen in Mediterranean-type ecosystems.

The techniques discussed above have been used in numerous studies to determine the role which symbiotic nitrogen fixation may play in replacing nitrogen lost via volatilization in Mediterranean-type ecosystems. Most of these studies have concentrated on the vegetation and nitrogen-fixing species, both legume and non-legume, of California and south Western Australia.

In California, the major putative nitrogen-fixing legumes include *Lotus scoparius* and various non-legume genera, *Ceanothus*, *Cerocarpus* (Rhamnaceae) and *Chamaebatia* (Rosaceae) which form symbiotic associations with the actinomycete *Frankia* (Virginia *et al.* 1989). Delwiche *et al.* (1965) found that 12 *Ceanothus* species growing in northern California were able to fix nitrogen at ecologically significant rates. Fixation rates for *Ceanothus velutinus* growing in Oregon have been calculated at 101 kg $N.ha^{-1}.y^{-1}$ (McNabb & Cromack 1983), 71.5 to 108.0 kg $N.ha^{-1}.y^{-1}$ (Youngberg *et al.* 1976). This contrasts strongly with the much lower nodulation and annual fixation found in southern California for *Ceanothus tomentosus* and *C. leucodermis* seedlings, which had rates of 0.38 kg $N.ha^{-1}.y^{-1}$ and 0.12 kg $N.ha^{-1}.y^{-1}$, respectively, (Ellis & Kummerow 1988) and a value of 0.10 g $N.ha^{-1}.y^{-1}$ for fixation by *Ceanothus greggii* (Kummerow *et al.* 1978). These authors suggest that the low moisture content of the soils of this area may be an important factor leading to lower fixation rates than those recorded in northern California.

The $\delta^{15}N$ natural abundance method has been used to survey plants of various Californian ecosystems for possible nitrogen-fixing ability. Delwiche *et al.* (1979) examined 54 native species and found that most of the suspected nitrogen fixers had lowered $^{15}N:^{14}N$ ratios. In a further study in various habitats in California, Virginia and Delwiche (1982) examined 176 plant species and found that presumed nitrogen-fixing plants in this study had lower ^{15}N abundance values (atom % ^{15}N values) as well as significantly higher nitrogen content than non-fixing counterparts. Neither of these studies attempted to quantify the amount of nitrogen which was fixed in these habitats, nor have any manipulative studies been done in the chaparral using this technique.

The manipulative and observational studies of nitrogen fixation in natural ecosystems which have been conducted in south Western Australia have concentrated mainly on legumes of the genera *Acacia* and *Kennedia* although the

cycad, *Microzamia riedlei* has also been shown to have ecologically significant nitrogen fixation rates (Halliday & Pate 1976; Grove *et al.* 1980).

Acacia spp. growing in various habitats in Australia have not proved to be exceptionally high in nitrogen fixation. For example, *A. pulchella, A. alata*, and *A. extensa* in Western Australian jarrah (*Eucalytus marginata*) forest near Perth fixed an average of less than 1.0 kg N $ha^{-1}.y^{-1}$ (Hansen *et al.* 1987b) while *A. pulchella* was recorded as fixing 2.2 kg N $ha^{-1}.y^{-1}$ in deep coastal sands near Perth (Monk *et al.* 1981). Lawrie (1981) has also reported rates below 1 kg N $ha^{-1}.y^{-1}$ for various *Acacia* spp. in the nutrient-poor heathlands of Victoria. These values should be compared to the 12.0 kg N $ha^{-1}.y^{-1}$ for *A. pellita* and 6.4 kg N $ha^{-1}.y^{-1}$ for *A. holosericea* measured on restored mining sites in the Northern Territory, Australia (Langkamp *et al.* 1979; Langkamp *et al.* 1982).

One of the reasons for the low nitrogen fixation rates found in plants growing in south Western Australia may be climatic. High soil temperatures and low soil moisture severely inhibit nitrogen fixation during summer as the nodules atrophy (Hansen & Pate 1987). Most nitrogen fixation takes place during the wetter months, reaching a peak during the warm, wet months of spring. Experimental additions of water to pot- and field-grown plants increases the nitrogen fixation in some of these species even during winter (Nakos 1977; Monk *et al.* 1981; Hansen & Pate 1987). Although the seasonality also applies to the Northern Territory sites mentioned before, the wet season is during the summer and the combination of higher temperatures and soil moisture may lead to higher fixation rates. The lower fixation rates in the Victoria heathlands may also be due to generally lower temperatures in this temperate region (Lawrie 1981).

Mineral nutrient content of the soils, especially paucity of phosphorus, may also restrict rates of fixation. In Western Australia it was shown experimentally that the maximum acetylene reduction for field grown *A. pulchella* was equivalent to pot-grown plants raised on very low phosphorus levels (Hansen & Pate 1987). Application of phosphorus to plants in the field greatly increased nodulation and acetylene reduction (Hingsten *et al.* 1982; Langkamp & Dalling 1982; Hansen & Pate 1987). Ecological studies have not shown other nutrients to be an important environmental factor for nitrogen fixation; *A. pulchella, Kennedia prostrata* and *K. coccinea*, for example, did not respond to additions of molybdenum or cobalt, and, in jarrah forests, molybdenum concentrations were not related to acetylene reduction rates (Hingston *et al.* 1982). Thus it appears that the soil moisture and

soil nutrient status may be important factors limiting nodule formation and the rate at which nitrogen is fixed in Mediterranean-type environments.

Few studies on nitrogen fixation have been undertaken in southern Africa in general. Fixation rates of between 6.3-8.5 kg N $ha^{-1}.y^{-1}$ were obtained for *Eragrostis pallens-Burkea africana* savanna in the Transvaal by the acetylene reduction method. Most of this fixation was attributed to legume-*Rhizobium* associations (Grobbelaar & Rosch 1981; Zietsman *et al.* 1988). Along an aridity gradient in Namibia, the contribution of fixed nitrogen to total-leaf nitrogen in 11 Mimosaceae species was estimated to be 30 % (Schulze *et al.* 1991). Apart from these three published studies no others have measured nitrogen fixation in southern Africa and no studies have previously been attempted in the south-western Cape. Extensive investigations into the nodulating ability of different southern African legumes have, however, been undertaken including many species from the Cape floristic region (Grobbelaar, *et al.* 1967; Grobbelaar & Clarke 1972; 1974; 1975; Grobbelaar & Van Rooyen 1979).

5. Methods for measuring nitrogen fixation.

A range of methods for measuring nitrogen fixation exists and the interpretation of nitrogen input results is often technique specific and thus should be borne in mind when attempting inter-study comparisons.

5.1. The acetylene reduction method.

The acetylene reduction method for determining nitrogen fixation has been used widely in agricultural as well as natural ecosystems. This is because it is simple, sensitive and of short duration. The test relies on the fact that the enzyme nitrogenase (which reduces N_2) is not specific for N_2 but will also reduce acetylene (C_2H_2) to ethylene (C_2H_4) (Hardy *et al.* 1968; 1973). The ethylene can be measured after a set time using gas chromatography. Normally, during nitrogen fixation, two protons, or sometimes more, are reduced for each N_2 molecule reduced. This is not the case with acetylene reduction where all the protons passing through the nitrogenase enzyme are reduced to ethylene (Gibson & Jordon 1982). Theoretically the ratio of acetylene reduced to N_2 reduced should be 3:1. This value has often been tested experimentally by comparing the total nitrogen accumulated by plants

dependent only on symbiosis with estimated acetylene reduction over the same time period. The ratio determined often differs significantly between and within species as well as by the way in which the calibration was conducted, for example whether the acetylene reduction was measured for whole plants, whole root systems or just the nodules (detached nodules have slower acetylene reduction rates (Bergensen 1970; Hansen *et al.* 1987a)). In order to obtain a more valid application of the acetylene reduction assay in the field, conditions should be highly standardized, for instance whether nodules or whole roots are used, etc.. The pot-cultured plants dependent on fixation for their nitrogen supply, on which the calibration is based, should have host-*Rhizobium* combinations and ages similar to the plants which are encountered in the field (Hansen *et al.* 1987a). These conditions are not easily met.

Many studies have used the acetylene reduction method to estimate the amount of nitrogen which fixing plants contribute to an ecosystem (See section 4.2.2.). In these studies the acetylene reduction measurements of samples of root nodules are taken and estimates of the total reduction over a time period made. Diurnal and seasonal variations in acetylene reduction should also be taken into account. In order to calculate the amount of nitrogen fixed for the nodule samples, the theoretical acetylene reduction to nitrogen reduction ratio of 3:1 is used or, in more thorough studies, the ratio is determined experimentally. The nodular biomass per plant or per ha. is then used to calculate the amount of nitrogen fixed in the area over the time period.

5.2. The $\delta^{15}N$ natural abundance method.

This method relies on the assumption that if a plant receives all its nitrogen from the air, it should reflect the ^{15}N:^{14}N ratio of the atmosphere whereas if it absorbs its nitrogen from the soil, it should reflect the ratio present in the soil (Shearer & Kohl 1986; 1989). (The ratio of ^{15}N to ^{14}N may be expressed for convenience as a $\delta^{15}N$ value (Formula 2.2.)). The δ15N value of the atmosphere is relatively constant (Mariotti 1983). A different signature may be induced artificially in the soil by adding small amounts of ^{15}N rich fertilizer (Ledgard *et al.* 1985 a,b,c), a method which is most suited to small field experiments or pot culture.

Ecological studies have relied on the fact that soils have naturally higher $\delta^{15}N$ values than the atmosphere owing to the fractionation during chemical and metabolic processes taking place in the soil. This has been found across a number of different environments, for example in the twenty states in the U.S.A. sampled by Shearer *et*

al. (1978) and the 18 sites within a 400 km^2 catchment site in Australia (Ledgard *et al.* 1984). A large variability in $\delta^{15}N$ values has been found in some studies but this appears to be atypical (Kohl *et al.* 1981). It is thus expected that the nitrogen-fixing plants will have a lower $\delta^{15}N$ than plants which obtain their nitrogen from the soil. As nitrogen-fixing plants in the natural environment do not obtain all their nitrogen from the atmosphere, they will probably have an isotopic signature somewhere between that of symbiotically dependent plants which are grown hydroponically on nitrogen-free nutrient solution and non-fixing reference plants. The fractional contribution of biologically fixed nitrogen to the nitrogen-fixing plant (FNdfa) may be calculated from the following formula (Shearer & Kohl 1986; 1989):

$$FNdfa = \frac{\delta^{15}N_o - \delta^{15}N_t}{\delta^{15}N_o - \delta^{15}N_a} \qquad \textbf{Formula 2.1.}$$

where $\delta^{15}N$ is the delta notation of ^{15}N abundance i.e.

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{\text{sample}}}{^{15}N/^{14}N_{\text{standard}}} - 1 \right) \times 1000\ \% \qquad \textbf{Formula 2.2.}$$

$\delta^{15}N_o$ is the $\delta^{15}N$ of plants which receive their nitrogen from the soil only (reference plants).
$\delta^{15}N_a$ is the $\delta^{15}N$ of the nitrogen-fixing plant grown hydroponically on nitrogen-free nutrient solution.
$\delta^{15}N_t$ is the $\delta^{15}N$ of the nitrogen-fixing plants growing in the field, i.e. plants which rely on both fixed nitrogen and soil nitrogen.

It is important to note that the $\delta^{15}N$ natural abundance method has the disadvantage that the difference in the abundance of ^{15}N between the nitrogen-fixing and the reference plant may be very small depending on the ^{15}N signature of the soil. Isotopic fractionation associated with various processes in the plant therefore becomes important. Three processes in the plant are of particular importance when using the $\delta^{15}N$ method; these are nitrate assimilation, nitrogen fixation and metabolism and transport of nitrogen within the plant (Shearer & Kohl 1989). The magnitude of this discrimination may be measured by the isotopic discrimination factor. In an open system where the substrate is essentially infinite or supplied and consumed at the same rate, the isotopic discrimination may be calculated from the following formula

$$\beta = \frac{{}^{15}N / {}^{14}N_{substrate}}{{}^{15}N / {}^{14}N_{product}}$$

Formula 2.3.

In closed systems where the ^{15}N abundance of both the substrate and the product increase during the reaction, the extent of the reaction must also be taken into account in Formula 2.3. leading to various modifications (see Shearer & Kohl 1986).

Isotopic fractionation associated with nitrate assimilation - The isotopic fractionation factor (ß) associated with this process is quite substantial in some cases. Kohl & Shearer (1980) observed values for ß of between 1.0067 and 1.0019 for soya (*Glycine max*), rye grass (*Lolium perenne*) and marigold (*Tagetes erecta*) seedlings. Mariotti *et al.* (1982) found that the isotopic fractionation was nil in mature pearl millet (*Pennisetum americanum* and *P. mollissimum*). In further experiments Shearer & Kohl (1989) found an average value of 1.00025 for ß in 38 species of two-month-old non-fixing higher plants. They concluded that isotopic fractionation in the nitrogen-fixing and non-fixing reference plant is not a problem as long as they are relatively mature plants actively growing in high light intensity and at moderate nitrate concentrations. The importance of any fractionation taking place is also lessened as the $\delta^{15}N$ value is measured directly, i.e. after absorption by the reference plant.

Isotopic fractionation associated with nitrogen fixation - Measurements of the isotopic fraction associated with nitrogen fixation have shown ß to lie between 0.988 and 1.002 in in soya, *Glycine max*, *Prosospis glandulosa* and *Dalea* spp. (Amarger *et al.* 1979; Shearer *et al.* 1980; 1984; Mariotti *et al.* 1980 In: Shearer *et al.* 1989; Shearer *et al.* 1989). Although these values are small, they are sufficiently large to introduce a significant error in the estimates of nitrogen fixation unless the $\delta^{15}N$ value of the nitrogen-fixing plant solely reliant on the atmosphere for its nitrogen supply (hydroponically grown and given nitrogen-free nutrient solution) is taken into account.

Isotopic fractionation associated with metabolism and transport - Shearer and Kohl (1989) note that the fraction associated with transport is probably nil but discrimination associated with various metabolic processes preceding transport may lead to differences in the distribution of isotopes across the plant. Distribution of ^{15}N across different non-nodular tissues has been found to be fairly uniform in

greenhouse and field grown annual plants. For instance, Shearer *et al.* (1980) found that in greenhouse-grown, nodulated as well as non-nodulated soya plants, different tissues deviated by only 2 ‰ from the values for the entire plant. Similar results have been obtained in a variety of other annual genera e.g. *Lupinus, Pisum, Vicia, Medicago, Trifolium, Cyamopsis* and *Dalea* spp. (Shearer & Kohl 1989). In the woody perennial, *Prosopis glandulosa*, however, Shearer *et al.* (1983) found that the trunkwood was depleted by 3.4 ‰. Values for other above-ground tissues in this species were similar to atmospheric values. Nodular tissue on the other hand has been found to be greatly enriched in ^{15}N in some species, e.g. 8.3 ‰ excess ^{15}N in soya (*Glycine max*) (Shearer *et al.* 1984), various crop plants and annuals, *Vigna ungiculata* (cowpea), *Phaseolus* spp. (beans), *Lupinus luteus* and *Trifolium subterraneum* (Shearer *et al.* 1982; Turner & Bergensen 1983) as well as trees, *Olneya tesota* and *Prosopis glandulosa* (Shearer *et al.* 1982; Virginia *et al.* 1984). The details of the mechanism for this enrichment remain unknown (Shearer & Kohl 1989). Unless the nodules form a large fraction of the plant's total nitrogen, this enrichment is unlikely to have a large effect on the $\delta^{15}N$ of the whole plant (Shearer & Kohl 1989). The modest difference in other plant parts may, however, still introduce significant errors into the estimation of the amount of nitrogen which is fixed when using the natural abundance method. Shearer and Kohl (1989) present evidence that the distribution of the ^{15}N in the nitrogen-fixing and reference plants is probably similar as long as similar tissues are used for the assessment of the $\delta^{15}N$ values of the reference and nitrogen-fixing plants. In this way errors due to metabolism and transport are kept to a minimum.

The choice of an appropriate reference plant is crucial as the method assumes that the ^{15}N abundance of nitrogen sources other than fixed nitrogen should be the same for the fixing and reference plants. If possible, the reference plant should be the same species grown on the same soil but without fixing nitrogen. This is not often possible due to the lack of nodulating isolines for most legumes, and therefore non-nodulating species from the same soil environment as the fixing plant have to be used. In this case it is preferable to choose plants with similar growth habits, e.g. rooting morphology and phenology, to the fixing plants. Virginia *et al.* (1989), for example, showed that *Ceanothus cuneatus* and *C. leucodermis* had lower $\delta^{15}N$ values and a higher nitrogen content than deep-rooted reference species from chamise chaparral but not the shallow-rooted reference species. In this case $\delta^{15}N$ decreased towards the soil surface and thus comparison with a shallower rooted reference species would have been inappropriate as this would lead to the conclusion that the plants were not fixing nitrogen. Virginia *et al.* (1989) also note the

importance of choosing the correct reference plant in chaparral systems as the $\delta^{15}N$ value of the soil in some of these environments may be very low. In such circumstances the determination of $\delta^{15}N$ of the nitrogen-fixing plant solely reliant on fixed nitrogen (i.e. hydroponically-grown on nitrogen-free nutrient solution) is critical for determining whether nitrogen fixation is taking place. This value takes into account fractionation in the plant resulting from the nitrogen-fixing process and is of great importance in cases where the difference between the $\delta^{15}N$ value of a plant receiving all its nitrogen from the soil and another receiving all its nitrogen from the atmosphere may be very small.

The advantage of the $\delta^{15}N$ natural abundance method is that it does not rely on the application of ^{15}N fertilizers which are used in other dilution methods. When compared with the acetylene reduction method it has the distinct advantage that it is an estimation of the fixation which has taken place since the tissues being measured were formed. It is not an instantaneous method which must be extrapolated over time. Dead or dried plant material may also be used. Like the acetylene reduction method, the $\delta^{15}N$ natural abundance method may also be used to estimate the amount of nitrogen fixed per plant or per unit area over a given time period. This is obtained by using the ratio of fixed nitrogen to soil obtained nitrogen, if the total increase of nitrogen for the plants over the same period is also determined. It has been used to calculate nitrogen fixed by soybeans (Amarger *et al.* 1979) and *Prosopis* woodland in the Sonoran Desert (Rundel *et al.* 1982).

The $\delta^{15}N$ natural abundance method has been compared with other methods of determining nitrogen fixation. Most of these tests have been in the form of pot experiments due to the difficulty of controlling the different variables in the field. For example, the results obtained for fixation via the natural abundance technique were strongly correlated with those of a nitrogen-yield experiment for nodulating and nonnodulating soybeans (*Glycine max*) cultivars grown on nutrient-poor soil in pot culture, field results however, gave a weaker correlation (Kohl *et al.* 1980). In soybeans, acetylene reducing activity and nitrogen fixation measured by means of the $\delta^{15}N$ natural abundance method were correlated (Amarger *et al.* 1979) as were the ^{15}N natural abundance estimates and estimates from a ^{15}N fertilizer application method for field-grown clover (*Trifolium subterraneum*) using rye grass (*Lolium rigida*) as a reference plant (Ledgard 1958b).

Some of the most notable studies in natural ecosystems in which use has been made of the ^{15}N natural abundance method are those based in the *Prosopis glandulosa*

dominated ecosystems of the Sonoran Desert of the U.S.A.. Nodules are not always found on the roots of *Prosopis* trees in the wild, especially in the drier, western parts of their range (Johnson & Mayeux 1990). The high production of these trees, which rely on deep supplies of ground water, suggests that the trees are able to fix nitrogen with nodules which are located at depth. The $\delta^{15}N$ natural abundance method was used to confirm the fact that these trees may well fix nitrogen (Shearer *et al.* 1982), as well as to calculate the amount of nitrogen which they fix, 40 kg $N.ha^{-1}.y^{1}$ (Rundel *et al.* (1982)). The N_2-fixing ability of the trees was confirmed by removing the surface roots that were in contact with most of the soil nitrogen. The ^{15}N content of the trees decreased as the trees then had to rely to a greater extent on fixed nitrogen (Virginia *et al.* 1989). These studies illustrate the usefulness and versatility of the $\delta^{15}N$ natural abundance method for environmental investigations.

It is important to note that for both the acetylene reduction method or the $\delta^{15}N$ natural abundance method, meaningful interpretation of the results relies on measurement of the nitrogen cycling being made through the ecosystem, otherwise it is difficult to determine whether the amount fixed is a meaningful contribution to the ecosystem's nitrogen balance. Fine examples of such thorough studies are those mentioned above on *Prosopis* ecosystems, and those of Vitousek *et al.* (1987) and Vitousek & Walker (1989). In these studies it was shown that *Myrica faya*, an invading species on newly formed volcanic rock in Hawaii, can quadruple the quantity of nitrogen entering the ecosystem. This extra nitrogen then facilitates the colonisation of a range of other species. This illustrates the influence nitrogen-fixing species may have on ecosystem composition and development.

6. Legumes in fynbos ecosystems.

6.1. The importance of legumes, in particular the genus *Aspalathus* in the Cape Flora.

Legume species are a common feature of the Cape Floristic Region of South Africa (Lamont 1982). The Fabaceae form the fourth largest family in the region as a whole, with 644 species in 38 genera. There is also a high degree of endemism and speciation with 525 of these species from eight genera endemic to the region (Bond & Goldblatt 1984). In the floras of six different areas within the Cape Floristic

Region, the Fabaceae feature as the second largest family in four areas and third largest in two (Cowling & Holmes 1992).

The genus *Aspalathus* is of particular interest as it is the most commonly represented genus in the Fabaceae and the second largest in the Cape floristic Region. It holds this position in four out of the six local flora investigated by Cowling & Holmes (1992). In his revision, Dahlgren (1988) divided *Aspalathus* into 274 species. Many of these species occur over a limited geographical range and appear to be very particular about the edaphic conditions in which they occur (Dahlgren 1963; 1968).

Initial work has been done on the ability of *Aspalathus* to form nodules and the specificity of the nodule-forming bacteria. Fifty species of *Aspalathus* were investigated in the field (Grobbelaar & Clarke 1972) and found to contain nodules. The *Rhizobium* strain found in these nodules appears to be specific to the genus as the bacteria from *A. linearis* would not induce nodule formation in 15 other legume genera tested nor could 14 stains of bacteria from other legume species induce nodule formation in this species (Staphorst & Strijdom 1975). Five out of six *Rhizobium* strains from different *Aspalathus* species were able to induce nodule formation in *A. linearis* indicating that the *Rhizobia* may not be very specific between species within the genus (Deschodt & Strijdom 1976). Apart from these studies little is known about the ability of *Aspalathus* spp. to fix nitrogen.

6.2. Legumes and fynbos fire ecology.

6.2.1. Fire survival strategies of *Aspalathus* and other fynbos legumes.

Fire has not always been considered an appropriate treatment for fynbos vegetation (e.g. Levyns 1929). Authors in fynbos ecology in the earlier part of this century recognised the importance of understanding plant succession after fire and determining whether the resulting community would eventually resemble the plant community present before the fire. (Levyns 1929; Adamson 1935). Wicht (1948) came to the conclusion that fire may be a natural process in the fynbos and that it is responsible for its maintenance.

It now appears that fynbos plants have adapted in different ways to survive fire - so-called survival strategies or life forms. As the vegetation ages after a fire, the relative abundance of these different life forms changes with some plants reaching

the end of their life spans as other, slower growing plants reach their full adult size (see section 5.2.2.).

Noble & Slatyer (1980) categorised species according to the vital attributes which help them survive a disturbance: firstly by their mechanism of persistence during a disturbance, or the arrival of propagules after the disturbance; secondly by their ability to establish either directly after the disturbance or later in the succession; and thirdly, by the timing of critical events in their life histories. Bell *et al.* (1984), on the other hand, have placed species into five categories according to their survival response to fire.

Few studies have examined survival mechanisms of different fynbos plant species in detail. Van Wilgen (1981) and Van Wilgen & Kruger (1981) classified plant survival strategies only as germinative, vegetative or unknown, while others have concentrated on the survival type in a single family such as the Proteaceae (Bond *et al.* 1984)). In a more wide-ranging study, Van Wilgen & Forsyth (1992) examined the species in ten, permanently marked plots in Swartboskloof and noted the vital attributes (Noble & Slatyer 1980) and the response to fire (Bell *et al.* 1984) for each species. In terms of fire response (Bell *et al.* 1984), most species (62%) were auto-regenerating, long-lived sprouters (ALS). As far as the Fabaceae of Swartboskloof are concerned, this included the genera *Cyclopia, Indigofera, Otholobium, Podalyria, Psoralea, Rafnia* and *Rhynchosia*. So-called fire ephemerals made up 6% of the species and included the genus *Aspalathus*. According to Noble & Slatyer's (1980) first two categories, the most common vital attribute type was (UI) - plants which sprout from mature tissue and do not establish later in the succession. This included all the Fabaceae genera mentioned above as ALS. *Aspalathus* fell into the (SI) group which made up 12% of species, i.e. those species which regenerate from soil-stored seed banks and do not regenerate later in the succession. According to the timing of critical life history events, most species (76%) fell into the category which matures within the youth phase of the succession (see below). This includes many species classified as ALS by their fire response and UI in terms of their vital attributes. *Aspalathus* fall into a group in which life-cycles are completed before the mature phase is reached. Their seed remains in the soil from which they regenerate after the next fire.

Van Wilgen & Forsyth (1992) note in their study that *Aspalathus ciliaris* present at Swartboskloof had a life-span of six years and a seed survival time in excess of 44 years. The life-span of the plant and the life-span of the seed are important in

determining whether a species will survive in an area under different fire regimes. If a species is short-lived, it must either have a long-lived seed bank, or it must be reintroduced from elsewhere if it is to reappear in the succession after fire. Longer lived species, however, may have seed with a much shorter life-span. Proteas which have canopy-stored seed banks (CI) are a well studied example of the interaction of fire frequency and seed attributes. Under short rotation fires, the plants do not have sufficient time to produce a large enough seed bank in their canopy before the next fire and will rapidly die out (Van Wilgen 1981). Should the interval be too long, however, the seed reserves appear to be reduced with the senescence of the parent plants and regeneration is reduced (Bond 1980). Legumes and *Aspalathus* species in general have very hard, long-lived seeds. These plants would therefore probably be most affected by very short fire intervals which would not allow the plants enough time to mature and produce seeds (intervals of less than two years between fires are unlikely). This would lead to the eventual depletion of the seed bank (Kruger 1984).

6.2.2. Legumes and pyric succession in fynbos ecosystems.

Kruger (1979) and Kruger & Bigalke (1984) recognise five phases in the succession of fynbos vegetation after fire:

1. Immediate post fire stage (0-1(2) years): - Seed germination and vegetative sprouting of almost all species. Various geophytes and most of the annuals reproduce in this phase.
2. Youth phase (2-4(5)years): - The community becomes dominated by restionaceous and graminoid shrubs (Restionaceae and Cyperaceae especially). Sprouting shrubs reach reproductive maturity. Opportunistic shrubs (e.g. *Aspalathus* spp., *Othonna quinquedentata*) set seed and die. Taller shrubs begin to emerge from the canopy which begins to approach pre-burn levels.
3. Transitional phase (4-10 years): - All plants now attain reproductive maturity. Tall shrubs emerge and start to adopt ascending branched habit. ·
4. Mature phase (10-30 years): - Tall shrubs reach their maximum height and rounded form. Seed regenerating low shrubs (e.g. *Erica* spp.) begin to die. Litter accumulates rapidly and there is a negligible amount of seed germination or seedling survival.
5. Senescent phase - Accelerated mortality in seed regenerating shrubs, their crowns become sparse and they begin to collapse. There may be very limited seed regeneration in open spaces.

These successional stages have been observed in various mountain fynbos communities, although they may differ in terms of their floristics and the time over which the succession takes place (e.g. Levyns 1929, Adamson 1935, Martin 1966, Wicht 1948, Taylor 1978). In these studies the succession at a particular site was observed as it occurred over a number of years. Others have compared different aged sites in similar habitats and inferred successional patterns. Van Wilgen (1981) for example, compared plots of different ages at Swartboskloof : four years (youth phase), 21 years (mature phase) and 37 years (senescent phase). There appeared to be greater cover of Restionaceae and Cyperaceae in the young plots when compared to the mature and senescent sites and greater cover of Proteaceae (*Protea neriifloia* and *P. repens*) in the mature and senescent plots than in the young plots, as would be expected from Kruger's (1979) successional stages. Asteraceae and Ericaceae did not fall into the predicted pattern. These families had a greater cover in the young site when compared to the mature site but there was no difference between the young and old sites. Hoffman *et al.* (1987), in a similar study on sandplain lowland fynbos at Pella, found Restionaceae to be more important in terms of cover in the older stages, and annuals to be more important throughout the succession at this site. These two studies illustrate the important information about pyric succession which may be gathered in this manner (comparing sites of different ages), especially when comparing the early to the very late phases in succession. It must be borne in mind, however, that they cannot replace observing the succession itself in any particular ecosystem. Both these approaches have been used in studies which have made special mention of legumes in pyric succession in the fynbos and other Mediterranean-type ecosystems.

Many of the species in the family Fabaceae appear to be either short-lived fire ephemerals which complete their life cycles in or before the transitional phase of the succession, or are longer lived shrubs capable of resprouting (Van Wilgen & Forsyth 1992). The large genus *Aspalathus* falls into the first group and may form dense stands in the first year after fire and die out after a few years. This has been observed in a number of different studies.

Adamson (1935) described *Aspalathus chenopoda* as being present in considerable numbers in the vegetation 4½ years after a fire on Table Mountain in 1927. About 6½ years later, the *Aspalathus* had died away completely. Levyns (1935) described the succession after a veld-burning experiment in the Renosterbosveld near Riversdale. *Aspalathus spinosa* formed the dominant species 3 years after a fire and *A. microdon*, a year later, when *A. spinosa* was already showing signs of

senescence. The experiment was terminated but she speculated that *A. microdon* would rapidly be replaced by renosterbos (*Elytropappus rhinocerotis*).

Apart from *Aspalathus*, other genera in the Fabaceae belonging to different regenerational types have been reported as less common in older vegetation when compared to similar, recently burnt areas. Martin (1966), for example, reported a 245 % increase in the density of the Fabaceae genera, *Amphithalea*, *Indigofera*, *Psoralea* and *Tephrosia* in a one-year old, burnt area of grassy fynbos near Grahamstown. Van Wilgen (1981) observed a mean cover of 4.5 and 3.3 % of *Rafnia capensis* for two four-year old sites; this species was absent from the nearby mature (21 years old) and senescent (37 years old) sites. Few studies in the fynbos or elsewhere have examined the cover of Fabaceae or legumes as succession proceeds or between sites of different ages. Hoffman *et al.* (1987) recorded a mean percentage cover for Fabaceae between 3 and 8 % for the first five years after fire and 1 and 2 % for sites aged 12, 15 and 19 years after fire in sandplain fynbos near Pella.

Dense stands of legumes which die out rapidly as well as a decrease in legumes in older vegetation have been observed in other Mediterranean-type ecosystems. Hanes (1971) reported a cover of 13.1 % for *Lotus scoparius* in coastal facing stands of chaparral, 26 % of these plants were dead in 2 - 8 year old plots. In plots older than nine years its cover had dropped to 0.2 % with up to 50 % of the plants having died. Westman (1981), working in Californian sage scrub, reported a drop in cover of five legume species (three species of *Lupinus* and two species of *Lotus*) from 11.5 % to 1,5 % between the first and seventh year after fire, respectively. After 21 years, legume cover had decreased to almost zero. Short-lived, fire ephemeral legumes are also important in the jarrah (*Eucalyptus maginata*) forest in south Western Australia. For example, the disappearance of *Acacia*, *Bossiaea* and *Kennedia* approximately six years after fire is responsible for the decrease in diversity of these forests seen at such times (Shea *et al.* 1979; Bell & Koch 1980). In general it appears that little is known about the importance of these stands in the functioning of Mediterranean-type ecosystems, especially in fynbos.

7. Conclusion.

In this literature review I have tried to set a background for this thesis from the present scientific knowledge of nitrogen fixation in Mediterranean-type ecosystems. I have highlighted areas where there is insufficient knowledge and understanding at present. This thesis aims to further our understanding by investigating aspects of the ecology and nitrogen-fixing ability of *Aspalathus* spp..

Chapter 3

Study sites.

1. Introduction.

In order to conduct this study, nine study sites were selected in the fynbos biome of the south-western Cape. The study sites were of two different types:

Firstly, there were seasonal study sites at which data were collected several times during the course of a year in order to determine seasonal trends. These study sites were at Cape Point I, Scarborough and Silvermine all of which, for logistic reasons, were on the Cape Peninsula. Their location is indicated in Figure 3.1. The study sites were visited every six weeks (May 1991 - June 1992) for collection of nodule cores, and three times, at six-monthly intervals, in order to collect vegetation and soil nutrient data (See Methods in Chapters 4, 5 and 6).

Secondly, there were geographic survey study sites which were included to increase the number of species and habitats sampled so that the study would be more representative of the fynbos biome as a whole. These study sites were visited on a once-off basis in spring 1991 and nodule cores, vegetation data and soil nutrient data were collected. The geographic survey sites included Bainskloof, Cape Point II, DuToitskloof, Groot Hagelkraal, Jonkershoek I and II, Orangekloof and Pella. The location of these sites is indicated in Figure 3.1..

Each of the study sites, for both the seasonal and geographic survey, had to comply with certain conditions. Each had a fairly recently burnt stand of vegetation (1-3 years previously) which contained a dense stand of *Aspalathus* plants. Such an area was chosen to be as close as possible to an unburnt area of older vegetation.

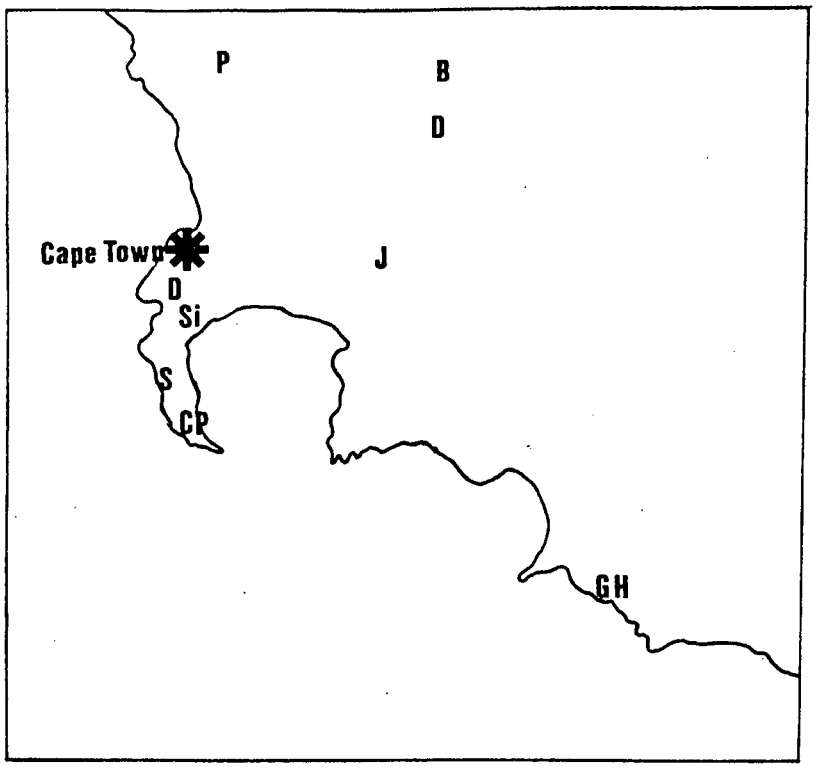


Figure 1. The location of the 11 study sites in the south-western Cape Province, South Africa. The letters represent the following study sites: i. Seasonal study sites: CP - Cape Point I; S - Scarborough and Sil - Silvermine. ii. Geographic survey study sites: B - Bainskloof; CP - Cape Point II; D - DuToitskloof; GH - Groot Hagelkraal; J - Jonkershoek I and II; O - Orangekloof and P - Pella.

The study sites at Silvermine and Scarborough had stands of vegetation of three different ages. The vegetation at each site could fall into one of three age categories; these were young (1- 3 years post-fire), middle-aged (4 - 9 years post-fire), and old (10+ years post-fire). The soil forms used in the study site description conform to the South African binomial system as described in the Soil Classification Group (1991).

2. Bainskloof.

Bainskloof Pass crosses the Boland mountains from Wellington to Woolsley. A geographic survey study site was situated at Eerste Toll, at the top of the pass on its western side (33° 6' S; 33° 37' E). A large section of these mountains was burnt by a wild fire in the first half of 1989. The study site was a fairly level area on the mountain spur (altitude 650m). The burnt, western side of the spur provided a stand of young vegetation while the unburnt, eastern side provided a middle-aged site. The study area was very rocky (Table Mountain Sandstone). The Mispah form soils were very shallow consisting of coarse, acid sand weathered from Table Mountain Sandstone.

The Mesic Mountain Fynbos (Moll *et al.* 1984) of the area does not appear to have been studied in detail. Using Campbell's (1985) classification (see 6) the site could be classified as Mesic Restioid Fynbos. Restioid Fynbos is of short stature with a high graminoid cover, especially Restionaceae, and a low shrub cover. Mesic Restioid Fynbos occurs in moister environments and has a higher ericaceous and total cover than other types of Restioid Fynbos.

3. Cape Point Nature Reserve.

Cape Point Nature Reserve comprises the most southern portion of the Cape Peninsula, which juts out about 30 km from the Cape Coast into the South Atlantic Ocean (34° 15' S; 18° 25' E). Two study sites were selected within the reserve: a seasonal site (Cape Point I) and a geographic survey site (Cape Point II).

3.1. Cape Point I.

The seasonal study site was on both sides of the road to the Point and about four kilometers from the reserve gate (34° 17' S; 18° 27' E). It lay across two fire management blocks. The western side of the road formed the young site which was last burnt in April 1989. The other side of the road was middle-aged, last burnt in February, 1986. The landscape in this part of the reserve consists of low, rocky hills which lie on the plateau west of the coastal mountains of the western edge of the peninsula (altitude 140m). The study site was rocky with much exposed Table Mountain Sandstone. The Mispah form soils were shallow, fairly well drained, coarse-grained, acid sands weathered from Table Mountain Sandstone (Smith-Baillie *et al.* 1976).

The vegetation of the reserve was described as Mesic Mountain Fynbos (Moll *et al.* 1984) and has been described in detail by Taylor (1983; 1984). The vegetation at this site is described by Taylor (1984) as Upland Mixed Fynbos which, when it is mature, has three layers. The tallest layer is of scattered, broad-leafed shrubs (e.g. *Leucadendron laureolum* and *Leucospermum conocarpodendron*). Under this is a layer of fine-leafed, ericaceous shrubs mixed with taller, restionaceous species (e.g. *Staberoha* spp., *Thamnocortus* spp. etc.). The third or ground layer is comprised of tufted Restionaceae and Cyperaceae. In its newly burnt state, this community

consists of emergent shrubs, annuals, geophytes and a dominant Cyperaceae and Restionaceae element.

3.2. Cape Point II.

The geographical survey study site at Cape Point was situated south of the road leading to Olifantsbos (34° 15' S; 18° 27' E). This site consisted of a middle-aged site only as there was no nearby unburnt area. The area was last burnt in February 1986. The site lies on a plateau (altitude 50 m) with little exposed rock in the area. The soils are of the Fernwood form consisting of fairly deep colluvial sands weathered from Table Mountain Sandstone while the shallower soils are of the Mispah form (Smith-Baillie *et al.* 1976; Taylor 1984).

The vegetation at this site is what Taylor (1984) described as Restionaceous Fynbos. This is a mosaic of Upland Mixed Fynbos and Tussock Marsh communities. The hill flora predominates on the slightly higher ground and the marsh flora on the lower ground which is seasonally inundated with water.

4. DuToitskloof.

This geographic survey study site lies on the old section of the Dutoitskloof Mountain Pass above the Huguenot tunnel, a major thoroughfare though the Boland Mountains (33° 44' S; 19° 5' E). A wildfire in the first half of 1990 burnt a large section of the vegetation of the mountains in this vicinity providing a stand of young vegetation. A stand of old-aged vegetation was on an adjacent area which had been protected from the fire by a shallow gully. The study site is a fairly steep scree slope (altitude 550m) strewn with rocks and boulders of Table Mountain Sandstone. The soil is shallow and a product of the underlying granite and the Table Mountain Sandstone of the upper slopes of the mountain.

The vegetation of the site was described as Mesic Mountain Fynbos by Moll (1984). No detailed vegetation studies have been done in the immediate area. Using Campbell's (1985) classification of mountain vegetation of the fynbos biome, however, the vegetation at the site may be classified as Talus Asteraceous Fynbos. Asteraceous Fynbos has a high grass and non-ericaceous shrub cover. Talus Asteraceous Fynbos is characteristic of many stabilized scree slopes. The presence

of *Protea nitida* provides this fynbos type with a 2 - 5 m stratum not found in other Asteraceous fynbos. This community is therefore similar to the "Waboomveld" found at Jonkershoek I and Orangekloof.

5. Groot Hagelkraal.

A geographical survey study site was selected on the farm Groot Hagelkraal which lies 22 km south-east of Gansbaai on the Cape south-west coast (34° 40' S; 19° 35' E). A wild fire which burnt most of this farm in February 1989 provided two stands of young vegetation. One of these was on acid sands and the other on the northern slope of a nearby limestone outcrop. An unburnt section of vegetation across a small dirt track provided a corresponding old-aged stand for the acid sand community. Unfortunately there was no corresponding old-aged site for the limestone community.

The area of the farm consists of an undulating plain (altitude 50m) which is bordered on the landward side by low, steep-sided, limestone hills and outcrops. The soils at the site are derived from Miopliocene dune limestones of the Bredasdorp group. The limestone outcrops are very rocky with Mispah form soils which are very shallow and alkaline to neutral. At the foot of the outcrops are deep colluvial sands which tend to be acidic (Thwaites & Cowling 1988).

Cowling *et al.* (1988) described the vegetation of this area according to the approach of Campbell (1985) in which structural characters, higher taxa and dominant species are used to classify mountain vegetation of the fynbos biome, the vegetation for both the limestone outcrops and the acid sands falls into Cowling *et al.*'s (1988) Proteoid Fynbos category. However, the limestone and the acid sand have very different communities. In its mature state the limestone community is characterised by the Proteaceae *Protea obtusifolia* and *Leucadendron meridianum* whereas the acid sand community has *P. sussanae* and *L. coniferum*. The latter differs from the former structurally in sometimes having a higher cover of ericoids and restioids. Floristically, there is an exceptionally high species turnover between these communities (Thwaites & Cowling 1988).

6. Jonkershoek.

The Jonkershoek State Forest Reserve lies 15 km from Stellenbosch. Jonkershoek is a steep-sided valley cut into the Hottentots' Holland Mountains by the Eerste River. A wild fire in December 1988 provided two geographic survey study sites, the first on a north-facing slope at the furthest end of the ring road into the valley (33° 59' S; 18° 58' E) (Jonkershoek I), the second site on a gently sloping, south-facing slope further into the valley (33° 59' S; 18° 58' E) (Jonkershoek II). The soils and vegetation of the nearby (1.5 km) Swartboskloof, which is an offshoot of the main Jonkershoek valley, have been well studied as this was one of the Fynbos Biome Project's intensive study sites (Lambrechts *et al.* 1986 (soils); Weger *et al* 1972; McDonald 1985 and Van Wilgen & McDonald 1992 (vegetation)).

6.1. Jonkershoek I.

This site was on the lowest, boulder-strewn slopes of the valley (altitude 375m). The young vegetation was on the mountain side of the ring road while an unburnt area over the road had old-aged vegetation. The study site was a scree slope strewn with large boulders of Table Mountain Sandstone. The soils consist of a mix of underlying granite and upper slope Table Mountain Sandstone. This gives rise to yellow-brown soils of the Oakleaf form (McDonald 1983; Lambrechts *et al.* 1986).

The vegetation of the valley is classified as Mesic Mountain Fynbos according to Moll *et al.* (1984). The community at this site corresponds well with the *Ischyrolepis gaudichaudiana - Myrsine africana*, high closed shrubland described by McDonald (1983; 1985) for Swartboskloof. This community, in its mature state, has a high (2 - 5 m) stratum with *Protea nitida* and *Protea nerifolia*, a tall (1 - 2 m) stratum of, for example *Cliffortia cuneata, Diospyros glabra*, and a short (< 1 m) stratum with, for example, *Montinia caryophyllaceae, Ischyrolepis gaudichaudiana*. It is also referred to as Waboomveld which is characteristic of scree slopes. In its post-fire state the tall and high strata are less evident and the *P. nitida* are resprouting.

6.2. Jonkershoek II.

This site was south-facing and more level than Jonkershoek II (altitude 400 m). There was no corresponding old-aged vegetation. The site contained no rock cover

and has soils which correspond to the Champagnes soil form described for Swartboskloof (McDonald 1983; 1984). These are derived from a mixture of granite and Table Mountain Sandstone and are deep, moderately drained, very humus-rich and black.

The vegetation at this site will probably develop into the *Myrsine africana - Olea europeae* subsp. *africana* community which grows on the Champagne soil form in Swartboskloof (McDonald 1983; 1985) and shares some of the species present at the site. However, the high cover of *Aspalathus* and the lack of a nearby unburnt site make comparisons difficult.

7. Orangekloof.

A geographic survey study site was located at Orangekloof, a valley situated at the southern side of the Table Mountain Massif (34° 0' E; 18° 24' S). A service road runs around the sides of the valley at about mid-slope (altitude 300 m). A wild fire in 1989 burnt a large area of vegetation on the mountain above this road. The vegetation below the road has been protected from fire for approximately 30 years. An area above the road on a west-facing slope contained a stand of young vegetation while the opposite side of the road had old-aged vegetation. The study site has a fairly steep gradient with a high rock cover of Table Mountain Sandstone. The Mispah form soils at the site are generally shallow and sandy, being weathered from a mixture of Table Mountain Sandstone and the underlying Cape Series shales.

The vegetation of Orangekloof is Mesic Mountain Fynbos (Moll *et al.* 1984). It was described in detail by McKenzie *et al.* (1977). The community present at the study site is the *Ischyrolepis gaudichaudiana - Lobostemon glaucophyllus* community. In its mature state it has a tall, woody component including *Protea nitida*; a restioid-shrub layer including *Ischyrolepis gaudichaudiana* and *Lobostemon glaucophyllus* and a low, graminoid-cyperaceous layer. It is therefor similar to the "Waboomveld" at Jonkershoek and DuToiskloof. In recently burnt vegetation the *P. nitida* and restionaceous elements are resprouting.

8. Pella.

This geographical survey study site was on the farm, Burgher's Post, near the Moravian village of Pella, 40 km north of Cape Town and 15 km inland of the Atlantic Ocean (33° 31' S; 18° 32' E). Part of this farm comprised one of the intensive study sites for the Fynbos Biome Project. An extensive wild fire in November 1986 provided a middle-aged stand of vegetation while a small, unburnt section provided an old-aged stand. The latter area was last burnt in November 1980. The landscape at Pella is undulating and gently sloping (altitude 200m). There was no surface rock present at the study site. The soils at Pella consist mainly of reworked parent material and medium sands of aeolian and later colluvial origin. This has been subjected to weathering and the later accumulation of iron (Lambrechts & Fry 1988). The Clovelly soil form is present at the study site.

The vegetation of the area around Pella was described by Moll *et al.* (1984) as Sand Plain Lowland Fynbos. Boucher and Shepherd (1988) described in detail the vegetation at the Pella Fynbos Biome Project intensive study site. Their *Thamnocortus punctatus - Leucospermum parile* community is present at the study site. In its mature state this community consists of three layers: a sparse mid-high shrub layer of broad and narrow-leafed, sclerophyllous shrubs (e.g. *Anthospermum aethiopicum, Passerina vulgaris and Leucospermum parile*); a mid-dense graminoid-low shrub layer dominated by Restionaceae (e.g. *Thamnocortus punctatus, Wildenowia sulcata* and *Staberoha distachya*) and lastly, a herb-dwarf shrub layer of narrow-leafed, sclerophyllous shrubs (e.g. *Phylica* spp. and *Stoebe fusca*) and herbaceous plants. When young, these layers cannot always be distinguished. If they can, the Proteaceae tend to be less prominent and contribute, with the Restionaceae, to the middle stratum along with various sprawling shrubs which are commoner in young vegetation.

9. Scarborough.

The village of Scarborough lies on the western side of the Cape Peninsula, on the border of the Cape Point Nature Reserve, approximately 20 km from Cape Point (34° 12' S; 18° 22' E). A seasonal study site was selected on the hillside above the houses (altitude 100 m). The vegetation was of three ages owing to the burning of

two fire breaks. Closest to the houses (± 50 m away) was a section of vegetation which had not been burnt for 12 - 18 years, and thus provided a stand of old-aged vegetation. Above this was the first fire-break which was burnt in May 1990, forming a stand of young vegetation and the second fire-break, burnt in 1987, forming a middle-aged stand. The study area has a gentle slope and surface rocks occurred in the stand of young vegetation. The middle-aged and old-aged stands had less exposed rock. The Mispah form soils present consist of coarse, acid sand weathered from Table Mountain Sandstone (Smith-Baillie 1976). They were shallowest under the young stand and slightly deeper in the other two stands of vegetation.

The vegetation of the area is Mesic Mountain Fynbos (Moll *et al.* 1984) and similar to that of the Cape Point Nature Reserve. It has not been described in any published phytosociological study. In its mature state, the community at the study site appears to be similar in structure to that found at Cape Point I and these sites have many species in common (see section 3.1) i.e. Upland Mixed Fynbos (Taylor 1983; 1984).

10. Silvermine.

Silvermine Nature Reserve includes an area of uplands and mountains which lies midway along the mountain chain of the Cape Peninsula (34° 6' S; 18° 25' E). The area is rugged and only accessible via Ou Kaapse Weg Mountain Pass. A seasonal study site was chosen at the top of the pass, on the crest of a north-facing saddle which lies between Muizenberg Mountain and Dassenberg Mountain (altitude 300m).

A wild fire in 1990 provided a stand of young vegetation, while an adjacent area which was not burnt during this fire but about five years before, provided a middle-aged stand. The area of vegetation on the opposite side of the road, which had not been burnt for more than ten years, provided a stand of old-aged vegetation. The young and middle-aged stands tended to have a higher rock cover than the old-aged stand. The Mispah form soil of the rocky slopes consists of fairly shallow, coarse sand weathered from Table Mountain Sandstone. The soil is deeper and of the Houhoek form in the rockless areas. (Smith-Baillie *et al.* 1976)

The Mesic Mountain Fynbos (Moll *et al.* 1984) of this area of Silvermine was described by Kathan (1981). The communities present at the site corresponded well with his *Leucadendron laureolum - Erica plukenetti - Mastersiella digitata* community, which was on sandy areas, and the *Leucadendron laureolum - Mastersiella digitata - Lobelia pinifolia* community, which was present on the rocky areas. This is very similar to the previous community except for the addition of *Lobelia pinifolia, Saltera sarcocolla* and *Chondropetalum tectorum*. These communities consist of two strata in their mature state: a shrub stratum of *L. lareolum* and a lower ericaceous - restionaceous stratum. In younger vegetation the cover is much reduced and the Restionaceae are dominant.

11. Climate.

The climate of Southern Africa is dominated by the presence of three anticyclones or high pressure cells. Two of these are situated over the oceans on either side of the sub-continent and the third, over the land. The south Atlantic anticyclone is situated further south and closer to the land during the summer. The descending air leads to the aridity and the presence of strong, southerly winds in the south-western Cape during summer. In the winter, this anticyclone tends to move about 4° to the north. The circumpolar westerly winds are thus able to spread their influence further northward allowing the cyclones, or low pressure cells, with their associated cold fonts, to bring cold and inclement weather with north-westerly winds to the south-western Cape (Fuggle 1981).

The diverse topography of the south-western Cape with its various intersecting mountain ranges also strongly affects climate at regional level. Mountainous areas receive a higher rainfall owing to orographic effects as well as a higher cloud cover during summer caused by the south-easterly winds blowing over the mountains, e.g. Table Mountain's "Table Cloth".

Table 1.1 shows available climatic data for seven study sites (Weather Bureau, Department of Environmental Affairs). Note, that the sites which are not near high mountains receive a lower annual rainfall (Cape Point, Pella and Groot Hagelkraal). Two of the areas near the sea, Groot Hagelkraal and Cape Point, were generally cooler in summer owing to the cooling effects of ocean breezes. Table Mountain House is situated on top of Table Mountain and is probably much cooler than the

study site in Orangekloof. Constantia, which is lower than Silvermine, is probably warmer than the site at Silvermine which is more exposed.

Table 1.1 Climatic data for study sites or nearest weather station (in brackets). (Scarborough would be similar to Cape Point. There were no data available for Bainskloof).

Site	Average temperature (max+min/2°C)		Sunshine (hours.day^{-1})		Rainfall Mean Annual (mm)
	Summer	Winter	Summer	Winter	
Cape Point	18.1	13.2	-	-	355
Silvermine (Constantia)	20.2	12.9	9.2	5.0	1282 (Silvermine)
Orangekloof (Table Mountain House)	15.6	9.8	8.6	4.5	1459 (Orangekloof)
Pella (Darling)	21.8	13.0	10.7	5.7	598
Jonkershoek	20.5	11.5	8.4	4.5	1459
DuToitskloof	22.3	12.2	-	-	991
Groot Hagelkraal (Dangerpoint)	18.7	13.7	-	-	454

Chapter 4

Nodulation in various species of *Aspalathus*.

1. Introduction.

Fynbos vegetation is periodically destroyed by fire and this has a major influence on the cycling of nutrients in a fynbos environment. One of the important effects of fire may be a significant loss of nitrogen via volatilization (See Chapter 2 for a review of nitrogen and phosphorus cycling and the role of fire in Mediterranean-type ecosystems). In the nutrient-poor, fynbos environment, where nitrogen is in short supply, it is of interest to determine how this nitrogen is replaced. Atmospheric deposition, for example, is very low in fynbos environments with levels of 1.99 $kg.ha^{-1}.y^{-1}$ recorded at Pella (Stock & Lewis 1986a) and 3.87 $kg.ha^{-1}.y^{-1}$ at Jonkershoek (Van Wyk *et al.* 1992). Schlesinger *et al.* (1982) calculated that at a deposition rate of 1.5 $kg.ha^{-1}.y^{-1}$ measured in the Californian chaparral, it may take 70 years to replace the nitrogen lost in a single fire. Nitrogen fixation, both symbiotic and non-symbiotic, could therefore play an important role in replacing lost nitrogen as well as in adding to the general nitrogen supply in these communities. What little is known about symbiotic nitrogen fixation in Mediterranean-type ecosystems relates to *Ceanothus* spp. from California, and *Acacia* spp. from south Western Australia (See Chapter 2 for a review of nitrogen fixation in Mediterranean-type ecosystems). Very little is known about symbiotic nitrogen fixation in fynbos in terms of the quantity of nitrogen fixed and the biology of the nitrogen-fixing species themselves.

Aspalathus, which is often dominant after fires in fynbos environments (Kruger 1979), has been suggested by a number of authors as being a potentially important nitrogen - fixing species in fynbos environments (Lamont 1982, Lamont 1983, Read & Mitchell 1983, Rundel 1983). They suggest that these plants are able to take advantage of the lowered nitrogen, and especially the raised phosphorus content, of recently burnt areas to grow and nodulate vigorously, so that they become dominant in the community. They later die out when the soil nutrient status changes as the community matures. There is very little data on the biology of *Aspalathus* spp. relating to their ability to nodulate and fix nitrogen in natural ecosystems, which makes it difficult to validate this

theory. It has, however, been established that several *Aspalathus* spp. have the ability to nodulate (Grobbelaar & Clarke 1972).

In this chapter, the following questions concerning the occurrence of stands of *Aspalathus* in recently burnt vegetation will be considered.

1. Are there any detectable differences between the soil nutrient status of recently burnt stands and nearby, older, unburnt vegetation which could be important in determining the establishment success of *Aspalathus* plants in the early successional stages? An alternative hypothesis is that seeds are stimulated to germinate by fire and soil nutrient status has no effect on the success of *Aspalathus* species (This idea is dealt with in Chapter 7).

2. If soil nutrients, particularly nitrogen, play a role in determining the success of *Aspalathus* plants in post-fire environments, this should be reflected in the plants' pattern of nodulation, i.e. do low nitrogen soils stimulate prolific nodulation and is this seasonally variable and potentially species dependent ?

3. Is the amount of nodulation in different *Aspalathus* species determined by various environmental factors or is it solely a function of the species being examined ?

2. Methods.

2.1. Field data collection.

2.1.1. Study sites.

The study sites are described in detail in Chapter 2. They were of two types :

1. Seasonal study sites - Cape Point I, Scarborough and Silvermine.
At these sites vegetation data (see Chapter 6) and soil nutrient samples were collected at six-monthly intervals over an eighteen month period (May 1991, December 1991 and June 1992). Nodule cores and soil moisture samples were collected every six weeks (May, June, August, September and December 1991 and February, April and June 1992).

2. Geographical survey study sites - Jonkershoek I and II, DuToitskloof, Bainskloof, Cape Point II, Groot Hagelkraal and Orangekloof.
At these sites, vegetation data, soil nutrient and moisture samples and nodule cores were collected on a once-off basis between July and November 1991.

2.1.2. Data collection.

Each study site had both recently burnt vegetation (0 - 3 years) and older, unburnt vegetation situated close together. The older vegetation fell into two age classes, 4 - 9 years (middle-aged) and 10 + years (old). In all cases the recently burnt vegetation contained stands of *Aspalathus* plants (exceptions were Bainskloof where these were in the older vegetation which fell into the 4 -9 years age class and Pella and Cape Point II where the recently burnt vegetation fell into the 4 - 9 years age class). Two sites had vegetation of only one age: Cape Point II and Jonkershoek II. Another two sites had vegetation of all three age categories young, middle-aged and old. At Silvermine the vegetation of intermediate age contained no *Aspalathus* plants while at Scarborough, *Aspalathus* plants were present.

Five plots of 25 m^2 were selected at random in the vegetation of different ages at each site. The following samples were collected at each plot:

1. Soil nutrient and moisture samples - Five samples were collected per plot using a plastic cylinder (5 cm diameter) which was hammered into the ground to a depth of 10 cm. Samples were placed in small plastic bags (10 cm x 5 cm) and sealed tightly.

2. Nodule cores - A 10 cm diameter plastic cylinder was used to collect the soil around the base of an *Aspalathus* plant by hammering it into the ground to a depth of 20 cm and carefully removing the cylinder and the soil it contained. Five samples were taken per plot. The plants were cut off at ground level and the soil was then removed and placed with its associated plant in a 20 cm x 30 cm plastic bag. In the case of rocky sites, the core was hammered in as deeply as possible and the remaining soil removed with a handtrowel.

3. Vegetation data were also collected (see Chapter 6).

2.2. Laboratory analysis.

2.2.1. Sorting, drying and weighing procedures.

1. Soil nutrient and moisture samples - These were sieved through a 2 mm mesh sieve, weighed fresh (wet mass $\pm$ 150.0 g) and again after being dried at 40 °C to give the equivalent of an air-dried mass. This was done in order to give an indication of soil moisture at each site (% dry mass). The use of higher temperatures was avoided as it could have affected the total nitrogen content of the soil sample (Bremner 1965) which was also used to determine total nitrogen and phosphorus. Soils are normally dried at 105 °C to obtain the dry mass. Once dried, the soil samples were used to determine pH, total nitrogen, phosphorus and organic matter content using the methods given in section 2.2.3. Only one sample per plot was analysed for pH and for total organic matter content.

2. The nodule sample cores - The plant belonging to each nodule core was dried at 80 °C for one week and weighed. The soil cores consisting of soil, stones and root material were air dried until they could be easily sieved through a 2 mm sieve. This removed the root material with its attached nodules, if present, and the stones. The root material was carefully sorted by hand and all the nodules removed and dried at 80 °C for one week prior to being weighed.

Using known volumes of soil, a standard curve of soil mass against volume was determined for the soil at each study site. This was used to calculate the volume of sieved soil in each nodule core. This was necessary as, although a specific volume of soil was collected in the field, the amount of sieved soil contained in each sample varied greatly owing to the inclusion of different amounts of stones and plant material. From the mass of nodules (mg), the mass of the plant (g) and the volume of soil (cm^3), the amount of nodulation per sample could be obtained as
mg nodules.g^{-1} plant.100 cm^{-3}.

2.2.3. Chemical analysis.

2.2.3.1. Soil total nitrogen.

Micro-Kjeldahl digest for the determination of total nitrogen in soil.

The detailed method followed was described by Stock (1985). One gram of sieved soil was placed in a 75 ml, thick-walled, digestion tube. To this was added 1 ml of distilled water and 5 ml of concentrated, nitrogen-free sulphuric acid containing 34 $g.l^{-1}$ of salicylic acid. A selenium catalyst tablet containing 0.05 g selenium and 1 g sodium sulphate were then added together with 0.2 g of sodium thiosulphate. The tubes were placed in an aluminium heating block and heated to 150 °C for at least two hours to drive off all water. The temperature was raised gradually over the next two hours to 375 °C where it remained for two hours until the digests were clear. In the micro-kjeldahl digest method all nitrogen in the soil sample is converted to ammonia by sulphuric acid. Selenium digest tablets are added in order to raise the temperature of the digest and catalyse the reaction (Bremner & Mulvaney 1982). Salicylic acid and sodium thiosulphate are then added to the digest to quantitatively convert all nitrate in the sample to ammonia (Bremner 1965). The samples were then cooled and made up to 50 ml with distilled water and thoroughly mixed.

Each batch of samples was accompanied by three blanks (which contained all the reagents but no soil) and a range of standards. The standards contained an ammonium sulphate solution which replaced the 1 ml of water in each case. The standards were set up so that they contained the following amounts of nitrogen per tube: 0.1, 0.3, 0.5, 0.7, 1.0, 1.5, and 2.0 mg.

Colorimetric determination of ammonia content of micro-Kjeldahl digests.

A 1 ml aliquot was taken from each digest. To this was added 25 ml of a 1.2 % EDTA solution, 2 ml of a sodium nitroprusside-phenol reagent and 5 ml of a phosphate buffer - sodium hypochlorite reagent. The mixture was then made up to 50 ml with distilled water.

The sodium nitroprusside-phenol reagent consisted of 0.5 g sodium nitroprusside dissolved in 100 ml water and 100 ml of a 10 % w/v solution of phenol in ethanol. The phosphate buffer-sodium hypochlorite reagent consisted of four parts phosphate buffer-solution (1 l aqueous solution of 6.93 g sodium hyperphosphate and 20.65 g sodium hydroxide) and one part of a 1.5 % w/v aqueous solution of sodium hypochlorite.

The tubes were allowed to stand for one hour at room temperature for colour development to take place. Their absorbance was then read on a spectrophotometer set at 635 nm which was zeroed on distilled water. The average of the three blanks was subtracted from all sample readings which were then converted to mg nitrogen using the standard curve.

2.2.3.2. Soil total phosphorus.

In a similar way to the determination of total nitrogen, acid digests were used to release all the phosphorus in the soil in the form of phosphates. The phosphate content of the acid digests was then determined using the single solution colorimetric method of Murphy & Riley (1962).

Nitric, sulphuric, perchloric acid digestion.

Dried soil (0.2 g) was placed in 50 ml thick-walled boiling tubes to which 3.5 ml of acid solution was added. This consisted of five parts nitric acid, one part sulphuric acid and one part perchloric acid (60 %). The samples were then gently boiled (180 °C) in a digestion block to remove mainly the nitric acid. The temperature was raised slightly (220 °C) to remove the perchloric acid. This process took approximately 1.5 hours. The nitric, sulphuric and perchloric acid digestion is described by Grimshaw (1985) who found it to be "totally satisfactory" for the recovery of phosphorus from several different soil types. The samples were cooled and made up to 50 ml with distilled water

and allowed to stand before aliquots were taken. Three blanks consisting of only the acid solution were digested with each batch.

Colorimetric determination of phosphate content of total phosphorus digest.

A 10 ml aliquot was taken from each of the digest solutions. To each aliquot 25 ml of distilled water (to dilute the acid of the solution) and 8 ml of Murphy and Riley's solution (described below) were added. The solution was then made up to 50 ml with distilled water. The samples were left to stand for an hour at room temperature to allow for colour development after which their absorbances where read on a spectrophotometer set at 882 nm.

A standard curve was run for each batch of samples using known phosphate amounts in place of aliquots of digest solution. A solution of potassium dihydrogen phosphate with 1 mg $P.ml^{-1}$ was used and 2, 4, 6, 8, 10, 15, 20, 25 and 30 mg $P.ml^{-1}$ added to the different standard solutions.

Murphey and Riley's solution (1962)
This solution was made up of the following aqueous solutions (Murphey & Riley 1962): 250 ml of 5 M sulphuric acid, 75 ml ammonium molybdate (40 $g.l^{-1}$), 25 ml potassium antimony tartrate (1 mg $Sb.ml^{-1}$ or 0.2743 $g.100\ ml^{-1}$) and 150 ml ascorbic acid (0.1 M or 1.64 $g.150\ ml^{-1}$)

2.2.3.3. Total soil organic matter content.

About 5.0 g of dried soil was placed in a small crucible which was then dried at 105 °C for 24 hours and weighed. Following this, the organic matter was removed by firing the crucible in a Muffle furnace for 16 hours. The crucibles were then reweighed. The total organic matter content of the soil was expressed as a percentage of the soil dry mass.

2.2.3.4. Soil pH.

A 20 g sample of dried soil was placed in a glass jar together with 50 ml of a 0.01 M $CaCl_2$ solution. The jars were placed in a mechanical shaker and shaken for one hour after which the pH of the solution in the jars was measured using an electronic pH meter.

3. Results.

3.1 *Aspalathus* species nodule production.

3.1.1. Nodule production of different species in various environments.

The average nodulation data showed considerable variation between the 15 species at the eight different geographical survey sites (Figure 4.1.). Two species, *Aspalathus abietina* at Silvermine and *A. ciliaris* growing on the acid sands at Groot Hagelkraal, were much more productive than the species at the other sites. *Aspalathus microphylla* and *A. spinescens* at Pella and *A. laricifolia* at Jonkershoek I showed very low nodulation with less than 0.1 mg nodules.g^{-1} plant.100 cm^{-3}.

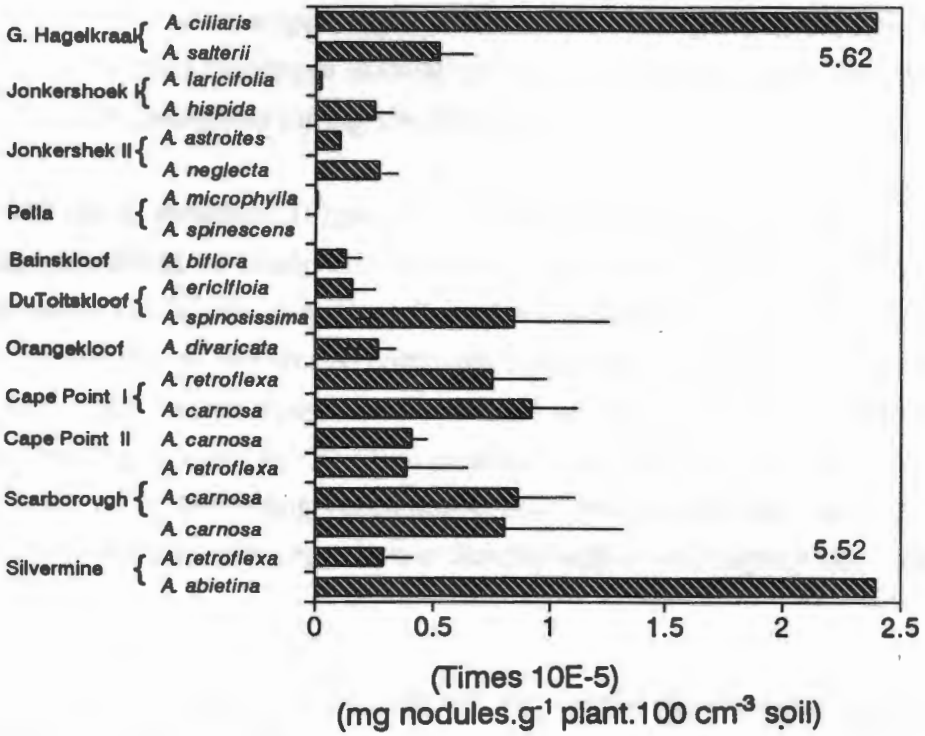


Figure 4.1. The average amount of nodulation for 15 *Aspalathus* spp. at eight geographical survey sites as well as data from the three seasonal sites (Cape Point I, Scarborough and Silvermine).

3.1.2. Seasonal nodule production.

At Silvermine, the two *Aspalathus* species present were the prostrate creeper, *A. retroflexa* (which was also present at Cape Point and Scarborough) and the low, procumbent bush (20 cm high), *A. abietina*. Both these species showed much higher average nodulation than *A. retroflexa* and *A. carnosa* at Cape Point and Scarborough (Figure 4.2.). *A. abietina* appears to nodulate very profusely and exhibited average nodulation of an order of magnitude greater than the other two species at all sampling times and at all three sites (note that the y-axis differs on the graph for Silvermine). The average nodulation at Cape Point I and Scarborough was similar. The same two species were present in the young vegetation at these sites, *A. retroflexa* and *A. carnosa*, a succulent bush about 50 - 100cm high. At Scarborough, stands of *A. carnosa* were also present in the middle-aged vegetation. At this site the average nodulation was higher for *A. carnosa* from the young vegetation than for the same species from the middle-aged vegetation and for *A. retroflexa* in the first year. At Cape Point I the average amount of nodulation for *A. carnosa* was again somewhat higher than that of *A. retroflexa* during the first year.

The data for *A. retroflexa* (Figure 4.2.) showed a slight trend towards an increase in the average amount of nodulation in the second year for all three sites where this species was present (i.e. Silvermine, Cape Point I and Scarborough). Note the very much higher nodulation at Silvermine compared to the other two sites. *A. carnosa*, however, showed a much clearer trend with the amount of nodulation increasing during winter and decreasing in summer. This was evident at all three sites for *Aspalathus* plants at Cape Point I. For the young vegetation at Scarborough the peak was in August, but for those in the middle-aged vegetation at Scarborough it was slightly later, during September.

The average soil moisture at the seasonal sites for the eight months when nodules were sampled showed that the soil was drier at all three sites during the hot, dry summer months and wetter during the cool, wet, winter months, as could be expected in a Mediterranean climate (Figure 4.3). The peaks in April for Scarborough indicate heavy, early rains which fell shortly before sampling took place at this site.

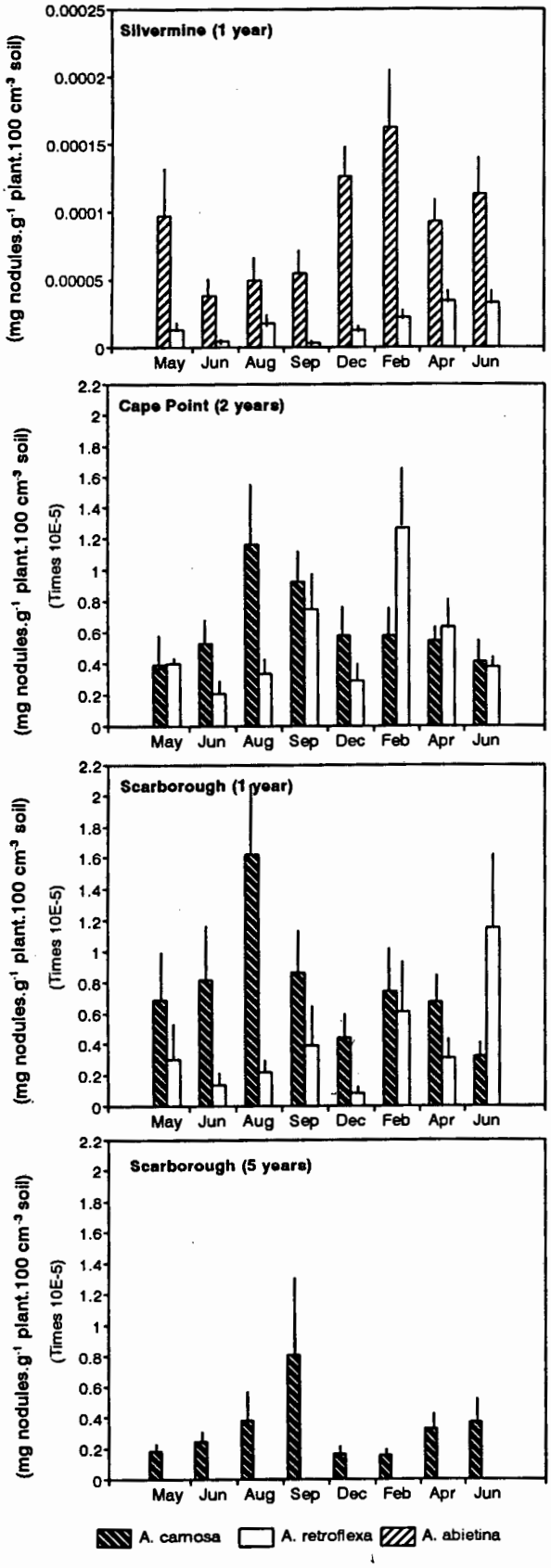


Figure 4.2. The average nodulation for *Aspalathus abietina*, *A. retroflexa* and *Aspalathus carnosa* present at the seasonal study sites Silvermine, Cape Point I and Scarborough (one year old and five year old vegetation) for the eight months sampled (May, June, August, September and December 1991 and April, February and June 1992). Standard error bars are shown.

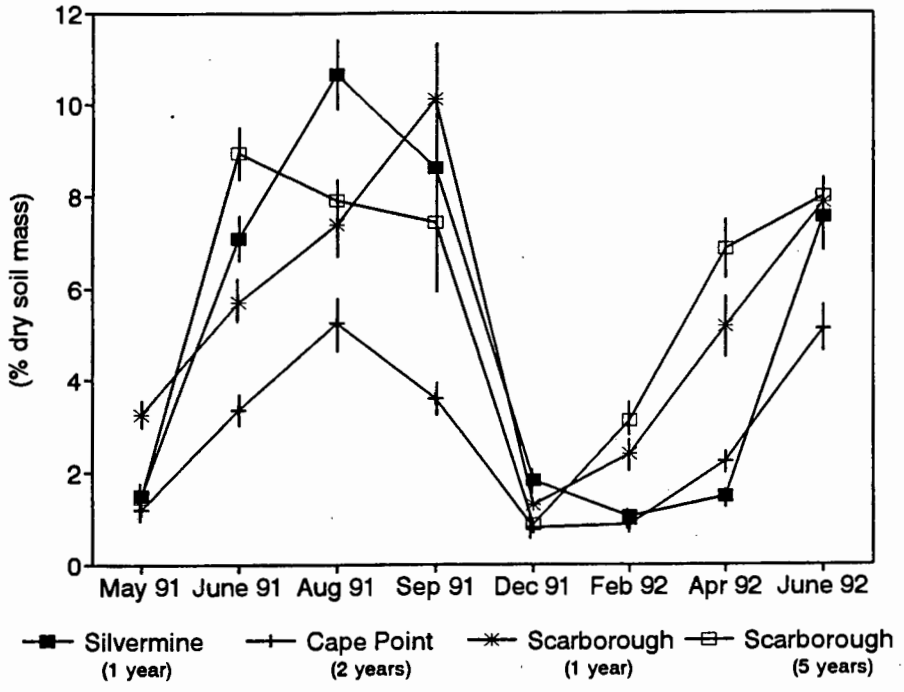


Figure 4.3. The average soil moisture at the three seasonal study sites for the eight months (May, June, August, September and December 1991 and April February and June 1992).

In general, Cape Point I appeared to be a drier site than the other two sites. The nodulation for *A. carnosa* at Cape Point I and Scarborough (young and middle-aged sites, Figure 4.2.) showed peaks during the wetter months of August and September (Figure 4.3.).

3.2. Total soil phosphorus and nitrogen.

The averages of soil total nitrogen and phosphorus for the geographic survey sites which had vegetation of two age categories were compared using t-tests (Figure 4.4. and Figure 4.4.). Significant differences between the average soil total nitrogen content of the young and older vegetation were found for Groot Hagelkraal acid sands ($t = 12.716$, $p < 0.001$), Pella ($t = 6.090$, $p < 0.001$), Bainskloof ($t = -3.202$, $P < 0.001$), Orangekloof ($t = -2.787$, $p < 0.001$) and Jonkershoek I ($t = 2.925$, $p < 0.001$) but not for DuToitskloof.

The average soil total phosphorus showed significant differences at Groot Hagelkraal for acid sands ($t = 10.458$, $p < 0.001$), Pella ($t = 15.442$, $p < 0.001$) and at DuToitskloof ($t = 5.282$, $p < 0.001$), but not at Bainskloof, Orangekloof or Jonkershoek I. Note that those sites with soils which have a granite or shale component i.e. Jonkershoek I, Dutoitskloof and Orangekloof, tended to have a higher total nitrogen and phosphorus content than the Table Mountain Sandstone derived soils of Pella, Cape Point II and the seasonal sites or the limestone soils of Groot Hagelkraal.

The Table Mountain Sandstone soils of Bainskloof appear to lie between these two groups. The very humus-rich Champagne-form soils of Jonkershoek II had a much higher total nitrogen and phosphorus content than any of the other soils. The sites with vegetation of 1-3 years old (generally those with stands of *Aspalathus*) did not appear to have a consistent trend of either increased or decreased average soil total

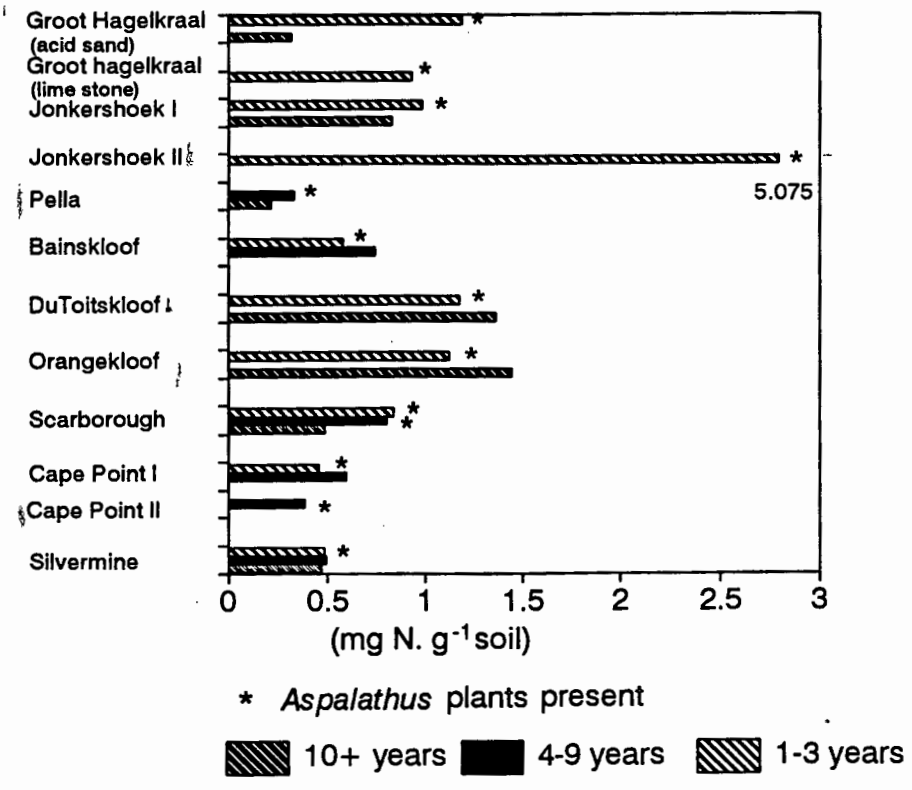


Figure 4.4. The average soil total nitrogen for vegetation of different ages at eight geographic survey sites and three seasonal sites (added for comparison). The places where *Aspalathus* plants were present are indicated (*). Standard error bars were too small to illustrate.

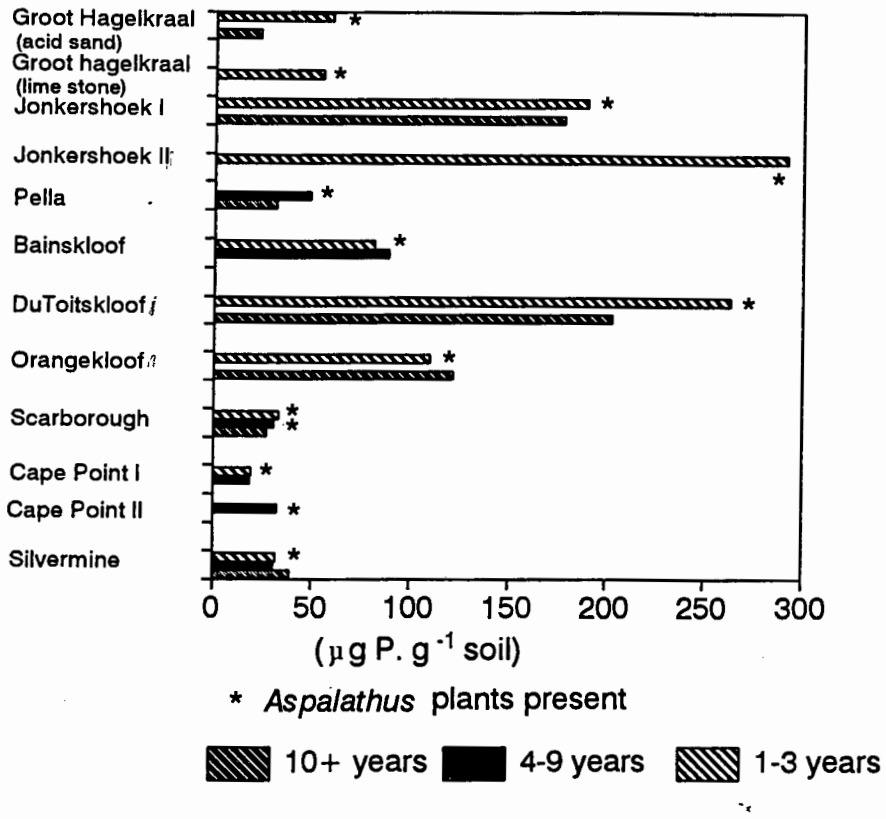


Figure 4.5. The average soil total nitrogen for vegetation of different ages at eight geographic survey sites and three seasonal sites (added for comparison). The places where *Aspalathus* were present are indicated (*). Standard error bars were too small to illustrate.

nitrogen content when compared to the older vegetation at each site. The average soil total phosphorus content of the younger vegetation at each site was, however, either greater than or the same as that of the older vegetation with the exception of Silvermine.

Total soil nitrogen and phosphorus data for the three sampling times (May 1991, December 1991 and June 1992) were grouped together to give one average per vegetation age at the three seasonal study sites (Figure 4.6. and Figure 4.7.). There was a significant difference (one way ANOVA) in the average soil total nitrogen content at Cape Point I ($F_{1,142} = 18.670$, $P < 0.001$) and Scarborough ($F_{2,219} = 54.923$, $P < 0.001$), but not at Silvermine. The soil total phosphorus content showed significant differences at Silvermine ($F_{2,215} = 37.082$, $p < 0.001$) and Scarborough ($F_{2,206} = 19.758$, $p < 0.001$) but not at Cape Point I. The data for the seasonal sites did not show any trend, i.e. vegetation of younger ages, which contained stands of *Aspalathus* plants, had either higher or lower average soil total nitrogen or phosphorus contents.

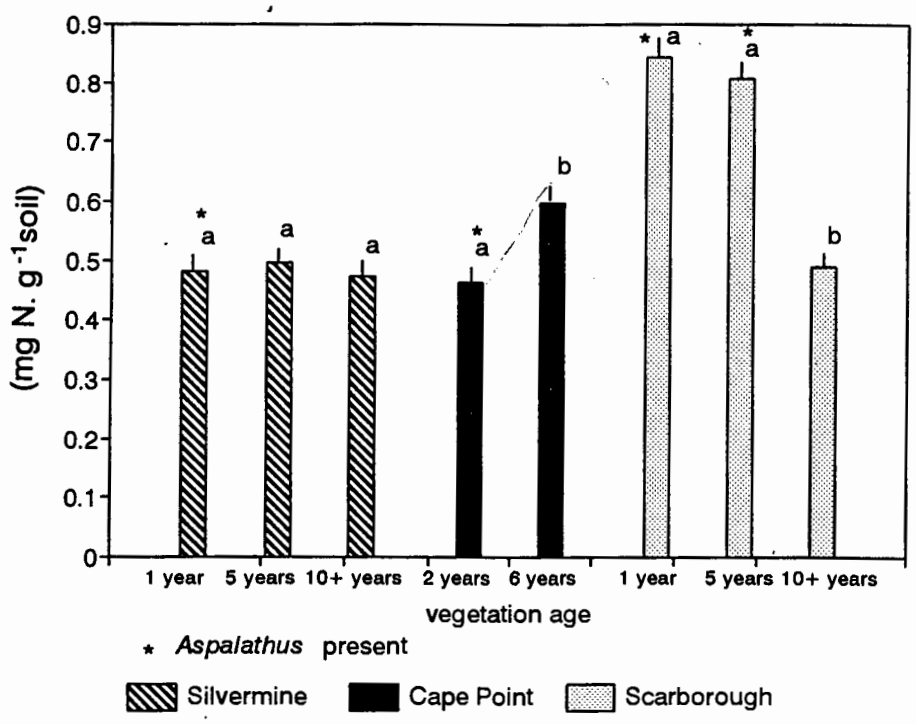


Figure 4.6. The average soil total nitrogen for the three seasonal study sites. The letters a or b indicate whether there was a significant difference between the vegetation ages at each site using a one-way ANOVA. Standard error bars and the presence of stands of *Aspalathus* are shown.

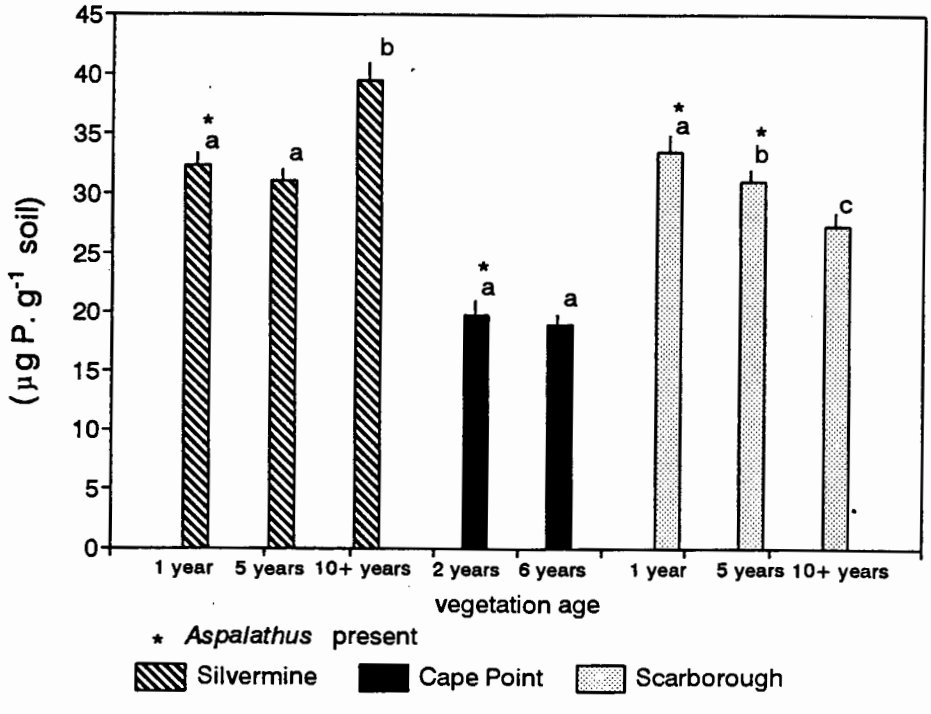


Figure 4.7. The average amount of soil total phosphorus for the three seasonal study sites. The letters a or b indicate whether there was a significant difference between the vegetation ages at each site using a one-way ANOVA. Standard error bar and the presence of stands of *Aspalathus* plants are shown.

3.3. Regression analysis of nodulation and six environmental factors.

A multiple regression using a step-wise variable selection approach was run on the average per plot data of nodulation, soil total nitrogen and phosphorus, pH, total organic content, soil moisture at the time of sampling and monthly temperature for the geographic survey sites (Table 4.1.). Data from the three seasonal sites (Silvermine, Cape Point I and Scarborough) were added for comparison and to increase the size of the data set. The data from September were selected for this purpose as the collection date was the closest to that of the geographic survey sites. The average nodulation data were log transformed to improve the normality of their distribution. In the final regression model (Table 4.2.), average pH, soil moisture and temperature were excluded. Phosphorus and total organic matter were found to be negatively correlated while average nitrogen was positively correlated with average nodulation. The regression as a whole was significant and these three factors appear to explain 12.61% of the variation in the average amount of nodulation across the eleven sites and containing a total of fifteen different species.

The data for the three seasonal sites (Silvermine, Cape Point I and Scarborough), i.e. average nodulation, soil total nitrogen and phosphorus, pH, total organic content, soil moisture at time of sampling and monthly temperature at site, are presented in Table 4.3. for the three, six-monthly sampling times (May, December 1991 and June 1992) when soil nutrient data were collected. The averages per plot were used in a multiple regression using a step-wise variable selection approach (Table 4.4.). The average nodulation data was transformed using a log transformation in order to improve the normality of the distribution. Average pH, total soil organic content, temperature and soil moisture were not found to be significant and were excluded from the model. There was a significant negative correlation with total nitrogen and a positive correlation with total soil phosphorus and average nodulation. An analysis of variance of the regression was significant; total soil nitrogen and phosphorus may thus explain 12.97% of the variation in the average amount of nodulation produced.

Table 4.1. The average (standard error below) nodulation for different *Aspalathus* species at eight geographic survey sites and three seasonal sites (included for comparison) with the average (standard error below) soil total nitrogen, phosphorus, soil moisture, pH and organic matter content as well as average monthly temperature for each site.

Study site (month sampled)	*Aspalathus* species	Average amount nodules (mg nodules. g^{-1} plant. $100cm^{-3}$)	Average soil total nitrogen (mg N. g^{-1} soil)	Average soil total phos-phorus (mg P. g^{-1} soil)	Average soil moisture (% dry mass soil)	Average monthly temper-ature (°C)	Average pH	Average soil organic content (% dry mass soil)
Groot Hagelkraal (July)								
acid sands	*A. ciliaris*	5.62E-05 9.52E-06	1.189 0.065	59.67 4.041	14.542 0.719	12.85	5.26 0.074	0.961 0.0505
limestone	*A. salterii*	5.28E-06 1.35E-06	0.93 0.035	55.05 3.324	4.969 0.481	12.85	7.15 0.103	2.035 0.04553
Jonkershoek I (September)	*A. laricifolia*	2.68E-07 1.16E-07	0.983 0.041	189.941 7.728	9.922 0.265	13.05	4.18 0.058	1.570 0.0492
	A. hispida	2.53E-06 9.21E-07	0.983 0.041	189.941 7.728	9.922 0.265	13.05	4.18 0.058	1.570 0.0492
Jonkershoek II (October)	*A. astroites*	1.03E-06 3.51E-07	5.075 0.165	292.186 10.374	21.898 0.897	16.15	4.58 0.032	7.906 0.2900
	A. neglecta	2.72E-06 7.95E-07	5.075 0.165	292.186 10.374	21.898 0.897	16.15	4.58 0.032	7.906 0.2900
Pella (August)	*A. microphylla*	1.39E-07 8.68E-08	0.335 0.014	49.276 0.905	2.289 0.254	10.89	4.43 0.051	1.240 0.2522
	A. spinescens	1.92E-08 1.2E-08	0.335 0.014	49.276 0.905	2.289 0.254	10.89	4.43 0.051	1.240 0.2522
Bainskloof (August)	*A. biflora*	1.28E-06 6.99E-07	0.582 0.028	81.700 4.346	6.676 0.706	11.7	3.71 0.056	1.148 0.09954
DuToitskloof (October)	*A. ericifolia*	1.59E-06 8.99E-07	1.177 0.11	263.505 9.385	5.221 0.386	17.95	4.18 0.081	2.327 0.1356
	A. spinossisima	8.48E-06 3.98E-06	1.177 0.11	263.505 9.385	5.221 0.386	17.95	4.72 0.081	2.327 0.1356
Orangekloof (October)	*A. divaricata*	2.65E-06 6.94E-07	1.124 0.054	109.941 5.821	7.155 0.449	14.2	4.41 0.119	2.093 0.1526
Cape Point I (September)	*A. retroflexa*	7.51E-06 2.36E-06	0.444 0.023	23.859 1.199	3.591 0.252	14.0	4.12 0.071	1.956 0.1513
	A. carnosa	9.22E-06 1.97E-06	0.444 0.023	23.859 1.199	3.591 0.252	14.0	4.12 0.071	1.956 0.1513
Cape Point II (August)	*A. carnosa*	4.07E-06 6.54E-07	0.39 0.031	32.694 10.381	10.114 0.596	14.2	3.72 0.052	1.451 0.2289
Scarborough (September)	*A. retroflexa*	3.89E-06 2.47E-07	1.011 0.043	34.757 1.2861	10.109 1.091	14.0	4.03 0.072	1.903 0.1525
	A. carnosa	8.65E-06 1.41E-07	1.011 0.043	34.757 1.286	10.109 1.091	14.0	4.03 0.072	1.903 0.1525
	A. carnosa	8.08E-06 3.57E-07	0.988 0.043	31.942 0.677	7.433 1.390	14	4.08 0.128	2.413 0.1113
Silvermine (September)	*A. retroflexa*	2.94E-06 1.24E-06	0.428 0.023	31.062 0.838	8.636 0.753	14.2	3.846 0.016	1.249 0.1488
	A. abietina	5.52E-05 1.36E-05	0.428 0.023	31.062 0.838	8.636 0.753	14.2	3.846 0.016	1.249 0.1488

Table 4.2. The final multiple regression model of average amount of nodulation against the averages of six soil environmental factors at different geographical survey sites.

Variable	t-value	Significance
Average soil total phosphorus	-2.0753	$p < 0.05$
Average soil total nitrogen	3.2146	$p < 0.01$
Average soil pH	excluded	
Avergae soil total organic content	-3.2140	$p < 0.01$
Average monthly temperature	excluded	
Average soil moisture	excluded	
r^2 for regression	**ANOVA of full regression**	
0.1298	$F_{3,59} = 4.68416$	
n = 63	$p < 0.01$	

Table 4.3. The average (standard error below) nodulation at three seasonal study sites for three species with the accompanying average (standard error below) soil total nitrogen, phosphorus, and soil moisture, pH, organic matter content as well as average monthly temperature.

Study site	Month	Average amount nodules (mg nodules.g^{-1} plant.100 cm^{-3})			Average soil total nitrogen (mg N. g^{-1} soil)	Average soil total phos-phorus (mg P. g^{-1} soil)	Average soil moisture (% dry soil mass)	Average monthly temper-ature (°C)	Average pH	Average soil total organic content (% dry soil mass)
		Aspalathus abietina	*Aspalathus retroflexa*	*Aspalathus carnosa*						
Silvermine young	May 91	9.75E-05 3.41E-05	1.28E-05 4.55E-06		0.421 0.025	32.66 0.895	1.499 0.164	17.8	3.87 0.096	1.759 0.2097
	December 91	0.000126 2.18E-05	1.22E-05 2.9E-06		0.428 0.023	31.062 0.838	1.820 0.251	17.7	3.85 0.016	1.249 0.1488
	June 92	0.000113 2.64E-05	3.3E-05 8.62E-06		0.599 0.040	33.172 1.364	7.555 0.615	12.6	3.95 0.108	1.851 0.1447
Cape Point young	May 91		4.02E-06 1.95E-06	3.49E-06 8.38E-07	0.320 0.024	19.793 1.528	1.191 0.111	16.8	4.00 0.052	1.903 0.1212
	December 91		2.87E-06 1.21E-06	5.82E-06 1.9E-06	0.444 0.023	23.859 1.199	0.80 0.053	16.6	4.12 0.071	1.956 0.1513
	June 92		3.76E-06 8.36E-07	4.11E-06 1.44E-06	0.612 0.041	16.222 0.8644	5.121 0.425	13.5	3.84 0.046	2.108 0.2016
Scarborough young	May 91		6.88E-06 2.31E-06	3.12E-06 3.02E-06	0.786 0.030	36.004 1.504	3.232 0.288	16.8	4.16 0.023	2.150 0.1962
	December 91		8.44E-07 2.47E-07	4.42E-06 1.41E-07	1.011 0.043	34.757 1.286	1.296 0.163	16.6	4.03 0.072	1.903 0.1525
	June 92		1.15E-05 4.87E-06	3.19E-06 8.62E-07	0.734 0.043	29.739 1.4781	7.872 0.408	13.5	4.03 0.055	1.891 0.2916
Scarborough middle-aged	May 91			1.81E-06 4.77E-07	0.662 0.036	29.070 1.065	1.445 0.120	16.8	4.12 0.083	1.694 0.2552
	December 91			1.64E-06 3.37E-07	0.988 0.043	31.942 0.6767	0.864 0.058	16.6	4.08 0.128	2.413 0.1113
	June 92			3.71E-06 1.43E-06	0.792 0.053	32.042 7.743	8.004 0.279	13.5	4.19 0.054	1.921 0.2284

Table 4.4. The final multiple regression model using stepwise variable selection for average nodulation against the averages of six soil environmental factors for the seasonal study sites during the months, May and December 1991 and June 1992.

Variable	t-value	Significance
Average soil total phosphorus	3.0674	$p < 0.05$
Average soil total nitrogen	-2.4852	$p < 0.05$
Average soil pH	excluded	
Avergae soil total organic content	excluded	
Average monthly temperature	excluded	
Average soil moisture	excluded	
r^2 for regression	**ANOVA of full regression**	
0.1297 n = 60	$F_{2,57} = 5.39923$ $p < 0.01$	

Only the soil moisture data for the three occasions on which nutrient sample were collected were used in the previous regression. To further test the negative result for soil moisture all the average soil moisture and average nodulation data (log transformed) for all eight six-weekly sampling times were regressed (averages per plot were used). No significant correlation was found using the data for the three seasonal study sites, Silvermine, Cape Point I and Scarborough, together. There was a significant correlation between the average nodulation and the soil moisture for the data from Scarborough when tested separately, however ($r^2 = + 0.107$, $n = 70$, $p < 0.05$).

In order to test whether there was any relationship between the amount of nodulation and the ratio of soil total nitrogen to phosphorus, the per plot averages of these factors were regressed against each other for both the geographic survey sites and the seasonal sites. The average amount of nodulation data was log transformed. No significant correlation could be found between these two factors in the case of the geographic survey sites. There was, however, a significant negative relationship for the data from the three seasonal sites although this ratio only explains 17.23 % of the variance (Figure 4.8.).

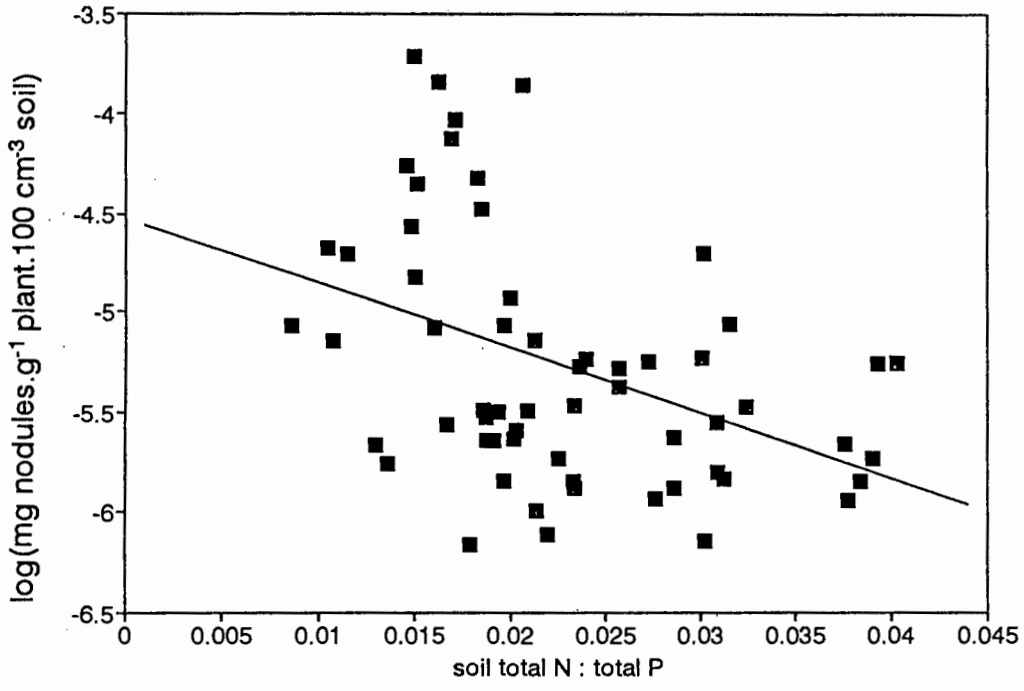


Figure 4.8. The average ratio of soil total nitrogen to phosphorus against the log. of the average amount of nodulation per plot at the three seasonal sites, Silvermine, Cape Point I and Scarborough (one year old and five year old vegetation) for the three sampling times (April and December 1991 and June 1992) when the data were collected ($r^2 = 0.1723$, $n = 60$, $P < 0.05$).

4 Discussion

Trends in *Aspalathus* nodule production.

All the *Aspalathus* spp. at both the seasonal sites and the geographical survey sites were found to nodulate well when grown in pot culture on acid-washed sand and fed with a nitrogen-free nutrient solution (see Chapter 5). Exceptions were *A. carnosa* and *A. salterii* which could not be grown as no seed could be found. Most of these species, as well as the two species which could not be grown in pot culture, were mentioned by Grobbelaar and Clarke (1972) as nodulating species. These included *A. abietina, A. asteroides, A. biflora, A. carnosa, A. ciliaris, A. divaricata, A. ericifolia, A. hispida, A. laricifolia, A. microphylla, A. retroflexa, A. salterii and A. spinescens*. It is thus evident that all the species included in this study have the ability to nodulate well under laboratory conditions. Under field conditions all species were able to nodulate although the amount of nodulation varied greatly. *Aspalathus spinescens* and *A microphylla* at Pella, for instance, showed very low average nodulation whereas others, for example *A. abietina* at Silvermine and *A. ciliaris* at Groot Hagelkraal, showed levels of nodulation several orders of magnitude greater. That all the species nodulate well when grown in pot culture suggests that environmental factors may play an important role in determining the amount of nodulation in the different species under field conditions. Further evidence demonstrating the importance of environmental factors in nodulation is that *A. retroflexa* at Silvermine consistently showed higher levels of nodulation than at the other two seasonal study sites at which it was found viz. Scarborough and Cape Point I. Some species, however, still appear to be able to nodulate better than others under the same conditions. Compare, for example, *A. retroflexa* and *A. abietina* over the year at Silvermine; *A. ciliaris* and *A. salterii* for the one-off collection at Groot Hagelkraal; and *A. spinosissima* and *A. ericifolia* at DuToitskloof. Lawrie (1981), working in Victoria, Australia, also found large differences in the amount of nodulation between different legumes of the same genus at the same site: *Acacia mearnsii*, for example, produced 0.451 g nodules. plant^{-1} and *A. paradoxa*, 0.977 g nodules.plant^{-1}.

Where seasonal trends in nodulation are concerned, this is strongest in *Aspalathus carnosa* with the highest number of nodules being present during the wet, winter months at the two sites, Cape Point I and Scarborough (young- and middle-aged vegetation). Similar seasonal patterns of nodule production have been found in south Western Australia with, for example, two year old *Acacia pulchella* growing on coastal

sands (Monk *et al.* 1981) and the understory legume species in jarrah (*Eucalyptus marginata*) forest, e.g. *Acacia alata* and *A. pulchella* (Hansen & Pate 1987) and *A. pulchella*, *Kennedia prostrata* and *K. coccinea* (Hingston *et al.* 1982). This trend was not visible in *Aspalathus retroflexa* which was present in the young vegetation at Silvermine, Cape Point I and Scarborough, possibly because this species has larger nodules than *A. carnosa*. The average individual masses were 3.23mg $\pm$0.218 and 0.752 mg $\pm$0.0375 for *A. retroflexa* and *A. carnosa*, respectively. This may lead to an accumulation effect as the larger nodules of *A. retroflexa* formed in the warm, wet months of spring and observed in the summer months' samplings may not be able to decompose as quickly as the much smaller nodules of *A. carnosa* lost from the plant in the hot, dry, summer months.

Differences in soil nutrients between vegetation of different ages.

Total nitrogen content has been found to be higher in burnt compared to unburnt fynbos and chaparral (Christensen & Muller 1975, Stock & Lewis 1986b). This increase in total nitrogen was found to decrease slowly over the months following the fire as nitrogen-containing ions and compounds were leached to lower levels or taken up by regrowing vegetation. At Pella, the total nitrogen content at the soil surface measured by Stock & Lewis (1986b) after a fire was 0.372 $mg.g^{-1}$ and dropped to 0.283 $mg.g^{-1}$ during the following nine months. As most of the young vegetation (i.e. recently burnt) was more than a year old, the flush of total nitrogen had probably passed at most of these sites. It is therefore not surprising that no consistent increase in average soil total nitrogen levels could be found in the soil of the young vegetation when this was compared to that of the old vegetation. Levels had most probably dropped back to those present before the fire. The increased nutrient levels seen at Scarborough (one year after the fire) may well, however, reflect a flush of total nitrogen still present at the site. The decreases in total nitrogen at younger as opposed to older vegetation at two-year-old sites (Cape Point I, Bainskloof and Orangekloof) may reflect losses of nitrogen owing to the fire. The increased levels of total nitrogen in the younger vegetation at Pella (4.5 years), Jonkershoek I (2.5 years) and Groot Hagelkraal (2.0 years), on the other hand, may reflect inherent differences between the soil of the younger and older vegetation at these sites.

Total phosphorus has also been found to be higher in chaparral immediately after fire (Christensen & Muller 1975, Debano & Conrad 1978). This was not the case for total

phosphorus measured by Brown and Mitchell (1986) in sandplain lowland fynbos at Pella. However, a flush of resin-extractable phosphorus found here after the fire returned to pre-fire levels within four months of the fire (Brown & Mitchell 1986). Christensen and Muller (1975) found an increase in water soluble phosphates in chaparral. What happened in the case of total phosphorous was not mentioned. In this study, increased total phosphorus levels were found in the soil of the young vegetation at Groot Hagelkraal, Pella, DuToitskloof, and Scarborough. Other sites (Cape Point I, Jonkershoek I, Orangekloof and Bainskloof) showed no significant differences between the phosphorus levels in soils of the young and old vegetation. The increase at the youngest sites, DuToitskloof and Scarborough, may well indicate a flush of increased phosphorus owing to deposition by the fire. At older sites it is more doubtful that the increases are owing to the last fire. At only one site, Silvermine, was the amount of total phosphorus significantly less in the younger vegetation. This may reflect inherent differences between the soil of the young and middle-aged vegetation as opposed to the old vegetation at this site. There was no difference in the levels between and the young- and middle-aged vegetation.

It was not obvious from the total nitrogen data whether a difference in nitrogen content of the soil accounts for the occurrence of stands of *Aspalathus* plants in younger vegetation at a specific site and their absence in nearby older vegetation. In the case of phosphorus, however, the soils where the plants occurred showed a total phosphorus content which was either equal to, or higher than that of the older vegetation at the site.

The effect of environmental factors on nodulation.

Phosphorus has been found to be an important element affecting the extent to which legumes nodulate. Hingston *et al.* (1982) found the application of 50 kg $P.ha^{-1}$ in jarrah forest increased *Acacia pulchella* nodule production. These plants produced 600 $mg.plant^{-1}$ of nodules in plots without phosphorus application and 1620 $mg.plant^{-1}$ in plots with phosphorus application. In pot experiments, both *Kennedia coccinea* and *Acacia pulchella* increased their nodule production with increased application of phosphorus. At the highest phosphorus applications, however, nodule production was reduced. Similar results were obtained by Hansen and Pate (1987) when they added phosphorus and water to field-growing *A. pulchella*. Wienhold and Klemmedson (1991) have shown that *Cerocarpus betuloides*, an actinorhizal shrub from the western U.S.A.,

also responds to phosphorus application with increased nodulation and nitrogen fixation (acetylene reduction) in pot experiments.

In this study, the multiple regression of the data at the seasonal sites showed a positive correlation with phosphorus and a negative correlation with nitrogen (Table 4.2.). The ratio of nitrogen to phosphorus gave a negative correlation with the amount of nodulation. Smith (1992) stresses the importance of nitrogen to phosphorus ratios in nitrogen fixation ecology. He noted that there was an increase in annual production of pasture legumes in New Zealand with increasing phosphorus fertilization rates. In a multiple regression of data from various studies of the same pasture legumes, initial soil total nitrogen was negatively correlated with the annul rate of nitrogen fixation but positively correlated with a dummy variable accounting for phosphorus fertilization. Similar results were obtained in other studies, e.g. Guofan and Tingxiu's (1991), which explored the relationship between nitrogen fixation and various soil properties in black locust (*Robinia pseudoacacia*) and found a negative correlation between nitrogen fixation (acetylene reduction) and total nitrogen to available phosphorus ratio.

There was no similar correlation between nitrogen to phosphorus ratio and the amount of nodulation at the geographic survey sites. Both total phosphorus and organic content were negatively correlated with the amount of nodulation across at the geographical sites in the multiple regression (Table 4.4.). This may result from differences in the soil types at the geographical survey as opposed to the seasonal sites. Unlike the multiple regression for the seasonal sites, which was across similar soils derived from Table Mountain Sandstone, the regression was across a number of soils of different parent materials in the case of the geographical survey sites. There were sites with soils of shale (e.g. Orangekloof) granite (Jonkershoek I and DuToitskloof) and Table Mountain Sandstone origin. Phosphorus was correlated with the organic content of the geographic survey soils ($r^2 = 0.398$, $n = 60$, $p < 0.05$) but there was no similar correlation at the seasonal sites. It is therefore possible that, although high levels of total phosphorus are associated with lower amounts of nodulation at the geographical sites, this phosphorus is trapped in the high organic matter content and not available to the plants.

The effect of climatic factors on the ability of legumes to nodulate has been noted in other studies. *Prosopis glandulosa* which generally nodulates well on a range of different soils in pot culture does not nodulate at all in the more arid western part of its

range (Johnson & Mayeaux 1990). Various *Ceanothus* species in northern California were estimated to have much higher amounts of nodulation (100 $g.m^{-2}$) (Delwiche *et al.* 1965) than *C. greggii* in the south with an average of 1-2 $g.m^{-2}$ and *C. tomentosa* which did not produce nodules (Kummerow *et al.* 1978). In this study, however, moisture was not correlated with amount of nodulation in the multiple regression for the geographical survey sites or the seasonal sites. Neither could a correlation be found at the seasonal sites when all the data for the six-weekly samplings were used. This is possibly because of the trend in *A. retroflexa* and *A. abietina* which showed their highest amount of nodulation during the summer months. It may also be a result of accumulation of nodules in the soil as explained earlier. The positive correlation found for the data from Scarborough alone is probably owing to *A. carnosa* which showed a strong trend towards higher nodulation during the wetter, winter months and which was prominent in the data from this site. Further study is needed to determine the activity of the nodules on an annual and seasonal basis.

In conclusion, the *Aspalathus* spp. which were examined were all able to nodulate under field conditions. This nodulation varied at different localities and over the course of the year. Environmental factors such as soil nutrient status and moisture may play a role in determining the amount of nodulation in *Aspalathus* plants. However limited relationship with nutrients and moisture suggests that other factors such as differences between species are also important in determining the amount of nodulation. Soil nutrients did not seem to be decisive in determining whether or not *Aspalathus* plants are present in a community some time after the last fire, although soil total phosphorus levels were never lower in the younger vegetation which contained stands of *Aspalathus* plants than in older sites which did not.

Chapter 5

Nitrogen fixation in *Aspalathus* as measured by the $\delta^{15}N$ natural abundance method.

1. Introduction.

In Chapter 4 of this thesis various aspects regarding the nodulation of *Aspalathus* species from a variety of fynbos environments were discussed. This chapter is a preliminary investigation to assess the significance and magnitude of nitrogen fixation occurring in the *Aspalathus* species present in these environments. Stable isotopes of nitrogen, ^{14}N and ^{15}N, were used to determine the proportion of nitrogen being fixed by these plants by means of the $\delta^{15}N$ natural abundance method. This method relies on the fact that the $\delta^{15}N$ value of the soil is higher than that of the atmosphere in most environments. Nitrogen-fixing plants thus tend to have a lower $\delta^{15}N$ value than plants which rely on the soil for their nitrogen supply. This has been documented for various Mediterranean ecosystems (Delwiche *et al.* 1979; Virginia & Delwiche 1982; Pate *et al.* 1993), although there may be notable exceptions. Pate *et al.* (1993), for instance, found that non-nitrogen-fixing, resprouting shrubs in *Banksia* woodland in south Western Australia had $\delta^{15}N$ values similar to those of nitrogen-fixing plants.

A field-grown plant may obtain its nitrogen both from the atmosphere and the soil. Therefore in determining the extent to which it is reliant on atmospheric nitrogen it must be compared to a plant reliant solely on nitrogen from the atmosphere and one reliant solely on nitrogen from the soil. The first value may be obtained by growing the nitrogen-fixing species in acid-washed sand and fed on nitrogen-free nutrient solution. The second value is usually obtained by measuring the $\delta^{15}N$ value for a plant of a presumed non-fixing species growing in the same environment as the plant of the fixing species, the so-called reference species (Shearer & Kohl 1986; 1989). The $\delta^{15}N$ of the atmosphere and the soil cannot be used instead of these two values as isotopic fractionation is associated with both nitrogen-fixing and nitrate absorption and metabolism. The fractionation associated with nitrogen fixation is small (Amarger *et al.* 1979; Shearer *et al.* 1980; 1984; Shearer & Kohl 1989) as is that associated with nitrate absorption and metabolism (Shearer *et al.* 1980; Mariotti *et al.* 1982; Shearer & Kohl 1989). The fractionation associated with both these

processes is large enough, however, to introduce a significant error into the estimation of nitrogen fixation. Fractionation is also associated with transport and metabolism of nitrogen in the plant and results in some tissues having lower $\delta^{15}N$ values, e.g. trunk wood in *Prosopis glandulosa* (Shearer *et al.* 1983), and others having higher $\delta^{15}N$ values, e.g. nodules of many species (Shearer *et al.* 1980; 1982; Turner & Bergensen 1983; Virginia *et al.* 1984). This problem may be overcome by sampling the same tissues for all the plants for which $\delta^{15}N$ values are obtained.

When choosing a reference plant species, the assumption is implicit that the nitrogen-fixing species and the reference plant species will have similar fractionation associated with metabolism and transport of nitrogen within the plants. The two species must also compare favourably in terms of growth habitat and phenology so that it may be assumed that the plants of both species are receiving their nitrogen from the same source and at the same time in seasonal studies. Shearer and Kohl (1989) recommend collecting several reference species in order to reduce the effect of error in the matching of nitrogen-fixing species and reference plant species. In this chapter a different method for obtaining the $\delta^{15}N$ value of the reference plant was used. The nitrogen-fixing species was grown on its native soil which was supplied with oxygen in order to prevent, or reduce, nodulation (Dakora & Atkins 1989; 1990a; 1990b). The $\delta^{15}N$ value of a plant of the nitrogen-fixing species reliant on its native soil as its only source of nitrogen was thus obtained. This method has the advantage of removing possible error in the choice of the reference plant species and eliminates the need to sample more than one species.

The use of this method gives insight into the nitrogen fixation which has taken place in the plant over the past growing seasonas it is an accumulative as opposed to an immediated measure. It can thus be used for comparing the amount of nodulation in the different *Aspalathus* species from different fynbos environments (Chapter 4) as these include both live and dead nodules present in the soil which formed during the past growing season.

2. Methods.

In order to determine the fractional contribution (FNdfa) of fixed nitrogen to the nitrogen content of field-growing *Aspalathus* plants by means of the natural abundance method, it is necessary to obtain data from three sources:
a. The $\delta^{15}N$ value of the field-grown *Aspalathus* plants ($\delta^{15}N_t$).
b. The $\delta^{15}N$ value of the same species grown on its native soil (i.e. that of the field-grown plants ($\delta^{15}N_o$)).
c. The $\delta^{15}N$ value of the same species grown hydroponically in acid-washed sand and fed nitrogen-free nutrient solution. These plants are thus reliant only on the nitrogen they are able to fix ($\delta^{15}N_a$).
From these $\delta^{15}N$ values (a, b and c) the percentage contribution of fixed nitrogen FNdfa, to the field-grown plants may be calculated:

$$\%FNdfa = \frac{\delta^{15}N_o - \delta^{15}N_t}{\delta^{15}N_o - \delta^{15}N_a} \times 100 \qquad \textbf{Formula 5.1.}$$

As the techniques used are very costly, the $\delta^{15}N$ value of only one sample from each of these three sources was determined for the 13 *Aspalathus* species. The methods used for the determination of $\delta^{15}N$ are given in section 2.2..

2.1. Collection and growth of *Aspalathus* plant samples needed for $\delta^{15}N$ natural abundance technique of determining nitrogen fixation.

2.1.1. Field-grown *Aspalathus* plants ($\delta^{15}N_t$).

The samples used were from plants which had been collected for determing the number of nodules produced. These plants were collected at the geographical survey study sites (Groot Hagelkraal, Jonkershoek I and II, Orangekloof, Pella, Bainskloof, DuToitskloof and Cape Point II) between July and November 1991, and at the seasonal study sites (Scarborough and Silvermine) where plants collected during September 1991 were used. Only those species for which seed could be collected and was therefore available for growth in the laboratory for the determination of $\delta^{15}N_o$ and $\delta^{15}N_a$ were included. These were: *Aspalathus* species: *A. ciliaris* (acid sand at Groot Hagelkraal), *A. hispida* and *A. laricifolia* (Jonkershoek I), *A. neglecta* and *A. astroides* (Jonkershoek II), *A. ericifolia* and *A. spinosissima* (DuToitskloof), *A. biflora* (Bainskloof), A. *divaricata* (Orangekloof), *A. microphylla* and *A. spinescens* (Pella), *A. abietina* (Silvermine) and *A. retroflexa* (Scarborough). The methods used to collect these plants and

determine their amount of nodulation are described in Chapter 4. From these nodulation data it was possible to select a plant which had above average nodulation in the field for each species. Young shoot material from these plants was ground in a Wiley mill using a 60 μm mesh in preparation for $\delta^{15}N$ analysis.

2.1.2. Aspalathus plants grown on native soil ($\delta^{15}N_o$).

Seeds of the *Aspalathus* species mentioned in section 2.1.1. were collected at the study sites where they occurred. The seeds were acid scarified for half an hour in concentrated sulphuric acid and then washed thoroughly and allowed to germinate on filter paper in a Petri dish. A commercial fungicide (Benylate) was added to each Petri dish to prevent fungal infection. After approximately one week the seedlings were planted out into pots containing soil which was collected from their place of origin (A total of four plants, two per plot, were used for each species). The bottom of each pot was connected to an oxygen source by means of a thin plastic pipe. The pots were placed in a glass house in shallow trays which could be flooded with water and then drained. Once every day for 20 minutes the trays were flooded and the pots supplied with oxygen through the pipe. The water in the tray forced the oxygen to rise upwards through the soil in the pot. The soil in the pot was disturbed from time to time by gently compressing the sides of the pots in order to promote the movement of oxygen through the soil. The plants were watered every four days with deionised water. After four months the plants were harvested and dried at 80 °C for a week. The mass of the roots, stems and nodules was then determined. In most species the amount of nodule formation was very low (see Results). The plant with the lowest nodule mass to shoot mass ratio was selected for each species as it would be least like to have fixed nitrogen. The shoots of this plant were ground in a Wiley mill using a 60 μm mesh prior to $\delta^{15}N$ analysis.

2.1.3. *Aspalathus* plants grown in acid-washed sand on nitrogen-free growth medium ($\delta^{15}N_a$).

Seeds of the *Aspalathus* species mentioned in section 2.1.1. were germinated as described in section 2.1.2.. These were transplanted into pots containing acid-washed sand and placed in a glass house. Four pots containing two to four seedlings per species were planted depending on the amount of seed available. Every four days the pots were given 50 ml of a standard long Ashton solution diluted to 10 % from which nitrogen was excluded (See recipe below). The pots were watered every second day with distilled water. After five months the plants were harvested and

then dried at 80 °C for a week. The mass of the roots, shoots and nodules was then determined. The plants generally nodulated very well. The plant with the highest nodule mass to shoot mass ratio was selected for each species as it would be the most like to have fixed nitrogen. The shoots of this plant were ground in a Wiley mill using a.60 μm mesh prior to $\delta^{15}N$ analysis.

(10 % dilute standard long Ashton solution:
The following were added to one litre of distilled water -
1 ml each of the following two solutions: $NaH_2PO_4.2H_2O$ (0.133 M) and $Na_2HPO_4.12H_2O$ (.0133 M) combined and $CaCl_2.2H_2O$ (0.4 M) as well as 2 ml of a solution of $MgSO_4.7H_2O$ (0.15 M), K_2SO_4 (0.2 M) and micronutrients together.)

2.2. Stable isotope analysis of nitrogen.

The method used for the preparation of the samples for stable isotope analysis was described by Sealy (1984; 1989). Approximately 10 mg of dried leaf material was weighed out into a small piece of quartz tube, sealed at one end, which acted as a "sample boat". The sample boat together with approximately 0.5 g of copper oxide, some pieces of copper metal ($\pm$ 2 cm^2) and a small piece of silver foil ($\pm$0.025 cm^2) was placed in a 9mm quartz tube. The tubes were evacuated to less than 10^{-2} torr and sealed using a glass blower's torch.

The tubes were then baked over night at 800°C and allowed to cool slowly. The halides and sulphur produced during the burning react with the silver foil, while the nitrogen oxides are reduced to N_2 gas by the copper metal and the sulphur dioxide combines with copper oxide to give copper sulphate (Sofer 1980) if the tubes are allowed to cool slowly. After combustion the tube contains water vapour and CO_2, N_2 in the gaseous phase.

The gasses were separated in a stainless steel gas-separation line which was first evacuated to less than 10^{-4} Torr. The mixture of gasses was introduced to the line from the quartz tube which was first scored and placed in a "cracker" similar to that described by Desmarais and Hayes (1976).

The gas was allowed to flow into the first trap which was immersed in liquid nitrogen to allow freezing of the water vapour and carbon dioxide. A period of five minutes was allowed for this process to take place. The gas was then passed into the second trap where the procedure was repeated. The free gas at this stage, which is

mainly nitrogen, was trapped in a thin quartzite tube immersed in liquid nitrogen. A few pieces of activated coconut charcoal had been placed into the quartz tube and thoroughly cleansed of impurities by using a blow torch to drive off any other gasses. Note: the nitrogen gas will freeze in liquid nitrogen only if it is provided with a molecular sieve such as the coconut shell. The tube containing the nitrogen gas was then sealed off using a blow torch. The $^{15}N:^{14}N$ ratio of the nitrogen gas was measured on a VG Micromass 60 E mass spectrometer. The results are expressed in the δ notation. This refers the gas to a standard, in this case the laboratory standard, which is a commercially produced nitrogen gas that has been calibrated against atmospheric nitrogen and IAEC standards N-1 and N-2.

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{standard}} - 1 \right) \times 1000\ ‰$$ **Formula 5.2.**

3. Results

3.1. Average nodule mass to shoot mass ratios.

The 13 *Aspalathus* spp. nodulated well when grown on acid-washed sand inoculated with a wash of soil from their site of origin and fed nitrogen-free nutrient medium as they had large nodule to shoot mass ratios (Figure 5.1.). There was considerable variation in these ratios for individual plants of some of the species e.g. *A. ciliaris, A. microphylla* and *A. retroflexa*. Thus, when choosing plants from those grown on acid-washed sand for the analysis of $\delta^{15}N$, healthy plants with the largest nodule to shoot ratios were selected.

When compared to the plants grown on acid-washed sand in Figure 5.1., the 13 *Aspalathus* spp. grown on their native soil and supplied with oxygen gas for 20 minutes every day showed extremely low nodule to shoot mass ratios in seven of the 13 species and greatly reduced nodule formation in a further four species (Figure 5.2.). Two species, however, showed no decrease: *A. abietina* had a similar nodule to shoot mass ratio when compared with plants grown on acid-washed sand (Figure 5.1.), although the high average in Figure 5.2. is largely the result of one very well nodulated plant; *A. retroflexa* actually showed a large increase in the average nodule to shoot mass ratio in the oxygen-supplied plants (Figure 5.2) when compared with the plants grown on acid-washed sand (Figure 5.1.). In most cases it was possible to choose a healthy plant with no nodules for the $\delta^{15}N$ analysis. In those cases where there was no plant without nodules, the plant with the lowest nodule mass to shoot mass ratio was chosen for use as reference plant.

3.2. The $\delta^{15}N$ values of individual plants.

The $\delta^{15}N$ values were determined for only a single plant of the three categories, i.e. plants of each species grown on their native soil and supplied with oxygen; plants grown on acid-washed sand; and field-grown plants (Table 5.1.).

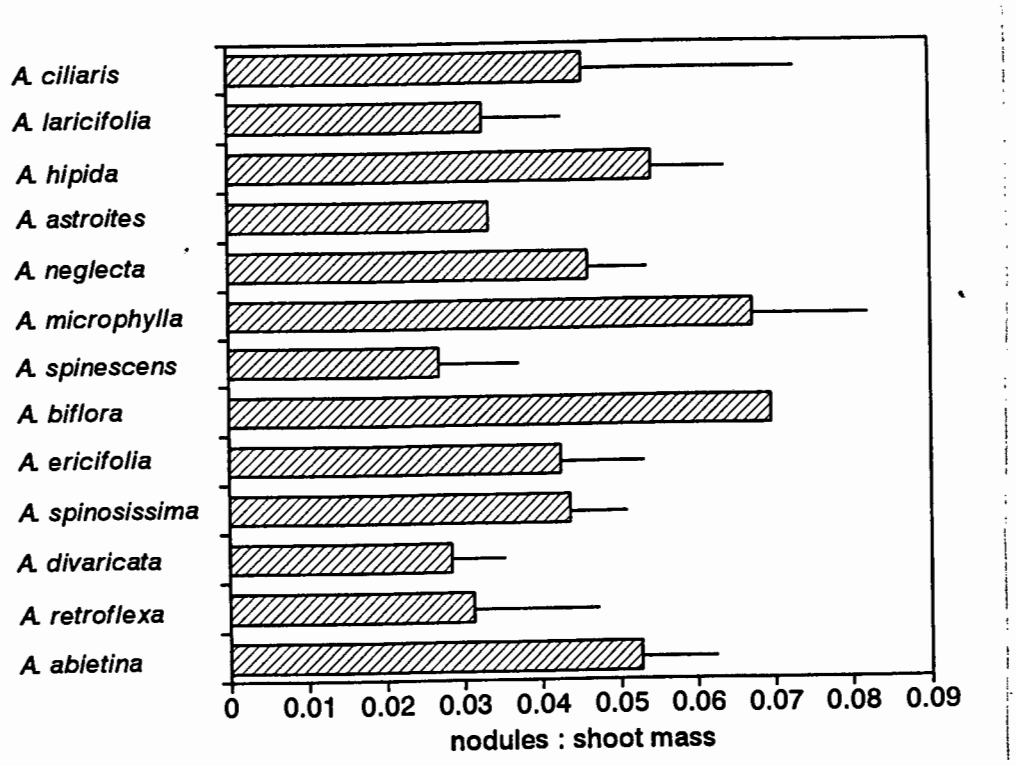


Figure 5.1. The average ratio of nodule to shoot mass per plant for 13 *Aspalathus* species which were grown for five months on acid-washed sand inoculated with a wash of the soil from their site of origin and fed nitrogen-free, 10 % dilute long Ashton solution. (The average for *A. biflora* is of two plants while that for *A. asteroides* is one plant's value. The other values are based on 6 - 10 plants per species.)

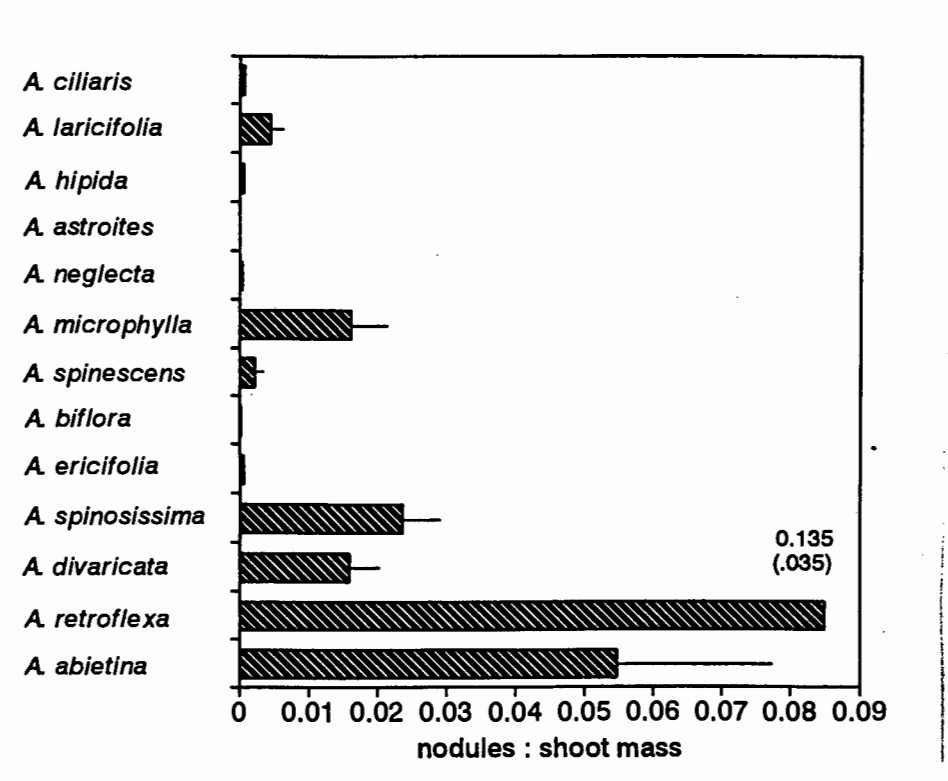


Figure 5.2. The average ratio of nodule mass to shoot mass per plants for 13 *Aspalathus* species grown on the soil from the site where the seed was collected. The plants were supplied with oxygen for 20 minutes every day for four months. (The averages are based on 6 -10 plants per species).

Table 5.1. The $\delta^{15}N$ values of leaves and nodule to shoot mass ratio of the individual analysed plants of 13 *Aspalathus* species which were grown on their native soil and supplied with oxygen, grown on acid-washed sand and fed nitrogen-free nutrient medium or field growing plants. The amount of nodulation is given for the individual field-grown plants and average for the study site (mg nodules.g^{-1} plant.100 cm^{-3} soil). (The average pH, soil total nitrogen and organic matter content are given for comparison. See chapter 4 for further details.)

Study site (month sampled)	*Aspalathus* spp.	non-N_2-fixing plants (O_2-supplied) $\delta^{15}N$ - nodule: shoot mass (individual)	N_2 fixing plants (acid-washed sand) $\delta^{15}N$ - nodule: shoot mass (individual)	Field-grown plants $\delta^{15}N$ - amount nodulation/ plant (individual) (mg nodules.g^{-1} plant.100cm^{-3} soil)	%Fndfa (Formula 5.1.) - average nodulation/ plant (site average) (mg nodules.g^{-1} plant.100cm^{-3} soil)	- Av.soil pH - Av. soil total nitrogen (mg N.g^{-1}) - Av. soil total organic matter (% dry mass)
Groot Hagelkraal	*A. ciliaris*					5.26
(acid sand)	$\delta^{15}N$	3.01	-0.81	1.31	44.50	1.189
(July 1991)	nodule:shoot	0	0.0337	5.94E-05	5.62E-05	0.961
Jonkershoek I	*A. laricifolia*					4.18
(September 1991)	$\delta^{15}N$	4.73	-0.42	3.04	32.81	0.983
	nodule:shoot	0	0.0717	6.85E-07	2.68E-07	1.570
	A. hispida					
	$\delta^{15}N$	12.04	0.26	1.31	91.07	
	nodule:shoot	0	1.1020	5.59E-06	2.53E-06	
Jonkershoek II	*A. asteroides*					4.58
(October 1991)	$\delta^{15}N$	2.90	0.06	0.07	99.65	5.075
	nodule:shoot	0	0.0334	4.42E-06	1.03E-06	7.906
	A. neglecta					
	δ15N	6.13	-2.19	-2.18	99.88	
	nodule:shoot	0	0.0730	4.05E-06	2.72E-06	
Pella	*A. microphylla*					4.43
(August 1991)	δ15N	-0.73	-1.31	-0.06	0	0.335
	nodule:shoot	0	0.1254	3.87E-07	1.39E-07	1.240
	A. spinescens					
	δ15N	-0.13	-0.65	3.38	0	
	nodule:shoot	0	0.0717	5.17E-08	1.92E-08	
Bainskloof	*A. biflora*					3.71
(August 1991)	δ15N	-1.26	-0.67	-1.06	100	0.582
	nodule:shoot	0	0.0700	5.21E-06	1.28E-06	1.148
DuToitskloof	*A. ericifolia*					4.18
(October 1991)	δ15N	-1.04	-0.94	-0.97	70.00	1.177
	nodule:shoot	0	0.0986	5.18E-06	1.59E-06	2.327
	A. spinosissima					
	δ15N	-0.120	-2.64	-0.96	0	
	nodule:shoot	0	0.0642	1.14E-05	8.48E-06	

...continued

Table 5.1. continued ...

Orangekloof	*A. divaricata*					4.41
(October 1991)	δ15N	6.54	-0.47	-0.90	100	1.124
	nodule:shoot	0.0012	0.0619	5.08E-06	2.65E-06	2.093
Scarborough	*A. retroflexa*					4.03
(September 1991)	δ15N	0.18	0.51	0.40	66.67	0.444
	nodule:shoot	0	0.0717	3.18E-07	3.89E-06	1.956
Silvermine	*A. abietina*					3.85
(September 1991)	δ15N	3.19	-1.34	-0.68	85.43	0.428
	nodule:shoot	0.0043	0.0662	0.000125	5.52E-05	1.249

It was possible to analyse a healthy plant with no visible nodules in 11 of the 13 species supplied with oxygen. The analysed plants of the remaining two species, *A. divaricata* and *A. abietina*, both had very low nodule to shoot mass ratios. In the case of the plants grown on acid-washed sand it was possible to choose well-nodulated plants in all cases.

The $\delta^{15}N$ values for the oxygen-supplied plants were generally higher than for the plants grown on acid-washed sand, exceptions being *A. retroflexa* and *A. ericifolia* where these values were lower. The $\delta^{15}N$ values for *A. microphylla* and *A. spinescens* from Pella and *A. ericifolia* and *A. spinosissima* from DuToitskloof were below zero. These low $\delta^{15}N$ values did not appear to be related to the soil pH, soil total nitrogen or soil total organic matter at these study sites.

The plants grown on acid-washed sand gave negative $\delta^{15}N$ values for the shoots in all but three cases: *A. hipida* and *A. asteroides*, where the values were still well below that of oxygen-supplied plants, and *A. retroflexa*, where it was not.

In six cases, the value for the field-grown plants fell between that of the oxygen-supplied plants and the plant grown on acid-washed sand; and the $\delta^{15}N$ value for oxygen-supplied plants was greater than that for the plants grown on acid washed sand. This resulted in a %FNdfa (Formula 5.1.) value between 0 and 100 which is the desired result. The closer the %FNdfa value is to 100, the greater the contribution of fixed nitrogen to the plant. In three cases, *A. microphylla* and *A. spinescens* at Pella and *A. spinosissima* at DuToitskloof, the value for field-grown plants was greater than that for the oxygen-supplied plants. This leads to a negative FNdfa value and suggests that no nitrogen fixation is taking place. On the other hand, in a further two cases, the *A. divaricata* at Orangekloof and *A. biflora* at

Bainskloof, this value was greater than 100 suggesting that nitrogen fixation was taking place but there is an inconsistency with one of three $\delta^{15}N$ values.

In all species, except *A. retroflexa*, the amount of nodulation of the field-grown plant used for analysis was higher than the average for its study site. For plants where the amount of nodulation was to the -7 exponent or below, a low FNdfa value was obtained (e.g. *A. laricifolia* at Jonkershoek I and *A. microphylla* and *A. spinescens* at Pella), whereas in cases where this value was equal to or greater than -6, FNdfa values close to or greater than 100% were obtained. This was not true, however, for *A. ciliaris* from the acid sand at Groot Hagelkraal and *A. spinosissima* at DuToitskloof, both of which had high amounts of nodulation but low FNdfa values. These nodules may have been ineffective.

4. Discussion.

All species, with the exception of *Aspalathus asteroides*, grew well in acicd-washed sand and nodulated very well when inoculated with a wash of their native soil. More interestingly, however, the plants grown in soil from their natural environment and supplied with oxygen showed reduced nodulation in most cases. The use of non-nodulated plants of the same species, reliant on soil nitrogen only is preferable to the use of reference plants of other species for the determination of nitrogen fixation by the $\delta^{15}N$ natural abundance method. This eliminates possible differences in fractionation of soil nitrogen between the reference plant species and the nitrogen-fixing species and difficulties in the choice of a reference plant owing to differences in fractionation between possible reference plant species (Shearer & Kohl 1989).

Reduction in the amount of nodulation has been observed in the cowpea, (*Vigna unguiculata*) when grown for extended periods (69 days) with high concentrations of oxygen around its roots. At ambient oxygen concentration, plants with 181 mg^{-1} nodules.$plant^{-1}$ were produced. At 80 % oxygen in nitrogen (v/v), the figure was reduced to 73.2 mg^{-1} nodules.$plant^{-1}$ (Dakora & Atkins 1989). This species also showed reduced rates of acetylene reduction, with 8.7 μmol $C_2H_4.h^{-1}.plant^{-1}$ for plants grown at ambient oxygen concentration and 1.4 μmol $C_2H_4.h^{-1}.plant^{-1}$ for plants grown at 80 % oxygen for 28 days (Dakora & Atkins 1990a). Similar results were obtained for soybean (*Glycine max*) grown for 27 days where the acetylene reduction was 4.2 μmol $C_2H_4.h^{-1}.plant^{-1}$ in plants at ambient oxygen concentration and 1.0 in plants at 80% oxygen in nitrogen (v/v) (Dakora & Atkins 1990b). The success of supplying oxygen to reduce nodulation and/or inhibit nitrogenase acitivy in this experiment also appeared to depend on the species, e.g. *A. abietina* and *A. retroflexa* nodulated well when compared with the plants grown on acid-washed sand. It was unlikely that this effect was due to a lack of penetration of the soil by the oxygen as both these species were growing in sand of a coarser texture than many of the other soils in the experiment although it may have resulted from the oxygen being able to leave to the soil more rapidly.

The oxygen-supplied plants gave positive $\delta^{15}N$ values with a broad range of 1.26‰ and 12.04 ‰. Plants from Pella and DuToitskloof, however, gave negative values. Shearer *et al.* (1983) found the range of non-fixing shrubs and herbs at Baja California to be between 7‰ and 12‰. In a fire-prone *Banksia* woodland in south Western Australia obligate seeders had the highest $\delta^{15}N$ values with a range of

between 1 ‰ and 4 ‰ (Pate *et al.* 1993). These species had active nitrate reductase and nitrate storage. Similarly, the highest $\delta^{15}N$ values in various northern Californian ecosystems were associated with annual herbaceous species (Virginia & Delwiche 1982). Except in the case of *A. ericifolia* the negative $\delta^{15}N$ values of the oxygen-supplied plants from Pella and DuToitskloof were still higher than those for plants grown on acid-washed sand. Virginia *et al.* (1989) have noted that certain Californian soils have very low $\delta^{15}N$ signatures making it difficult to differentiate between nitrogen-fixing and reference plants. This problem will still arise should the fixing plant be grown on its native soil and prevented from nodulating. The negative $\delta^{15}N$ values for Pella are not related to the soil, however, as values of 4.99 ‰ and 4.03 ‰ have been measured by W.D. Stock (unpublished data) but no similar $\delta^{15}N$ values are available for DuToitskloof. It is possible that the plants were absorbing nitrogen in the form of ammonia in these two cases. Low $\delta^{15}N$ values were found to be associated with resprouting shrubs with low nitrate reductase activity by Pate *et al.* (1992).

In the plants grown on acid-washed sand, $\delta^{15}N$ values ranged between 0.26 ‰ and -2.64 ‰ and there was less variation than in the oxygen-supplied plants. The variation observed was possibly a result of differences in the amount of fractionation occurring in different species, although nitrogen present in the plants obtained from the seed could, conceivably, also have lead to variation.

The $\delta^{15}N$ values for the field-grown plants ranged between 3.38 ‰ and -2.18 ‰ which is a much broader range for nitrogen-fixing species than the values obtained by Pate *et al.* (1993) for *Banksia* woodland (0.29 ‰ to 0.79‰). Shearer *et al.* (1983), however, obtained values of between -0.1 ‰ and 11 ‰ for a number of legumes in Baja California. W.D. Stock (unpublished data) has measured $\delta^{15}N$ values for several other *Aspalathus* species and these fell within the range obtained in this study. He recorded the following values at Scarborough -0.10 ‰ for *A. carnosa* in 18-month vegetation and -0.86 ‰ for it in 15-year-old vegetation and -0.92 ‰ and -0.64 ‰ for *A. callosa* and *A. capensis* respectively. At Jonkershoek -2.04 ‰ was recorded for *A. coronata*. Thus the need to compare the values for field-grown plants, which vary greatly between each other, with solely nitrogen-fixing plants and reference plants is clear.

In only six of the species did the $\delta^{15}N$ value for the field-grown plants fall between the two poles of the plant grown on acid-washed sand and the oxygen-supplied plants. The $\delta^{15}N$ value for the oxygen-supplied plants was also greater than that for

the plants grown on acid-washed sand. The negative $\delta^{15}N$ values obtained for the oxygen-supplied plants from DuToitskloof and Pella causes interpretation of the results from these two sites difficult. Nitrogen fixation seems unlikely for *A. spinescens* at Pella given the very much higher $\delta^{15}N$ of the field-grown plants (3.38 ‰) which were very poorly nodulated. In *A. microphylla* from Pella, the $\delta^{15}N$ value (-0.06 ‰) lies well above that of the oxygen-supplied plant. This together with the low levels of nodulation in this species, suggests that nitrogen fixation was minimal in this plant. This is not true for *A. ericifolia* and *A. spinosissima* from DuToitskloof where the $\delta^{15}N$ values for the field-grown and the oxygen-supplied plants are very close to one another. The high degree of nodulation in these species also suggests that nitrogen fixation may be taking place.

A. retroflexa showed a lower $\delta^{15}N$ value for the oxygen-supplied plant. In this case fixation may well have been taking place as the plant used was the only plant found without visible signs of nodulation. The other plants of this species were very heavily nodulated (see section 3.1.). Given the much lower $\delta^{15}N$ value of *A. biflora* and *A. divaricata*, it is possible that fixation was taking place in these field-grown plants.

In conclusion, it appears that the $\delta^{15}N$ natural abundance method for determining nitrogen fixation shows promise for the fynbos and other Mediterranean ecosystems. The use of oxygen to reduce nodulation and inhibit nitrogenase activity could be a more useful and accurate method than the use of unrelated reference plants as a means of determining $\delta^{15}N$ values for nitrogen-fixing plant species that are reliant on soil nitrogen for their nitrogen supply as it avoids the assumption that the nitrogen-fixing species and the reference plant species respond in the same way to different conditions. Low $\delta^{15}N$ signatures of the soil will, however, still be reflected in the $\delta^{15}N$ values for the oxygen-supplied plants and if these are similar to those of the field-grown plants it will make the determination of nitrogen fixation difficult.

Chapter 6

Aspalathus species growth, senescence and interaction with vegetation composition.

1. Introduction.

Dense stands of *Aspalathus* plants are a fairly common sight in fynbos environments about 3-5 years after a fire. They may blanket whole mountain sides forming almost impenetrable thickets. These plants grow and thrive during the first phases of pyric succession (Kruger 1979) but, as the vegetation approaches maturity, they die in large numbers. Although this situation is common, it has seldom been reported in fynbos literature in any detail. Adamson (1935) and Levyns (1935), for example, are among the few botanists who, in their studies, mention large stands of *Aspalathus* being found. Thus little quantitative data and few descriptions of the significance of this phenomenon exist.

In the literature review of this thesis (Chapter 2), the fire survival strategies of legumes in fynbos were discussed and many members of the Fabaceae appear to be so-called auto-regenerating, long-lived sprouters (Bell *et al.* 1984). *Aspalathus* species, however, are different as they appear to fall into the fire ephemeral category. In fynbos vegetation it appears that *Aspalathus* as well as other auto-regenerative sprouting Fabaceae become less common as the vegetation ages (Levyns 1929; Adamson 1935; Levyns 1935; Martin 1966; Van Wilgen 1981; Hoffman *et al.* 1987). There are little data, however, with which to substantiate this hypothesis. It may also be possible that the Fabaceae, which are the forth largest fynbos family (Cowling & Holmes 1992), may have been overlooked in vegetation analysis in terms of cover as the above-mentioned studies concentrated on older vegetation. In this chapter, I offer a test of the hypothesis that the Fabaceae become less common as the vegetation ages by comparing stands of differing ages. I also investigate the growth and senescence of *Aspalathus* species which differ in possible survival strategies over the course of a year at different fynbos sites.

Finally, it may be argued that dense stands of *Aspalathus* have an influence on the surrounding vegetation and may lead to lower point, or alpha, diversity. This has been found to be true for the Proteaceae in a number of studies (Campbell es & van der Meulen 1980; Cowling & Gxaba 1990; Esler & Cowling 1990). I therefore examined the species diversity of a number of stands of vegetation with different densities of *Aspalathus* in order to determine whether members of this genus influence alpha diversity.

2. Methods.

2.1. Study sites.

The study sites were of two types. These are described in detail in Chapter 3.

1. Seasonal sites - Cape Point I, Scarborough and Silvermine. At these sites vegetation and nodulation data (see Chapter 4) were collected at six-monthly intervals over one year (May 1991, December 1991 and June 1992).

2. Geographical survey sites - Jonkershoek I and II, DuToiskloof, Bainskloof, Cape Point II, Groot Hagelkraal and Orangekloof. Data at these sites were collected on a once-off basis between July and November 1991.

2.2. Data collection.

At each of the study sites the young (i.e. recently burnt) vegetation contained stands of *Aspalathus*, the exception was Bainskloof where these were located in the middle-aged vegetation. The young vegetation which contained stands of *Aspalathus* was located next to older (i.e. unburnt) vegetation at very close proximity (5-30m) (see Chapter 3). Two of the seasonal sites had vegetation of three ages: Silvermine, where the so-called middle-aged vegetation did not contain stands of *Aspalathus*; and Scarborough, where it did. Cape Point II and Jonkershoek II had only middle-aged and young vegetation respectively and no old vegetation.

Five plots where laid out in the different aged vegetation at each site. The same plots were used for both the assessment of the vegetation and the collecting of nodule cores (the data are presented in Chapter 4). Thus, at the seasonal sites which were sampled at three, six-monthly intervals, new plots adjacent to the previously sampled plots were laid out for each sample.

At each plot a visual estimate was made of the percentage-cover of all the plant species found in the plot. Note was also taken of the plot's aspect, rock cover and slope. Samples were taken of all the plant species occurring at each of the sites so that they could be identified in the Bolus Herbarium at the University of Cape Town. Most plants species were identified to species level. However, in some cases this was not possible and in such instances the plants could only be identified to the generic or, more rarely, the family level.

The size of each *Aspalathus* plant within the plots was measured and the viability estimated for the plots in the new vegetation at each study site. All the *Aspalathus* species, which ranged from small to large shrubs, erect or spreading, were measured in three dimensions: height and two width measurements. In the case of *A retroflexa*, which is a prostrate creeper, this measurement was not practical and thus the length of each plant's main branches was measured. In plots where the stands of *Aspalathus* were very dense a representative subsample of the plot was measured. The viability of the plant was assessed as the percentage of the plant perceived to be dead and each plant was placed in one of eight categories, i.e. 0-12; 13-25; 26-33; 34-50; 51-66; 67-75; 76-87; 88-100 % dead.

2.3. Data analysis

2.3.1. Species composition

The total percentage-cover of each species was calculated and a species list compiled for the different aged vegetation at each site. The total cover of all the families occurring in the vegetation of different ages at each site was then calculated by adding the total percentage-cover of all the different species belonging to that family.

The species lists with associated cover values were used to calculate two diversity indices for the young plots at both the seasonal and geographical survey sites. The indices calculated were -

a. Simpson's dominance index

$$\hat{\lambda} = \sum_{i=1}^{s} \frac{n_i(n_i-1)}{n\,(n-1)}$$

where n is the total cover of all species, s, in the sample and n_i is the cover of the i^{th} species. This index, which varies between 0 and 1, gives the probability that two individuals drawn from the same data set will belong to the same species. The higher this probability, the lower the diversity of the data set (Ludwig & Reynolds 1988).

b. Shannon-Wiener index.

$$\hat{H} = -\sum_{i=1}^{s} \left[\left(\frac{n_i}{n} \right) \ln \left(\frac{n_i}{n} \right) \right]$$

where n is the total cover of all species, s, in the sample and n_i is the cover of the i^{th} species. This index is a measure of the uncertainty in predicting that a species drawn from the data set at random will belong to a particular species in a data set. The uncertainty will increase as the number of species increases and also as the cover of the different species becomes more similar. Thus the Shannon Wiener index is maximal when all the species have the same cover and one if, and only if, there is only one species in the sample (Ludwig & Reynolds 1988).

3. Results.

3.1. Contribution of major fynbos families to vegetation of different ages.

Vegetation age is important when interpreting the data of percentage-cover and number of species in five major plant families at various study sites (Table 6.1.,). The original species lists have been placed in the Appendix. The vegetation of different ages at the study sites was divided into three age categories: young vegetation (1-3 years post-fire); middle-aged vegetation (4-9 years post-fire); and old vegetation (10+ years post-fire). All the study sites had young vegetation and at Jonkershoek II and Groot Hagelkraal on the limestone soils, only vegetation of this category was present while at Pella the younger vegetation fell into the middle-aged category. Old vegetation was present at most sites but at Cape Point I and Bainskloof, however, the older vegetation fell into the middle-aged category. Only at Silvermine and Scarborough was vegetation of all three categories present.

The cover of the Proteaceae and Ericaceae showed an increase between the young and the old vegetation at each study site (Table 6.1.). This trend was not found for the Proteaceae in the six-year-old vegetation of Cape Point I or in the acid sand vegetation at Groot Hagelkraal for either family. The Asteraceae had the lowest cover in the vegetation of about six years of age i.e. Silvermine, Cape Point I and II and Bainskloof. Stands of old vegetation at the different study sites showed neither an increase nor decrease in common when compared with the young vegetation at each study site for this family. In the middle-aged vegetation other than at Bainskloof, a large increase in the cover of Restionaceae is evident. Stands of old vegetation again had neither an increase nor decrease in common between the study sites for this family. The "other" families included a broad spectrum. Some of the better represented families in this group were herbaceous bushes (Geraniaceae, Campanulaceae, Mesembrianthemaceae etc.), sclerophyllous shrubs (Rutaceae, Rhamnaceae, Rosaceae, Thymeliaceae etc.) and geophytes (Iridaceae, Asphodelaceae and Haemodoraceae etc.). The cover of these species was generally higher or the same as that of the old vegetation with the exception of the acid sand vegetation at Hagelkraal and at Jonkershoek I where there was an increase. In a number of cases the young sites also contained a greater variety of species compared to the older vegetation.

Table 6.1. The percentage-cover and number of species in the families Fabaceae, Proteaceae, Ericaceae, Asteraceae, Restionaceae and Cyperaceae and "others" in vegetation of three age categories (young, middle-aged and old) at each of the eight geographical survey sites (data collected from July 1991 to November 1991) and three seasonal sites (data collected during December 1991).

Site	Age	Family												Total	
	young: 1-3 years middle-aged: 4-9 years old: 10+ years	Fabaceae		Proteaceae		Ericaceae		Asteraceae		Restionac. & Cyperac.		Other families			
		% cover	No. spp.	% cover	No. spp.	% cover	No. spp.	% cover	No. spp.	% cover	No. spp.	% cover	No spp.	% cover	No spp
Groot Hagelkraal															
acid sands	young	3.6	1	4.0	5	5.8	7	11.0	7	40.6	11	8.2	15	73.2	46
	middle-aged	-		-		-		-		-		-			
	old	0		2.8	1	3.2	3	4.4	5	43.2	15	13.6	14	67.2	38
limestone	young	28.4	4	3.4	4	1.4	1	5.8	10	23.0	11	10.4	21	72.4	51
Jonkershoek I	young	9.4	3	4.2	4	0.4	1	7.0	9	30.2	11	25.8	37	77.0	65
	middle-aged	-		-		-		-		-		-			
	old	1.4	3	9.2	3	10.8	5	14.8	5	9.0	8	30.6	26	75.8	50
Jonkershoek II	young	28.6	4	0.4	2			24.2	6	6.2	2	30.4	22	89.8	36
Pella	young	-		-		-		-		-		-			
	middle-aged	24.6	4	2.6	6	0		15.4	11	14.8	9	14.4	19	71.8	49
	old	3.6	5	12.2	4	7.2	2	13.4	12	24.0	8	15.0	12	75.4	43
Bainskloof	young	0.6	1	0.6	3	1.2	3	28.2	11	7.6	10	6.0	17	44.2	45
	middle-aged	7.2	1	1.0	1	4.6	6	5.6	9	40.8	9	4.4	10	63.6	36
	old	-		-		-		-		-		-			
Dutoitskloof	young	13.2	3	0.6	1	0		12.6	7	15.6	4	23.0	27	65.0	42
	middle-aged	-		-		-		-		-		-			
	old	0.6	2	3.0	2	19.6	2	15.6	6	4.6	3	22.2	28	65.6	43
Orangekloof	young	24.6	4	3.4	4	0		8.4	9	9.8	9	39.2	51	85.4	77
	middle-aged	-		-		-		-		-		-			
	old	0.4	2	6.0	4	6.4	5	6.0	10	24.2	10	32.6	35	75.6	66
Scarborough	young	16.4	6	1.6	3	1.6	2	17.6	17	10.6	13	19.4	32	67.2	73
	middle-aged	9.4	4	2.6	3	11.4	7	13.4	19	23.0	16	18.0	31	77.8	80
	old	0.8	3	3.8	2	20.2	5	15.8	11	17.6	11	16.8	31	75.0	63
Cape Point I	young	5.3	4	2.0	4	1.8	4	9.2	12	11.8	14	11.8	23	41.9	61
	middle-aged	3.0	3	1.6	3	23.0	5	4.4	6	28.8	11	5.8	13	65.8	41
	old	-		-		-		-		-		-			
Cape Point II	middle-aged	20.0	1	1.4	2	8.6	8	3.2	2	21.0	8	6.2	12	60.4	33
Silvermine	young	6.0	2	0.4	1	0		6.4	10	18.2	18	21.2	26	52.2	57
	middle-aged	0		4.4	2	12.8	3	1.4	3	51.0	11	4.2	10	73.8	29
	old	0.2	1	4.2	2	15.6	3	12.8	7	19.4	14	10.8	19	63.0	46

The high cover of Fabaceae in the young vegetation at all the study sites is expected as these sites were chosen because they contained stands of *Aspalathus*. Bainskloof and Pella are exceptions as the *Aspalathus* spp. were present in the middle-aged vegetation at these study sites. Higher cover of Fabaceae was also present at Cape Point II and in the old vegetation of Cape Point I which were both approximately six years old. In vegetation older than 12 years the cover of Fabaceae was below 1.5 % and at Pella (10 years old), 3.6 %. Thus, to summarise, it appears that the Fabaceae formed a small percentage of the cover of the older vegetation at the study sites. This was especially the case where the vegetation was located in close proximity to young vegetation rich in Fabaceae was older than 12 years.

3.2. The phytomass and viability of stands of *Aspalathus* plants over one year.

Phytomass is here defined as the whole standing plant, i.e. the live parts or biomass and the dead parts or necromass. The average total phytomass of the plants occurring in a plot was calculated for the seasonal sites, Cape Point, Scarborough and Silvermine for the three sampling times, May 1991, December 1991 and June 1992. In the case of the small shrub species, *Aspalathus carnosa* and the spreading bush species, *A. abietina*, the total phytomass as volume (m^3) of the plants in a plot was calculated. With the prostrate creeper, *A. retroflexa*, however, the phytomass in terms of total branch length (m) of the plants in a plot was calculated. The two measurements are thus not directly comparable. As the total phytomass (Figure 6.1. and 6.2.) is the average of only five plots per study site, these values could not be tested for significant differences between the different sampling times at a study site.

Aspalathus carnosa (Figure 6.1.), at the three sites where it was present, showed an increase in phytomass over the spring growing season. Note that the larger phytomass at Scarborough is associated with older vegetation (five years) whereas the young vegetation at this site was only one year old and at the Cape Point site, two years old. *A. abietina* at Silvermine showed a large increase in phytomass over the spring period. By June 1992 of the following year, however, the standing phytomass of plants had decreased substantially. *Aspalathus retroflexa* (Figure 6.2.) showed a marked increase in phytomass over the spring season at the three study sites where it was present. By June 1992 the phytomass had decreased at all three sites to near its initial level.

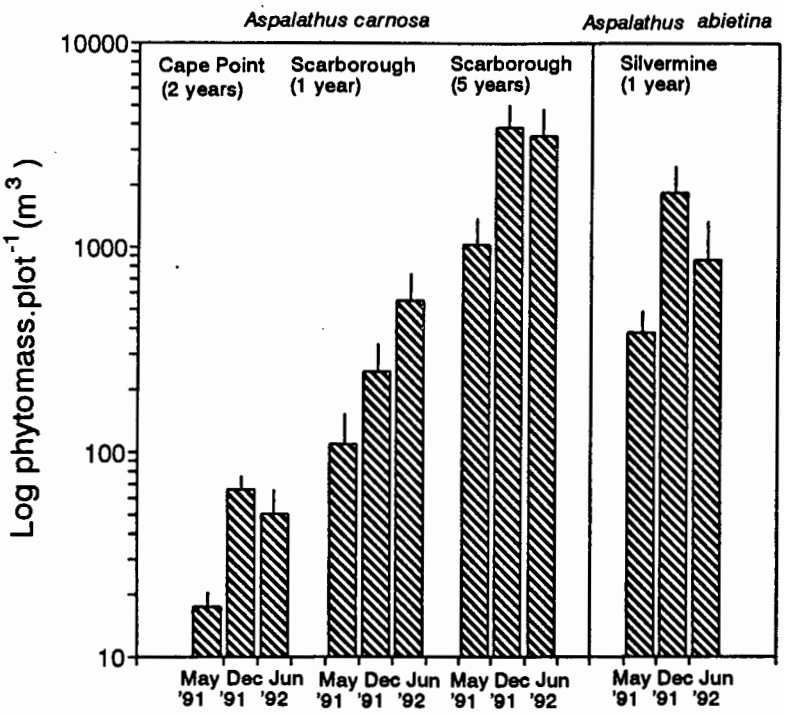


Figure 6.1. The average total phytomass per plot (m^3) (with standard error bars) of *Aspalathus carnosa* at the seasonal study sites, Cape Point (two year post-fire) and Scarborough (one and five years post-fire) also included is *Aspalathus abietina* at Silvermine (one year post fire). Data were collected during May 1991, December 1991 and June 1992.

The average size or phytomass of the individual plants of *A. carnosa* showed an increase in size over the spring season but not over the rest of summer at Cape Point or in the middle-aged vegetation at Scarborough (Figure 6.3.). In the young vegetation at Scarborough there was also a small but significant difference in size over the first half of the year. In the second half of the year there was a large and significant increase. A. *abietina* also showed a significant increase in plant size over the spring growing season (Figure 6.3.).

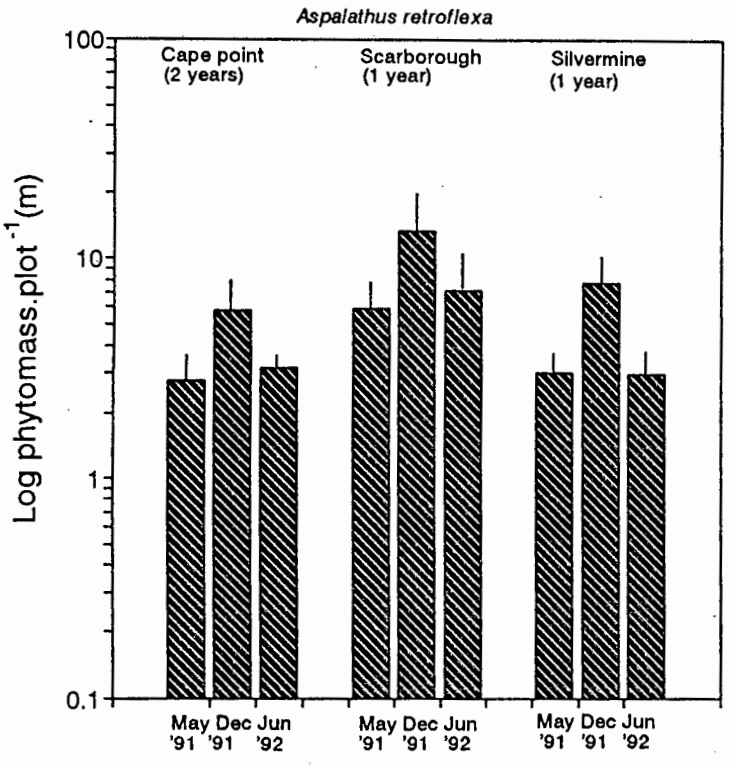


Figure 6.2. The average total phytomass per plot (m) (with standard error bars) of *Aspalathus retroflexa* at the seasonal study sites, Cape Point (two year post-fire), Scarborough (one year post-fire) and Silvermine (one year post-fire). Data were collected during May 1991, December 1991 and June 1992.

A. retroflexa at Cape Point I showed a significant increase in average plant phytomass over the first half of the year but by June 1992, however, there was a decrease in average plant size possibly due to parts of the plants having broken off. At Silvermine there was a significant increase in plant size by June 1992, but not over the spring growing season. The significant decrease in average plant phytomass observed over the spring growing season in the young vegetation at Scarborough appeared, with closer examination of the data, to be a spurious result of the chance exclusion of smaller plants which were included in the following two samplings (December 1991 and June 1992).

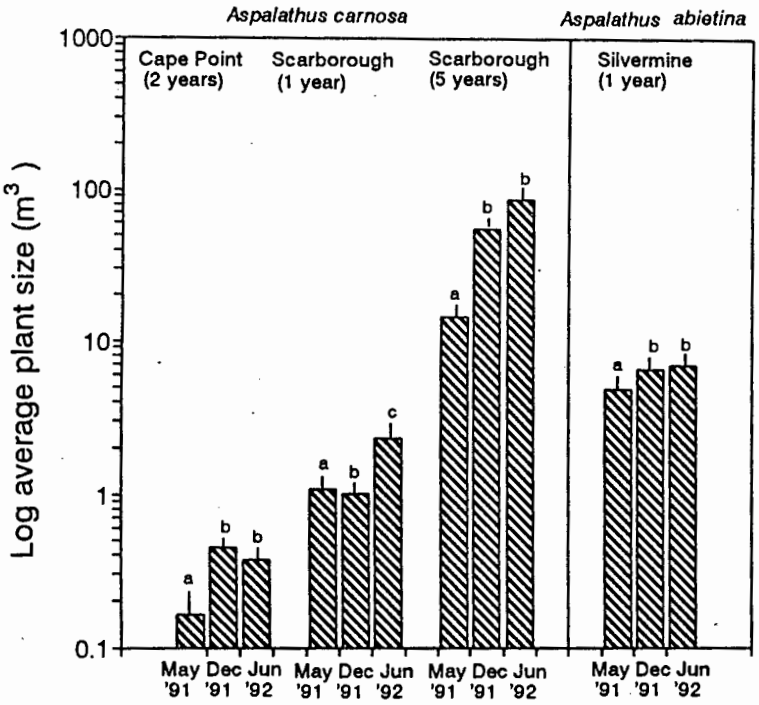


Figure 3. The average plant size or phytomass (m^3) (with standard error bars) of *Aspalathus carnosa* at the seasonal study sites, Cape Point I (two years post-fire) and Scarborough (one and five years post-fire). Also included is *Aspalathus abietina* at Silvermine (one year post-fire). Data were collected during May 1991, December 1991 and June 1992. The letters a, b and c denote significant differences (one-way ANOVA, $P < 0.05$) between the average plant sizes for the three samplings at each study site.

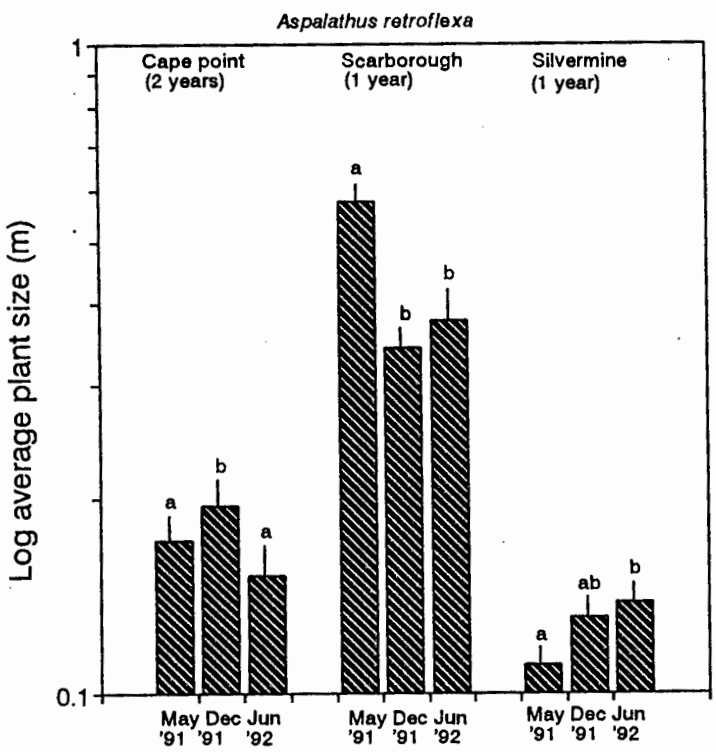


Figure 4. The average plant size or phytomass (m) (with standard error bars) of *Aspalathus retroflexa* at the seasonal study sites, Cape Point I (two years post-fire), Scarborough (one year post-fire) and Silvermine (one year post-fire). The letters a, b and c denote significant differences (one-way ANOVA, $p < 0.05$) between the average plant sizes for the three samplings at each study site.

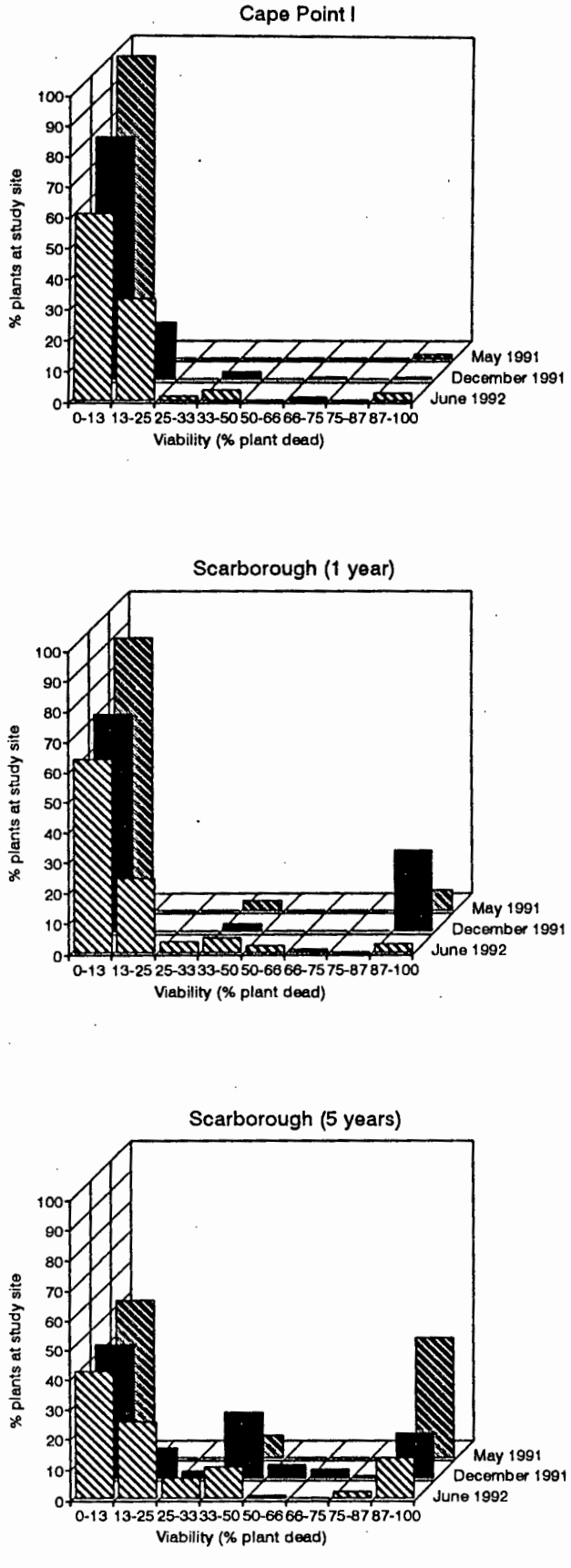


Figure6.5.. The percentage of *Aspalathus carnosa* plants in eight viability classes (% plant judged to be dead) at Cape Point I (two years post fire) and Scarborough (one and five years post fire).

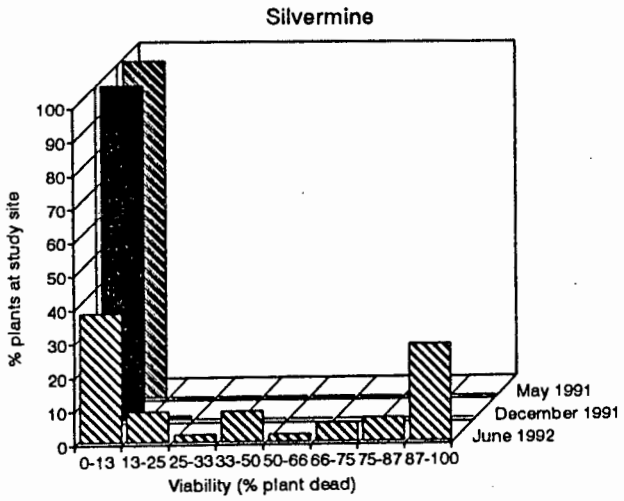


Figure 6.6. The percentage of *Aspalathus abietina* plants in eight viability classes (% plant judged to be dead) at Silvermine (one year post-fire).

The viability of the *Aspalathus carnosa* plants is illustrated in Figure 6.5. The percentage of plants which fell into the first viability class (0 - 13 % dead) dropped considerably over the year to almost 60 % at both Cape Point I and for the one year old vegetation at Scarborough. In the five-year-old vegetation at Scarborough, a larger percentage of the vegetation was senescent from May 1991 to June 1992 with only 40 - 50 % of the plants falling into the 0 - 13 % dead category. The high number of dead plants in May 1991 was due to one dense stand of very small, dead plants having been incorporated into one of the plots. *Aspalathus abietina* at Silvermine (Figure 6.6.) showed a very large increase in senescence over the summer growing season with almost 100 % of the plants being located in the 0-13 % dead category for the May and December 1991 samplings. This figure dropped drastically to less than 40 % by June 1992 when there was also a very large increase in the 87 - 100 % dead category. *Aspalathus retroflexa* at all three sites where it was sampled (Figure 6.7.) showed the most dramatic increase in the amount of senescence. Over summer, by June 1992, a very large proportion of the plants fell into the higher percentage dead categories. About 25 % of the plants at Silvermine fell into the 87 - 100 % dead category and about 10 % of the plants at Scarborough fell into this category in both December 1991 and June 1992.

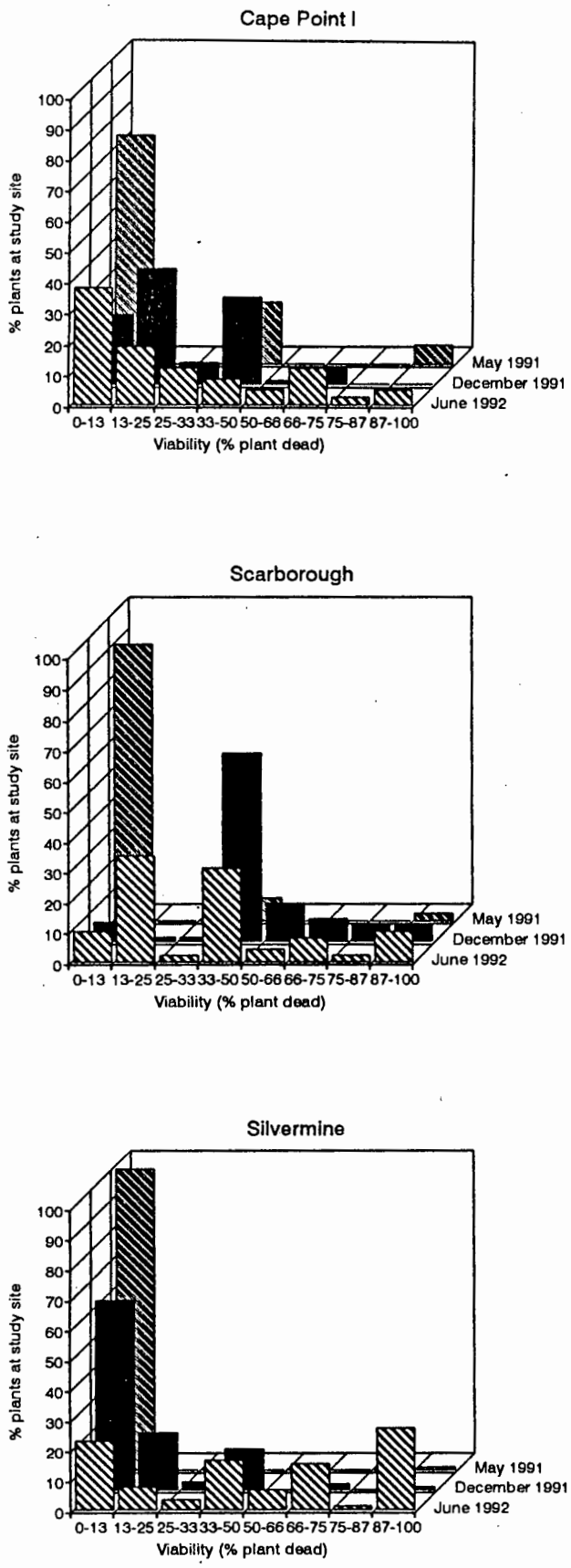


Figure 6.7. The percentage of *Aspalathus retroflexa* plants in eight viability classes (% plant judged to be dead) at Cape Point I (two years post-fire) and Scarborough (one year post-fire) and Silvermine (one year post-fire).

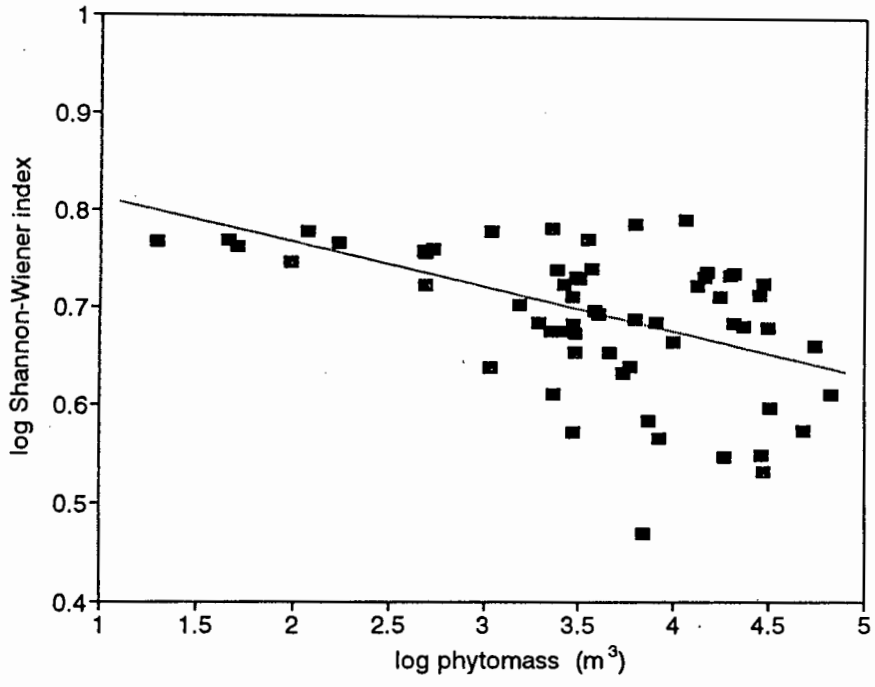


Figure 8. The log of the total phytomass of *Aspalathus* per plot against the log of the Shannon-Wiener index of the other species occupying the plot. The data are those for the vegetation which contained stands of *Aspalathus* plants of the geographic survey sites and the seasonal sites for the December 1991 samplings. $r^2 = 0.177$; $p < 0.01$.

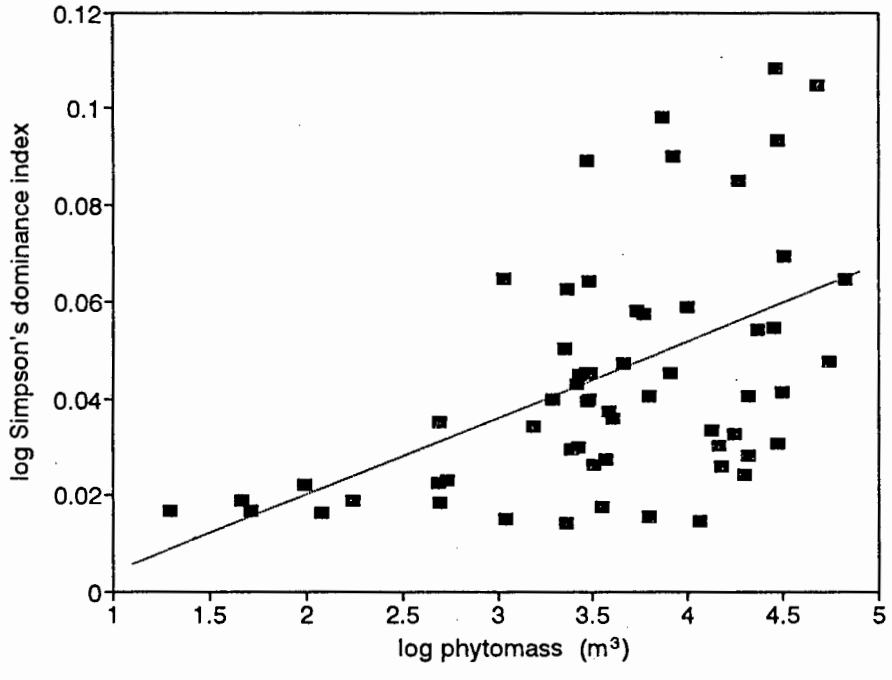


Figure 9. The log of the total phytomass of *Aspalathus* per plot against the log of the Simpson's dominance index of the other species occupying the plot. The data are those for the vegetation which contained stands of *Aspalathus* plants at the geographic survey sites and the seasonal sites for the December 1991 sampling. $r^2 = 0.195$; $p < 0.01$.

3.3. Correlations between total *Aspalathus* phytomass and various species diversity parameters.

The effect of increasing density of *Aspalathus* plants on the associated vegetation's diversity and cover was investigated. The total phytomass of *Aspalathus* plants per plot (m^3) was calculated for the young vegetation of the geographical survey sites (the middle-aged vegetation in the case of Bainskloof) as well as the young vegetation (and the middle-aged vegetation at Scarborough) of the seasonal sites. The data for December 1991 were used. These sites all contained stands of *Aspalathus* plants. Only the *Aspalathus* species which are erect or reclining shrubs and which were measured in three dimensions were included. Thus *A. retroflexa* which is a prostrate creeper and difficult to compare with the other species was not included in the phytomass measurement. The percentage-cover data of the all species present in the plot, with the exception of the *Aspalathus* species for which phytomass data were collected, were used to calculate diversity indices (Shannon Wiener and Simpson's dominance), percentage-cover and the number of species per unit area for each plot.

The log of the total phytomass of *Aspalathus* is plotted against the log of the Shannon-Wiener index for each plot (Figure 6.8.). The use of the log value improved the normal distribution of the data. There was a negative correlation between these two factors. The log of Simpson's dominance index was positively correlated with the log of the total phytomass of *Aspalathus* per plot (Figure 6.9.). The log of the total phytomass of *Aspalathus* was negatively correlated with the number of species per unit area in each (Figure 6.10.). Finally, the log of the phytomass of *Aspalathus* was positively correlated with the log of the total cover of other species in the plot although less of this variation was explained by the total phytomass of *Aspalathus* in the plot than in the other three cases ($r^2 = 0.070$; Figure 6.11.).

The four factors, Shannon-Wiener index, Simpson's dominance index, species per unit area and total cover of other species in each plot were most strongly correlated with the total phytomass of *Aspalathus*. The inclusion of a viability factor by multiplying the phytomass of the plants by their respective percentage viabilities (which excluded dead plants and reduced the size of partly dead plants) did not increase the r^2 values of these correlations. This is illustrated in Table 6.2.

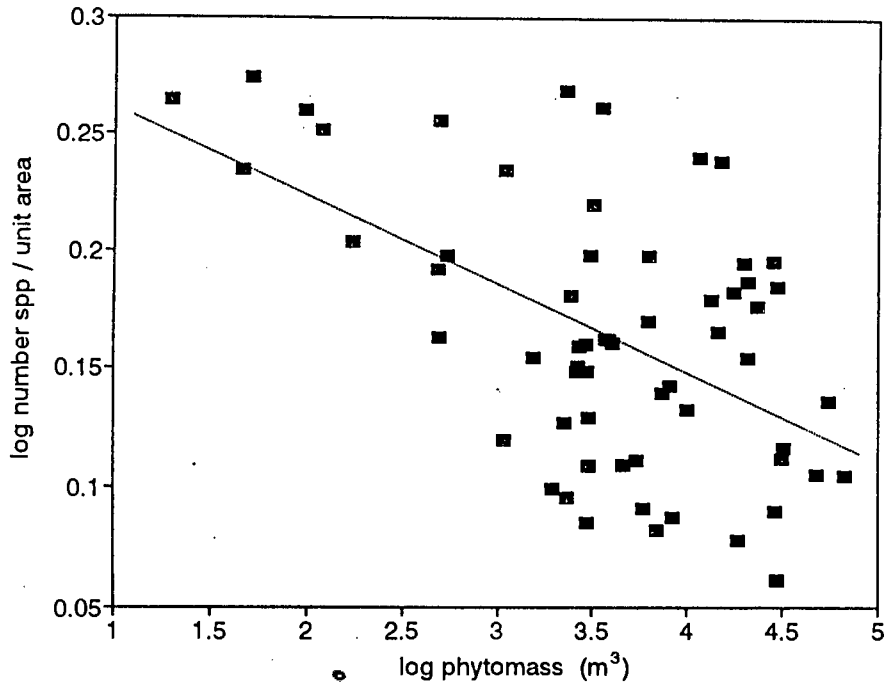


Figure 10. The log of the total phytomass of *Aspalathus* per plot against the log of the number of species per unit area in the plot. The data are those for the vegetation which contained stands of *Aspalathus* plants at the geographic survey sites and the seasonal sites for the December 1991 sampling. $r^2 = 0.282$; $p < 0.01$.

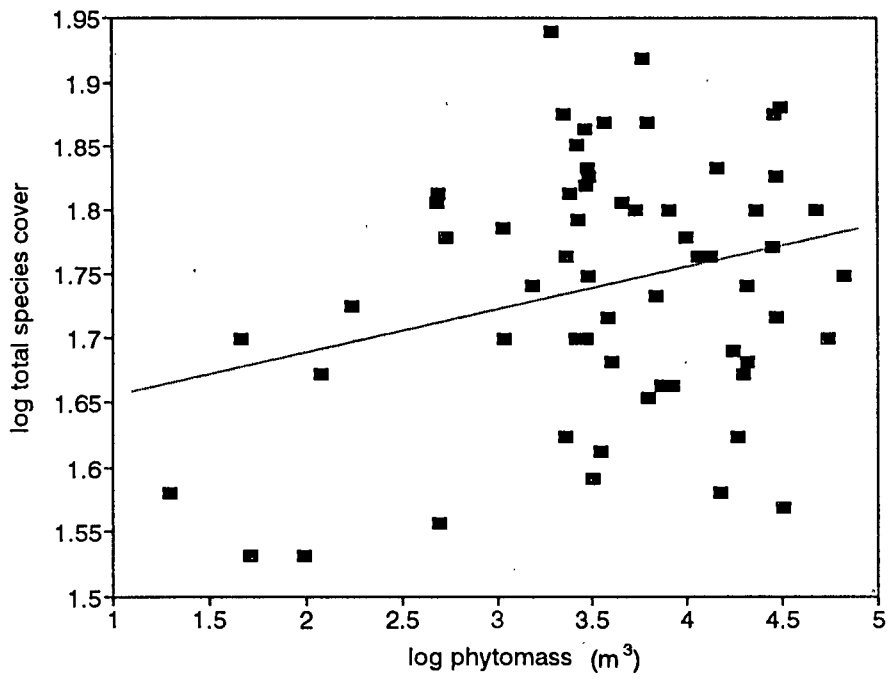


Figure 11. The log of the total phytomass of *Aspalathus* per plot against the log of the total cover of other species if the plot. The data are those for the vegetation which contained stands of *Aspalathus* plants at the geographic survey sites and the seasonal sites for the December 1991 sampling. $r^2 = 0.070$; $p < 0.01$.

Table 6.2. The r^2 values for the correlations of the log of the total *Aspalathus* phytomass per plot or the log of the product of the total *Aspalathus* phytomass and the percentage viability of each plant against the log of the Shannon Wiener index, Simpson's dominance index, species per unit area and cover of the other species occupying the plot. The + or - in parentheses indicate whether the correlation was positive or negative.

	Log total plot phytomass r^2	**Log total plot phytomass x % viability r^2**
Shannon Wiener index	(-) 0.177 ($p < 0.01$)	(-) 0.070 ($p < 0.05$)
Simpson dominance index	(+) 0.195 ($p < 0.01$)	(+) 0.073 ($p < 0.05$)
Species per unit area	(-) 0.282 ($p < 0.01$)	(-) 0.062 ($p = 0.05$)
Species total cover	(+) 0.070 ($p < 0.05$)	(+) 0.009 (not significant)

4. Discussion

Family composition of vegetation of different ages.

When comparing the percentage cover of different families in stands of vegetation of differing ages it is implicitly assumed that the two sites which are being compared proceed through successional stages in which the composition of the vegetation at sites is identical. This is not necessarily true as there may be biotic and abiotic differences between the sites. In this study the two areas being compared were as close to each other as possible, in most cases being separated only by a fire break or the width of a road (see Chapter 2). They should therefore have had most abiotic factors, such as the nature of the soil, microclimate, aspect etc., in common. It was not, however, possible to control biotic factors such as the nature of the fire and its effects on the previous stand of vegetation and its propagules, these could have exerted various influences on the stands of vegetation being compared. Heat from fire, for instance, may determine the number of seeds which germinate in some species (Auld & O'Connell 1991) while recent evidence suggests that smoke may also be a factor influencing germination (Brown & Botha 1993). The nature of the previous stand of vegetation is also important as differences in its age when burnt may have a significant effect seed set and seedling recruitment in certain species, e.g. *Protea* spp. (Bond 1980). The most effective way of overcoming such constraints would be to compare the same area over the course of time which is impossible in a short-term study. Instead a number of study sites in different fynbos vegetation types were examined and characteristics inferred that were true for most of the sites.

Most of the young vegetation of the geographical survey sites (Table 1) fell, according to age, into Kruger's (1979) regenerating phase category of pyric succession in the fynbos. The old vegetation fell mainly into his mature phase category and the middle-aged vegetation, into his transitional phase category. The middle-aged vegetation at Cape Point I and Bainskloof, however, appeared to show more of the characteristics of the early mature phase. At none of the study sites had the vegetation reached the senescent phase. The vegetation at most of the study sites showed an increase in Proteaceae and Ericaceae between the young and the old vegetation in accordance with the characteristics of Kruger 's (1979) successional phases. The increase in ericaceous cover was accompanied in most cases by an increase in species richness. The

Asteraceae and Restionaceae, on the other hand, showed little trend of either increased or decreased cover between young and old vegetation. These families also did not necessarily contain more species in the young vegetation compared to the older vegetation. Van Wilgen (1981), however, found that these two families were important contributors of both species and cover to young vegetation (four-year-old fire break) compared to mature vegetation (21-year-old stand) at Jonkershoek. As mentioned, this was not generally true for all the sites but held in the case of species number for Asteraceae and cover and species number for Restionaceae at Jonkershoek I. Hoffman *et al.* (1987) found an increase in the Restionaceae cover and a slight decrease in Asteraceae cover at Pella between plots aged five and ten years, this concurs with the findings in this study.

Thus it appears that the Ericaceae and the Proteaceae show a predictable increase in cover with increasing vegetation age after fire, and that the Fabaceae decrease very sharply in cover, especially in those sites which are within Kruger's (1979) mature phase in fynbos pyric succession. (The high cover of Fabaceae in the young vegetation is expected as vegetation containing stands of *Aspalathus* were chosen for the study. In nearby stands of older vegetation, however, the contribution was much reduced.) The Fabaceae may still form an important element of vegetation in the transitional phase (4-10 years). Decrease in the cover and prominence of legumes has been noticed in other studies of Mediterranean-type ecosystems. Hoffman *et al.* (1987) recorded a reduction in Fabaceae from between three and eight percent in five year old sites to between one and two percent in sites aged 12, 15 and 19 years at Pella. Westman (1981), working in sage scrub in California, described a drop in Fabaceae cover from 11.5 % to 1.5 % in the first and seventh years after fire respectively and a virtual absence from areas aged 21 years. In jarrah (*Eucalyptus marginata*) forest in southwestern Australia, peak richness and diversity of the vegetation was associated with the presence of legume species such as *Acacia pulchella, A. browniana, Bossiaea aquifolium* and *Kennedia coccinea* three to five years after fires. The decline in diversity after this time was due to the disappearance of these species (Bell & Koch 1980). Most studies have, however, concentrated on the cover and occurrence of specific legumes in vegetation of different ages rather than on the group as whole. From the findings in this and other studies it does appear that the bulk of Fabaceae cover consists of short-lived species which take advantage of the condition present in an environment after fire.

Growth and senescence of *Aspalathus* plants over one year.

The disappearance of *Aspalathus* from the vegetation during Kruger's (1979) maturing phase of pyric succession in fynbos has been noted but not described in detail (see, for instance, Adamson (1935); Levyns (1935) and Kruger (1979)). The disappearance of stands of legumes as the vegetation ages has also been noted in other Mediterranean-type ecosystems, for example Californian sage scrub (Westman 1981) and chaparral (Hanes 1971; Keeley *et al.* 1981). *Lotus scoparius* is often highlighted as this species may play an important role in nutrient conservation in Californian chaparral (Nilsen 1982). *L. scoparius*, along with two other species, formed 50 % of the seedling crop after a fire in chaparral (Keeley *et al.* 1981). Its cover soon dropped off dramatically, i.e. from 13.1 % in two to eight year old vegetation where 26 % of the plants were dead to 0.2 % with 50 % of the plants dead in nine year old vegetation. *L. scoparius* was the only species in the study of succession in the chaparral by Hanes (1971) to show a clear inverse relationship with vegetation age. Reduction in cover of legumes has been noted in jarrah forest (Shea *et al.* 1979; Bell & Koch 1980) and coastal dune system vegetation (Monk *et al.* 1981) in south Western Australia. Monk *et al.* (1981) observed an initial density of 10130 plants.ha^{-1} for *A. pulchella* which were halved during the first year after fire. Comparisons with plots of other ages indicated that 75 % may be dead by the fourth year with only 10 % remaining by the 11th year.

The three *Aspalathus* species which were examined in this study have very different life strategies. *A. retroflexa* appears to be a very early successional species although it showed some increase over the spring growing season. By December 1991 the plants were showing strong signs of stress and had greatly reduced viability and biomass by the following year. *A. retroflexa* thus dies off quickly in the succession and was only very seldom found in older vegetation. *A. abietina* showed a similar response to that of *A. retroflexa* but the die-back in this species took place over the second-half of summer. Both these species take advantage of the open conditions after fire. *A. retroflexa* is a prostrate creeper and *A. abietina*, a low, spreading bush, hence these species would soon be overtopped by taller species and are probably not able to cope well later in the succession. They may also not be able to compete as efficiently for water in the maturing community if it becomes limited during summer. Note that most senescence in these two species took place after summer.

Aspalathus carnosa occurs later in the succession than *A. retroflexa* and *A. abietina*. In the young vegetation these plants were still seedlings whereas the *A. retroflexa* present

at the same sites were mature plants. *A. carnosa* is a well-branched, succulent shrub, 0.5 - 1.0 m high, and may be able to compete better in the maturing vegetation. It was still present in large stands in the middle-aged vegetation but not in old vegetation. The dry summer period also appeared to have a negative effect on this species, especially in the middle-aged vegetation at Scarborough where the small crowded plants died in large numbers during the summer.

Summer drought may thus also play a role in reducing the number of these three species as succession proceeds. The importance of moisture stress in seedling death and senescence has been observed in several studies in similar environments. Auld (1987) noted that moisture stress was a major factor limiting seedling survival in *Acacia suaveolens* which established after fire in New South Wales. Wellington and Noble (1985) found that the seedling mortality (75 % in two years) in the mallee (*Eucalyptus incrassata*) of semi-arid south-eastern Australia was density dependent and confined to the dry, summer season. The mortality of these seedling could be reduced by supplying them with extra moisture. Water stress during summer is possibly a major factor leading to death and senescence in these *Aspalathus* species as well.

Influence of *Aspalathus* density on vegetation diversity.

Do large dense stands of *Aspalathus* have any effect on the small scale species diversity (alpha diversity) of the vegetation in which they occur? Specht and Morgan (1981) found a negative relationship between species richness and overstory cover in heathland in South Australia and sclerophyllous communities in Queensland. Cowling *et al.* (1992) include overstory-understory interactions as an important determinant of alpha diversity in the fynbos. They note that a number of studies have shown a negative relationship between the density of cover of Proteaceae and species richness. Campbell and van der Meulen (1980) argue for instance that overtopping is an important feature in reducing diversity in old compared to young stands of fynbos.at Jonkershoek. Esler and Cowling (1990) found species richness and density of *Protea lepicarpodendron* stands to be negatively related, while Cowling and Gxaba (1990) had similar results for *Leucadendron laureolum*. The latter authors suggest that these stands are important in maintaining species richness in an area as they provide a number of different habitats for understory species. These habitats vary considerably with space and time.

In this study, the density of various *Aspalathus* species has been shown to be negatively related to species richness over a range of geographic localities. It was negatively related to the Shannon-Wiener index which is a measure of the average degree of uncertainty when predicting to what species an individual will belong (Ludwig & Reynolds 1988). Simpson's dominance index, however, is the probability that two plants drawn at random from the community will belong to the same species. It is therefore expected that *Aspalathus* density should show the opposite relationship with this factor.

These *Aspalathus* species do not form an overstory as in the case of Proteaceae. It is more likely that they exert their effect on species richness by occupying space that would otherwise be available for other species. No relationship should then be expected between species per unit area and *Aspalathus* density i.e. as less space becomes available, fewer species occupy it. The negative relationship between number of species per unit area and *Aspalathus* density, however, suggest that *Aspalathus* species have a negative effect on species diversity other than simply a spatial one. This would be a spurious result as albeit not a strong correlation the area covered by other species in dense plots also increased with *Aspalathus* density which could lead to the negative correlation with species per unit area. Thus it appears that the main influence of *Aspalathus* plants on alpha species diversity is a spatial one.

The four correlations are strongest if total *Aspalathus* phytomass is used. In order to ascertain whether the correlation improves when only live biomass of the *Aspalathus* plants is used instead of standing phytomass, viability was included by using the product of the percentage viability of each plant and its phytomass (which removed dead plants and reduced the effective size of dying plants). These correlations were weaker than those which used standing phytomass. It therefore appears that the *Aspalathus* have exercised an effect on the community structure which is lasting and not better correlated with plants which are still alive. The effect of the *Aspalathus* plants on the vegetation is likely to last after the plants have died and their standing phytomass has reduced because seedling recruitment is very low between fires in the fynbos. Any seedlings of slower maturing species, which are choked out by dense stands of *Aspalathus* plants during the first few seasons after fire, are not likely to be replaced later in the succession.

In summary it appears the Fabaceae have evolved a strategy which enables them to take advantage of the open conditions after fire in the fynbos. Fast growth rates enable them to quickly reach maturity and set seed before they are out-competed by faster growing plants. Different species vary with respect to their approach within this strategy. For instance species with very rapid growth which do not survive longer than the youth stage of pyric succession (Kruger 1979) and those which may form an imporant part of the transitional phase. By the mature phase their contribution to the community is minimal, however. Very dense stands of *Aspalathus* plants may have an important effect on the community reducing its alpha diversity which lasts into later succesion once they have died away.

Chapter 7

Heat triggered germination in *Aspalathus* seed.

1. Introduction.

The survival of many plant species in Mediterranean-type ecosystems is dependent on dormant seeds remaining in a soil seed bank. These seeds germinate after fire replacing adults killed and thus contributing to the pyric succession (Christensen & Muller 1975; Keeley *et al.* 1981; Kruger 1984; Bell *et al.* 1993). Although many large genera of non-sprouting shrub species appear to survive fire in a particular environment via a soil seed bank, e.g. *Agathosma*, *Erica*, *Phylica*, *Muraltia*, *Aspalathus* and *Cliffortia* (Pierce 1987) soil stored seed banks have not been well studied in the fynbos. The seed banks of six dune fynbos species have been examined in detail by Pierce and Cowling (1991) who found them to be fairly small in comparison to seed banks of shrubs from other fire-prone vegetation. A few studies in the fynbos have observed recruitment from soil stored seeds as succession proceeded in the vegetation (Levyns 1929; Adamson 1935; Levyns 1935; Wicht 1948; Martin 1966).

Fynbos Fabaceae species may fall into one of two groups according to their fire survival strategy. The first group is fire ephemerals (*sensu* Bell *et al.* 1984). These species are members of the SI group according to Noble and Slatyer (1980), i.e. species which only regenerate from soil stored seed after fire. Most *Aspalathus* species appear to fall into this category. The second group are the so-called auto-regenerative long-lived sprouters, ALS (Bell *et al.* 1984) which Noble and Slatyer (1980) classify as UI, that is, species capable of sprouting from mature tissue and which do not establish in later succession. The fire ephemeral species are totally reliant on their soil stored seed bank for survival in an area after fire while the resprouting (ALS) species are less so but must still rely on soil stored seed for the replacement of individuals which die or may have been killed by the fire. Fabaceae of both these regenerating types have seeds with hard seed coats and a long life span, for instance >44 years for *Aspalathus ciliaris* (Van Wilgen & Forsyth 1992) and >30 years for *Virgilia oroboides* (Phillips 1926). The seeds of many species of Fabaceae and other families, e.g. the Rhamnaceae, possess a very hard seed coat.

This not only protects the seed from decay in the soil but also stops the seeds from absorbing water thereby preventing germination (Rolston 1978).

In order for seeds from the seed bank to germinate after fire their dormancy must be broken. Recent evidence has shown that the smoke from fires may play an important role in the stimulation of seed germination of many fynbos genera for example in many Restionaceae genera and in *Erica* spp. (Brown & Botha 1993). Germination in the case of the Fabaceae, however, appears to be possible only after damage to the seed coat, especially in the area of the strophiole, which allows the absorption of water (Cavanagh 1980). These seeds can withstand fairly rigorous treatment in order to break their dormancy; hot water, dry heat, freezing, acid scarification, mechanical scarification, microwave energy, organic solvents and oxidising agents are all effective (Cavanagh 1980, Pieterse & Cairns 1986a).

In legume species and other plants with hard seed coats from Mediterranean-type and other fire-prone ecosystems, heat from fires appears to play an important role in the stimulation of germination. This has been observed in many laboratory-based studies. Keeley (1987) investigated 45 tree, shrub, sub-shrub and liana species from fire-prone coastal sage scrub in California. He found that about a quarter of these species exhibited heat stimulated germination, especially those with hard seed coats e.g. *Ceanothus* species (Rhamnaceae). *Lotus scoparius*, a species in the Fabaceae from Californian chaparral, has also been shown to respond to heat treatment (Christensen & Muller 1975). In 18 legume species from south-eastern U.S.A., seven species showed increased germination after moist heat and eight species after dry heat (Martin *et al.* 1975). Other studies have concentrated on Australian legumes. Amongst these, *Acacia* and *Kennedia* spp. from wet sclerophyll forests in northern New South Wales are stimulated by heating (Floyd 1966; 1976) as are the invasive *Acacia* species from Australia in South Africa e.g. *A. cyclops* (Jeffery *et al.* 1988). Auld and O'Connell (1991) examined 35 Fabaceae species belonging to 15 genera from south-eastern Australia in one of the most comprehensive laboratory-based studies. These species showed heat stimulated germination when tested over a range of different temperature and durations.

These laboratory-based studies have been complemented by field based studies which have demonstrated the connection between temperatures which reached in the soils during fires, seed bank distribution in the soil and germination and distribution of the seedlings. It has been demonstrated that many of these species are fire-cued in their natural environment. Examples include the legumes from jarrah

natural environment. Examples include the legumes from jarrah (*Eucalyptus marginata*) forests of Western Australia (Shea *et al.* 1979), the wet sclerophyll forest of northern New South Wales (Floyd 1966; 1976), and the *Acacia longifolia* communities of New South Wales (Weiss 1984). In South Africa, field studies have examined seed banks and germination in invasive Australian *Acacias* spp.. in order to improve understanding of their control and eradication (Milton & Hall 1981: Pieterse & Cairns 1986b).

Although there is good evidence for heat stimulated germination caused by fire in legumes from other Mediterranean-type ecosystems, there are very little data for legume species native to the Fynbos Biome. This chapter investigates the germination response of selected *Aspalathus* spp. to differing heat exposures. These data are then related to the embryo to seed coat ratio to determine whether the thickness of the seed coat could be used to predict the level of heat exposure required to break dormancy in other members of this family.

2. Methods.

2.1. The collection of seed.

Seeds of Fabaceae species, *Aspalathus* in particular, were collected at the geographical survey study sites (Orangekloof, DuToitskloof, Bainskloof, Jonkershoek I and II, Groot Hagelkraal and Pella) as well as at the seasonal study sites (Cape Point I, Scarborough and Silvermine). Where possible, seeds of additional *Aspalathus* species and other members of the Fabaceae were collected at these study sites. The *Aspalathus* seeds were collected by bagging the flower-bearing parts of the plant with net bags after the pods had begun to form and leaving the bags on the plants until the pods had released their seeds. Seeds of additional Fabaceae species were collected from ripe pods. Large numbers of seed are very difficult to obtain in the case of *Aspalathus* species as in many species only one seed is produced per pod and the pods are often highly predated (personal observation). In this study the most problematic *Aspalathus* species from which to collect seeds were those which had few flowers scattered far apart along their branches e.g. *A. retroflexa*, *A. microphylla* and *A. biflora*.

Of the fifteen species of *Aspalathus* examined in chapter 4, no seeds could be obtained in the case of *Aspalathus salterii* and *A. carnosa* while too few seeds were obtained for the heat treatment experiments in the case of *A. laricifolia*, *A. microphylla*, *A. retroflexa* and *A. biflora*. Sufficient seeds of the following additional species were, however, obtained: *A. capensis* (Scarborough), *A. radiata* (Bainskloof), *A. albens* (Pella), *A. araneosa* (Jonkershoek II), *A. cephalotes* (Jonkershoek II) and *Psoralea affinis* (Groot Hagelkraal). Seed of *Podalyria canescens* and *P. sericea* were obtained from Kirstenbosch Botanical Gardens.

2.2 Heat exposure, germination trials.

The fact that the amount of seed was limited for all species had to be taken into account in the experimental design. The methods of Auld and O'Connell (1991) who had similar constraints were used as a guide.

Four temperatures, (60 °C, 80 °C, 100 °C and 120 °C) at three durations, (5, 15 and 30 minutes) were tested as was a control at ambient temperature. A batch of

25 seeds was tested for each species temperature-time combination except for *A. divaricata* where the few seeds available necessitated the omission of four time temperature combinations. A small volume of soil from the study sites where the seeds were collected was placed in a Petri dish and heated to the required temperature in an oven. The seed was then quickly added to the soil in the Petri dish which was replaced in the oven for the required length of time. The seeds were removed from the soil using a sieve and placed in a sterile Petri dish on Whatman number 4 filter paper. The filter paper was moistened with distilled water to which a commercial fungicide (Benylate) was added to prevent possible fungal infection. The Petri dishes were placed in a growth cabinet set for 12 hours light at 25 °C and 12 hours dark at 20 °C. Control seeds were handled in the same manner but were not heated in the oven. Germination of the seeds was followed for four weeks. A seed was considered to have germinated if the radicle extended $\pm$ 2 mm beyond the seed coat. Seeds were taken as dead if they imbibed water but went soft. After four weeks all imbibed seeds which were still firm were tested with tetrazolium to determine if they were still viable. This involved cutting the seeds through the embryonic axis and placing them on a thin layer of a 1 % aqueous solution of tetrazolium for 24 hours after which all seeds in which the embryonic axis appeared as bright red were regarded as still viable and counted as seeds which had germinated. Seeds which had not imbibed water and were thus still small and hard were acid scarified and allowed to germinate in order to determine the viability of the seed lot of each species.

Where possible, batches of seed which showed 50 % germination or germination in the control were replicated as recommended by Auld and O'Connell (1991). This was not possible for a number of combinations due to shortage of seed. The percentage germination for every temperature-time combination was calculated for each species. In order that the data only reflected seed simulated to germinate owing to the influence of heat, the data were corrected for germination in the control. Correction for seed lot viability was undertaken in order that seed germination could be compared between the species tested. The percentage of seeds killed at each temperature-time combination (i.e. imbibed water and went soft) was also calculated for each species. These values were corrected for the number of seeds which reacted in the same way in the control. The average germination rate (days per seed) was calculated for the temperature-time combination which showed the highest percentage gemination for each of the 16 species examined. The following weighted average formula was used:

3. Results.

The percentage viability was above 90 % in 11 of the species while in the remaining five species it was over 70 % (Table 7.1.). Most of the *Aspalathus* species had a low non-dormant seed fraction ranging between 0 - 4 %. Only *Aspalathus spinosissima* had a fairly high non-dormant fraction at 12 % with *Podalyria canescens* and *Psoralea affinis* both having high non-dormant seed fractions at 34 and 24 %, respectively. The viability of the seed lot and the non-dormant fraction were used to adjust the germination results so that they could be compared across species.

If dormancy is considered to be broken when 10 % of the seed germinates (a conservative estimate), eight species showed enhanced germination at 60 °C (Figure 7.1.). However, in only four species, *Aspalathus hispida*, *A. capensis*, *A. araneosa* and *Podalyria canescens*, was the germination above 10 % when averaged over the three durations of exposure to this temperature. These species may have shown some germination at 40 °C, especially *A. capensis* and *A. hispida*. Unfortunately, germination at this temperature could not be measured owing to the limited amount of seed available. Five species had their dormancy broken at 80 °C, according to the above definition and one, *A. radiata*, at 100 °C. *A. divaricata* could not be assessed since some of the temperature-time combinations were not tested.

Despite the heat treatment, highest percentage germination was below 50 % in seven of the species (Figure 7.1.). In only two cases, *Aspalathus hispida* and *A. neglecta*, was the highest percentage germination above 80 %. Ten species had their highest percentage germination at 100 °C for a time exposure of 5 or 15 minutes. There was a reduction in germination in these cases for an exposure time of 30 minutes at 100 °C, except in the case of *Aspalathus divaricata* which showed its highest germination at this combination. Five species showed their highest germination at 80 °C: *A. astroites*, *A. capensis*, *A. ericicfloia*, *A. spinescens*, *A. spinosissima* and *Psoralea affinis*. Apart from *A. spinosissima*, these species all showed their highest germination at exposures of 30 minutes and reduced germination at shorter durations (Figure 7.1.).

Table 7.1. The viability of the seed lot, non-dormant seed fraction, the temperature and durations at which the highest germination was recorded and the number of days for these seeds to germinate.

Species	Study site	viability of seed lot (%)	non dormant seed fraction (%)	Germination highest % germ.	temp. (°C)	time (min.)	rate (days seed^{-1})	seed mass (mg) (st. error)	seed coat :embryo ratio
Aspalathus abietina	Silvermine	94.7	0	27.3	120	5	19.2	3.12 (0.098)	3.40 (.419)
A. albens	Pella	90.0	2	28.6	100	15	20.0	2.63 (0.16)	3.28 (.172)
A. araneosa	Jonkershoek II	72.0	2	58.9	100	5	7.6	3.7 (0.14)	4.11 (.251)
A. astroites	Jonkershoek II	96.7	2	76.2	80	30	7.36	8.66 (0.27)	2.06 (.363)
A. capensis	Scarborough	72.1	0	71.68	80	30	28.1	7.0 (0.18)	3.77 (.151)
A. cephalotes	Jonkershoek II	68.0	4	63.4	100	15	8.8	5.6 (0.32)	3.58 (.560)
A. divaricata	Orangekloof	97.0	0	41.2	100	30	15.7	6.2 (0.28)	2.21 (.327)
A. ericifolia	DuToiskloof	69.4	4	57.6	80	30	13.2	1.6 (0.046)	2.57 (.237)
A. hispida	DuToitskloof	83.8	2	100	100	15	11.3	1.2 (0.052	3.49 (.527)
A. neglecta	Jonkershoek II	96.6	0	98.88	120	15	6.8	6.3 (0.33)	4.13 (.177)
A. radiata	Bainskloof	90.4	4	17.44	100	15/30	13.2	2.4 (0.069)	3.55 (.400)
A. spinescens	Pella	98.0	0	36.7	80/100	30/5	26.9	4.4 (0.15)	2.74 (.489)
A. spinosissima	DuToiskloof	91.4	12	26.2	80	5	25.6	7.5 (0.17)	3.19 (.272)
Podalyria canescens	Kirstenbosch	93.5	34	44.5	100	5	24.0	26.0 (1.73)	3.21 (.172)
P. sericea	Kirstenbosch	96.71	4	65.3	100	15	32.0	24.8 (1.0)	2.57 (.083)
Psoralea affinis	Groot Hagelkraal	100	24	56.0	80	30	8.1	12.5 (0.38)	2.14 (.060)

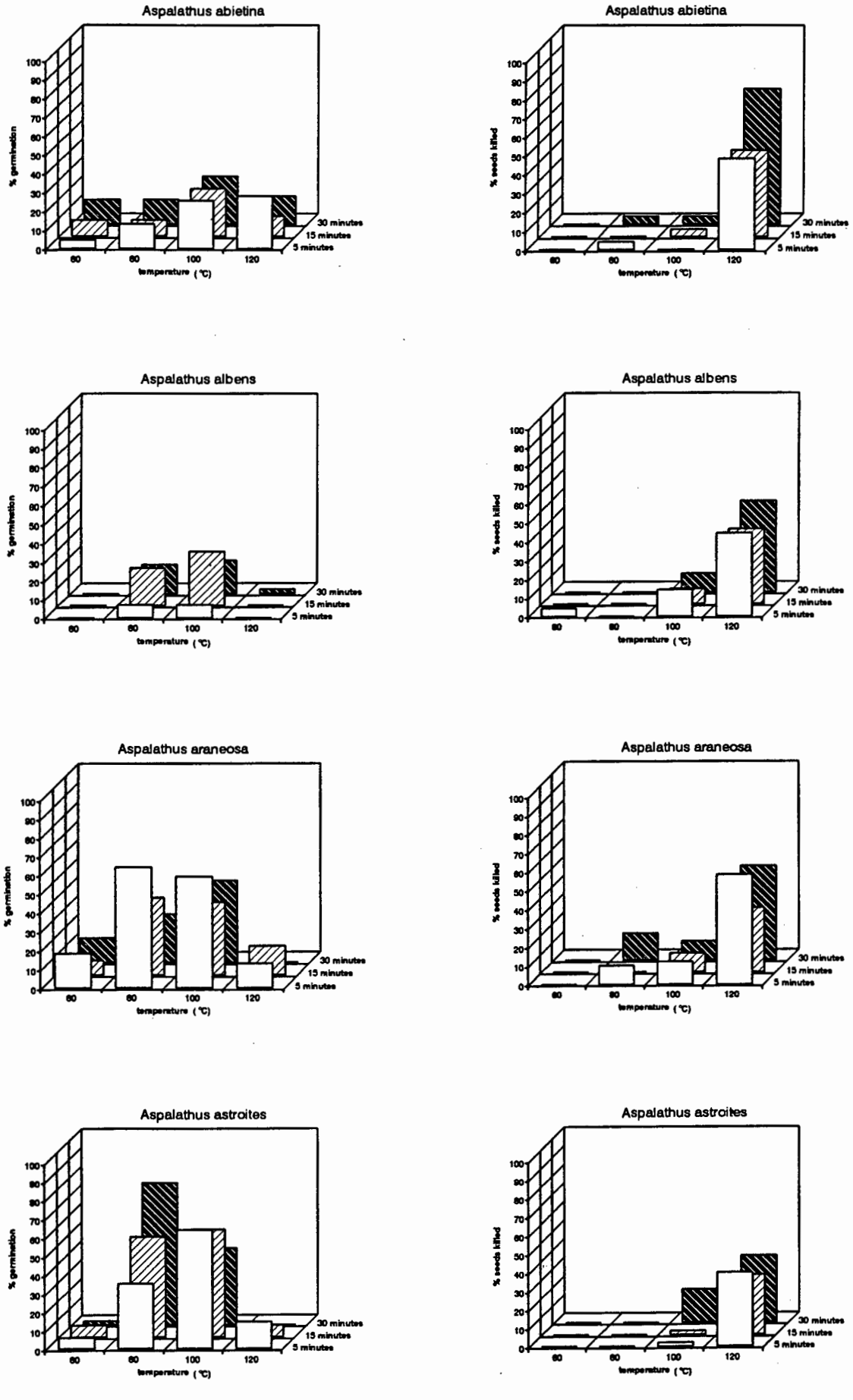
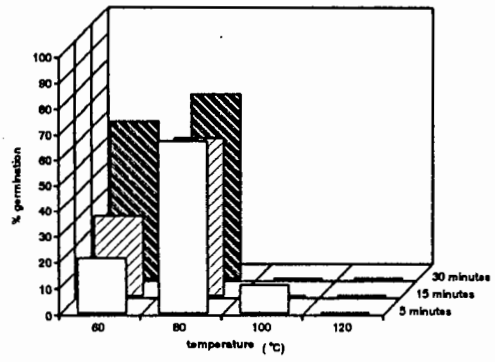
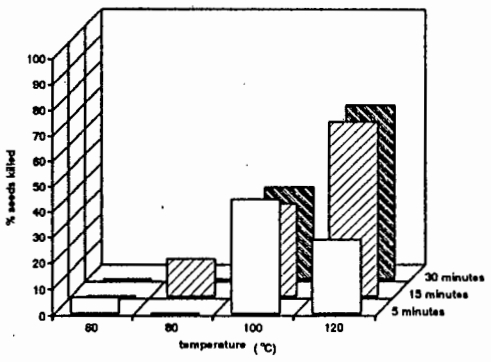


Figure 1. The percentage germination and the percentage of seeds killed (imbibed water but went soft) of 13 species of *Aspalathus* and three other Fabaceae species at different temperature (°C) and time (minutes) combinations. (The data were corrected for seed lot viability and non-dormant seed fraction for percentage germination results and dead fraction in the control for percentage seeds killed results.)

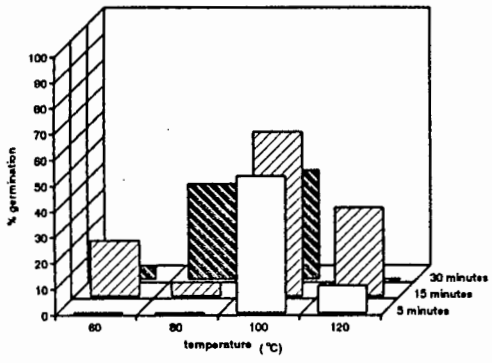
Aspalathus capensis



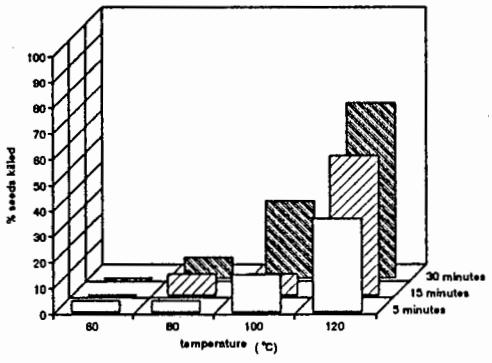
Aspalathus capensis



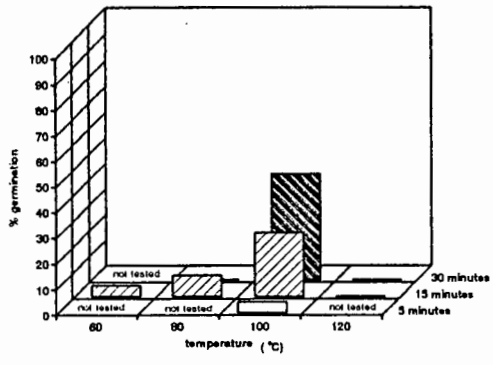
Aspalathus cephalotes



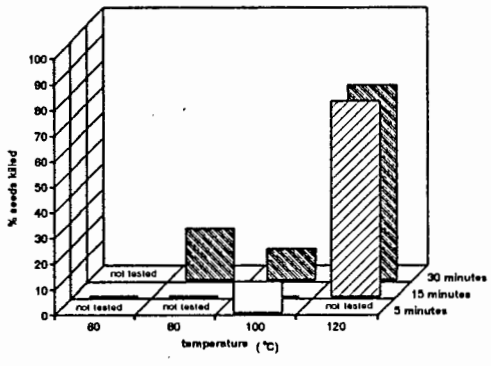
Aspalathus cephalotes



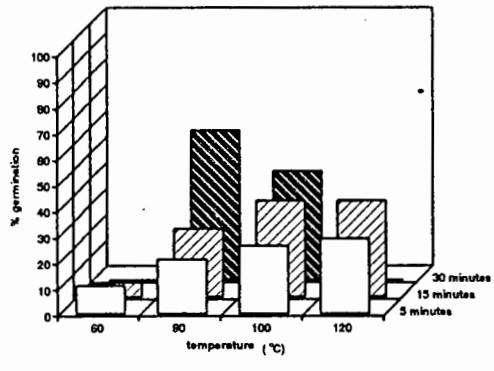
Aspalathus divaraicata



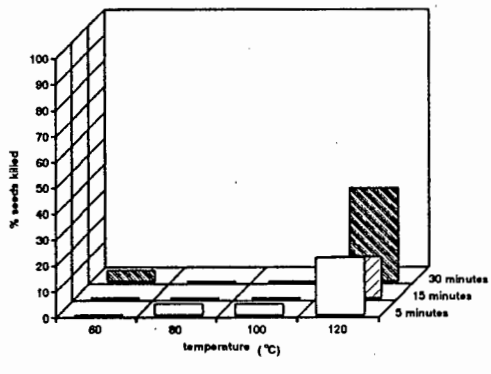
Aspalathus divaricata



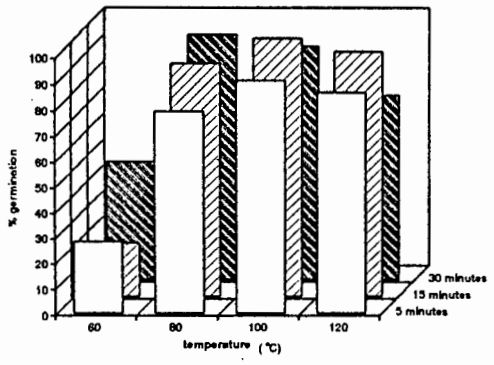
Aspalathus ericifolia



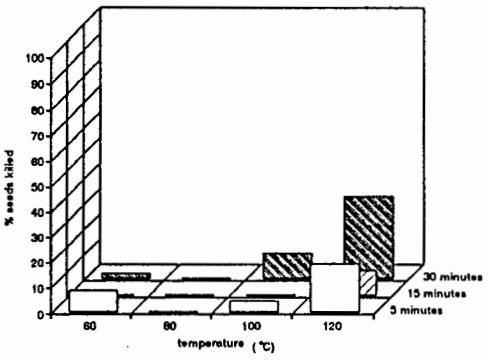
Aspalathus ericifolia



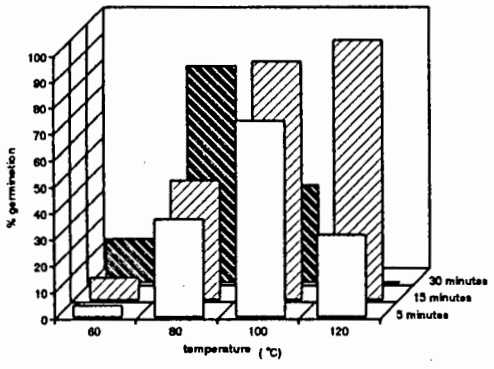
Aspalathus hispida



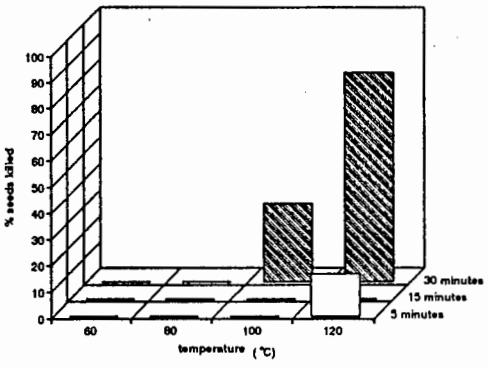
Aspalathus hispida



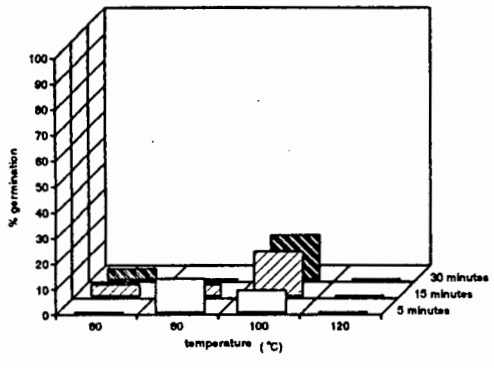
Aspalathus neglecta



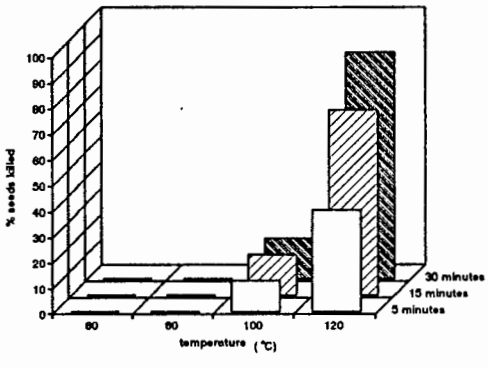
Aspalathus neglecta



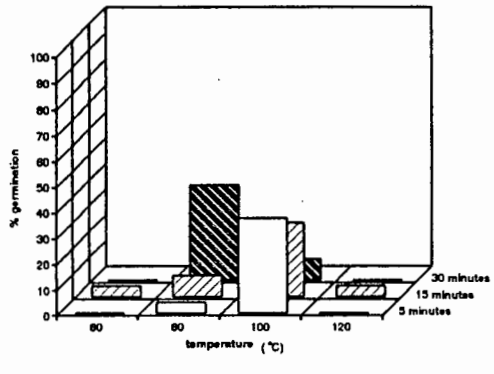
Aspalathus radiata



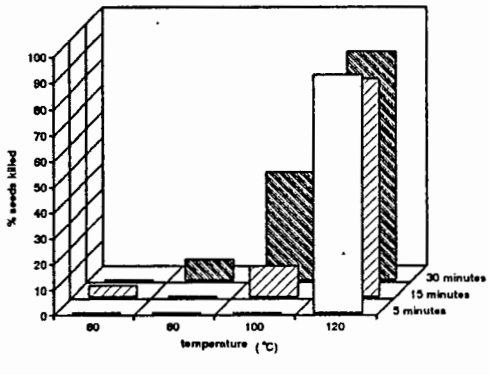
Aspalathus radiata



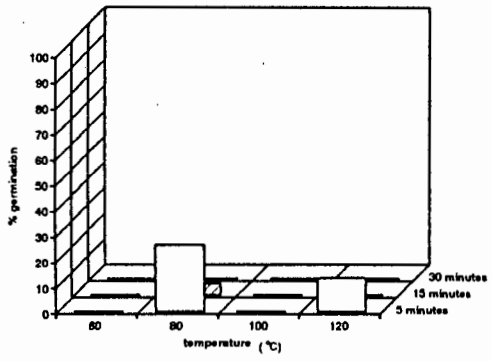
Aspalathus spinescens



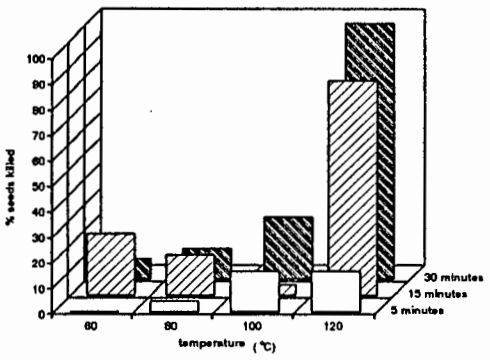
Aspalathus spinescens



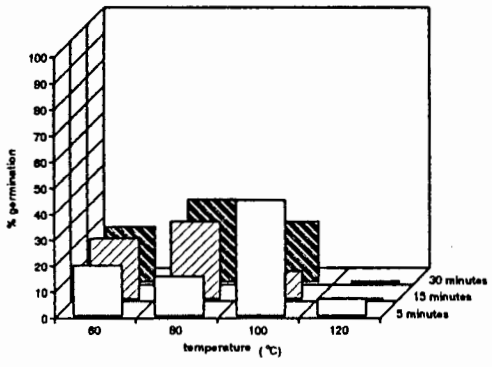
Aspalathus spinosissima



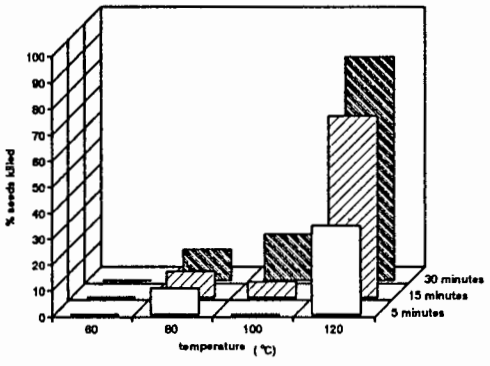
Aspalathus spinosissima



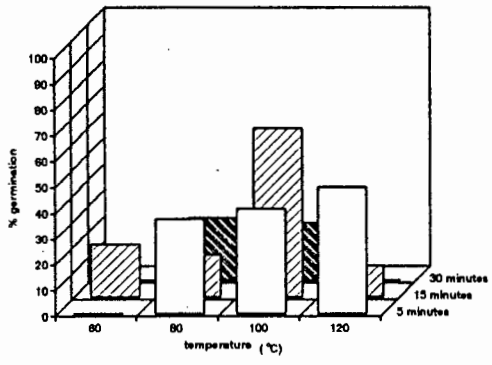
Podalyria canescens



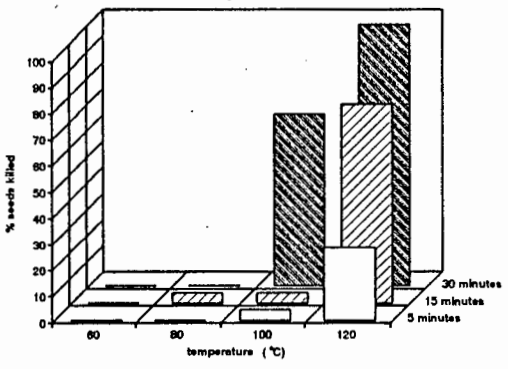
Podalyria canescens



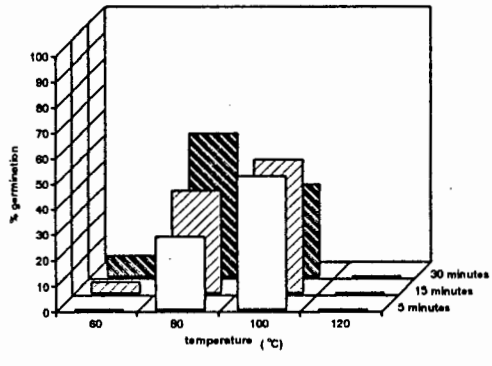
Podalyria sericea



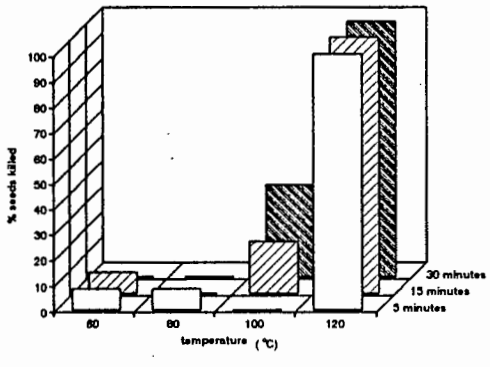
Podalyria sericea



Psoralea affinis



Psoralea affinis



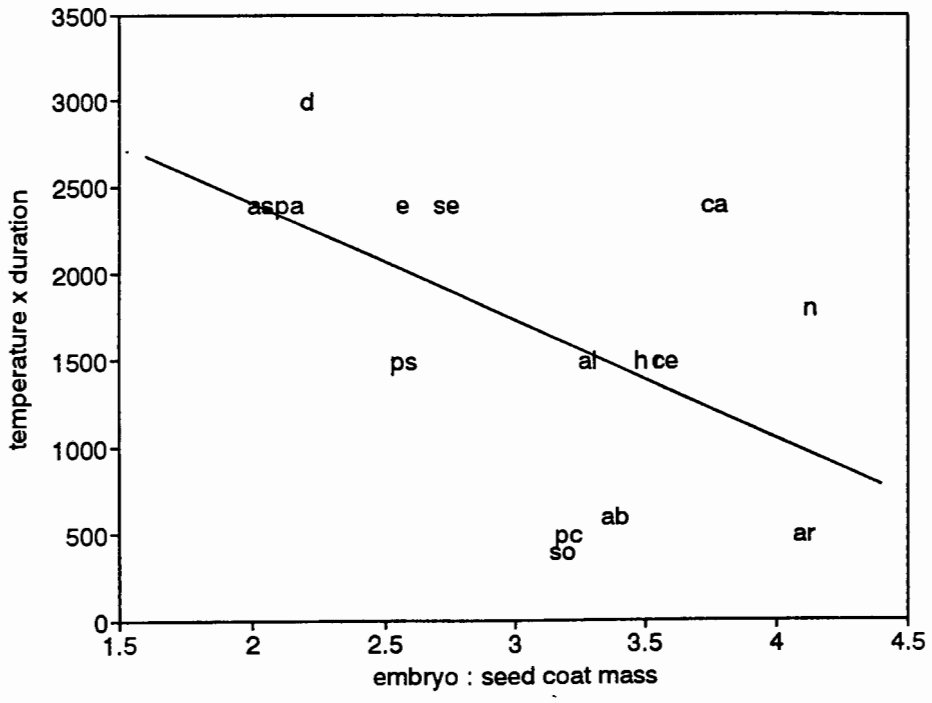


Figure 7.2. The ratio of embryo to seed coat mass against the product of the temperature and time-duration which showed the highest percentage germination. The letter represent the following species ab - *Aspalathus abietina*, al - *A. albens*, ar - *A. araneosa*, as *A. astroites*, ca - *A. capensis*, ce - *A. cephalotes*, d - *A. divaricata*, e - *A. ericifolia*, h - *A. hispida*, n - *A. neglecta*, r - *A. radiata*, se - *A. spinescens*, so - *A. spinosissima*, pc - *Podalyria canescens*, ps - *P. sericea* and pa - *Psoralea affinis*. (r^2 = 0.3069, n =16, p, 0.05).

Other species which showed good germination at 80 °C for 30 minutes also showed reduced germination for shorter exposure times, i.e. *A. cephalotes*, *A. hispida*, *A. neglecta*, *Podalyria canescens*, *Psoralea affinis*. However, in a number of cases, higher germination was recorded for shorter exposure times at 80 °C, i.e. *A. araneosa*, *A. albens*, *A. abietina* and *Podalyria sericea*. Most species showed reduced germination at 120 °C, especially at exposure for 30 minutes. *A. ericifolia*, *A. cephalotes*, *A. neglecta* and *Podalyria sericea* showed good germination at exposures of 5 and 15 minutes at this temperature, while *A. abietina* and *A. hispida* still showed good germination at 120 °C for 30 minutes. Most species showed little response at 60 °C. Some notable exceptions were *A. capensis*, *A. hispida* and *Podalyria canescens*.

Also illustrated in Figure 7.1. are the percentages of seeds of each species which were killed. These were seeds which imbibed water but failed to germinate and went soft as opposed to seeds which showed no response to the heat treatment and remained hard. In all species, the highest number of seeds killed was at 120 °C, especially when exposed at this temperature for 30 minutes. Ten species also showed greater than 10 % seed mortality at 100 °C when exposed for 30 minutes.

The ratio of embryo to seed coat mass was plotted against the temperature multiplied by the time duration. This showed the highest percentage germination for each species (Figure 7.2.). There was a significant negative correlation between these two factors ($r^2 = 0.306$, $n = 16$, $p < 0.05$). If *A. capensis* and *A. neglecta* which appear as partial outliers are excluded from the correlation, it is improved ($r^2 = 0.585$, $n = 14$, $p < 0.05$).

4. Discussion.

The fraction of non-dormant seeds among the *Aspalathus* species in this study was low (0 - 4 %) except for *A. spinosissima* (12 %.), Auld and O'Connell (1991) recorded non-dormant fractions of 0 - 19 % for 11 *Acacia* species and 1.1 - 58.9 % for seven species of *Pultenaea*. Shea *et al.* (1979) recorded fractions of 3.8 - 18.7 % for five *Acacia* species from south Western Australia.

For most of the species in this study, dormancy, as defined in the results, was broken between 60 °C and 80°C (In two species 40 °C may have been effective but this temperature was not included in the study). Auld and O'Connell (1991) suggested that they could divide the 35 Fabaceae species which they examined into three groups by the temperatures at which dormancy was considered to be broken, i.e. 40 °C, 60 °C and 80 °C. Unfortunately, they were unclear as to when they considered dormancy to be broken. Martin *et al.* (1975) considered dormancy to be broken at the temperature at which there was significantly higher germination than in the controls and found this occurred at between 45 °C and 80 °C in *Lespedeza* spp..

Auld and O'Connell (1991) found that most of the 35 Fabaceae species they examined showed highest germination at 80 °C but that it was still high at 100 °C in many species. This is similar to the findings of this study where the highest germination in most local species was either at 80 °C or 100 °C. Their data also showed a trend towards increased germination with increased exposure at 80 °C and decreased germination with increased exposure at 100 °C in certain species. In this study germination was reduced at 120 °C with most species showing either no germination or only at one minute exposure. Germination was still fairly high in a number of species at 5 and 15 minutes. Jeffery *et al.* (1988) found that *Acacia saligna*, an Australian invasive tree in South Africa, showed similar percentage germination patterns with a maximum around 80 °C - 100 °C. *Acacia cyclops*, on the other hand, did not show much response to heating. The two indigenous species which they included for comparison, *Virgilia oroboides* and *Podalyria calyptrata*, responded well in terms of percentage germination to temperatures between 80 °C - 100 °C for durations of 5 and 15 minutes respectively. *Podalyria calyptrata* showed higher percentage germination than the two species *P. canescens* and *P. sericea* in this study but the temperature and durations over which germination was recorded were similar. Shea *et al.* (1979) found

that five *Acacia* species from the jarrah (*Eucalyptus marginata*) forests of Western Australia had percentage germination values ranging from 62 - 89 % at 90 °C for 15 minutes compared to the controls of 4 - 19 %. Among various legume species from the south-eastern U.S.A. pine forests the peak percentage germination in eight species stimulated by dry heat lay between 80 - 90 °C for four minutes, the only duration which was tested (Martin et al. 1975). Other examples of hard-seeded species are five *Ceanothus* species from Californian chaparral, which also showed increases over the control at 70 °C for one hour, 100 °C for five minutes and 120 °C for five minutes, the only combinations which were tested (Keeley 1981).

In this study a better understanding was obtained of the temperature-time combinations which show the highest percentage germination. It appears that the thickness of the seed coat relative to the embryo is important in determining this combination. The seeds of species with small embryo to seed coat ratios, i.e. thicker seed coats relative to the embryo, showed higher germination at higher temperature and longer time durations than seeds with lower ratios. It may thus be possible to predict the response of other *Aspalathus* spp. without needing to test their germination reponse to exposures at differeing temperature, time combinations.

Auld and O'Connell (1991) used data on percentage germination at different temperature-time combinations to determine the number of seeds which would germinate under different fire conditions in the 35 Fabaceae species. Data from Auld (1986a) on temperatures reached in the soil during "cool" and "hot" burns, as well as the assumption that seeds of the 35 species would be distributed similarly to *Acacia suaveolens* seeds observed by Auld (1986b), were used to calculate the proportion of each of the 35 species seeds which were likely to germinate after such fires. They found that the proportion of seeds likely to germinate under a low intensity burn was much lower than the proportion which would germinate after hotter fires. Few seeds would be likely to germinate below 2 cm after a "cool" fire, whereas after a "hot" fire, seed at deeper levels would germinate, especially that concentrated at a depth of 3 cm although a few would germinate down to a depth of 7 cm.

In order to understand these results it is necessary to digress a little and examine the effect of fires on soil heating. Soil is a poor conductor of heat and there is a steep heat gradient with depth in the soil. The temperatures reached below the soil during a fire therefore are far below those reached at the surface and are raised only for brief

periods. Soil moisture increases the conductivity of the soil but, owing to the latent heat of evaporation, lowers the maximum temperature that can be atttained to 100 °C until the water is removed. Beadle (1940) found that in *Eucalyptus* forest fires, when the trees are not burned down, the surface temperatures were > 250 °C but only 111 - 114 °C at one inch (2.5 cm) below the surface and 59 - 67 °C at 3 inches (7.6 cm). If the trees burn (a very intense fire), temperatures ranged from 175 - 180 °C at one inch (2.5 cm) for a fire burning for two hours. Similar results were obtained by Shea *et al* (1979) for high intensity fires in jarrah forests. Floyd (1966), working in wet sclerophyll forests of northern New South Wales, recorded lower temperatures over a range of fires with three fuel loads. Temperatures never rose above 100 °C at one inch below the surface. Auld (1986a) warns that fires under artificial fuel loads such as this are difficult to compare with natural fires where fuel load will probably be much lower. Comparisons between fires are also difficult as different soils may have different thermal properties. In fynbos soils, Martin (1966) recorded temperatures of 550 °C at the surface but less of than 43 °C at 12 mm below the surface. These examples serve to illustrate the point that the proportion of the seed bank for which dormancy is broken will depend heavily on the nature of the fire and the depth at which the seed is distributed in the soil.

To apply predictions of temperature that will be reached during fires and on germination of seeds in the field is difficult as the distribution of seed in the soil will be poorly known or understood in many cases. For instance in *Aspalathus*, nothing is known about the distribution of seed in the soil, or any detail about its dispersal (e.g. if there is any interaction with ants). Some studies have assumed seed distribution to be the same as that measured in other species (Auld & O'Connell 1991) but this could lead to spurious results. Seed is not necessarily evenly distributed throughout the soil. The seed may be concentrated at deeper levels in ant nests, for example, and this will also affect seed germination in the field. Field studies on seedling emergence in some *Acacia* species, however, have shown emergence to be concentrated over a narrow range of depths, as would be expected if germination was stimulated by heat from fires. Auld (1986a) found that although after a fire seedlings of *Acacia suaveolens* may germinate at a depth of 9 cm in the soil, most are concentrated between 1 and 3 cm. Monk *et al* (1981) obtained similar results for *A. pulchella* in which emergence was between 0 and 3 cm and nothing occurring below 6 cm. This was confirmed experimentally by Portlock *et al* (1990) who showed that the highest germination in this species occurred at depths of 1 or 4 cm. After an intense fire in jarrah forest in Western

Australia, Shea *et al.* (1979) found similar results for this species (between 1 and 4.5 cm), although a few emerged from as deep as 9 cm. They note that broad scale emergence of legume species does not occur in jarrah forest during normal low intensity prescription burns as much of the seed is distributed too deeply in the soil where it is not heated sufficiently to break dormancy. Further evidence by Pieterse and Cairns (1986) on *Acacia longifolia* seed in South Africa showed that seed buried at 1 cm below the surface was destroyed by fire, that dormancy was broken between 2 and 3 cm and that below 3 cm the seed was largely unaffected. Milton and Hall (1981) caution that although mild fires may not stimulate germination in *Acacia* species in Australia, in South Africa the seed is not redistributed by ants to lower levels in the soil and light fires are therefore likely to stimulate germination as much of the seed is near the surface. They recommend hot fires which would kill the bulk of this seed.

The results in this chapter show that seeds of various *Aspalathus* species from the fynbos are stimulated to germinate by temperatures similar to those recorded for other legume species. The temperature-time combination which is most effective at stimulating germination in a particular species is also correlated with the thickness of the seed coat. Hot fires are thus likely to break the dormancy of seeds at lower levels in the soil to a depth of 3 cm as shown in the studies mentioned above. Conclusive evidence on the proportion of *Aspalathus* seed which is likely to germinate after fires of different intensity will depend on data on the distribution of their seed in the soil.

Chapter 8

General discussion.

1. Synthesis and summary.

The aim of this thesis was to investigate aspects of the ecology of the *Aspalathus* spp. which may help to elucidate the role they play in the nitrogen economy of fynbos. As members of the Fabaceae with the ability to nodulate and perhaps fix nitrogen, it has often been suggested that these plants may play an important role in fynbos environments by replacing nitrogen lost during fires.

Nitrogen and phosphorus are important to the functioning of Mediterranean-type ecosystems and fynbos in particular, where these nutrients are not abundant. As decomposition rates are slow, fire is generally regarded as playing a pivotal role in cycling nutrients trapped in the vegetation back into the soil (Chapter 2). This comes at a price, as nitrogen is easily volatilized from the soil and vegetation and lost to the atmosphere during fire. The lost nitrogen is not replaced by precipitation (wet or dry) which is low in the fynbos and in other Mediterranean-type ecosystems (Stock & Lewis 1986b). The role of symbiotic and non-symbiotic nitrogen fixers is thus of particular interest in these environments as a means of explaining how lost nitrogen is replaced. This study has attempted to increase our understanding of nitrogen fixation in the Mediterranean-type areas of South Africa. The large fynbos genus *Aspalathus* provides excellent material for research as it contains a large variety of species which occur in many different fynbos ecosystems and often form dense stands after fires.

Aspalathus spp. have the ability to nodulate in the field, as demonstrated both across a number of species in different fynbos environments and in three species over one year (Chapter 4). All these species also nodulated well in pot culture but in the field there was considerable variation in the amount of nodulation both across the different species and at the various localities (Chapter 4). From the evidence of one of the species which was examined over the year (*A. carnosa*), a strong seasonal trend in nodulation was apparent, with peak nodulation in the cool wet winter months. Similar trends have been observed in the legume species of south Western Australia (Hingston *et al.* 1982; Hansen & Pate 1987). The other two species which

were examined over one year, *A abietina* and *A. retroflexa*, did not show any strong seasonal trends. The higher nodulation present in these two species near the end of the year, may have been due to an accumulation of nodules which were formed during the warm, wet months of spring in the soil, thus here seasonal trends also seem likely. Unfortunately the scope of this study was too broad to enable examination of the activity of the nodules, a problem that should be addressed in future research.

Although an indication of whether nitrogen fixation is taking place in the field may be obtained by examining nodulation, it cannot be assumed that it is necessarily taking place. In order to examine nitrogen fixation as such in the various *Aspalathus* spp. which were examined for nodulation, the $\delta^{15}N$ natural abundance method of measuring nitrogen fixation was used (Chapter 5). In most cases the estimation of the fractional contribution of nitrogen fixation of the field-growing plants corresponded well to their nodulation observed in the field. Mass spectrometry of the stable isotopes of nitrogen is expensive however, and therefore a limited number of samples were run in this preliminary study which was designed to test the suitability of the method in fynbos ecosystems.

Studies which have examined nitrogen fixation in Mediterranean regions have concentrated on legumes, e.g. *Acacia* spp. in Australia and *Ceanothus* spp. in California (Rhamnaceae). Apart from inter-specific differences, climatic factors and soil nutrient status, specifically phosphorus, appear to be important in determining the amount of nitrogen which is fixed in these environments. In general these environments are not conducive to high levels of nitrogen fixation due to an unfavourable climate which has long, dry summers as well as the low phosphorus content in many of the soils (Chapter 2).

Both phosphorus and nitrogen appear to be important variables in determining the amount of nodulation at the seasonal sites which had very similar soil types (Chapter 4). In a multiple regression total phosphorus was positively correlated whereas total nitrogen was negatively correlated with nodule production across the different seasonal sites. The ratio of total nitrogen to phosphorus was also negatively correlated to the nodulation in the species at the different sites. Thus, when similar soils are compared, the ratio of nitrogen to phosphorus appears to be important in determining the amount of nodules produced and possibly quantity of nitrogen fixed. This would ultimately have an influence on the success of the species in the community. For instance nitrogen to phosphorus ratios have been

found to be important in determining fixation rates and production in pasture legumes (Smith 1992). The other variables which were included in the multiple regression analysis soil pH, total organic matter, mean monthly temperature and soil moisture showed no correlation except for organic matter which was negatively correlated with nodulation across the geographical survey sites which had a variety of different soils. At the geographic survey sites phosphorus was negatively correlated with nodulation and nitrogen positively correlated with nodulation. There was, however, no correlation between the ratio of total nitrogen to phosphorus and nodulation across these sites. The negative correlation with organic matter may indicate that there are discrepancies between total and available phosphorus and nitrogen which may be responsible for the opposite correlations, to those at the seasonal sites, found for soil total nitrogen and phosphorus and nodulation. I am thus still confident that when similar soils are compared nitrogen to phosphorus ratios will prove to be important in determining nitrogen fixation and possibly competitive success.

A change in the nutrient status of the soil may therefore be fundamental to the eventual demise of dense stands of *Aspalathus* plants as the vegetation proceeds though the pyric succession. Although total nitrogen did not show any trend between younger vegetation containing stands of *Aspalathus* plants and nearby older vegetation which did not, phosphorus on the other hand did (Chapter 4). Phosphorus was always the same or greater in the soils of the young vegetation which contained *Aspalathus* than those of the nearby older vegetation. This suggests that phosphorus and its ratio to nitrogen may play a role in determining when *Aspalathus* plants die out during succession after fire.

From the literature on fire survival strategies of fynbos plants it appears that the largest proportion of fynbos species are capable of resprouting after fire but not able to establish from seed in later successional phase after fire, this includes many legume genera. *Aspalathus* spp. are mainly fire ephemerals i.e. species which germinate and grow rapidly after fires from a soil stored seed bank and usually die out early in the pyric succession (Chapter 2). Dense stands of short-living, leguminous plants, appearing after fire, have been noted in fynbos, and have been investigated in other Mediterranean-type ecosystems e.g. California and Australia. Few studies on fynbos have documented change in the vegetation during pyric succession, as it occurs over a number of years, or inferred successional phase changes in the vegetation from differences between sites of differing ages (Chapter 2). There is thus a lack of information on the occurrence of legumes in the

successional stages after fire. The ecology of dense stands of legumes is also poorly understood.

From this study it appears that, with succession after fire in fynbos, prominence of legumes in the vegetation decreases. Two other prominent fynbos families, the Ericaceae and the Proteaceae, on the other hand, showed increased cover in the older vegetation, whereas no clear pattern emerged in a further two families (Restionaceae and Asteraceae).

Circumstantial evidence indicates that moisture levels during summer may be important in determining the survival of these plants in the short term although nutrient levels in the soil particularly phosphorus, may ultimately be responsible for die-off of these plants and the disappearance of the dense stands in older vegetation. This was observed in the growth and senescence of three *Aspalathus* spp. at three intervals during the year. *A. abietina* and *A. retroflexa* present in vegetation of one to two years post fire were very early successional species. Both these species appeared to be reaching the end of their life span and showed increased senescence after the dry summer season. The third species *A. carnosa*, however, appears to be a later successional species. This species was mainly present as seedlings but senescence and death of plants was also observed in this species after the summer season.

The nature of the previous fire and the amount of heat which was transferred to the soil will determine the number *Aspalathus* seeds which germinate and, in turn, the density of the resulting stand of plants. Although studies in other Mediterranean-type ecosystems have demonstrated the importance of heat from fire in stimulating legume germination there is little evidence for heat stimulated germination in legumes from fynbos ecosystems. However, a few studies have investigated heat stimulated germination in invasive Australian *Acacia* spp. (Jeffery *et al.* 1980). In this study temperatures of 80 °C and 100 °C at various durations caused the highest percentage germination of *Aspalathus* seeds (Chapter 7). Exposure to 120 °C killed the seeds of most species, especially for longer durations. A negative correlation between the ratio of embryo to seed coat mass and the product of the temperature and duration at which the highest percentage germination occurred suggests that a thick seed coat relative to a small embryo mass may be important in determining the temperature and duration which stimulate germination. This could possibly be used to predict an *Aspalathus* spp. germination response to heating.

Once formed the dense stands of *Aspalathus* plants appear to interact with the vegetation around them having a negative effect on the diversity of the accompanying vegetation. This was seen in the higher Simpson's dominance and lower Shannon-Wiener index with increasing *Aspalathus* biomass (Chapter 6). In dense stands of Proteaceae this has been shown to be due to an overtopping effect of the Proteaceae plants which changes the characteristics of the underlying habitats (Cowling & Gxaba 1990; Esler & Cowling 1991). In *Aspalathus* it is probably due to the repression of other species as they occupy space which would have been occupied by other species. It is possible that this has a lasting effect on vegetation diversity as seedlings of species which mature slowly will be lost due to competition early in the succession and replacements of these plants via seedling recruitment is unlikely between fires.

2. Scope for further study of *Aspalathus* spp..

It appears that *Aspalathus* spp. may play an important role in replacing nitrogen lost by fire in the fynbos. Their contribution, however, will vary from one environment to another and with the species being examined. More research is needed in order to quantify the amount of nitrogen which is being fixed. This is possible if the proportion of nitrogen fixed is determined by the $\delta^{15}N$ natural abundance method and related to the accumulation of nitrogen in the plants over the time period of interest. Once fixed in dense stands of *Aspalathus* plants more investigation is needed into the cycling of the nitrogen back into the soil as such processes are only poorly understood for fynbos (Stock & Allsopp 1992).

As this was a preliminary study only three samples per species were used for $\delta^{15}N$ analysis and only investigated trends in *Aspalathus* nitrogen fixation. In order to obtain a more accurate picture of what is taking place the analysis of more samples is necessary. The natural abundance method relies on the comparison of the field-grown plant's $\delta^{15}N$ value to that of a plant reliant solely on nitrogen fixation and a reference plant (a non-fixing plant from the same locality as the field grown plant). Unfortunately assumptions about the similarity in growth habits and metabolism of the field grown plant and its reference plant must be made. In this study a new method was used to obtain the $\delta^{15}N$ value for a non-fixing reference plant. The same species grown in pot culture in its native was used. Oxygen was allowed to filter through the soil to prevent nodulation. This new method of determining the $\delta^{15}N$ value for non-fixing plants appears to be very successful. The use of oxygen to suppress nodulation and obtain a reference plant of the same species should increase

the precision of nitrogen fixation estimates and prevent inaccuracy due to the choice of reference plants. This may be very useful when using the $\delta^{15}N$ natural abundance method, not only in *Aspalathus* spp. from fynbos ecosystems but for other legume spp. from other ecosystems as well. In-depth studies of the seasonal and daily changes in the rate of nitrogen fixation of *Aspalathus* spp. in the field could also be obtained by using the acetylene reduction methods. It would be interesting to relate this information, to that obtained using stable isotopes.

The role of phosphorus and nitrogen in determining the amount of nodulation and nitrogen fixation needs further attention both in laboratory and field studies. It appeared from this study that the ratio of soil total phosphorus to nitrogen is important in determining the amount of nodulation in Table Mountain Sandstone soils. Pot experiments using different supply ratios of these two nutrients could provide valuable insight into their importance in determining both nodule formation and nitrogen fixation. Further information from natural ecosystems is required on nitrogen fixation both long term (using the $\delta^{15}N$ natural abundance method) or instantaneous and how this is related to the available nitrogen and phosphorus in the soil.

Although *Aspalathus* seed dormancy is broken at similar temperatures to those of other legumes it is difficult to predict how their seed will respond to fires of differing intensities. Further information is required on the distribution of *Aspalathus* seed in the soil. This may be used to predict how many seeds will germinate after fires of differing intensities. For instance, hotter fires in certain ecosystems may lead to a higher percentage germination of *Aspalathus* seed in the seed bank. As these plants are able to grow quickly, they will dominate the community in early succession and, as shown in this study, this may have important effects on species diversity in the developing community. Further investigation however, is necessary into the role of *Aspalathus* density in determining plant species diversity in fynbos ecosystems as this study has only shown interesting trends between *Aspalathus* density and alpha diversity. Circumstantial evidence suggested that water limitation may be the immediate cause of death and senesence in *Aspalathus* plants in dense stands, more research is needed to understand this and the role which plant architecture (creepers versus shrubs) may play in the survival of *Aspalathus* plants in the different phases of pyric succession in fynbos.

In summary, this study has provided insight into the nitrogen fixation of legumes in Mediterranean South Africa. It has shown trends in the nodulation of these species and how this may be related to soil factors. The preliminary investigation into nitrogen fixation using the natural abundance method with non-nodulated reference plants of the same species was successful and holds promise for future studies of nitrogen fixation in natural ecosystems. It has also layed a foundation for the understanding of how dense stands of legumes form in fynbos ecosystems, how they effect the surrounding vegetation and why they may die out.

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Appendix.

Species list of vascular plants recorded in the five 25 m² plots layed out in vegetation of different ages at the three seasonal and eight geographic survey study sites (Chapter 3).

Bainskloof species list. (August 1991)

Family	Species	Total % cover young site per species	young site per family	middle-aged site per species	middle-aged site per family
Adiantiaceae	Schizaea pectinata (L.) Sw.			0.2	0.2
Apiaceae	Peucedanum sieberanum Sonder	0.4	0.4		
Asteraceae	Arctotis angustifolia L.	1.4	28.2	0.8	5.6
	Berkheya barbata (L. f.) Hutch.			0.2	
	Corymbium africana L.	1.8			
	Corymbium congestum E. Meyer ex DC.	6			
	Corymbium glabrum L.			0.2	
	Cullumia ciliaris (L.) R. Br.			0.2	
	Euryops abrotanifolius (L.) DC.	0.2		0.2	
	Gazania serrata DC.	9			
	Haplocarpha lanata (Thunberg) Less.	0.6			
	Helichrysum dasyanthum (Willd.) Sweet	0.4			
	Helichrysum stoloniferum (L.f.) Willd.	6.4		0.8	
	Heterolepis aliena (L.f.) Druce	0.6			
	Stoebe plumosa (L.) Thunberg			2	
	Stoebe spiralis Less.			1	
	Ursinia abrotanifolia (R. Br.) Spengel	0.2		0.2	
	Ursinia sp.	1.6			
Brassicaceae	Heliophila scoparia Burchell ex DC.			0.6	0.6
Campanulaceae	Prismatocarpus altiflorus L'Her	0.2	0.2		
Crassulaceae	Crassula sp.	0.2	0.2	0.2	0.2
Cyperaceae	Ficicnia duesta (Bergius) Levyns	0.4	3		2.2
	Ficinia cf ixiodes Nees	0.6			
	Ficinia nigrescens (Schrader) Raynal	0.4			
	Tetraria cuspidata (Rottb.) C.B. Clarke	1.2		0.2	
	Tetraria ustulata (L.) C.B. Clarke	0.4		2	
Droseraceae	Drosera sp.	0.6	0.6		
Ebenaceae	Diospyros glabra (L.) de Winter	0.2	0.2		
Ericaceae	Eremia totta (Thunberg) D. Don	0.2	1.2	1.2	4.6
	Erica articularis L.	0.8			
	Erica hispidula L.			0.4	
	Erica plukenetti L.			0.6	
	Erica quadrangularis Salisb.			0.2	
	Erica cf setacaea	0.2		1.8	
	Erica sp.			0.4	
Euphorbiaceae	Clutia polifolia Jacq.	0.2	1	0.6	1.4
	Clutia rubricaulis Ecklon & Zeyher ex Sonder	0.8		0.8	
Fabaceae	Aspalathus biflora E. Meyer		0.6	7.2	7.2
	Rafnia amplexicaulis Thunberg	0.6			
Geraniaceae	Pelargonium pinnatum (L.) L'Her	0.2	0.2		
Iridaceae	Aristea racemosa Baker	0.2	1.6		
	Babiana sp.	0.2			
	Micranthus sp.	0.6			
	Tritoniopsis sp.	0.6			
Mesembrianthemace	Lampanthus section Adunci			0.4	0.4
Poaceae	Pentachistis sp.	0.4	0.6	0.2	1

	Pseudopentameris macrantha (Schrader) Conert	0.2		0.8	
Polygalaceae	Muraltia heisteria (L.) DC.	0.4	0.4	0.2	0.2
Proteaceae	Leucadendron salignum Bergius	0.2	0.6	1	1
	Protea acaulos (L.) Reichard	0.2			
	Protea nitida Miller	0.2			
Restionaceae	Hypodiscus aristatus (Thunberg) Masters	0.2	4.6	5.4	38.6
	Ischyrolepis curviramis (Kunth) Linder	0.2		16	
	Ischyrolepis gaudichaudiana (Kunth) Linder	0.2		4.8	
	Restio sp.	3.4		1.8	
	Staberoha cernua (L. f.) Durand & Schinz	0.6		4	
	Thamnocortus lucens Poir.			6	
	Wildenowia glomerata (Thunberg) Linder			0.6	
Rosaceae	Cliffortia atrata Weim.		0.2	0.4	0.4
	Cliffortiua ruscifolia L.	0.2			
Rutaceae	Agathosma sepyllacea Licht. ex Roemer & Schultes	0.4	0.4		

Cape Point I species list. (December 1991)

Family	Species	Total % cover young site		Total % cover middle-aged site	
		per species	per family	per species	per family
Asphodelaceae	Trachyandra divaricata (Jacq.) Kunth.	0.4	0.4		
Asteraceae	Anaxeton laeve (Harvey) Lundgren	0.4	9.2		4.4
	Corymbium enervae Markotter	0.2			
	Edmondia sesamoides (L.) Wild.	1		0.4	
	Elytropappus scaber (L.f.) Levyns	0.6		0.2	
	Helichysum latorale Bolus	0.8			
	Metalasia cephalotes (Thunberg) Less.	1		3	
	Metalasia muricata (L.) D. Don	0.6		0.4	
	Osteospermum pogaloides L.	1.2			
	Phaenocoma prolifera (L.) D. Don	0.4			
	Senecio umbellatus L.	0.2			
	Syncarpha vestita (L.) B. Nord.	2		0.2	
	Ursinia palaceae (L.) Moench	0.8		0.2	
Campanulaceae	Lobelia coronopifolia L.	1	3.8		1.6
	Lobelia pinifolia L.			0.8	
	Lobelia setaceae Thunberg	0.8		0.4	
	Roella recurvata A. DC.	1			
	Roella triflora (Good) Adamson	1		0.4	
Crassulaceae	Crassula fascicularis Lam.			0.2	0.2
Cyperaceae	Ficinia duesta (Bergius) Levyns	0.2	4.8		5
	Ficinia pinguior C.B. Clarke	0.2			
	Tetraria cf compacta Levyns	0.6		0.4	
	Tetraria compar (L.) Lestib.	0.8		0.2	
	Tetraria eximia C.B. Clarke	0.2			
	Tetraria microstachys (Vahl.) Pfeiffer	0.8			
	Tetraria cf padulosa Levyns			1.2	
	Tetraria pygmaea Levyns	0.4			
	Tetraria thermalis (L.) C.B. Clarke	1.6		3.2	
Ericaceae	Blaeria ericoides L.		1.8	3.6	23
	Coccosperma hexandra (Klotzsch) Druce	0.4		5.6	
	Erica articularis L.	1		2.2	
	Erica imbricata L.	0.2		1	
	Sympieza labialis (Salisbury) Druce	0.2		10.6	
Fabaceae	Aspalathus carnosa Bergius	1.4	5.3	2.4	3
	Aspalathus retroflexa L.	3.1			
	Aspalathus serpens Dahlg.	0.4			
	Indigofera brachystachya E. Meyer	0.4		0.4	
	Liparia parva Vogel ex Walp.			0.2	
Haemodoraceae	Dilatris pillansii W. Barker	0.2	0.2	0.2	0.2
Iridaceae	Bobartia gladiata (L. f.) Ker Gawler	0.6	0.6	0.6	0.6
Linaceae	Linum thunbergii Ecklon & Zeyher	0.2	0.2		
Mesembrianthemace	Erepsia anceps (Haw.) L. Bolus	0.2	0.2		
Penaeaceae	Penaea mucronata L.		0.8	0.6	1.4
	Saltera sarcocolla (L.) Bullock	0.8		0.8	

Poaceae	Pentachistis cf clorata (Steudel) Stapf.	0.2	0.6		
	Pseudomentameris macrantha (Shrader) Conert	0.4			
Proteaceae	Diastella divaricata (Bergius)	0.4	2	1	1.6
	Leucadendron coniferum (L.) Meissner			0.4	
	Lecadendron cf floridum R. Br.			0.2	
	Leucadendron sp.	1			
	Protea cynaroides (L.) L.	0.4			
	Serruria vallosa (Lam.) R. Br.	0.2			
Restionaceae	Elegia stipularis Masters	1	7	3.4	23.8
	Hypodiscus aristatus (Thunberg) Masters	2.2		2.8	
	Ischyrolepis capensis (L.) Linder	0.4			
	Ischyrolepis cincinnata (Masters) Linder	0.8		6	
	Mastersiella digitata (Thunberg) C. Benedict			0.4	
	Staberoha cernua (L. f.) Durand & Schinz	0.4		7.8	
	Thamnocortus gracilis Masters	2.2		3.2	
	Wildenowia sulcata Masters			0.2	
Rutaceae	Adenandra villosa (Bergius) Lichtenstein ex Roemer	0.6	1	0.6	0.6
	Agathosma hookerii Sonder	0.4			
Santalaceae	Thesium acuminatum A.W. Hill	0.8	1.4	0.6	1
	Thesium sp.	0.4		0.4	
	Thesium virgatum Lam.	0.2			
Scrophulariaceae	Harveya purpurea (L.f.) Harvey	0.2	1.2		0.2
	Agathelpis angustifolius Choisy	0.8		0.2	
	Selago spuria L.	0.2			
Thymelaeaceae	Struthiola cf ciliata (L.) Lam.	1.4	1.4	0.4	0.4

Cape Point II species list. (August 1991)

Family	Species	Total % cover middle-aged si per species	per family
Anacardiaceae	Rhus laevigata L.	1	1
Asteraceae	Helipterum vestitum (L.) Schrank	2.2	3.2
	Metalasia muricata (L.) D. Don	1	
Cyperaceae	Ficinia duesta (Bergius) Levyns	0.2	0.4
	Tetraria eximia C.B. Clarke	0.2	
Ebenaceae	Diospyros glabra (L.) de Winter	0.6	0.6
Ericaceae	Blaeria ericoides L.	0.2	8.6
	Coccosperma hexandra (Klotzsch) Druce	0.2	
	Erica articularis L.	0.2	
	Erica imbricata L.	1	
	Erica plukenetti L.	0.4	
	Erica sessiliflora L.	0.2	
	Erica sp.	0.2	
	Scyphogyne muscosa (Aiton) Steudel	6.2	
Fabaceae	Aspalathus carnosa Bergius	20	20
Geraniaceae	Pelargonium triste (L.) L'Her	0.2	0.2
Mesembrianthemace	Erepsia anceps (Haw.) L. Bolus	0.2	0.6
	Ruschia sarmentosa (Haw.) Schwantes	0.4	
Poaceae	Pentachistis sp.	0.2	2.6
	Pseudomentameris macrantha (Shrader) Conert	2.4	
Polygalaceae	Muraltia demissa Wolley-Dod	0.2	0.2
Proteaceae	Lecadendron laureolum (Lam.) Fourc.	1.2	1.4
	Mimetes hirtus (L.) Salisbury ex J. Knight	0.2	
Restionaceae	Hypodiscus aristatus (Thunberg) Masters	1	20.6
	Ischyrolepis capensis (l.) Linder	5.6	
	Ischyrolepis cincinnata (Masters) Linder	2	
	Ischyrolepis tenuissimus (Kunth) linder	0.4	
	Staberoha cernua (L.f.) Durand & Schinz	11.4	
	Thamnocortus gracilis Masters	0.2	
Rosaceae	Cliffortia atrata Weim.	0.2	0.2
Rutaceae	Diosma oppositifolia L.	0.4	0.4
Thymelaeaceae	Struthiola cf ciliata (L.) Lam.	0.4	0.4

DuToitskloof species list. (October 1991)

Family	Species	Total % cover young site per species	young site per family	old site per species	old site per family
Adiantiaceae	Cheilanthes hirta Sw.	0.8	0.8	0.2	0.2
Amarylladaceae	Haemanthus sp.			0.2	0.2
Aizoaceae	Aizoaceae sp.	0.2	0.2		
Anacardiaceae	Heeria argentea (Thunberg) Meissner	0.4	0.4	0.2	1
	Rhus rosmariniflius Engl.			0.4	
	Rhus tomentosa L.			0.4	
Apiaceae	Glia gummifera (L.) Sonder		0.2	0.4	0.4
	Peucedanum galbanum (L.) Benth. & Hook F.	0.2			
Asparagaceae	Protasparagus rubicundus (Bergius) Oberm.	0.2	0.2	0.4	0.4
Asphlodelaceae	Albuca sp.	0.4	0.4		
Asteraceae	Alciope tabularis (Thunberg) DC.		12.6	0.6	15.6
	Berkheya barbata (L. f.) Hutch.			0.6	
	Chrysocoma ciliata L.	0.2			
	Gazania serrata DC.	0.2			
	Helichysum dasyanthum (Willd.) Sweet	3.2			
	Metalasia cephalotes (Thunberg) Less			8.8	
	Senecio pinnulatus Thunberg	3.6			
	Senecio umbellatus L.	0.8			
	Stoebe cinerea Thunberg			2	
	Stoebe plumosa (L.) Thunberg	1.4		3	
	Ursinia cf nudicaulis (Thunberg) N.E. Br.	3.2		0.6	
Boragiunaceae	Lobostemon glaucophyllus (Jacq.) Buek	0.4	0.4		
Campanulaceae	Cyphia volubulis (Burm. f.) Willd.			0.2	0.2
Celastraceae	Maytenus oleoides (Lam.) Loes.			0.6	0.6
Cyperaceae	Ficinia bergiana Kunth		10.4	0.2	0.4
	Ficinia nigrescens (Schrader) Raynal	8.8			
	Tetaria ustulata (L.) C.B. Clarke			0.2	
	Cyperaceae sp.	1.6			
Ebenaceae	Diospyros glabra (L.) de Winter			2.6	2.6
Ericaceae	Eremia totta (Thunberg) D. Don			19.4	19.6
	Erica grandiflora L. f.			0.2	
Euphorbiaceae	Clutia polifolia Jacq.	0.8	0.8		0.6
	Euphorbia genistoides A. Berger			0.6	
Fabaceae	Aspalathus spinosissima R. Dahlgren	3	13.2		0.6
	Aspalathus cymbiformis DC.	9.8			
	Indigofera digitata Thunberg	0.4		0.4	
	Indigofera incana Thunberg			0.2	
Gentianaceae	Sebaea exacoides (L.) Schinz			0.8	0.8
Geraniaceae	Pelargonium myrrhifolium (L.) L'Her	0.4	1.2		0.2
	Pelargonium rapaceum (L.) L'Her	0.4			
	Pelargonium tabularae (Burm. f.) L'Her	0.4			
	Pelargonium ternifolium Vorster			0.2	
Hyacinthaceae	Trachyandra sp.			0.4	0.4
Iridaceae	Geissorhiza aspera Goldb.			0.4	0.4
Mesembrianthem	Erepsia cf anceps (Haw.) L. Bolus	0.8	0.8		
Montiniaceae	Montinia caryophylaceae Thunberg	3.4	3.4	0.6	0.6

Orchidaceae	Satyrium bicorne (L.) Thunberg			0.2	0.2
Poaceae	Themeda triandra Forsskal	2.2	2.2	2.2	2.2
Polygalaceae	Muraltia alopecuroides (L.) DC.		1	0.2	0.2
	Muraltia divaricata Ecklon & Zeyher	1			
Proteaceae	Protea laurifolia Thunberg		0.6	0.4	3
	Protea nitida Miller	0.6			
Restionaceae	Ischyrolepis gaudichaudiana (Kunth) Linder	1	5.2		4.2
	Ischyrolepis sp.	4.2		4.2	
Rhamnaceae	Phylica atrata Lichtenst. ex Roemer & Schultes		0.2	1	1
	Phylica imberbis Bergius	0.2			
Rosaceae	Cliffortia atrata Weim.			0.2	0.6
	Cliffortia ruscifolia L.	3.8	3.8	0.4	
Rubiaceae	Anthospermum cf aethiopicum L.	1.8	2.4	1.6	2
	Anthospermum spatulatum Spengel			0.4	
	Anthospermum sp.	0.6			
Rutaceae	Agathosma hispida (Thunberg) Bartling & Wendl.		0.2	4	4
	Agathosma serpyllacea Licht. ex Roemer & Schultes	0.2			

Groot Hagelkraal species list. (July 1991)

Family	Species	Total % cover limestone young site per species	per family	acid sand young site per species	per family	old site per species	per family
Aizoaceae	Polpoda capensis Presl					0.4	0.4
Anacardiaceae	Rhus laevigata L.	0.2					
Apiaceae	Centella difformis (Ecklon & Zeyher) Adamson	0.8	0.8				
Asteraceae	Athanasia quinquedentata Thunberg	0.2	5.8	0.6	11		4.4
	Berkheya barbata (L. f.) Hutch.	0.2					
	Corymbium glabrum L.					0.2	
	Disparago anomala Schltr. ex Levyns	0.4		8.6		3.4	
	Edmondia sesamoides (L.) Willd.			0.2		0.6	
	Haplocarpha lanata (Thunberg) Less.			0.2			
	Helichrysum stolonifera (L.f.) Willd.	0.8					
	Metalasia muricata (L.) D. Don	2.2		0.2			
	Oedera imbricata (Lam.)	0.4				0.2	
	Phaenocoma prolifera (L.) D. Don			0.4			
	Senecio sp.	1					
	Syncarpha paniculata (L.) B. Nord.	0.6		0.8			
Bruniaceae	Berzelia abrotanoides (L.) Brongn.	0.2	0.2		0.2	0.2	0.2
	Brunia laevis Thunberg			0.2			
Campanulaceae	Lobelia coronopifolia L.		0.4	0.2	0.2		
	Roella cf lightfootioides schltr.	0.4					
Cyperaceae	Ficinia duesta (Bergius) Levyns		12.6		15.6	0.2	3.4
	Ficinia lateralis (Vahl.) Kunth	1					
	Ficinia praesmorsa Nees	5.2					
	Ficinia tenuifolia Kunth	0.2					
	Ficinia truncata (Thunberg) Schrader	0.6					
	Tetraria compar (L.) Lestib.					0.6	
	Tetraria cuspidata (Rottb.) C.B. Clarke	5.6		6.2			
	Tetraria flexuosa (Thunberg) C.B. Clarke			7.2			
	Tetraria pleosticha C.B. Clarke					2.6	
	Tetraria sp.			2.2			
Ericaceae	Blaeria ericoides L.		1.4	0.2	5.8	0.8	3.2
	Erica corifolia L.			0.2			
	Erica imbricata L.			0.8		1.8	
	Erica lineata Benth.			0.6			
	Erica pulchella Houtt.			0.4		0.6	
	Erica sp.	1.4		0.4			
	Ericaeae (minor genus) sp.			3.2			
Fabaceae	Aspalathus ciliaris L.		28.4	3.6	3.6		
	Aspalathus ramulosa E. Meyer	1.4					
	Aspalathus salteri L. Bol.	23					
	Aspalathus repens Dahlg.	3.2					
	Indigofera brachystachya E. Meyer	0.8					
Geraniaceae	Pelargonium betulinum (L.) L'Her.	0.2	0.2				
Iridaceae	Aristea cf anceps Ecklon ex Klatt.				0.2	1.4	1.4
	Aristea cf confusa Goldbl.			0.2			

Juncaceae	Juncus rigidus Desf.			0.2	0.2		
Mesembrianthemace	Carpobrotus acinaciformis (L.) L. Bolus			1.8	2		
	Erepsia sp.			0.2			
Myrsinaceae	Myrsine africana L.					0.2	0.2
Polygalaceae	Muraltia collina Levyns	0.2	0.2				
Penaeaceae	Penaea mucronata L.					0.8	0.8
Proteaceae	Leucadendron liniflolia		1.8	0.8	4		2.8
	Leucadendron meridianum Williams	1					
	Leucadendron salicifolium (Salisb.) I.J. Williams			1.8			
	Leucadendron salignum Bergius			0.4		2.8	
	Leucospermum patersonii Phill.	0.6					
	Mimetes cucullatus (L.) R. Br.			0.4			
	Protea obtusifolia Buek ex Meisn.			0.6			
	Protea susannae E. Phillips	0.2					
Poaceae	Pseudopentameris macrantha (Schrader) Conert	1.6	1.8		2		
	Poaceae sp.	0.2		2			
Restionaceae	Chondropetalum microcarpum (Kunth) Pill.	3.8	10.4		25		39.8
	Chondropetalum nudum Rottb.			2		6	
	Elegia stipularis Masters	0.8				4	
	Elegia sp.			3.2		4	
	Hypodiscus rigidus Masters	1.6				4	
	Hypodiscus wildenowia (Nees) Masters			5.6		4.4	
	Ischyrolepis sp.					0.2	
	Mastersiella digitata (Thunberg) C. Benedict			4			
	Restio filiformis Poir			0.6		6.6	
	Restio triticeus Rottb.	0.6		1			
	Staberoha multispicula Pill.					4.6	
	Thamnocortus fraternus Pill.	3.2					
	Thamnocortus pellucidus Pill.					4	
	Wildenowia teres Thunberg	0.4				0.2	
	Restionaceae sp.			8.6		0.4	
Rhamnaceae	Phylica ericoides L.	0.8	1		0.4		2.6
	Phylica imberbis Bergius			0.4			
	Phylica stipularis L.					2.6	
	Phylica sp.	0.2					
Rosaceae	Cliffortia stricta Weim			0.6	0.6	2	2
Rubiaceae	Anthospermum aethiopicum L.	0.2	0.6	0.2	0.4	0.2	0.2
	Anthospermum sp.	0.4		0.2			
Rutaceae	Agathosma cerefolium (Vent.) Bartling & Wendl.		1.6	0.2	0.2	0.4	2
	Agathosma geniculata Pill.	0.2					
	Diosma haelkraalensis I.J. Williams	1.2				1.6	
	Euchaetes cf scabricosta I.J. Williams	0.2					
Santalaceae	Thesium cf capitatum L.	0.6	1.2	0.6	0.6		
	Thesium sp.	0.6					
Schizaeaceae	Schizeae pectinatus (L.) Sw.					0.6	0.6
Sterculiaceae	Hermannia trifoliata L.	0.4	0.4	0.2	0.2		
Thymelaeaceae	Gnidia juniperifolia Lam.	0.6	1.8		1		3
	Gnidia sp.			0.2		0.2	
	Passerina paleacea Wikstrom	0.2					
	Passerina vulgaris Thoday			0.8		2.8	
	Struthiola myrsinates Lam.	1					
Zygophyllaceae	Zygophyllum flexuosum Ecklon & Zeyher					0.2	0.2

Jonkershoek I species list. (September 1991)

Family	Species	Total % cover young site per species	young site per family	old site per species	old site per family
Amaryllidaceae	Haemanthus sanguineus Jacq.	0.2	0.2	0.2	0.2
Anacardiaceae	Rhus rosmarinifolia Vahl.	0.4	0.4		
Apiaceae	Centella glabrata L.	0.8	0.8	0.2	0.2
Asparagaceae	Protoasparagus rubicundus (Bergius) Oberm.	0.2	0.2	0.4	0.4
Asphodelaceae	Trachiandra muricata (L. f.) Kunth	0.4	0.4	0.6	0.6
Asteraceae	Corymbium glabrum L.	0.4	7		14.8
	Elytropappus glandulosus Less.	1.2		10	
	Euryops linifolius (L.) DC.			0.2	
	Gazania serrata DC.	0.2		0.4	
	Gerbera crocea (L.) Kuntze	0.8			
	Helichrysum sp	0.6		0.2	
	Othonna quinquedentata Thunberg	2.8			
	Stoebe plumosa (L.) Thunberg			4	
	Senecio sp	0.2			
	Stoebe fusca Thunberg	0.2			
	Stoebe plumosa (L.) Thunberg	0.6			
Campanulaceae	Prismatocarpus diffusus (L. f.) A. DC.	0.2	0.4	0.2	0.2
	Roella ciliata L.	0.2			
Celastraceae	Maytenus oleiodes (Lam.) Loes	1.2	1.2	0.4	0.4
Crassulaceae	Crassula fascicularis Lam.	0.8	0.8		
Cyperaceae	Ficinia bergiana Kunth		7.4	0.2	3.2
	Ficinia duesta (Bergius) Levyns	0.6			
	Ficinia nigrescens (Schrader) Raynal	1.8		0.6	
	Tetraria cuspidata (Rottb.) C.B. Clarke	0.6		0.8	
	Tetraria ustulata (L.) C.B.Clarke	4.4		1.6	
Dennstaedtiaceae	Pteridium aquilinum (L.) Kuhn			1.4	1.4
Dipsacaceae	Cephalaria rigida (L.) Roemer & Schultes	1	1	0.8	0.8
Ebenaceae	Diospyros glabra (L.) de Winter	1.6	1.6		
Ericaceae	Blaeria cf dumosa Wendl.		0.4	0.2	10.8
	Erica cf plukenetii L.			3.6	
	Erica hispidula L.			5.6	
	Erica sp.	0.4		0.4	
	Scyphogyne muscosa (Aiton) Steudel			1	
Euphorbiaceae	Clutia alaternoides L.	0.2	0.4		0.2
	Euphorbia genistoides A. Berger	0.2		0.2	
Fabaceae	Aspalathus hispida Thunberg	4.6	9.4		1.4
	Aspalathus laricifolia Bergius	4.6			
	Indigofera mauritanica (L.) Thunberg			0.4	
	Otholobium fructicans (L.) Stirton			0.6	
	Tephrosia capensis (Jacq.) Pers.	0.2		0.4	
Gentianaceae	Chironia baccifera L.			0.2	0.2
Geraniacee	Pelargonium tabularae (Burm. f.) L'Her	0.2	1		0.6
	Pelargonium triste (L.) L'Her	0.6		0.6	
	Pelargonium sp.	0.2			
Iridaceae	Aristea africana (L.) Hoffsgg.	0.4	0.6		
	Watsonia borbonica (Pourr.) Goldbl.	0.2			

Montiniaceae	Montinia caryophylaceae thunberg	2.2	2.2	1.8	1.8
Oxalidaceae	Oxalis versicolor L.	0.4	0.4	0.8	0.8
Poaceae	Pentachistis colorata (Steudel) Stapf	0.4	5.8		12.2
	Themeda triandra Forsskal	5.4		9.2	
	Poaceae sp.			3	
Polygalaceae	Muraltia alopecuroides (L.) D.C.	0.2	0.4	0.4	0.8
	Muraltia heisteria (L.) D.C.	0.2			
	Muraltia pauciflora (Thunberg) D.C.			0.4	
Proteaceae	Hakea sp.	0.2	4.2		9.2
	Leucadendron salignum Bergius	1		5	
	Protea acaulos (L.) Reichard	2.6		1	
	Protea nitida Miller	0.4		3.2	
Restionaceae	Anthocortus ecklonii Nees	1	22.8		5.8
	Hypodiscus wildenowia (Nees) Masters	0.8		1	
	Ischyrolepis capensis (L.) Linder	4.8			
	Ischyrolepis gaudichaudiana (Kunth) Linder	7.2		3.4	
	Restio filiformis Poir.	7.4		1.2	
	Thamnocortus fructicosa Bergius	0.6		0.2	
	Thamnocortus lucens Poir.	1			
Rhamnaceae	Phylica stipularis L.	1.8	1.8	2.8	2.8
Rosaceae	Cliffortia cuneata Aiton	0.2	0.4	4.4	4.4
	Cliffortia ruscifolia L.	0.2			
Rubiaceae	Anthospermum aethiopicum L.	0.2	0.8		
	Anthospermum galioides Riechb.	0.6			
Rutaceae	Diosma hirsuta L.	1.2	1.2		
Santalaceae	Colpoon compressum Bergius	0.2	1.6		
	Thesium virgatum Lam.	1.4			
Schizaeaceae	Schizaea pectinatus (L.) Sw.			0.2	0.2
Scrophulariaceae	Agathelpis angustifolius Choisy	0.2	1.4		
	Selago spuria L.	1.2			
Thymelaeaceae	Struthiola ciliata (L.) Lam.	0.8	0.8		

Jonkershoek II species list. (October 1991)

Family	Species	Total % cover young site per species	per family
Anacardiaceae	Rhus angustifolia L.	2.2	2.2
Apiaceae	Lichtensteinia lacer Cham. & Schldl.	0.8	0.8
Asteraceae	Cullumia setosa (L.) R. Br.	0.4	24.2
	Helichrysum helianthymifolium (L.) D. Don	0.2	
	Metalasia cephalotes (Thunberg) Less.	2.6	
	Osteospermum ciliatum Bergius	20	
	Othonna quinquedentata Thunberg	0.8	
	Stoebe cinerea Thunberg	0.2	
Campanulaceae	Cyphia bulbosa (L.) Bergius	0.4	0.4
Cyperaceae	Ficinia bergiana Kunth	0.8	0.8
Dennstaedtiaceae	Pteridium aquilinum (L.) Kuhn	12.6	12.6
Ebenaceae	Diospyros glabra (L.) de Winter	0.6	0.6
Fabaceae	Aspalathus araneosa L.	0.2	28.6
	Aspalathus astroites L.	13	
	Aspalathus cephalotes Thunberg	0.4	
	Aspalathus neglecta Salter	15	
Iridaceae	Bobartia gladiata (L f.) Ker Gawler	0.4	0.6
	Watsonia borbonica (Pourr.) Goldbl.	0.2	
Mysinaceae	Myrsine africana L.	0.8	0.8
Polygalaceae	Muraltia alopecuroides (L.) D.C.	4.6	4.6
	Muraltia pauciflora (Thunberg) D.C.	1	
Proteaceae	Leucospermum grandiflorum (Salisb.) R. Br.	0.2	0.4
	Protea neriifolia R. Br.	0.2	
Ranuculaceae	Knowltonia vesicatoria (L. f.) Sims	0.2	0.2
Restionaceae	Elegia capensis (Burm. f.) Schelpe	5.4	5.4
Rosaceae	Cliffortia cuneata Aiton	0.6	0.8
	Cliffortia phillipsii Weim.	0.2	
Rubiaceae	Anthospermum aethiopicum L.	0.2	0.4
	Anthospermum galioides Riechb.	0.2	
Santalaceae	Thesium carinatum A. DC.	1	1.8
	Thesium virgatum Lam.	0.8	
Scrophulariaceae	Halleria elliptica Thunberg	2.4	3.6
	Selago spuria L.	0.2	
	Teedia lucida (Aiton) Rudolphi	1	

Orangekloof species list. (October 1991)

Family	Species	Total % cover young site per species	young site per family	old site per species	old site per family
Adiantiaceae	Pellaea pteroides (L.) Prantl.	4.2	5.4		
Alliaceae	Agapanthus africanus (L.) Hoffsgg.	0.4	0.4		
Amaryllidaceae	Haemanthus sanguineus Jacq.	0.4	0.4		
Anacardiaceae	Rhus laevigata L.	4.8	4.8	4.6	5.8
	Rhus tomentosa L.			1.2	
	Rhua rosmarinifolia Engl.	0.4	0.4	1.2	
Apiaceae	Glia gummifera (L.) Sonder	1.6	2.4		
	Hermas depauperata Thunberg	0.6			
	Lichtensteinia lacera Cham. & Schld.	0.2			
Asparagaceae	Protasparagus compactus (T.M. Salter) Oberm.	0.4	0.4		0.2
	Protasparagus rubicundus (Bergius) Oberm.			0.2	
Asphodelaceae	Trachyandra hirsuta (Thunberg) Kunth	0.2	0.2	0.2	0.2
	Trachyandra revoluta (L.) Kunth	0.2	0.2		
Asteraceae	Alciope tabularis (Thunberg) DC.	1	8.4	0.6	6
	Castalis nudicaulis (L.) Norlindh.	0.6			
	Chrysanthemoides monilifera (L.) Norlindh.	1.6			
	Chrysocoma cosma-aurea L.	0.2		0.2	
	Cullumia ciliaris (L.) R.Br.			0.4	
	Felicia aethiopica (Burm. f.) Adamson & T.M. Salter			0.2	
	Helichrysum cf cymosum (L.) D. Don			1.6	
	Helichrysum cf dasyanthrum (Willd.) Sweet			0.2	
	Helichrysum odoratissimum (L.) Sweet	3.8		0.2	
	Senecio bipinnatus (L.f.) Less.	0.2		0.4	
	Senecio umbellatus L.	0.2		0.8	
	Senecio elegans L.	0.2			
	Stoebe cinarea Thunberg	0.6		1.4	
Boraginaceae	Lobostemon glaucophyllus (Jacq) Beuk.	1	1	0.8	0.8
Bruniaceae	Brunia nodiflora L.	0.2	0.2	3.6	3.6
Campanulaceae	Cyphia bulbosa (L.) Bergius	0.4	1.4	0.2	0.2
	Cyphia volubilis (Burm. f.) Willd.	0.2			
	Lobelia pinifolia L.	0.8			
Celastraceae	Maytenus oleoides (Lam.) Loes.		0.2	3	3
Crassulaceae	Crassula fascicularis Lam.	0.2		0.2	0.2
Cyperaceae	Ficinia nigrescens (Schrader) Raynal	0.4	2.2	0.2	3.6
	Ficinia trichotes (Schrader) Benth. & Hook f.	0.4		0.2	
	Ficinia sp.	1			
	Tetraria thermalis (L.) C.B. Clarke	0.2		2.2	
	Tetraria cf compacta Levyns			0.4	
	Tetraria compar (L.) Lestib.	0.2			
	Tetraria ustulata (L.) C.B. Clarke			0.6	
Dennstaedtiaceae	Pteridium aquilinum (L.) Kuhn	0.8	0.8	6.2	6.2
Dipsacaceae	Cephalaria rigida (L.) Roemer & Schultes	2	2		
Ebenaceae	Diospyros glabra (L.) de Winter	0.6	0.6	0.8	0.8
Ericaceae	Erica baccans L.			0.4	6.4
	Erica conica Lodd.			0.2	
	Erica hirtiflora Curtis			4	

	Erica hispidula L.			1.4	
	Erica plukenetti L.			0.4	
Euphorbiaceae	Clutia alaternoides L.	0.6	0.6	0.2	0.2
Fabaceae	Aspalathus divaricata Thunberg	21	24.6		0.4
	Bolusafra bituminosa (L.) Kuntze	3.2		0.2	
	Indigofera mauritanica (L.) Thunberg			0.2	
	Lebeckia cf carnosa (E. Mey.) Druce	0.2			
	Podalyria sericea R. Br.	0.2			
Flacourtiaceae	Kiggalaria africana L.			0.2	0.2
Gentianaceae	Chironia baccifera L.			0.4	0.4
Geraniaceae	Pelargonium pinnatum (L.) L'Her	0.2	1.4		0.6
	Pelargonium senecioides L'Her	0.6			
	Pelargonium tabulare (Burm. f.) L'Her	0.2			
	Pelargonium sp.	0.4		0.6	
Haemodoraceae	Dilateris pillansii W. Barker		0.6	0.4	1.2
	Wachendorphia cf paniculata L.	0.6			
Iridaceae	Aristea major Andrews		3.8	0.6	1.6
	Bobartia gladiata (L.f.) Ker Gawler	0.4		0.2	
	Hesperantha fulcata (L. f.) Ker Gawler	0.2			
	Micranthus sp.			0.4	
	Watsonia angusta Ker Gawler	0.2		0.4	
	Watsonia borbonica (Pourret) Goldbl.	3			
Lamiaceae	Salvia Chamelaeagna Bergius	0.6	0.8		
	Salvia africana-caerulea L.	0.2			
Mesembriantrhemac	Erepsia gracilis (Haw.) L. Bolus	0.2	0.2		
Montiniaceae	Montinia caryophylaceae Thunberg	0.8	0.8	0.8	0.8
Myrsinaceae	Myrsine africana L.	0.6	0.6	0.2	0.2
Penaeaceae	Penaea mucronata L.	1	1	3.4	3.4
Poaceae	Merxmueller cf rufa (Nees) Conert	0.6	2.2	0.2	1
	Pentachistis sp.	0.2			
	Poaceae sp.	0.4			
	Pseudopentameris macrantha (Schrader) Conert	0.4		0.4	
	Themeda triandra Forsskal	0.6		0.4	
Polygalaceae	Polygala garcinii DC.	0.4	0.4		
Proteaceae	Leucadendron salignum Bergius	0.4	3.4	0.2	6
	Protea acaulos (L.) Reichard	0.2			
	Protea lepidocarpodendron (L.) L.			0.4	
	Protea nitida Miller	2.6		5.2	
	Protea sp.	0.2		0.2	
Ranuculaceae	Knowltonia vesicatoria (L.f.) Sims	0.2	0.2		
Restionaceae	Cannomois virgata (Rottb.) Steud.	4.6	7.6		20.6
	Elegia juncea L.	0.4		1.6	
	Hypodiscus albo-aristatus (Nees) Masters	0.2		0.8	
	Restio cf triceus Rottb.			12.4	
	Staberoha cernua (L.) Durand & Schinz			0.2	
	Wildenowia cf sulcata Masters			5.6	
	Restionaceae sp.	2.4			
Rhamnaceae	Phylica imberbis Bergius	0.2	0.6		
	Phylica stipularis L.	0.4			
Rosaceae	Cliffortia ruscifolia L.			0.4	0.4
Rubiaceae	Anthospermum aethiopicum L.			1.2	1.2
Rutaceae	Diosma oppositifolia L.	0.2	0.2		
Schizaeaceae	Schizeae pectinatus (L.) Sw.	0.4		0.8	0.8
Scrophulariaceae	Agathelpis angustifolia Choisy	0.2	5.4	0.4	0.4
	Dischisma ciliatum (Bergius) Choisy	3.4			
	Selago spuria L.	1.8			
Thymelaeaceae	Struthiola ciliata (L.) Lam.	0.4	0.4	0.4	0.4

Pella species list. (August 1991)

Family	Species	Total % cover middle-aged site		old site	
		per species	per family	per species	per family
Aizoaceae	Pharnaceum incanum L.	0.2	0.2		
Asparagaceae	Protoasparagus rubicundus (Bergius) Oberm.	0.4	0.4		
Asteraceae	Elytropappus glandulosus Less.	1.2	15.4	0.2	13.4
	Gazania ciliaris DC.	0.4		1	
	Gymnodiscus cappilaris (L. f.) Less	0.6		0.8	
	Helichrysum indicum (L.) Gierson	0.6			
	Helichrysum cf niveum (L.) Less			0.2	
	Ifloga ambigua (L.) Druce	0.6		0.8	
	Lachnospermum fasciculatum (Thunberg) Baillon	4		3.6	
	Metalasia adunca Less.	0.4		4.6	
	Metalasia capitata (Lam.) Less.	1.4		0.2	
	Metalasia muricata (L.) D. Don	2		0.6	
	Othonna stenophylla Levyns			0.2	
	Petalacte coronata (L.) D. Don	3.6		0.8	
	Senecio pubigerus L.	0.4			
	Stoebe cf fusca Thunberg			0.4	
	Stoebe leucocephala DC.	0.2			
Campanulaceae	Roella ciliata L.	0.4	0.4	0.2	0.2
Cyperaceae	Tetraria nigrovaginata (Nees) C.B. Clarke			0.4	0.4
Ericaceae	Erica mammosa L.			0.2	7.2
	Grisbachia plumosa Klotzsch			7	
Fabaceae	Aspalathus albens L.	1.4	24.6	1.4	3.6
	Aspalathus microphylla DC.	9.4		0.2	
	Aspalathus spinescens Thunberg	13.4			
	Indigofera sp.			0.2	
	Lotonotus sp.			0.6	
	Rafnia angulata Thunberg	0.4		1.2	
Geraniaceae	Pelargonium triste (L.) L'Her	0.2	0.2		
Iridaceae	Aristea africana (L.) Hoffsgg.	0.2	0.2	0.4	0.8
	Gladiolus gracilus Jacq.			0.4	
Lamiaceae	Salvia africana-caerula L.	0.4	0.4		
Mesembrianthemace	Lampranthus sp.	0.4	0.4		
Poaceae	Pseudopentameris macrantha (Schader) Conert	0.6	1		
	Poaceae sp.	0.4			
Polygalaceae	Muraltia dumosa (Poiret) DC.	1	1.2	0.8	0.8
	Muraltia brevicornu DC.	0.2			
Proteaceae	Leucadendron salignum Bergius	0.2	2.6	0.2	12.2
	Leucospermum hypophyllocarpodendron (L.) Druce	0.6		0.4	
	Leucospermum parile (Salisl. ex J. Knight) Sweet	0.8		11.4	
	Protea acaulos (L.) Reichard	0.2		0.2	
	Protea scolymocephala (L.) Reichard	0.4			
	Serruia fasciflora Salis. ex J. Knight	0.4			
Restionaceae	Calopsis impolitus (Kunth) Linder	0.6	14.8	3	23.6
	Cannomois parviflora (Thunberg) Pill.	2		2.2	
	Hypodiscus wildenowia (Nees) Masters	3			

	Ischyrolepis monanthos (Masters) Linder	0.6		1.4	
	Restio praeacutus Masters	0.2		0.4	
	Staberoha distachya (Rottb.) Kunth.	2.8		1.4	
	Thamnocortus punctatus Pill.	0.6		14.8	
	Wildenowia arescens Kunth	4.8		0.4	
	Wildenowia sulcata Masters	0.2			
Rhamnaceae	Phylica ericoides L.	4.2	6.4	8.4	10.8
	Phylica stipularis L.	2.2		2.4	
Rubiaceae	Anthospermum aethiopicum L.	1	1	0.8	0.8
Rutaceae	Agathosma imbricata (L.) Willd.	0.8	0.8	0.2	0.6
	Diosma dichotoma Bergius			0.4	
Thymelaeaceae	Passerina vulgaris Thoday		1	0.2	0.2
	Struthiola ciliata (L.) Lam.	0.2			
	Thesium densiflorum A. D.C.	0.8			
Zygophyllaceae	Zygophyllum spinosum L.	0.8	0.8	0.8	0.8

Scarborough species list. (December 1991)

Family	Species	Total % cover young site		middle-aged site		old	
		per species	per family	per species	per family	per species	per family
Apiaceae	Lichtensteinia lacera Cham. & Schldl.	0.4	0.4	0.2	0.2		
Asphodelaceae	Trachyandra brachypoda (Barker) Oberm.	0.4	0.4	1.2	1.4	0.4	
	Trachyandra hirsutiflora (Adamson) Oberm.					0.4	
	Tritoniopsis sp.			0.2		0.6	
Asteraceae	Anaxeton laeve (Harvey) Lundgren	2	17.6	0.6	13.4		15.8
	Corymbium enervae Markotter	0.2		0.6			
	Edmondia sesamoides (L.) Willd.	1.4		1.2			
	Elytropappus scaber (L.f.) Levyns	5.2		5.2		11.8	
	Euryops abrotanifolius (L.) DC.			0.4			
	Helichrysum indicum (L.) Grierson			0.2			
	Helichysum latorale Bolus					0.2	
	Ifloga ambigua (L.) Druce			0.2		0.4	
	Metalasia brevifolia (Lam.) Levyns			0.2		0.2	
	Metalasia cephalotes (Thunberg) Less.	2.6		0.4			
	Metalasia muricata (L.) D. Don	0.4		0.8		2	
	Oeroeda imbricata Lam.	0.8		1		0.2	
	Osteospermum pogaloides L.	0.2					
	Othonna quinquidentata Thunberg	0.2		0.2			
	Petalacte coronata (L.) D. Don			0.8		0.2	
	Senecio pubergerus L.	1				0.2	
	Senecio umbellatus L.	0.2		0.2		0.4	
	Stoebe cinerea Thunberg	0.8					
	Stoebe cf fusca Thunberg	1.4		0.4			
	Stoebe plumosa (L.) Thunberg			0.2			
	Stoebe sphaerocephala Schltr.	0.6		0.2			
	Syncarpha vestita (L.) B. Nord.	0.2		0.2		0.2	
	Ursinia palaceae (L.) Moench	0.4		0.4			
Brasicaceae	Heliophila linearis (Thunberg) DC.					0.2	0.2
Campanulaceae	Lobelia comosa L.	0.4	5.6		0.8		
	Lobelia setaceae Thunberg	1.6					
	Roella amplexicaulis Wolley-Dod	0.2					
	Roella ciliata L.	1.4		0.6			
	Roella prostrata E. Meyer ex A. DC.	0.2					
	Roella triflora (Good) Adamson	1.6		0.2			
	Lobelia coronopifolia L.	0.2					
Celastroides	Maytenus oleoides (Lam.) Loes					0.4	0.4
Crassulaceae	Crassula fascicularis Lam.	0.4		0.4	0.4	0.4	1
	Crassula nudicaulis L.					0.6	
Cyperaceae	Ficinia cf acuminata (Nees) Nees	0.2	2.4		2.2	0.2	1.2
	Ficinia duesta (Bergius) Levyns			0.2			
	Ficinia filiformis (Lam.) Schrader	0.2		0.2			
	Tetraria compar (L.) Lestib.			0.4			
	Tetraria cf compacta Levyns					0.6	
	Tetraria eximia C.B. Clarke	0.6		0.2			

	Tetraria microstachys (Vahl.) Pfeiffer	0.6		0.6		0.4	
	Tetraria sylvatica (Nees) C.B. Clarke	0.8		0.2			
	Tetraria sp.			0.4			
Ebenaceae	Diospyros glabra (L.) de Winter					0.2	0.2
Ericaceae	Blaeria ericoides L.	0.4	1.6	7	11.4	15.4	20.2
	Coccosperma hexandra (Klotzsch) Druce			2.8		3.2	
	Erica abaetina L.					0.6	
	Erica articularis	1.2		0.6			
	Erica imbricata L.			0.4		0.8	
	Erica plukenetti L.			0.4		0.2	
	Erica sesssilifolia			0.2			
	Scyphogyne mucosa (Aiton) Steudel			0.2			
Euphorbiaceae	Clutia Alternoides L.				0.4	0.4	0.8
	Euphorbia genistoides A. Berger			0.4		0.4	
Fabaceae	Aspalathus callosa L.		16.4	0.2	9.4		0.8
	Aspalathus carnosa Bergius	2.8		8.8			
	Aspalathus ericifolia L.					0.2	
	Aspalathus laricifolia	0.2					
	Aspalathus retroflexa L.	11					
	Indigofera brachystachya E. Meyer	0.4		0.2		0.2	
	Indigofera glomerata E. Meyer	0.4					
	Lebeckia meyeriana Ecklon & Zeyer	1.6		0.2		0.4	
Gentianaceae	Chironia baccifera L.		0.4		0.4	0.2	0.8
	Chironia linioides L.	0.4		0.4		0.6	
Geraniaceae	Pelargonium capitatum (L.) L'Her.	0.2	0.2	0.4	0.8		1.2
	Pelargonium cucullatum (L.) L'Her.			0.2		0.6	
	Pelargonium triste (L.) L'Her			0.2		0.6	
Hyacinthaceae	Albuca sp.			0.2	0.2		
Iridaceae	Bobartia indica L.	0.4	0.4	0.4	0.4	0.2	0.2
Mesembrianthemace	Erepsia anceps (Haw.) L. Bolus	1.2	1.2	0.4	0.6		0.4
	Ruschia sarmentosa (Haw.) Schwantes			0.2		0.4	
Penaeaceae	Stylapterus fructicosus (L. f.) Adr. Juss	0.2	0.2				
Poaceae	Pentachistis cf clorata (Steudel) Stapf.	0.4	2.8	1	4.2		0.6
	Pentachistis sp.	0.4		1		0.6	
	Pseudomentameris macrantha (Shrader) Conert	2		2			
	Tibolium uniolae (L. f.) Renvoize			0.2			
Proteaceae	Leucadendron salignum Bergius	0.4	1.6	2	2.6	0.4	3.8
	Leucadendron sp.	0.2		0.2			
	Serruria cf villosa (Lam.) R.Br.	1		0.4		3.4	
Restionaceae	Elegia stipularis Masters	1	8.4	7	21	2.2	15.8
	Hypodiscus aristatus (Thunberg) Masters			2			
	Hypodiscus wildenowia (Nees) Masters	0.4		0.6		0.6	
	Ischyrolepis capensis (L.) Linder	0.2		1.2		0.2	
	Ischyrolepis cincinnata (Masters) Linder	2		3.2		1.2	
	Staberoha cernua (L. f.) Durand & Schinz	1.4		1.2		3.4	
	Thamnocortus fructicosus Bergius	0.2		1.6		3.6	
	Thamnocortus gracilis Masters	2.4		3.2		2.4	
	Wildenowia sulcata Masters	0.8		1		2.2	
Rhamnaceae	Phylica imberbis Bergius	1.4	1.6	1.4	1.4	1	2.2
	Phylica stipularis L.	0.2				1.2	
Rosaceae	Cliffortia atrata Weim.	0.6	0.6		0.2	0.2	0.2
	Cliffortia cf polygonifoia L.			0.2			

Rutaceae	Adenandra villosa (Bergius) Lichtenstein ex Roemer	0.2	1.6		5		1.6
	Agathosma hookerii Sonder			0.2		0.2	
	Agathosma imbricata (L.) Willd.	0.2		2		0.2	
	Diosma oppositifolia L.	1		2.2		1	
	Macrostylis villosa (Thunberg) Sonder	0.2		0.6		0.2	
Santalaceae	Thesium acuminatum A.W. Hill	0.4	1.2		0.2		
	Thesium sp.	0.6		0.2			
	Thesium virgatum Lam.	0.2					
Scrophulariaceae	Agathelpis angustifolius Choisy	1.2	1.2	0.4	0.4	0.4	0.6
	Dischimia ciliatum (Bergius) Choisy					0.2	
Thymelaeaceae	Cryptadenia grandiflora (L. f.) Meissner		1.2		1.6	0.2	5
	Gnidia juniperifolia Lam.					0.2	
	Gnidia parvifolia Meissner	0.2		0.8		0.6	
	Gnidia pinifolia L.					0.2	
	Passerina cf vulgaris Thoday			0.4		3.8	
	Struthiola cf ciliata (L.) Lam.	1		0.4			

Silvermine species list. (December 1991)

Family	Species	Total % cover young site		middle-aged site		old site	
		per species	per family	per species	per family	per species	per family
Asphodelacceae	Trachyandra cilata (L. f.) Kunth	0.2	0.8			0.4	0.4
	Trachyandra tabularis (Baker) Oberm.	0.6					
Asteraceae	Corymbium africana L.	0.4	6.4	0.4	1.4		11.2
	Corymbium glabrum L.	0.6					
	Edmondia sesamoides (L.) Willd.	0.8		0.2		0.2	
	Elytropappas scaber (L. f.) Levyns	0.4		0.8		9.4	
	Euryops abrotanifoius (L.) DC.	0.4					
	Helichrysum litorale Bolus	1.6				0.2	
	Metalasia muricata (L.) D. Don	0.8				0.6	
	Phaenocoma prolifera (L.) D. Don	0.4					
	Senecio umbellatus L.	0.8				0.6	
	Stoebe sp.					0.2	
	Syncarpha vestita (L.) B. Nord.	0.2				1.6	
Brasicaceae	Heliophila scoparia Burchell ex DC.	0.2	0.2				
Campanulaceae	Lobelia coronopifolia L.		0.8		0.8	0.2	0.2
	Lobelia pinifolia L.	0.8		0.8			
	Roella triflora (Goo) Adamson	1	1				
Cyperaceae	Ficinia duesta (Bergius) Levyns	0.4	7.8		5.4	0.4	11.4
	Ficinia lateralis (Vahl.) Kunth.	0.6				0.8	
	Ficinia pinguior C.B. Clarke	0.4				0.2	
	Tetaria compar (L.) Lestib.	2.2		0.8		5.2	
	Tetraria crinifolia (Nees) C.B. Clarke					1.6	
	Tetraria cuspidata (Rottb.) C.B. Clarke					0.4	
	Tetraria eximia C.B. Clarke	0.6				0.2	
	Tetraria fasciata (Rottb.) C.B. Clarke			1		1	
	Tetraria flexuosa (Thunberg) C.B. Clarke	1				1	
	Tetraria thermalis (L.) C.B. Clarke	1.6		3.6			
	Tetraria macostachys (Vahl.) Pfeiffer	1				0.6	
Ericaceae	Blearia ericoides L.			5.8	12.8	5.4	15.6
	Erica coriifolia L.					0.2	
	Erica plukenetti L.			0.2			
	Erica pulchella Houtt.			6.8		10	
Fabaceae	Aspalathus abietina Thunberg	3.2	6				0.2
	Aspalathus retroflexa L.	2.8				0.2	3.2
Haemodoraceae	Dilatris pillansii W.F. Barker	1.4	1.8	0.4	0.4	1.2	
	Wachendorphia paniculata L.	0.4				2	
Hyacinthaceae	Albuca Cooperi Baker	0.2	0.2			0.6	
Iridaceae	Aristia dichotoma (Thunberg) Ker Gawler	0.4	1			0.2	1.2
	Bobartia filiformis (L. f.) Ker Gawler	0.4					
	Tritoniopsis cf parviflora (Jacq.) G. Lewis	0.2				0.2	
	Watsonia sp.					0.8	
Mesembrianthemace	Erepsia anceps (Haw.) L. Bolus	0.2	0.2			0.2	0.2
Penaeaceae	Penaea mucronata L.	0.2	0.6	0.6	1.2	1.6	1.6
	Saltera sarcocolla (L.) Bullock	0.4		0.6			

Poaceae	Merxmuellera cf rufa (Nees) Conert.	0.2	1.2		0.2		0.2
	Pentachistis colorata (Steudel) Stapf	0.8		0.2			
	Pentachistis curvifolia (Schrader) Stapf					0.2	
	Pseudopentameris macrantha (Schrader) Conert.	0.2					
Proteaceae	Lecadendron laureolum (Lam.) Fourc.		0.4	1.8	4.4	2.8	4.2
	Leucadendron cf Xanthoconus (Kuntze) K. Schum	0.4		2.6		1.4	
Restionaceae	Elegia stipularis Masters	0.6	10.4	13.4	36		9
	Hypodiscus aristatus (Thunberg) Masters	1.2		3		1.2	
	Ischyrolepis pratensis Esterh.	2		2.6			
	Restio filiformis Poir.			5		0.2	
	Restio sp.	1.2					
	Staberoha cernua (L. f.) Durand & Schinz	1.2		5.4			
	Thamnocortus gutheriae Pill.	1.4					
	Thamnocortus sporadicus Pill	1.8		5.2		3.4	
	Wildenowia glomerata (Thunberg) Linder	1		0.4			
	Wildenowia cf sulcata Masters			1		4.2	
Rosaceae	Cliffortia stricta Weim.			0.2	0.2		
Rutaceae	Adenandra villosa (Bergius) Lichten. ex Roemer & Sc	0.6	0.6	0.2	0.2	0.6	0.8
	Macrostylis villosa (Thunberg) Sonder					0.2	
Santalaceae	Thesium capitatum L.	0.2	1.4				
	Thesium sp.	1.2					
Scrophulariaceae	Agathelpis angustifolia choisy	1	1.8			0.6	0.6
	Selago spuria L.	0.8					
Thymelaeaceae	Cryptadenia grandiflora (L. f.) Meissner	0.2	1.8		1.2	0.2	1.8
	Gnidia pinifolia L.	0.8		0.4		0.8	
	Passerina vulgaris Thoday			0.2		0.6	
	Struthiola ciliata (L.) Lam.	0.6		0.6		0.2	
	Struthiola sp.	0.2					