ASPECTS OF HONEYBUSH TEA (*CYCLOPIA* SPECIES) PROPAGATION

by

MONGEZI MORRISON MBANGCOLO

Thesis presented in partial fulfillment of the requirements for the degree Master of Science in Agriculture at Stellenbosch University

Study Leader: Dr. E. Reinten

Co-Study Leader: Prof. G.A. Agenbag

Department of Agronomy

Stellenbosch University

December 2008

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 10 November 2008

ABSTRACT

Honeybush (*Cyclopia* spp. Fabaceae) is indigenous to the fynbos botanical biome of the Eastern and Western Cape of South Africa. The increase in the international demand for honeybush tea for health benefits, concern over exploitation of wild populations and the lack of published agronomic information necessitated this study to evaluate different aspects of honeybush propagation. The main objectives of this study were to evaluate the effect of species and cutting position on rooting of cuttings of *Cyclopia* species using different rooting hormones, to evaluate the effect of an organic plant fertilizer and cutting position on growth and establishment of rooted cuttings and to study the influence of different seed pre-treatments on germination of *Cyclopia* species.

Terminal and sub-terminal cuttings of *C. intermedia* and *C. genistoides* treated with different rooting hormones were rooted under day/night temperature controlled glasshouse conditions. Intermittent mist was used as means of moisture supply to the cuttings for 45-60 seconds daily every 30 minutes. *C. genistoides* rooted significantly better compared to *C. intermedia* as measured by rooting percentage, number of roots per cutting, length of longest root and mean root length during the summer season. The cutting position had a significant effect on rooting of the cuttings in summer compared to winter and spring season. The interactive effect of species, treatment and cutting position resulted into 86% of rooting in summer from the terminal cuttings of *C. genistoides*, while only 4% was recorded as the highest rooting percentage in both winter and spring seasons. The highest number of roots and the greatest root length per cutting were obtained with 2 and 4 g L^{-1} IBA from terminal cuttings of *C. genistoides* and these hormone concentrations were not significantly different to each other.

To evaluate the effect of an organic plant fertilizer and cutting position on plant growth and establishment, rooted cuttings of two *Cyclopia* species (*C. intermedia* and *C. genistoides*) from two cutting positions (terminal and sub-terminal) were transferred to pots (576 cm^3) and treated with Nitrosol®

fertilizer at application rates of 3.33 $ml.L^{-1}$, 1.67 $ml.L^{-1}$ and 0 $ml.L^{-1}$ (control). *Cyclopia* plantlets were uniformly inoculated once with a symbiotic *Rhizobium* bacteria to improve the formation of nodules. Nitrosol® at 3.33 $ml.L^{-1}$ significantly affected fresh and dry plant weight, fresh and dry root weight, number of shoots and nodules per plant compared to either 1.67 $ml.L^{-1}$ or the control. Relative to species, *C. genistoides* performed better in terms of fresh and dry plant weight, fresh and dry root weight, and number of shoots and nodules per plant compared to *C. intermedia*. The origin of the cutting position did not significantly affect the above mentioned parameters. Plant mineral analysis revealed that most of the essential elements increased with increasing Nitrosol® application rates, with *C. genistoides* having higher levels of mineral elements than *C. intermedia*. This could be an indication of the differences between the two species in terms of nutrient uptake, utilization and distribution within the plant tissues.

In the germination studies, seeds obtained from different seed sources of *Cyclopia* species were subjected to different pre-sowing treatments. Seed treatments were sulphuric acid (95%), hot water (100°C), water with smoke paper disk, and demineralised water (control). The study revealed that all the treatments had a significant effect on germination with the exception of eight year old seeds obtained from *C. subternata* (seed source two). Although hot water treatment improved germination compared to smoked paper disk and the control, seeds treated with hot water degenerated rapidly. The highest overall germination (77.33%) was found with one year old seeds compared to other seed sources older than one year. Although smoked paper disks generally did not improve germination compared to the control, in one year old seeds from seed source one, this treatment greatly influenced germination, suggesting that seed age might have influenced germination of these seeds. In terms of germination rate, germination generally started after four days in most treatments.

OPSOMMING

Heuningbos (*Cyclopia* spp., Fabaceae) is inheems aan die fynbos bioom van die Oos- en Wes-Kaap. Die toename in internasionale vraag na heuningbostee met sy gesondheidseienskappe, oorontginning van natuuurlke hulpbronne en die tekort aan gepubliseerde agronomiese inligting, het hierdie ondersoek na verskillende ontkiemingsaspekte van heuningbos geïnisieer. Die doelwit van die ondersoek was om die effek van spesies en steggie-oorsprong met die bewortelings en vestiging van *Cyclopia* steggies te bepaal met verskillende groeihormoonbehandelinge en voedingstoedienings. Die invloed van 'n reeks voor-afbehandelinge vir die ontkieming van Cyclopia-saad is ook bepaal.

Terminale en subterminale steggies van *C. intermedia* en *C. genistoides* behandel met verskillende bewortelingshormone, is in dag/nag temperatuurbeheerde glashuistoestande bewortel. Steggies is daagliks van 08H00 tot 17H00 elke 30 minute vir 45-60 sekondes aan misbesproeiing blootgestel. Beworteling van *C. genistoides* in die somer was beduidend beter in vergelyking met *C. intermedia* ten opsigte van persentasie beworteling, aantal gevormde wortels per steggie en die gemiddelde wortellengte. Die oorsprong van die steggie het 'n beduidende effek op steggiebeworteling in die somer teenoor winter en lentesteggies getoon. Ongeag spesies, het steggies beter in die somer as in die winter of lente bewortel. Die interaktiewe effek van spesies, behandeling en steggie-oorsprong het gelei na 86% beworteling van terminale *C. genistoides* steggies terwyl slegs 4% in die winter of lente bewortel het. Die meeste wortels asook die langste wortellengte per steggie het met 2 en 4 g L^{-1} IBA by terminale steggies van *C. genistoides* gelei, maar was nie beduidend verskillend van die ander nie.

Om die effek van organiese plantvoeding en steggie-oorsprong op die groei en vestiging te bepaal, is gewortelde steggies van twee Cyclopia spesies (*C. intermedia* en *C. genistoides*) van twee oorsprongsnitte (terminaal en subterminaal) in potte (576 cm^3) geplaas en met Nitrosol® voedingstof met toedienings van 3.33 $ml.L^{-1}$, 1.67 $ml.L^{-1}$ en 0 $ml.L^{-1}$ (kontrole). Plante is

eenmalig uniform met simbiotiese *Rhizobium* bakterieë geïnokuleer om wortelknoppies te induseer.

Nitrosol® teen 3.33 $ml.L^{-1}$ het vars en droë gewig van plante, die aantal lote en die wortelknoppies per plant in vergelyking met 1.67 $ml.L^{-1}$ en die kontrole beduidend beïnvloed. Tussen spesies het *C. genistoides* in terme van vars en droë gewig, aantal lote en wortelknoppies per plant teenoor *C. intermedia* beter presteer. Die oorsprong van steggieposisie het nie bogenoemde parameters beduidend beïnvloed nie. Minerale plant ontledings het aangetoon dat die meeste essensiële elemente met toenemende Nitrosol® toedienings toegeneem het. *C. genistoides* het hoër vlakke van minerale elemente as *C. intermedia* in terme van minerale plantanalises getoon. Dit kan dui op die verskille tussen die twee spesies ten opsigte voedingsopname, verbruik en verspreiding in die plantweefsel.

Met die ontkiemingsstudies, is sade van verskeie oorsprong verkry en aan verskillende voorbehandelings onderwerp. Saadbehandelings het swaelsuur (95%), warm water (100°C), rookwater en gedeïoniseerde water (kontrole) ingesluit. Die ondersoek het aangetoon dat alle behandelings 'n beduidende effek op saadontkieming het met die uitsondering van agt jaar oue saad van *C. subternata* (saadbron twee). Alhoewel warm water behandelinge ontkieming verbeter het in vergelyking met rookwater en die kontrole, het sade met warm water behandelinge vinnig agteruitgegaan en is die ontkieming na 18 dae gestaak. Die beste algehele ontkieming is by eenjarige sade ondervind, in vergelyking met ouer sade. Rookwater in die algemeen in vergelyking met die kontrole, het nie ontkieming bevorder nie, maar met eenjarige saad van saadbron een, het rookwater behandelinge grootliks ontkieming bevorder, wat kan dui op die invloed van saadouderdom op ontkieming. In terme van kumulatiewe ontkieming, het ontkieming meestal na vier dae plaasgevind. Die ontkiemingskurwes dui aan dat ontkieming in die begin vinnig is en dan tydens die eksperimente 'n maksimum bereik het na 18 dae.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following individuals and institutions:

The Lord Almighty God, for giving me strength and perseverance without whom none of this would be possible;

Agricultural Research Council (ARC) for financial support and lending me the opportunity to further my studies;

Agribusiness in Sustainable Natural African Plant Products (ASNAPP) for financing the research project;

National Department of Agriculture for financial assistance;

My study leaders, Prof. G.A. Agenbag and Dr. E. Reinten for their invaluable guidance and help during the period of my studies;

Dr. H. De Lange, Miss. M. Joubert, Miss. J. Bloem and Mrs. M. Van Zyl for devoting their time and supplying me with the necessary materials for my study project;

ASNAPP South Africa and Department of Agronomy staff members in general for their support and assistance;

Marieta van der Rijst, ARC Agrimetrics Institute, for the statistical analysis of all the data;

Mr. D. Murray – ARC- Institute for Tropical and Subtropical Crops (ITSC) for his encouragement and support as my PDP mentor;

My family and friends, for their patience, devotion and moral support during this lengthy journey.

TABLE OF CONTENTS

Chapter 3

Effect of species, cutting position and exogenous rooting hormones on rooting of honeybush (*Cyclopia* spp.) cuttings

Chapter 4

Effect of an organic plant fertilizer and cutting position on the establishment of rooted cuttings of *Cyclopia* species

Chapter 5

Effect of different seed treatments on germination of *Cyclopia* spp. seeds

CHAPTER 1

PROBLEM STATEMENT

Introduction

Honeybush tea (*Cyclopia* spp.) is indigenous to the Eastern and Western Cape of South Africa. The foliage, fine stems and flowers of the *Cyclopia* species are used to make a beverage and a honey-like flavoured herbal infusion known as honeybush tea (Du Toit, Joubert & Brits, 1998). The tea has become internationally recognized as a substitute for ordinary tea like *Camellia sinensis* due to its health benefits (Dharmananda, 2004). There has been a dramatic growth in the use of honeybush tea over the last few years and the industry has a huge potential in the herbal tea category as it has no competition from other countries. Furthermore, the industry has the potential to position the tea within the major food trends as an organic specialty health product and value-added products such as ice tea, green tea and baby products (Wesgro, 2005). About 90 tons of honeybush tea was exported in 1999 and there has been a significant growth in exports over the years. It is estimated that about 300 tons of tea were exported in 2005 (SAHTA, 2007).

The honeybush plant is a shrub of the Fabaceae family that grows in the fynbos botanical biome of the Western and Eastern Cape Provinces of South Africa (Schutte, 1995). Honeybush plants can be easily recognized by trifoliate leaves, single-flowered inflorescences, and sweetly scented, bright yellow flowers. The plants have woody stems, a relatively low ratio of leaves to stems, and hard shelled seeds (Bond & Goldblatt, 1984). The most desirable components for the tea are the leaves and flowers, but the relatively tasteless stems are also included. Commercial supplies of honeybush tea are mainly obtained from *Cyclopia intermedia, C. subternata,* and *C. genistoides*. However, about 23 species of *Cyclopia* have been identified and most of the species have very limited distribution ranges and unique habitat preferences (Bond & Goldblatt, 1984; Schutte, 1995). Some are restricted to mountain peaks, perennial streams, marshy areas, or wet southern slopes. Some of the species, such as *C. maculata*, and *C. sessiflora*, have been used for home consumption. It appears that most of the species are suitable for tea, but the taste quality can vary and some species exist in very small quantities. The collection of honeybush in South Africa has shown a significant increase in recent years. A large amount of the tea is still collected from wild populations; hence cultivation has become necessary due to the rapid growth of the industry and the demand for a more uniform product (SAHTA, 2007).

The selection and breeding of improved quality plants necessitates vegetative propagation. Vegetative propagation of *Cyclopia* spp. by stem cuttings, requirements for seed germination and the response of rooted stem cuttings to varying fertilizer solution levels to enhance subsequent growth and development has not been reported in literature. Therefore, the main objectives of this study were:

- To determine the effect of species (*C. intermedia* and *C. genistoides*), cutting position (terminal and sub-terminal) and exogenous rooting hormone on rooting of honeybush (*Cyclopia* spp.) cuttings.
- To evaluate the influence of an organic plant fertilizer and cutting position on the establishment of rooted cuttings of *Cyclopia* species.
- To determine the effect of different seed treatments on germination of *Cyclopia* spp. under incubation chamber conditions.

REFERENCES

BOND, P. & GOLDBLATT, P., 1984. Plants of the Cape Flora. A descriptive Catalogue. pp. 285-286. CPT Book Printers, Cape Town, South Africa.

DHARMANANDA, S., 2004. Honeybush: Healthful beverage tea from South Africa. Institute for traditional medicine, Portland, Oregon, USA.

DU TOIT, J., JOUBERT, E, & BRITS, T.J., 1998. Honeybush tea - a rediscovered indigenous South African herbal tea. *J. Sust. Agric.* 12, 67-84.

SAHTA, 2007. South African Honeybush Tea Association, Notes compiled for Honeybush Farmers' Day, March 2007, Kanetberg, Riversdale. P.O. Box 663, Riversdale 6670.

SCHUTTE, A.L., 1995. A taxanomic study of the tribes Podalyrieae and Liparieae (Fabaceae). Ph.D. Dissertation, Rand Afrikaans University, Johannesburg, South Africa.

WESGRO, 2005. Agriculture: Natural Products. P.O. Box 1678, Cape Town 8000, South Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Honeybush (*Cyclopia* spp.) is used as an indigenous herbal tea in South Africa. The foliage, fine stems and flowers of the *Cyclopia* species are used to manufacture a sweetish, honey-like flavoured herbal infusion known as honeybush tea. The plant is known to have been in existence for centuries in South Africa. According to Kies (1951), the earliest mention of honeybush tea in botanical literature dates back as early as 1705. However, it is not clear whether the bush was used for consumption back in those days, but it can be stated that the local inhabitants realized the health giving properties of the tea in their search for natural herbs and medicines.

Cyclopia species are leguminous shrubs belonging to the Cape Fynbos biome and grow in the coastal districts of the Western and Eastern Provinces of South Africa, from Darling to Cederberg, Koue Bokkeveld, Klein Swartberg, Groot Swartberg and Kouga mountain ranges but the individual species seem to be fairly localized (Kies, 1951). According to Bond & Goldblatt (1984) and Schutte (1995), more than 20 species of *Cyclopia* have been described and they normally occur on the shady and cooler southern slopes of the mountain ranges. The pleasant sweet honey-like taste and anecdotal evidence on the beneficial qualities of honeybush tea resulted in this tea being an alternative to coffee and black tea (Du Toit, Joubert & Brits, 1998). Growing interest in herbal teas over the last few years, both locally and internationally has lead to the increased interest of the honeybush tea industry.

2.2 Botanical review

Cyclopia is a distinct genus of the tribe Podalyrieae and is classified as a member of the Fabaceae (Schutte, 1995). According to Bond & Goldblatt (1984), most of the bushes can grow up to 1.5 m high, but some can reach up to 3 m. The stems are woody with relatively low leaf-to-stem ratio and bearing pods with hard shelled seeds which germinate poorly if not scarified prior to germination (Welgemoed, 1993). Plant leaves are trifoliate (Levyns, 1920; Marloth, 1925) and leaf shape varies considerably between species, from pubescent, narrow-leafed (Bond & Goldblatt, 1984) to flattened (Kies, 1951). *C. intermedia* is a shrub with flat leaves (18-28 mm long, 2-5 mm wide), and *C. genistoides* has linear leaves (14-20 mm long, 1-2 mm wide). During the flowering period, bushes of honeybush tea are easily recognized in the field as they are covered with distinctive, deep-yellow flowers which have a characteristic sweet, honey scent. Flowering is usually in spring (September to October), with the exception of *C. sessiflora* which flowers during late autumn or early winter (May and June) (Schutte, 1995).

2.3 Health associated properties of honeybush tea

Honeybush tea is an herbal infusion and many of its health properties are associated with the regular consumption of the tea. The anecdotal evidence suggests that the infusion increases appetite (Watt & Breyer-Brandwijk, 1962) and is therefore known as “hungry tea” (Viljoen, 1994). A preliminary study on rats suggests that the infusion increases appetite (Stander & Morgenthal, 1995).

Honeybush tea has a very low tannin content and contains no caffeine. It is, therefore, especially valuable for children and patients with digestive and heart complexes where stimulants and tannins should be avoided (Marloth 1925; Terblance, 1982). The tea is often administered to babies and children with stomach problems and is also suitable as a remedy for calves with digestive problems. The lack of caffeine in honeybush tea could contribute to the calming effect of the tea and can help to combat sleeplessness (insomnia). According to Terblance (1982), the infusion stimulates milk production and is regarded as beneficial for breast-feeding women.

The extract can be used to help for certain skin ailments such as psoriasis. A decoction of the tea has been used as a restorative and an expectorant in chronic catarrh and pulmonary tuberculosis. Research by the Department of Chemistry of the University of the Free State (Bloemfontein, Free State Province) indicated that substantial amounts of (+)-pinitol is present in honeybush tea. Pinitol is used as an expectorant (Beecher, Farnsworth & Gyllenhall, 1989), and also has anti-diabetic activity (Narayan *et al*., 1987). *C. intermedia* extracts showed the presence of flavonoids, isoflavonoids, xanthones and cosmetans (Kamara, 1997). According to Joubert & Ferreira (1996), the electron-rich aromatic B-ring system of the flavones can supply electrons that are required for the reduction of the active oxygen species. These phenolic compounds, therefore, contribute towards the scavenging ability of herbal teas. De Nysschen *et al*. (1996) indicated that the major phenolic compounds of honeybush tea are xanthone, mangiferin and the flavones iso-sakuranetin and hesperitin. These compounds are present in varying quantities in all the 22 *Cyclopia* species that were examined. The contribution of these types of phytochemicals to the health-giving properties of plants has been given a special attention suggesting their importance in human consumption. The role of antioxidants has attracted much interest due to their protective role against free radical damage that may be the cause of many diseases including cancer (Nakayama *et al*., 1993). The antioxidative effects of green teas arise from their phenolic compounds such as the fluvanoids (Pietta, Simonetti & Mauri, 1998). Some fluvanoids and non-fluvanoid phenolic compounds have been reported to also show alkylperoxyl radical scavenging activity, thus reducing radical pathogenesis, e.g. carcinogenesis (Sawa *et al*., 1999).

2.4 Production of honeybush

C. intermedia is the most important species in terms of market share and is exclusively harvested in the natural fynbos areas and on private farms. According to SAHTA (2007), it is estimated that approximately 30 000 ha of fynbos, including Tsitsikamma, Kouga, Baviaans, Langeberg and Swartberg mountain ranges are the areas where honeybush grows sporadically (Kies, 1951). The growing demand of the honeybush tea market can therefore result into the extinction of the wild populations, which is mainly caused by unsustainable harvesting methods of the natural populations.

In terms of cultivation, *C. subternata* and *C. genistoides* are the two main cultivated species. Their cultivation is localized in the area of Overberg to the Langkloof, with approximately 200 ha under cultivation (SAHTA, 2007). *C. subternata* grows mainly on sandy loam soil in valleys in the Langkloof, Waaboomskraal near George and in the Riversdale area. *C. genistoides* grows naturally in the coastal sandy areas from the west coast to Mossel Bay, hence plantations have been established in the Overberg and Mossel Bay/Albertinia areas.

C. intermedia has been indicated to be a difficult species to cultivate commercially. This species can only be harvested every second or third year, making it uneconomical to cultivate for commercial purposes. Frequent harvesting does not allow plants to build up sufficient energy reserves causing a die-back. Currently, about 30 hectares of *C. intermedia* is planted in the Langkloof and Southern Cape areas (SAHTA, 2007).

Data collected by the Western Cape Department of Agriculture shows that the cost of establishing a honeybush plantation is estimated to be between R10 000 and R20 000 per hectare (Miss B. Matoti, pers. comm., 2007. Agricultural Economics, Department of Agriculture: Western Cape, Private Bag X1, Elsenburg, 7607). Yields of biomass depend on various factors, including species, climate, soil and management practices and may vary from 3 to 5 tons per hectare with fresh plant material being sold to processors for R2, 00 - R3, 00/kg.

2.5 Marketing of honeybush tea

Honeybush tea has strong cultural and historical roots in South Africa. The industry has huge potential in the herbal tea category as it has no competition from other countries. Furthermore, the honeybush tea industry has the potential to position the tea within the major food trends as an organic, specialty health products and value-added products such as ice tea, green tea and baby products (Wesgro, 2005). Since 1996, a private commercial company, Cape Natural Tea Products has been marketing honeybush tea. Initial indications were that this tea has great potential because of its pleasant taste and healthy properties. It is estimated that in 1997 about 30 tons of honeybush tea was processed with most of it sold in the local market. The 1999 market was about 50 tons and in 2000 approximately 150 tons of honeybush were harvested (Mr. D. De Villiers, pers. comm., 2006, Cape Natural Teas, P.O. Box 509, Brackenfell, 7561, South Africa). Table 1 shows the exported quantities of honeybush from 1999 to 2005. Through research and much trial and error, high quality products have been developed for both the local and export markets. Both these markets are showing continued growth and it is expected that sales will even grow stronger in the future.

Table 1 Honeybush tea exports from year 1999 to 2005 (SAHTA, 2007)

Period (Year)	Export (tons)
1999	50
2000	100
2001	60
2002	156
2003	163
2004	100
2005	300

According to Wesgro (2002), the market was found to be worth R3-million in 2002 and total production being 200 tons. Potential for expansion is substantial. Exports account for 80% of production and are primarily to health-conscious markets in Europe and the US. Studies show that expansion of the industry can be justified by growing demand. Large scale planting is, therefore, a necessity to ease the pressure on honeybush currently harvested by small farmers in its natural habitat (about 70% of total production) (Wesgro, 2002). The industry has shown a 20% annual growth around the beginning of the century and has the potential to emulate the rooibos tea industry of 4500 tons for the local consumption and 6500 tons for export within the next 20 years, following the trends as a health, caffeine-free, low-tannin herbal tea. Challenges include identifying and verifying chemical components that are linked to health aspects and finding alternative uses. Opportunities exist for medicinal and cosmetic value adding.

2.6 Propagation of honeybush plants

2.6.1 Propagation by stem cuttings

Propagation by cuttings is one of the most important means for clonal regeneration of many horticultural crops, ornamental shrubs, and deciduous species as well as broad and narrow-leaved types of evergreens. This method of propagation is reported to be an extensively practiced and economical method of vegetative propagation (Hartmann *et. al.*, 1997). Unlike other vegetative propagation techniques such as grafting, budding and micro-propagation, the cutting technique is relatively easy, inexpensive, and quick.

In propagating by stem cuttings, individual plants with superior performance in growth characteristics such as volume and form, field resistance to pests, diseases, or frost can be selected for these traits (Radke, 2005). Cutting technique is needed to develop new clones and bring new genetic material into breeding programs as well as for multiplying limited amounts of selected material. It avoids the graftage problems associated with rootstocks and poor graft union formation. Greater uniformity is obtained due to the absence of variation which sometimes appears as a result of variable seedling rootstocks grafted on plants. The parent plant is usually reproduced exactly with no alterations in the genetic material (Hartmann *et al*., 1997).

It is generally accepted that auxins play a central role in the process of root formation (Davis, Haissig & Sankhla, 1989). They induce root initials and influence the growth of the newly formed roots in the expressive phase of root development (Bellamine *et al*., 1998). Plants produce indole acetic acid (IAA) in the shoot apices and in young leaves, but to ensure successful rooting in difficult-to-root plant species, it is important to supply exogenous auxin. However, there is no direct evidence that synthetic auxins might replace natural production in the cells, but can contribute significantly to the plant's auxin pool and promote adventitious root formation (Spethmann & Hamzah, 1988). Investigations have shown that IBA (indole-3-acetic acid) has a greater ability to promote adventitious root formation (Spethmann & Hamzah, 1988) along with other artificial auxins such as NAA (naphthale acetic acid). IBA and NAA are used in combination in certain circumstances, but IBA is regarded as the best auxin for general use because it is nontoxic to plants over a wide concentration range, and is effective in promoting rooting of a large number of plant species (Hartmann *et al*., 1997).

2.6.2 Seed propagation of fynbos species

Fynbos is the dominant vegetation type in the Cape floristic region. It is exceptionally recognised for its richness in species and contributes most of the species to the flora of the region (Bond & Goldblatt, 1984). Fynbos species are adapted to recurrent fire cycles and characteristically experience intense recruitment after fire with little or no recruitment between fires. Recruitment into the post-fire environment can be achieved in several ways, including germination in response to cues associated with fire. Fire-stimulated germination of seed has been reported for a wide variety of fynbos species (Le Maitre & Midgely, 1992). A number of factors have been proposed as being responsible for the effects of fire on germination. These include dry heat fracturing of the seed coat in hard-seeded species (Gill, 1975; Jeffrey, Holmes & Rebelo, 1988), dry heat stimulating the embryo directly (Blommaert, 1972; Van der Venter & Esterhuizen, 1988; Musil & De Witt, 1991), high temperature desiccation of the seed coat (Brits and Brown, 1991), stimulation of germination by ethylene and ammonia contained in smoke (Van der Venter & Esterhuizen, 1988), and less specifically stimulation of germination by unknown chemical factors in plant-derived smoke extracts (De Lange &

Boucher, 1990). There can be complex, species specific interactions between smoke and other environmental factors. Factors such as seed age, light levels, temperature and hydration levels can also influence the extent of smoke-induced germination. Although the mechanisms are not well understood, it is clear that smoke's ability in enabling seeds to germinate is long lasting. Seed treated with smoke retain an enhanced ability to geminate even after one year of storage (Brown, Prosch & Botha, 1998).

Based on the ease with which their seed germinate, *Cyclopia* species can be divided into two different groups. But both groups exhibit a coat-imposed dormancy; seed of seeders like *C. subternata* germinate readily after scarification, while seed of resprouters such as *C. intermedia* exhibit additional embryo dormancy (Sutcliffe & Whitehead, 1995). Studies on germination *of C. intermedia* and *C. subternata* showed that seed germination was partially dependant on ethylene. The stimulating effect of smoke and ethylene was inhibited after exposure to 2,5-norbornadiene (NBD), indicating that ethylene in the smoke was responsible for the stimulation of germination. The involvement of ethylene in germination was demonstrated by treatment with aminooxyacetic acid (AOA), which then inhibited germination. The presence of AOA could be explained by its volatilization due to heat produced during the burning of the dried seedpods which contained relatively large quantities of the acid. When vegetation is burnt, both ethylene and short-chain fatty acids are released in the smoke. The presence of these compounds in smoke could stimulate seed germination in *Cyclopia* species and many other species (Whitehead & Sutcliffe, 1994).

2.7 Nutrient requirements of honeybush

2.7.1 Plant growth responses to different nutrients

Plant nutrition and growth are interdependent. Growth and development from germination through to senescence alter the nutrient requirements of a plant. On the other hand, the nutrient status of a plant alters the rate of development, the extent of growth and even specific morphological features

(Epstein & Bloom, 2005). A plant nutrient is a chemical element that is essential for plant growth and reproduction. Essential element is a term often used to identify a plant nutrient and in turn, the term nutrient implies essentiality. It is, therefore, redundant to call these elements, essential elements (Barker & Pilbeam, 2007). Based on the criteria used to classify plant nutrients, 17 elements are considered to have met the criteria for designation as plant nutrients. Elements that might enhance growth or have a function in some plants but not in all plants are referred to as beneficial elements (Barker & Pilbeam, 2007). For example, silicon (Si), cobalt (Co) and sodium (Na) are notable beneficial elements. Among the essential elements, nitrogen (N), phosphorus (P), potassium (K), Calcium (Ca), magnesium (Mg) and sulfur (S) are macro-nutrients, while iron (Fe), manganese (Mn), copper (Cu), boron (B), zinc (Zn), molybdenum (Mo), chlorine (Cl) and nickel (Ni) are classified as essential micro-nutrients (Reed cited by Barker & Pilbeam, 2007). Macro-elements are required in considerable quantities, generally accumulating to 0.1% and upward of the dry mass in plant tissues, and micro-elements generally accumulate to amounts less than 0.01% of the dry mass of plant tissues. These values can vary considerably depending on plant species, plant age and concentration of other mineral elements.

The main functions of mineral nutrients such as N, S, and P serve as constituents of proteins and nucleic acids. Other mineral nutrients, such as Mg, and the micro-nutrients (except Cl), may function as constituents of organic structures, predominantly of enzyme molecules, where they are either directly or indirectly involved in the catalytic function of the enzymes (Marschner, 1986). Potassium and presumably Cl, are the only mineral elements that are not constituents of organic structures. They function mainly in osmoregulation, the maintenance of electrochemical equilibria in cells and their compartments and the regulation of enzyme activities.

Recent studies on nutrition of honeybush indicated that the crop is likely to show positive growth responses to liming, phosphorus and treatment with molybdenum during *Rhizobial* inoculation (Joubert, Kotze & Woolridge, 2007). Consistent responses on nitrogen, magnesium and manganese were not observed. Another study by Joubert *et al*. (2007) confirmed the view that in low P soils, the growth of honeybush may be enhanced by the addition of phosphorus and indicated that *Cyclopia* species differ in their phosphorus requirements. At low phosphorus concentrations, and also in phosphorus supplemented *Cyclopia* plantations, mortality rates may be reduced by mulching with an organic material such as sawdust.

2.7.2 Nitrogen and biological nitrogen fixation in legumes

Depending on the plant species, development stage, and organ, the nitrogen (N) content required for optimal growth varies between 2 and 5% of the plant dry weight. When the supply is suboptimal, growth is retarded, N is mobilized in mature leaves and retranslocated to areas of new growth (Marschner, 1986). Typical N deficiency symptoms such as chlorosis and an etiolated habit resulting into retarded and slow growth are well known (Marschner, 1986; Epstein & Bloom, 2005). Nitrogen-deficient foliage is a pale colour of light green or yellow. An increase of the N supply, on the other hand, not only delays senescence and stimulates growth but also changes plant morphology in a typical manner, particularly if the N availability is high in the rooting medium during the early growth. Shoot elongation is enhanced and root elongation inhibited, a shift which is unfavourable for nutrient acquisition and water uptake in later stages (Epstein & Bloom, 2005).

The atmosphere contains about 80% of N gas (N_2), but this N cannot be used by higher plants until it is chemically combined with hydrogen (H), oxygen (O), or carbon (C). The process of combining N with another element is known as N fixation (Thompson & Troeh, 1978). Nitrogen fixation in nature is accomplished by certain micro-organisms and by lightning, but the amounts fixed are usually small. Nodulated legumes, such as soybean, faba bean, clover and alfalfa, in symbiosis with *Rhizobium* bacteria are among the most prominent N-fixing systems in agriculture (Marschner, 1986). These bacteria form nodules on the legume and initiate N fixation. The amount of N fixed by *Rhizobium* varies with the carbohydrate supply in the plant and the available N supply in the soil. The bacteria need the carbohydrates for energy to fix N.

But they will not fix much N when it is readily available in the soil even if the carbohydrate supply is high. It is important that a suitable strain *of Rhizobium* is added for the particular legume (Thompson & Troeh, 1978). A species that is suitable to nodulate alfalfa roots will not serve for a soybean crop, or vice versa. The *Rhizobium* bacteria can be applied to the seed in a simple process known as inoculation. Nodules that are no longer fixing N usually turn green and may be discarded by the plant. Pink or red nodules predominate on a legume in the middle of the growing season. If white, grey or green nodules predominate, little N fixation is occurring as a result of insufficient *Rhizobium* strain, poor plant nutrition or other plant stresses (Lindemann & Glover, 2003).

The Western Cape of South Africa is a distinctive phytogeographical unit known as the Cape Floristic Region. Much of this region is vegetated by a Mediterranean heathland called fynbos. The region is characterized by sandy, acidic, and low nutrient soils, with total N levels typically less than 0.1% (Cowling, Holmes & Rebelo, 1992). As a result, in the Western Cape, farming has largely depended on the use of chemical fertilizers, which may be costly to low-capital farmers and are environmentally unsuitable (Spriggs & Dakora, 2007). The solution to low soil productivity often lies in the use of N-fixing legumes. Honeybush tea is a symbiotic legume and a shrubby perennial endemic to the fynbos (Arnold & de Wet, 1994).

To domesticate and commercialise *Cyclopia* as a tea legume would require the selection of high performing genotypes of both legume and bacterial partners. Applying sufficient quantities of the selected rhizobial inoculant is also important for increasing N-fixation in legumes (Peterson & Loynachan, 1981, Brockwell, Bottomley & Thies, 1995). Glasshouse studies by Spriggs & Dakora (2007) on the nodulation competitiveness of three locally isolated *Cyclopia Rhizobia* (UCT40a, UCT44b and UCT61a) and the recommended strain for *Cyclopia* (PPRICI3) revealed some differences in competitive abilities for nodule formation. It was concluded that *Cyclopia* isolates were as competitive as the recommended strain PPRICI3 for nodule formation in *Cyclopia*. Furthermore, inoculating *Cyclopia* seedlings with the test strains in the nursery boosted their competitiveness in crown nodulation but not in number of nodules formed in the distal areas of the rootstock, where they were outcompeted by native soil *rhizobia* (Spriggs & Dakora, 2007).

Due to poor competitive ability of inoculants under field conditions, inoculation had no effect on *Cyclopia* yield, nodule number and nodule fresh mass. Many studies have revealed low competitiveness of inoculant strains for nodule formation in field experiments and this is attributed to the uneven distribution of introduced strains in the soil profile (Postma, Hok-a-Hin & Oude Voshaar, 1990; Brockwell *et al*., 1995; Patrick & Lowther, 1995). Poor occupancy of distal nodules formed in the field after transplanting from the nursery suggests that the test inoculants were unable to move out of the plug to compete with the endogenous soil R*hizobia* (Spriggs & Dakora, 2007).

2.8 Conclusions

The increase in the international demand for honeybush tea, concern over exploitation of wild populations and the lack of published agronomic information necessitated this study to evaluate different aspects of honeybush (*Cyclopia* spp.) propagation. Previous studies have focused more on the chemical and medicinal properties of this crop, leaving a wide gap with regard to the production practices including propagation. Amongst others, Joubert *et al*. (2007) reported on honeybush response to phosphorus fertilization and mulching, which indicates the importance of the production practices of the crop. Joubert *et al*. (2007) also surveyed the effect of liming and mineral nutrition on growth of honeybush (*Cyclopia* spp) plants. These recent studies indicate that the research on cultivation practices of this crop is still in its infancy.

2.9 REFERENCES

ARNOLD, T. & DE WET, B.C., 1994. Plants of Southern Africa. Memoirs of the Botanical Society of South Africa 62. National Botanical Institute, Pretoria, South Africa.

BARKER, A.V. & PILBEAM, D.J., 2007. Introduction. *In*: A.V. Barker & D.J Pilbeam (eds). Handbook of plant nutrition, pp 3-19. Taylor & Francis, London.

BEECHER, C.W.W., FARNSWORTH, N.R. & GYLLENHALL, C., 1989. Pharmacologically active secondary metabolites from wood. *In*: J.W. Rowe (ed.). Natural Products of Woody Plants II, pp 1059-1164. Springer-Verlag, Berlin.

BELLAMINE, J., PENEL, C., GREPPIN, H. & GASPAR, T., 1998. Confirmation of the role of auxin and calcium in the late phase of adventitious root formation. *Plant Growth Regul*. 26, 191-194.

BLOMMAERT, K.L.J., 1972. Buchu seed germination. *J. S. Afr. Bot*. 38, 237-239.

BOND, P. & GOLDBLATT, P., 1984. Plants of the Cape Flora. A descriptive Catalogue, pp. 285-286. CPT Book Printers, Cape Town.

BRITS, G. & BROWN, N.A.C., 1991. Control of seed dormancy in *Leucospermum*. Proceedings, Sixth conference of the International Protea Association. Perth, Western Australia, 323-333.

BROCKWELL, J., BOTTOMLEY, P.J. & THIES, J.E., 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant and Soil* 174, 143-180.

BROWN, N.A.C., PROSCH, D.S. & BOTHA, P.A., 1998. Plant-derived smoke: an effective pretreatment for seeds of *Syncarpha* and *Rhodocoma* and potential for many other fynbos species*. S. Afr. J. Bot*. 64, 90-92.

COWLING, R.M., HOLMES, P.M. & REBELO, A.G., 1992. Plant diversity and endemism. *In*: R.M. Cowling (ed.). The Ecology of Fynbos: Nutrients, Fire and Diversity, Oxford University Press, UK.

DAVIS, T.D., HAISSIG, B.E. & SANKHLA, N., 1989. Adventitious root formation in cuttings. Advances in Plant Sciences Series, Vol. 2. Dioscorides Press, Portland, Oregon, USA.

DE LANGE, J.H. & BOUCHER, C., 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). 1. Plant-derived smoke as a seed germination cue. *S. Afr. J. Bot*. 56, 700-703.

DE NYSSCHEN, A.M., VAN WYK, B.E., VAN HEERDEN., F. & SCHUTTE, A.L., 1996. The major phenolic compounds in the leaves of *Cyclopia* species (Honeybush tea*). Biochemical Syst. Ecol.* 24, 234-246.

DU TOIT, J., JOUBERT, E, & BRITS, T.J., 1998. Honeybush tea - a rediscovered indigenous South African herbal tea. *J. Sust. Agric*. 12, 67-84.

EPSTEIN, E. & BLOOM, A., 2005. Mineral Nutrition of plants: Principles and Perspectives, 2nd Edition. Sinauer Associates, Inc., Sunderland.

GILL, A.M., 1975. Fire and Australian flora: a review. *Australian Forestry* 38, 4-25.

HARTMANN, H.T., KESTER, D.E., DAVIES JR., F.T. & GENEVE, R.L., 1997. Plant Propagation: Principles and practices, 6th Edition. Prentice Hall, London.

JEFFREY, D.J., HOLMES, P.M. & REBELO, A.G., 1988. Effects of dry heat on seed germination in selected indegenous and alien legume species in South Africa. *S. Afr. J. Bot*. 54, 28-34.

JOUBERT, E. & FERREIRA, D., 1996. Antioxidants of rooibos tea – a possible explanation for its health promoting properties? *S. Afri. J. Food Sci. Nutr.* 8, 79-83.

JOUBERT, M.E., BOTMA, P.S., KOTZÉ, W.A.G., & WOOLDRIDGE, J., 2007. Honeybush (*Cyclopia* spp.) response to phosphorus fertilisation and mulching. *S. Afr. J. Plant Soil* 24(3), 176-177.

JOUBERT, M.E., KOTZÉ, W.A.G. & WOOLRIDGE, J., 2007. Effect of liming and mineral nutrition on growth of honeybush (*Cyclopia* spp.) plants. *S. Afr. J. Plant Soil* 24(3), 161-165.

KAMARA, B.I., 1997. Structure and synthesis of phenolic metabolites of honeybush tea (*Cyclopia intermedia*). M. Sc. Thesis, University of the Free State, Bloemfontein, South Africa.

KIES, P., 1951. Revision of the genus *Cyclopia* and notes on some other sources of bush tea. *Bothalia* 6, 161-176.

LE MAITRE, D.C. & MIDGLEY, J.J., 1992. Plant reproductive ecology, Pp. 135-174. *In*: R.M. Cowling (ed.). The ecology of fynbos. Oxford Univ. Press, Cape Town.

LEVYNS, M.R., 1920. A guide to the flora of the Cape Peninsula, p. 147. Juta & Co (Ltd), Cape Town.

LINDEMANN, W.C. & GLOVER, C.R., 2003. Nitrogen fixation by legumes, Guide – 129. New Mexico State University.

MARLOTH, R., 1925. The flora of South Africa with synoptical tables of the genera of the higher plants, pp. 69-72. Darter Bros & Co, Cape Town.

MARSCHNER, H., 1986. Mineral nutrition of higher plants. Academic Press, London.

MUSIL, C.F. & DE WITT, D.M., 1991. Heat-stimulated germination in two Restionaceae species. *S. Afr. J. Bot*. 57, 175-176.

NAKAYAMA, T., YAMADA, M., OSAWA, S. & KAWAKISHI, S., 1993. Suppression of active oxygen-induced cytoxicity by flavonoids. *Biochem. Pharmacol.* 45, 265-267.

NARAYAN, C.R., JOSHI, D.D., MUJUMDAR, A.M. & DHEKNE, V.V., 1987. Pinitol – a new anti-diabetic from the leaves of *Bougainvillea spectabilis*. *Curr. Sci*. 56, 139-141.

PATRICK, H.N. & LOWTHER, W.L., 1995. Influene of the number of rhizobia on the nodulation of and establishment of *Trifolium ambiguum*. *Soil Biol. & Biochem*. 27, 717-720.

PETERSON, H.L. & LOYNACHAN, T.E., 1981. The significance and application of *Rhizobium* nitrogen fixation by perennial legumes. *International Review of Cytology* 13, 311-331.

PIETTA, P., SIMONETTI, P. & MAURI, P., 1998. Anti-oxidant activity of selected medicinal plants, *J. Agric. Food Chem.* 46, 4487-4490.

POSTMA, J., HOK-A-HIN., C.H. & OUDE VOSHAAR, J.H., 1990. Influence of inoculum density on the growth and the survival of *Rhizobium leguminosarum* biovar *trifolii* introduced into sterile and non-sterile loamy sand and silt loam. *FEMS Microbiology Ecology* 73, 49-58.

RADKE, P., 2005. The role of clonal propagation in forestry and agriculture in Australia. *Combined proceedings: International Plant Propagators' Society* 55, 96-99.

SAHTA, 2007. South African Honeybush Tea Association, Notes compiled for Honeybush Farmers' Day, March 2007, Kanetberg, Riversdale. P.O. Box 663, Riversdale 6670, South Africa.

SAWA, T., NAKAO, M., AKAIKE, T., ONO, K. & MAEDA, H., 1999. Alkylperoxyl radical-scavenging activity of various flavonoids and other polyphenolic compounds: implications for the anti-tumor-promoter effect of vegetables, *J. Agric. Food Chem.* 47, 397- 402.

SCHUTTE, A.L., 1995. A taxanomic study of the tribes Podalyrieae and Liparieae (Fabaceae). Ph.D. Dissertation, Rand Afrikaans University, Johannesburg, South Africa.

SPETHMANN, W. & HAMZAH, A., 1988. Growth hormone induced root system types in cuttings of some broad leaved tree species. *Acta Hort*. 226, 601-605.

SPRIGGS, A.C. & DAKORA, F.D., 2007. Competitive ability of selected *Cyclopia* Vent rhizobia under glasshouse and field conditions. *Soil Biol. & Biochem*. 39, 58-67.

STANDER, R. & MORGENTHAL, J.C., 1995. Medisinale plante met verwysing na *C. intermedia*. Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch, South Africa.

SUTCLIFFE, M.A. & WHITEHEAD, C.S., 1995. Role of ethylene and short-saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. *J. Plant Physiol*. 145, 271-276.

TERBLANCE, S.E., 1982. Report on honeybush tea. Department of Biochemistry, University of Port Elizabeth, Port Elizabeth, South Africa.

THOMPSON, L.M. & TROEH, F.R., 1978. Soils and soil fertility. 4th Edition, McGraw-Hill, New York.

VAN DER VENTER, H.A. & ESTERHUIZEN, A.D., 1988. The effects of factors associated with fire on seed germination of *Erica sessiflora* and *E. hebecalyx* (Ericaceae). *S. Afr. J. Bot.* 54, 301-304.

VILJOEN, B., 1994. Honeybush tea for extra income. *Farmer's Weekly, March* 4, 24-25.

WATT, J. M. & BREYER-BRANDWIJK, M. G., 1962. The medicinal and poisonous plants of Southern and Eastern Africa: being an account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal, 2^{nd} ed. E. & S. Livingstone LTD. Edinburgh.

WELGEMOED, Z., 1993. Heuningtee straks kommersieel verbou. Landbouweekblad 792, 52-55.

WESGRO, 2002. Agriculture: Natural Products. P.O. Box 1678, Cape Town 8000, South Africa.

WESGRO, 2005. Agriculture: Natural Products. P.O. Box 1678, Cape Town 8000, South Africa.

WHITEHEAD, C.S. & SUTCLIFFE, M.A., 1994. Effect of low temperatures and different growth regulators on seed germination in *Cyclopia* spp. *Plant Physiol*. 147, 107-112.

CHAPTER 3

EFFECT OF SPECIES, CUTTING POSITION AND EXOGENOUS ROOTING HORMONE ON ROOTING OF HONEYBUSH (*CYCLOPIA* SPP.) CUTTINGS

Abstract

The effect of species (*Cyclopia intermedia* and *C. genistoides*), cutting position (terminal or sub-terminal) and rooting hormone was studied for three seasons (summer, winter and spring). Terminal and sub-terminal cuttings of *C. intermedia* and *C. genistoides* treated with different rooting hormones were rooted under glasshouse conditions where night and day temperature of the glasshouse was controlled. Intermittent mist was used as means of moisture supply to the cuttings for 45-60 seconds every 30 minutes, daily. *C. genistoides* rooted significantly better compared to *C. intermedia* as measured by rooting percentage, number of roots per cutting, length of longest root and mean root length during the summer season. Cutting position had a significant effect on rooting of the cuttings in summer compared to winter and spring seasons. Cuttings taken in summer rooted better than cuttings taken either in winter or spring irrespective of the species. The interactive effect of species, treatment and cutting position resulted into 86% of rooting in summer from the terminal cuttings of *C. genistoides*, while only 4% was recorded as the highest rooting percentage in both winter and spring seasons. In terms of number of roots per cutting, 2 and 4 g L^{-1} IBA resulted into the highest number of roots (3.28 and 3.64 respectively) from terminal cuttings of *C. genistoides* and these hormone concentrations were not significantly different from each other. Similarly, the greatest root length per cutting (29.8 and 29.96 mm respectively) was obtained from terminal cuttings of *C. genistoides* treated with 2 and 4 g L^{-1} IBA.

Key words: cutting, cutting position, *Cyclopia* species, honeybush, rooting

3.1 Introduction

Cyclopia is a very distinct genus of the Podalyrieae and is classified as a member of the Fabaceae family (Schutte, 1995). The stems and leaves of the *Cyclopia* species are used to manufacture a sweetish herbal infusion known as honeybush tea (Du Toit, Joubert & Brits, 1998). The species belong to the Cape fynbos biome and grow in the coastal districts of the Western and Eastern Cape Provinces of South Africa, from Darling to Port Elizabeth, being bounded in the north by the Cederberg, Koue Bokkeveld, Klein Swartberg, Groot Swartberg and Kouga mountain ranges. More than 20 species of *Cyclopia* have been described and the species are found to be fairly localized (Kies, 1951; Bond & Goldblatt, 1984; Schutte, 1995).

The bushes are normally found on the shady and cooler southern slopes of the mountain ranges and are about 1.5 m in height, but can reach up to 3 m (Bond & Goldblatt, 1984). The plants have woody stems with a relatively low leaf-stem ratio. The bushes can be easily recognized in the field as they are covered with distinctive, deep-yellow flowers with a characteristic sweet honey scent during the flowering period. Flowering period differs with species; some species flower during late autumn or early winter (May and June) while others flower in spring.

Vegetative propagation of plants by cuttings is mainly used to reproduce plants identical in genotype to a single source plant. There are a number of reasons for propagating plants vegetatively, e.g. uniformity of populations, fixing or maintaining superior genotypes, shortening time to flower, etc (Hartmann *et al*., 1997). Uniformity of individual plants is a major advantage in commercial production. Uniformity of plant size, growth rate, time of flowering, time of harvesting, and other phenotypic characteristics make economic production of many valued crops possible (Westwood, 1994). Variations in the quality of honeybush produced at present have prompted a need to find alternative means of propagation other than by seed. Vegetative propagation by cuttings can therefore be used as a tool in improving the quality of commercially produced species of honeybush (*Cyclopia* spp.).

Vegetative propagation procedures basically involve taking cuttings from the mother-plant and treating them with a hormone such as indole butyric acid (IBA) to stimulate root formation (Hammond & Polhamus, 1965). Terminal cuttings are commonly used in the propagation of *Leucadendron* spp. (Malan, 1992). According to Rodriguez-Perez *et al*. (2001), terminal cuttings are usually recommended for the propagation of *Leucospermum* spp. although some commercial nurseries also use basal cuttings. In proteas, the use of basal cuttings combined with some hormones has improved rooting in *Protea obtusifolia* (Rodriguez-Perez, 1990), in *Leucadendron* 'Safari Sunset' propagated in spring when rooting is more difficult, in *Leucandendron discolor* (Rodriguez-Perez & De Neon Hernandez, 1997) and in *Leucospermum* 'Sunrise' (Rodriguez-Perez *et al*., 1999). Season is considered to be one of the major factors that affect rooting success of cuttings (Klein, Cohen & Hebbe, 2000). Its effect on rooting efficiency is very common in woody plants and there is optimal time for root establishment for each species (Howard, 1996). Al-Barazi & Schwabe (1982) found that rooting of pistachio cuttings from mature trees was unsuccessful without considering season (Curir *et al*., 1993). Similarly, Puri & Vermat (1996) revealed that *Dalbergia sissoo* could be rooted in spring and monsoon seasons, while winter cuttings did not root at all. Hartmann *et al*. (1990) and Wilson (1993) also reported that simple rooting of softwood cuttings could only be achieved when taken during spring and summer than in winter. In contrast, Henry, Blazich & Hinesey (1992) revealed that root number and rooting percentage of *Eastern recedar* was higher throughout winter. On the other hand, rooting of Mytaceae family (*Chamaelaucium* sp.) is unaffected by season.

To date, the effect of species and cutting position on rooting of *Cyclopia* spp. cuttings is not known in literature. An experiment was conducted to evaluate the response of two *Cyclopia* species (*C. intermedia* and *C. genistoides*) cuttings to specific concentrations of indole butyric acid (IBA), a combination of IBA and naphthalene acetic acid (NAA) solutions, and IBA in powder form in three different seasons (summer, winter and spring).

3.2 Materials and methods

The aim of the experiment was to determine the effect of species and cutting position on rooting of honeybush (*Cyclopia* spp.) cuttings treated with different hormones and repeated for three different seasons. The first experiment was conducted during the summer season of 2006, while the second and third similar experiments were carried out in winter and spring of 2007, respectively. Two *Cyclopia* species with two cutting positions (terminal and sub-terminal) were examined using different rooting hormones. Mother stock plants of the two honeybush tea species (*Cyclopia intermedia* and *C. genistoides*) were collected randomly from a seven year old honeybush plantation by means of pruning shears from the experimental farm of the ARC Infruitec-Nietvoorbij Research Station situated in Stellenbosch, Western Cape, South Africa on December 13, 2006, 16 May and 27 September, 2007 for summer, winter and spring seasons respectively. These stock plants were collected during the early hours of the day (07H00 – 08H00) while they were still turgid and were immediately put into black polyethylene plastic bags to prevent moisture loss. Within one hour after cutting, the plant material was put into a coldroom (4-6°C) to prevent moisture loss and to decrease biochemical activity.

The cuttings were treated with Seradix® 2 powder (active ingredients - 4-indole-3-butyric acid at 3 g kg^{-1}), Dip & Root™ liquid rooting stimulator (active ingredients – a mixture of 4-indole-3-butyric acid at 10 g L^{-1} and 1-Naphthy-Acetic Acid at 5 g L^{-1}), 2 g L^{-1} IBA and 4 g L^{-1} IBA solutions (Mr. P. Breugem, 2006, PBR TRADING INT, P.O. Box 414, Pringlebaai, 7196, South Africa) and a control which did not contain the hormone. However, the cuttings were immersed in distilled water for ten seconds prior to sticking as a control treatment. The diameter of the cuttings varied between 3 and 4 mm and the length was ±8 cm. The bottom leaves of the cuttings were stripped and the basal 1 cm dipped in the IBA solutions for ten seconds based on the treatment. For the Seradix® 2 powder (4-indole-3-butyric acid) treatment, the base of the cuttings were dipped in a powder and the excess powder was removed by slightly tapping the cuttings prior to sticking. Immediately after the application of treatments, the cuttings were planted in polystyrene trays filled with moist growing medium of river sand and fine pine bark (1:1) as a substrate. After planting the trays were placed under controlled environment in a glasshouse. Misting was scheduled to irrigate for 30 seconds every 30 minutes from 07H00 to 18H00. The glasshouse was set at a 20/25°C night/day temperature and no bottom heating was applied. To evaluate the effect of the cutting position (terminal and sub-terminal) on rooting; the cuttings prepared from the terminal sections of the shoot were regarded as terminal and any other cutting taken below terminal section was considered a sub-terminal cutting.

The experimental design was a split plot with hormone as main plot treatment and species and cutting type as split plot factors. The main plot design was a randomized complete block with 5 treatments (Dip and Root™, 2 g L^{-1}, 4 g L^{-1} IBA, Seradix® and control) replicated at random in 5 blocks. The treatment design of the split plot factors was a 2x2 factorial with two species (*C. genistoides* and *C. intermedia*) and two cutting positions (terminal and sub-terminal), randomly allocated within each main plot treatment. Each experimental unit consisted of 10 cuttings. Number of cuttings rooted, percentage rooting, number of roots and root lengths were assessed after 62 days of growth. To do this, plants were carefully removed from the polystyrene trays before the growth media were washed from the roots.

Analysis of variance was performed, using GLM (General Linear Model) Procedure of SAS statistical software version 9.1 (SAS, 2000). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests. Data for different growing seasons were analyzed separately.

3.3 Results and discussion

Summer cuttings

Analysis of variance

Generally, the statistical analyses of the rooting experiments conducted in summer, winter and spring showed that the coefficient of variation (*CV*) was very high (>40%) (Tables 3, 6 and 8 respectively). These high *CV*s made it difficult to show statistically significant differences between treatment means and is most probably the result of large genetic variation in the source of the mother stock plants that were used in this study as they originated from seeds. Another possible factor that might have contributed to the large variation is the random sampling of the mother stock plants in the field due to the limited availability of the plant material. These mother plants were not necessarily grown under the same conditions as they were growing in an open field where differences due to the variation in soil physical and chemical properties might have caused differences with regard to their physiological properties.

Table 1 showed that rooting percentage, number of roots and length of longest root per cutting, and mean root length were not significantly affected by rooting hormone treatments. Rooting percentage, number of roots and length of longest root per cutting and mean root length were highly significantly affected by species. Cutting position had a significant effect on rooting percentage, number of roots and length of longest root per cutting, but the mean root length was not significantly affected by the cutting position. Significant interactions between species and cutting position occured for rooting percentage and number of roots per cutting, but not for length of longest root per cutting and mean root length. The interactive effects of treatment and species, treatment and position, and treatment, species and position were not significant on rooting percentage, number of roots, length of longest root per cutting or mean root length.

Table 1 Significant levels of the main factors on rooting of *Cyclopia* spp. cuttings during summer season

	Pr>F			
Source	Rooting Percentage	Number of roots	Longest root length	Mean root length
Hormone treatment (T)	ns	ns	ns	ns
Species (S)	<.0001	<.0001	<.0001	<.0001
Cutting position (P)	<.0001	<.0001	0.0360	ns
SXP	0.001	0.0016	ns	ns
TXS	ns	ns	ns	ns
TXP	ns	ns	ns	ns
TXSXP	ns	ns	ns	ns
CV (%)	45.04	68.11	64.23	64.34

Hormone treatments

No significant differences were found between the four hormone treatments in terms of rooting percentage, number of roots, length of longest roots and the mean root length. This is an indication that the cuttings have the potential to root with or without synthetic auxins during the summer season. According to George (1984), some species will produce roots with no auxin treatment, although better results were achieved with 3 500 ppm IBA for most *Banksia* spp. Akoumianaki-Ioannidou, Kravari & Chronopoulos (2000) reported that rooting of untreated cuttings of *Polygala myrtifolia* was highest (98.3±1.7%) in warm periods (summer) and lowest in (47.7±11.7%) the cold season (winter), while autumn resulted into 76.6% rooting.

Species

Although *C. genistoides* produced on average more roots, had a higher rooting percentage as well as longer roots than *C. intermedia*, these differences between species were affected by the cutting position. On average, a rooting percentage of 57.80% was recorded for *C. genistoides* compared to 28.60% for *C. intermedia* (Table 2). Brits (1986) found that cultivar differences in *Leucospermum* were also important as 75 and 30% rooting were obtained for ‘Caroline’ and ‘Hybrid T 75 11 24’, respectively. Sedgely (1995) reported that genotype influenced rooting, with variation from 0 to 80% success for different individuals of *Banksia hookeriana* and *B. prionotes*. This is a clear indication that differences exist in terms of rooting within different cultivars of the same species. In this study, it was also observed that the cuttings of *C. intermedia* had fewer but thick roots in comparison with many but thin roots in *C. genistoides*. On average, the root length of *C. genistoides* was 13.73 mm compared to 5.63 mm in *C. intermedia* (Table 2).

Table 2 Effect of species on rooting of honeybush (*Cyclopia* spp.) cuttings during summer season

Species	Rooting percentage (%)	Per cutting Number of roots	Length of longest root (mm)	Mean root length (mm)
C. genistoides	57.80a	2.01a	21.51a	13.73a
C. intermedia	28.60b	0.71b	7.59b	5.63b

Means with same letter are significantly different (LSD=0.05)

Cutting position

In terms of rooting percentage, number of roots per cutting, length of longest root per cutting and mean root length, significant differences were obtained between terminal and sub-terminal cuttings. These differences between the two cutting positions were, with exception of mean root length, affected by the species. On average, terminal cuttings had higher rooting percentage (53.80%) compared to sub-terminal cuttings (32.60 %) (Table 3). Terminal cuttings produced on average 1.94 roots with a longest root length of 16.56 mm compared to 0.79 roots with a longest root of 12.55 mm for sub-terminal cuttings. Similarly, the mean root length of terminal cuttings was 10.22 mm compare to 9.14 mm of the sub-terminal cuttings. Terminal cuttings are usually recommended for propagation of most cuttings, although some commercial nurseries also use sub-terminal cuttings with good results (Brits, 1986; Harré, 1988; Malan, 1992). Rodriguez-Perez & De Leon-Hernandez (1997) found that terminal cuttings of *Leucadendron discolor* rooted better than basal cuttings. They further concluded that the use of wounded terminal cuttings treated with 4000 ppm of IBA is recommended for the propagation of *L. discolor* by stem cuttings. Brits (1986) compared terminal and sub-terminal cuttings of *Leucospermum* and found that recently matured terminal cuttings taken in autumn rooted best.

Table 3 Effect of cutting position on rooting of honeybush (*Cyclopia*) *spp*. cuttings during summer season

		Per cutting		
Cutting position	Rooting percentage (%)	Number of roots	Length of longest root (mm)	Mean root length (mm)
Terminal (T)	53.80a	1.94a	16.56a	10.22a
Sub-terminal (ST)	32.60b	0.79b	12.55b	9.14b

Means with the same letter are not significantly different (LSD = 0.05)

Interactive effects

Significant species x cutting position interaction was found with regard to rooting percentage and number of roots (Table 1). No significant differences were found between the two cutting positions of *C. intermedia* in terms of rooting percentage and number of roots per cutting, but terminal cuttings of *C. genistoides* showed a higher rooting percentage, more roots per cutting as well as longer root lengths than sub-terminal cuttings of the same species (Table 4). Terminal cuttings of *C. genistoides* with a total rooting of 74.80%, 2.89 roots per cutting and longest root length of 25.32 mm were also better than terminal and sub-terminal cuttings of *C. intermedia.* Although sub-terminal cuttings of *C. genistoides* also showed a higher rooting percentage, more roots per cutting and on average longer roots compared to sub-terminal cuttings of *C. intermedia*, these results clearly showed that terminal cuttings of *C. genistoides* were the best to use under summer conditions.

Table 4 Interactive effect of species and cutting position on rooting of honeybush (*Cyclopia* spp.) cuttings during summer season

			Per cutting		
Species	Cutting position	Rooting percentage (%)	Number of roots	Length of longest root (mm)	Mean root length (mm)
C. genistoides	T	74.80a	2.89a	25.32	15.33
C. genistoides	ST	40.80b	1.13b	17.70	12.12
C. intermedia	T	32.80bc	0.98b	7.80	6.15
C. intermedia	ST	24.40c	0.44c	7.39	5.10

Means with the same letter are not significantly different (LSD = 0.05)

T = terminal cutting position; ST = sub-terminal cutting position

Although the interaction amongst hormone treatment, species and cutting position did not have a significant effect on rooting percentage, number of roots, length of the longest root per cutting and mean root length produced per cutting (Table 1), responses are summarized in Table 5. Terminal cuttings of *C. genistoides,* treated with 2 g L^{-1} and 4 g L^{-1} IBA tended to have the highest rooting percentage, produce the most roots per cutting, had the longest roots as well as greatest mean root length. Compared to the control (74% rooting), terminal cuttings of *C. genistoides* treated with 2 g L^{-1} and 4 g L^{-1} IBA resulted into 86 and 82% of rooting, respectively. Swamy, Puri & Singh (2002) reported that auxin treatment significantly enhanced the number of roots, root length, leaf number and leaf area in *Robinia pseudoacacia* cuttings, while Harré (1988) suggested an optimal level of 2000 ppm IBA for *Leucadendron.* Malan (1995) gave a general recommendation of 4000 ppm IBA (4 g L^{-1}) for proteas. In the case of sub-terminal cuttings of *C. genistoides,* the control treatment tended to give the best results.

Terminal cuttings from *C. intermedia* showed the best results when treated with Seradix®, but a rooting percentage of only 46%, 1.42 roots per cutting, a root length of 13.84 mm and a mean root length per cutting of only 8.15 mm were achieved. For the control treatment with terminal cuttings of this species a rooting percentage of 38%, 1.16 roots per cutting, a longest root of 9.76 mm and a mean root length per cutting of 7.14 mm were obtained. In terms of sub-terminal cuttings of this species, the highest rooting percentage (26%) was recorded from the control treatment as was also found with sub-terminal cuttings of *C. genistoides*.

Table 5 The interactive effect of hormone treatment, species and cutting position on rooting of honeybush (*Cyclopia* spp.) cuttings during summer season

Species	Cutting position	Treatment	Rooting percentage (%)	Per cutting: Number of roots	Per cutting: Length of longest root (mm)	Per cutting: Mean root length (mm)
C. genistoides	T	Control	74.00	2.28	23.38	14.57
C. genistoides	T	IBA 2 g L^{-1}	86.00	3.28	29.8	18.19
C. genistoides	T	IBA 4 g L^{-1}	82.00	3.64	29.96	17.42
C. genistoides	T	Seradix®	70.00	2.58	18.36	11.87
C. genistoides	T	Dip & Root™	62.00	2.68	25.10	14.61
C. genistoides	ST	Control	58.00	1.78	22.26	14.84
C. genistoides	ST	IBA 2 g L^{-1}	38.00	1.10	14.00	8.59
C. genistoides	ST	IBA 4 g L^{-1}	26.00	0.62	11.34	8.96
C. genistoides	ST	Seradix®	32.00	0.86	15.86	10.72
C. genistoides	ST	Dip & Root™	50.00	1.28	25.06	17.51
C. intermedia	T	Control	38.00	1.16	9.76	7.14
C. intermedia	T	IBA 2 g L^{-1}	32.00	1.06	6.80	4.32
C. intermedia	T	IBA 4 g L^{-1}	22.00	0.64	3.52	1.92

T = terminal cutting position; ST = sub-terminal cutting position

(Table 5 continued)

Species	Cutting position	Treatment	Rooting percentage (%)	Per cutting: Number of roots	Length of longest root (mm)	Mean root length (mm)
C. intermedia	T	Seradix®	46.00	1.42	13.84	8.15
C. intermedia	T	Dip & Root™	26.00	0.64	5.06	3.99
C. intermedia	ST	Control	26.00	0.56	10.84	8.46
C. intermedia	ST	IBA 2 g L^{-1}	20.00	0.34	5.08	4.55
C. intermedia	ST	IBA 4 g L^{-1}	24.00	0.32	6.62	5.98
C. intermedia	ST	Seradix®	26.00	0.50	9.26	7.05
C. intermedia	ST	Dip & Root™	26.00	0.50	5.10	4.70

T = terminal cutting position; ST = sub-terminal cutting position

Winter cuttings

Analysis of variance

The results from Table 6 indicate that the hormone treatments had no significant effect on rooting percentage, number of roots, length of longest root and mean root length. Similarly, species as a factor had no significant effect on the rooting parameters of the two *Cyclopia* spp. cuttings. Cutting position had no significant effect on rooting percentage, length of longest root, number of roots and the mean root length. The interaction between species and position is the only factor that had a significant effect on the above mentioned rooting parameters. No significant interactive effects were found between the species and treatment, treatment and cutting position, and species, treatment and cutting position.

Table 6 Significant levels of the main factors on rooting of *Cyclopia* spp. cuttings during winter season

	Pr>F			
Source	Rooting Percentage	Number of roots	Length of longest root	Mean root length
Treatment (T)	ns	ns	ns	ns
Species (S)	ns	ns	ns	ns
Cutting position (P)	ns	ns	ns	ns
SXP	0.0217	0.0411	0.0217	0.0241
TXS	ns	ns	ns	ns
TXP	ns	ns	ns	ns
TXSXP	ns	ns	ns	ns
CV (%)	424.26	479.16	424.288	432.19

Hormone treatments

No significant differences (Table 6) were found between the hormone treatments in terms of rooting percentage, number of roots, length of longest root per cutting and mean root length. The response of cuttings to the treatments was very poor compared to the results obtained during the summer season in terms of rooting percentage. A mean rooting percentage of 0.5% (Table 7) was obtained from winter season compared to 43.2% (Table 5) for summer cuttings. This clearly shows that the application of auxins failed to trigger and enhance rooting to the cuttings taken during this period of the year. Leaves on the cuttings began to die immediately after planting, terminal leaves of about half the cuttings died 5-10 days after planting. By the end of the experiment, the majority of the cuttings were dead especially cuttings of *C. intermedia*. In terms of percentage mortality, about 77% and 73.6% of the cuttings died from *C. intermedia* and *C*. *genistoides,* respectively. However, no roots or very few roots were found in the cuttings that remained green throughout the experiment.

Species

The analysis of variance (Table 6) indicated that species as a main factor had no significant effect on rooting percentage, number of roots per cutting, length of longest root and mean root length, but all responses are summarized in Table 7. On average only 0.6% rooting was achieved with *C. genistoides*, compared to 0.4% with *C. intermedia.* These figures are very low compared to the rooting percentage obtained during the summer season for both species and clearly illustrated the effect of season on rooting of these species. Season is considered to be one of the major factors that affect rooting success of cuttings according to Klein, Cohen & Hebbe (2000). Similarly, Puri & Vermat (1996) showed that *Dalbergia sissoo* could be rooted in spring and monsoon seasons, but not during the winter. Hartmann *et al*. (1990) and Wilson (1993) also reported that rooting of softwood cuttings could only be achieved when taken during spring and summer.

Cutting position

Due to the very low percentage rooting, no significant differences were found between terminal and sub-terminal cuttings with regard to the rooting percentage, number of roots, length of roots and mean root length (Table 6). On average only 0.6 of terminal and 0.4% of sub-terminal cuttings rooted. Significant interactions between species and cutting position, however, suggest that cuttings from the different species responded differently.

Interactive effects

Although very inconclusive because of very low rooting percentages, the results showed on average a 1.2% rooting with terminal cuttings of *C. genistoides* compared to no rooting of sub-terminal cuttings of this species. With *C. intermedia*, no rooting was achieved with terminal cuttings, while sub-terminal cuttings showed a 0.8% rooting on average. From Table 7 it is, however, clear that only terminal cuttings of *C. genistoides* treated with Seradix®, 4 g L^{-1} IBA or Dip and Root™ rooted (2% rooting for all treatments). Terminal cuttings from *C. intermedia* did not root at all, while sub-terminal cuttings rooted only (6%) where the control treatment was applied.

These results clearly confirmed previous studies (Bassuk & Howard, 1981; Harrison-Murray, 1991) which showed that seasonal timing, or the time of the year in which cuttings are taken can play an important role in rooting, but are in contrast to results of Davies, Jr. (1984) who reported that although seasonal changes influenced rooting of both juvenile and mature *Ficus pumila* cuttings, treating juvenile (easy-to-root) cuttings with IBA can override the seasonal effects.

Table 7 Interactive effects of species, treatment and cutting position on rooting of honeybush (*Cyclopia* spp.) cuttings during winter season

Species	Cutting position	Treatment	Rooting percentage (%)	Per cutting: Number of roots	Per cutting: Length of longest root (mm)	Per cutting: Mean root length (mm)
C. genistoides	T	Control	0.00	0.00	0.00	0.00
C. genistoides	T	IBA 2 g L^{-1}	0.00	0.00	0.00	0.00
C. genistoides	T	IBA 4 g L^{-1}	2.00	0.80	0.40	0.19
C. genistoides	T	Seradix®	2.00	0.08	0.48	0.23
C. genistoides	T	Dip & Root™	2.00	0.06	0.52	0.29
C. genistoides	ST	Control	0.00	0.00	0.00	0.00
C. genistoides	ST	IBA 2 g L^{-1}	0.00	0.00	0.00	0.00
C. genistoides	ST	IBA 4 g L^{-1}	0.00	0.00	0.00	0.00
C. genistoides	ST	Seradix®	0.00	0.00	0.00	0.00
C. genistoides	ST	Dip & Root™	0.00	0.00	0.00	0.00
C. intermedia	T	Control	0.00	0.00	0.00	0.00
C. intermedia	T	IBA 2 g L-1	0.00	0.00	0.00	0.00
C. intermedia	T	IBA 4 g L-1	0.00	0.00	0.00	0.00

T = terminal cutting position; ST = sub-terminal cutting position

(Table 7 continued)

Species	Cutting position	Treatment	Rooting percentage (%)	Per cutting: Number of roots	Per cutting: Length of longest root (mm)	Per cutting: Mean root length (mm)
C. intermedia	T	Seradix®	0.00	0.00	0.00	0.00
C. intermedia	T	Dip & Root™	0.00	0.00	0.00	0.00
C. intermedia	ST	Control	4.00	0.60	1.08	0.83
C. intermedia	ST	IBA 2 g L^{-1}	0.00	0.00	0.00	0.00
C. intermedia	ST	IBA 4 g L^{-1}	0.00	0.00	0.00	0.00
C. intermedia	ST	Seradix®	0.00	0.00	0.00	0.00
C. intermedia	ST	Dip & Root™	0.00	0.00	0.00	0.00

T = terminal cutting position; ST = sub-terminal cutting position

Spring cuttings

Analysis of variance

Table 8 indicates that the studied rooting parameters of *Cyclopia* spp. were not significantly affected by the hormone treatment in spring season. The effect of species, however, was significant for rooting percentage, length of longest root and mean root length, but not significant for number of roots per cutting. Cutting position had no significant influence on rooting of *Cyclopia* spp. cuttings during spring. Similarly, the interactive effects of species and cutting position, hormone treatment and species, hormone treatment and cutting position, as well as hormone treatment, species, and cutting position did not significantly influence rooting percentage, number of roots per cutting, length of longest root and mean root length.

Table 8 Significant levels of the main factors on rooting of *Cyclopia* spp. cuttings during spring season

	Pr>F			
Source	Rooting Percentage	Number of roots	Length of longest root	Mean root length
Treatment (T)	ns	ns	ns	ns
Species (S)	0.0162	ns	0.0345	0.0258
Cutting position (P)	ns	ns	ns	ns
SXP	ns	ns	ns	ns
TXS	ns	ns	ns	ns
TXP	ns	ns	ns	ns
TXSXP	ns	ns	ns	ns
CV (%)	314.27	370.91	450.58	425.41

Hormone treatment

As also shown with summer and winter cuttings, rooting of *Cyclopia* spp. cuttings in spring did not respond significantly to the different rooting hormone treatments with regard to rooting percentage, number of roots, length of longest root and mean root length. As also found with winter cuttings, all treatments resulted in very poor rooting.

Species

In contrast to the summer and winter cuttings, significant differences in rooting percentage, length of longest root and mean root length due to species as a main factor were found for the cuttings taken in spring (Table 9). On average, 1.60% of *C. genistoides* cuttings rooted with a mean root length of 0.29 mm and the longest root length of 0.47 mm compared to 0.20% rooting of *C. intermedia* cuttings with a longest root of 0.01 mm and a mean root length of less than 0.01 mm. No significant differences were, however, found between the two *Cyclopia* spp. regarding number of roots produced per cutting.

Table 9 Effect of species on rooting of *Cyclopia* spp. cuttings during spring season

Species	Rooting percentage (%)	Per cutting: Number of roots	Length of longest root (mm)	Mean root length (mm)
C. genistoides	1.60a	0.04a	0.47a	0.29a
C. intermedia	0.20b	0.01a	0.01b	0.00b

Means followed by the same letters are not significantly different (LSD=0.05%)

Cutting position

In contrast to summer cuttings, but as also found with winter cuttings, cutting position as main factor did not have a significant effect on rooting of spring cuttings. As a result, no significant differences were found between the two cutting positions (terminal and sub-terminal) with regard to rooting percentage, number of roots per cutting, length of longest root and mean root length.

Interactive effects

In contrast to summer and winter cuttings, no significant interaction between species and cutting position was recorded for spring cuttings (Table 8). Rooting responses due to hormone treatments, species and cutting position are however summarized in Table 10. Although not significant higher than other treatments, terminal cuttings of *C. genistoides* again tended to result in the highest percentage rooting and number of roots per cutting. Treatments with either IBA at 4 g L^{-1} or Dip & Root™ resulted in a 4.0% rooting percentage (Table 10). Both the control and the 2 g L^{-1} IBA treatments, however, resulted in a 2.0% rooting percentage. Similar rooting percentages were obtained with sub-terminal cuttings of *C. genistoides* treated with either 2 or 4 g L^{-1} IBA. Terminal cuttings of *C. intermedia* also resulted in a 2.0% rooting, but all terminal and sub-terminal cuttings of *C. intermedia* failed to root.

These results suggest that spring was not a good season for the collection of cutting material. Mother stock plants were in the flowering phase during the collection of the cutting material. According to Johnson (1970), flowering is a complex phenomenon and can serve as a competing sink to the detriment of rooting. O'Rourke (1940) found that hardwood cuttings of herbaceous dahlia cuttings bearing flower buds did not root as well as cuttings with only vegetative buds. Cuttings with flower buds are found to be more difficult to root than cuttings having only vegetative buds (Biran & Halevy, 1973).

Seasonal timing or the period of the year at which the cuttings are taken can, therefore, play an important role in rooting as also shown by Anand & Heberlein (1975), Bassuk & Howard (1981) and Harrison-Murray (1991). Nicola *et al.* (2005) suggested that the seasonal response affected rooting of medicinal and aromatic cuttings may be due to the changes of environmental factors such as temperature and light which had a direct effect on the physiology of the stock plants and rooting capacity of the cuttings themselves.

Table 10 Interactive effect of species, cutting position and treatment on rooting of honeybush (*Cyclopia* spp.) cuttings during spring season

				Per cutting		
Species	Cutting position	Treatment	Rooting percentage (%)	Number of roots	Length of longest root (mm)	Mean root length (mm)
C. genistoides	T	Control	2.00	0.02	0.20	0.20
C. genistoides	T	IBA 2 g L^{-1}	2.00	0.08	1.40	0.77
C. genistoides	T	IBA 4 g L^{-1}	4.00	0.10	0.58	0.39
C. genistoides	T	Seradix®	0.00	0.00	0.00	0.00
C. genistoides	T	Dip & Root™	4.00	0.14	1.64	0.90
C. genistoides	ST	Control	0.00	0.00	0.00	0.00
C. genistoides	ST	IBA 2 g L^{-1}	2.00	0.02	0.04	0.04
C. genistoides	ST	IBA 4 g L^{-1}	2.00	0.08	0.84	0.60
C. genistoides	ST	Seradix®	0.00	0.00	0.00	0.00
C. genistoides	ST	Dip & Root™	0.00	0.00	0.00	0.00
C. intermedia	T	Control	0.00	0.00	0.00	0.00
C. intermedia	T	IBA 2 g L^{-1}	2.00	0.08	0.06	0.04
C. intermedia	T	IBA 4 g L^{-1}	0.00	0.00	0.00	0.00

T = terminal cutting position; ST = sub-terminal cutting position

(Table 10 continued)

Species	Cutting position	Treatment	Rooting percentage (%)	Per cutting: Number of roots	Length of longest root (mm)	Mean root length (mm)
C. intermedia	T	Seradix®	0.00	0.00	0.00	0.00
C. intermedia	T	Dip & Root™	0.00	0.00	0.00	0.00
C. intermedia	ST	Control	0.00	0.00	0.00	0.00
C. intermedia	ST	IBA 2 g L^{-1}	0.00	0.00	0.00	0.00
C. intermedia	ST	IBA 4 g L^{-1}	0.00	0.00	0.00	0.00
C. intermedia	ST	Seradix®	0.00	0.00	0.00	0.00
C. intermedia	ST	Dip & Root™	0.00	0.00	0.00	0.00

T = terminal cutting position; ST = sub-terminal cutting position

3.4 Conclusions

Results indicated that stem cuttings of *C. genistoides* and *C. intermedia* taken in summer can be rooted more easily than during winter or spring. Because of the very poor rooting with winter and spring cuttings these seasons are not recommended for the making of *Clyclopia* cuttings. Although the use of exogenous auxins did not have a significant effect in any season, 2 and 4 g L^{-1} IBA tended to improve rooting and the highest rooting percentage (86%) was obtained from terminal cuttings of *C. genistoides* when treated with 2 g L^{-1} IBA during the summer season.

Terminal cuttings of *C. genistoides* rooted better than sub-terminal cuttings of the same species as well as both terminal and sub-terminal cuttings of *C. intermedia* during summer. No significant differences due to cutting position were found with *C. intermedia.* Although the same tendency was shown for winter and spring cuttings, less than 5.0% of all cuttings rooted irrespective of the cutting position, species or hormone treatment.

3.5 REFERENCES

AKOUMIANAKI-IOANNIDOU, A., KRAVARI, E. & CHRONOPOULOS, J., 2000. Propagation of *Polygala myrtifolia* by cuttings. *Acta Hort*. 541, 265-268.

AL-BARAZI, A. & SCHWABE, W.W., 1982. Rooting softwood cuttings of adult *Pistachio vera*. *J. Hort. Sci.* 57(2), 247-252.

ANAND, V.K. & HEBERLEIN, G.T., 1975. Seasonal changes in the effects of auxin on rooting in stem cuttings of *Ficus infectoria*. *Physiol. Plant* 34, 330-334.

BASSUK, N.L. & HOWARD, B.H., 1981. Seasonal changes in apple hardwood cuttings and their implications to nurserymen. *Comb. Proc. Intl. Plant Prop*. 30, 289-293.

BIRAN, I. & HALEVY, A.H., 1973. The relationship between rooting of dahlia cuttings and the presence and type of bud. *Physiol. Plant* 28, 244-247.

BOND, P. & GOLDBLATT, P., 1984. Plants of the Cape flora. A descriptive catalogue, pp. 285-286. CPT Book Printers, Cape Town.

BRITS, G.J., 1986. The influence of genotype, terminality and auxin formulation on the rooting of *Leucospermum* cuttings. *Acta Hort*. 185, 23-30.

CURIR, P., SULIS, S., MARIANI, F., VAN SMERE, C.F., MARCHESINI, A. & DOLCI, M., 1993. Influence of endogenous phenols on rooting ability of *Chamaelacium uncinatum* Schauer stem cuttings. *Sci. Hort*. 55, 303-314.

DAVIES, JR., F.T., 1984. Shoot RNA, cambial activity and indolebutyric acid effective in seasonal rooting of juvenile and mature *Ficus pumila* cuttings. *Physiol. Plant* 62, 571-575.

DU TOIT, J., JOUBERT, E, & BRITZ, T.J., 1998. Honeybush tea - a rediscovered indigenous South African herbal tea. *J. Sust. Agric*. 12, 67-84.

GEORGE, A.S., 1984. The remarkable banksias. Wild flowers of great potential. *Austr. Hort.* 82(10), 16-21.

HAMMOND, B.L. & POLHAMUS, L.G., 1965. Research on guayule (*Parthenium argentatum*): 1942-1959. USDA Tech. Bul.1327.

HARRÉ, J., 1988. The propagation and production of Proteaceae. Feilding, New Zealand.

HARRISON-MURRAY, R.S., 1991. A leaf-model evaporimeter for estimating potential transpiration in propagation environments. *J. Hort. Sci.* 66, 131-139.

HARTMANN, H.T., KESTER, D.E., DAVIES, F.T., JR. & GENEVE, L.R., 1990. Plant propagation: Principles and practices, 5th Edition. Prentice Hall International Editions, New Jersey, USA.

HARTMANN, H.T., KESTER, D.E., DAVIES, F.T., JR. & GENEVE, L.R., 1997. Plant propagation: Principles and practices, 6th Edition. Prentice Hall International Editions, New Jersey, USA.

HENRY, P.H., BLAZICH, F.A., & HINESEY, L.E., 1992. Influence of stock plant fertility on adventitious rooting of stem cuttings. *J. Am. Soc. Hort. Sci*. 117, 568-570.

HOWARD, B.H., 1996. Relation between shoot growth and rooting of cuttings in three contrasting species of ornamental shrubs. *J. Hort. Sci*. 71, 591-605.

JOHNSON, C.R., 1970. The nature of flower bud influence on root regeneration in the Rhododendron shoot. Ph.D. Dissertation. Oreg. State Univ. Corvalis, Oreg., USA.

KIES, P., 1951. Revision of the genus *Cyclopia* and notes on some other sources of bush tea. *Bothalia* 6,161-176.

KLEIN, J.D., COHEN, S. & HEBBE, Y., 2000. Seasonal variation in rooting ability of myrtle (*Myrtus communis* L.) cuttings. *Sci. Hort*. 83, 71-76.

MALAN, D.G., 1992. Propagation of Proteaceae. *Acta Hort*. 316, 27-34.

MALAN, D.G., 1995. Crop Science of Proteaceae in Southern Africa: Progress and challenges. *Acta Hort*. 387, 55-62.

NICOLA, S. FONTANA, E., HOEBERECHTS, J. & SAGLIETTI, D., 2005. Rooting products and cutting timing on Sage (*Salvia officinalis* L.) propagation. *Acta Hort*. 676, 135-141.

O'ROURKE, F.L. 1940. The influence of blossom buds on rooting of hardwood cuttings of blueberry. *Proc. Amer. Soc. Hort. Sci*. 40, 332-334.

PURI, S. & VERMAT, R.C., 1996. Vegetative propagation of *Dalbergia sissoo* Roxb. using softwood and hardwood stem cuttings. *J. Arid Envir*. 34(2), 235-245.

RODRIGUEZ-PEREZ, J.A., 1990. A technique to improve the propagation of stem cuttings of *Protea obtusfolia* Buek ex Meisn. *Acta Hort*. 264, 41-43.

RODRIGUEZ- PEREZ, J.A. & DE LEON HERNANDEZ, A.M., 1997. Influence of cutting position, wounding and IBA on the rooting of *Leucadendron discolor* stem cuttings. *Acta Hort*. 453, 29-34.

RODRIGUEZ-PEREZ, J.A., VERA BATISTA, M.C., DE LEON-HERNANDEZ, A.M. & ARMAS AMRMAS, P.C., 1999. Influence of cutting position, wounding and IBA on the rooting of *Leucospermum* 'Sunrise' stem cuttings. *J. Int. Protea Assoc*. 37, 4-11.

RODRIGUEZ-PEREZ, J.A., VERA BATISTA, M.C., DE LEON-HERNANDEZ, A.M. & ARMAS ARMAS, P.C., 2001. Influence of cutting position, wounding and IBA on the rooting of *Leucospermum cordifolium* 'California Sunshine' cuttings. *Acta Hort*. 545, 171- 177.

SAS, 2000. SAS/STAT Users Guide, Version 9.1, First Edition, Volume 2. SAS Institute Inc., Cary, NC, USA.

SCHUTTE, A.L., 1995. A taxanomic study of the tribes Podalyrieae and Liparieae (Fabaceae). Ph.D. Dissertation, Rand Afrikaans University, Johannesburg, South Africa.

SEDGELY, M., 1995. Breeding biology of *Banksia* species for floriculture. *Acta Hort.* 397, 155-162.

SHAPIRO, S.S. & WILK, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611.

SWAMY, S.L., PURI, S. & SINGH, A.K., 2002. Effect of auxins (IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia optiva*. *New Forests* 23(2), 143-157.

WESTWOOD, M.N., 1994. Temperate zone pomology. physiology and culture, 3rd edn. Portland, Timber Press, USA.

WILSON, P.J., 1993. Propagation characteristics of *Eucalyptus globules* Labill. stem cuttings in relation to their original position in the parent shoot. *J. Hort. Sci.* 68(5), 715-724.

CHAPTER 4

EFFECT OF AN ORGANIC PLANT FERTILIZER AND CUTTING POSITION ON THE ESTABLISHMENT OF ROOTED CUTTINGS OF *CYCLOPIA* SPECIES

Abstract

An experiment was conducted to evaluate the effect of an organic plant fertilizer solution (Nitrosol®), cutting position, and species on the establishment and growth of rooted cuttings of *Cyclopia* (Fabaceae) species. Rooted terminal and subterminal cuttings of two *Cyclopia* species (*C. intermedia* and *C. genistoides*) were transplanted into pots (576 cm^3) and treated with Nitrosol® fertilizer at application rates of 3.33 $ml.L^{-1}$ and 1.67 $ml.L^{-1}$ and 0 $ml.L^{-1}$ (control). To enhance the formation of nodules the cuttings were inoculated with symbiotic *Rhizobium* bacteria. Nitrosol® at 3.33 $ml.L^{-1}$ significantly increased fresh and dry plant weight, fresh and dry root weight, number of shoots and nodules per plant compared to the 1.67 $ml.L^{-1}$ and control treatments. *C. genistoides* performed better in terms of fresh and dry plant weight, fresh and dry root weight, and number of shoots and nodules per plant compared to *C. intermedia,* but cutting position did not significantly affect the above mentioned parameters. Plant mineral analysis revealed that most of the macro elements increased with increasing Nitrosol® application rates. Comparing the two *Cyclopia* species, revealed that *C. genistoides* had higher levels of mineral elements than *C. intermedia*. This could be an indication of the differences between the two species in terms of nutrient uptake, utilization and distribution within the plant tissues. Nitrosol® application rates had variable effects on micro-elements with only Mn that showed clear increasing trends with increasing application rates.

Keywords: *Cyclopia* spp., Nitrosol®, nodules, *Rhizobium*, shoots

4.1 Introduction

Honeybush (*Cyclopia* spp.) is a shrub belonging to the tribe Podalyrieae and classified as a member of the Fabaceae family growing in the fynbos botanical biome (Schutte, 1995). The leaves and flowers of most of the *Cyclopia* species are used to manufacture a herbal infusion known as honeybush tea (Du Toit & Joubert, 1998). *Cyclopia* species have been used for many years in areas where they occur naturally. The earliest mention of *Cyclopia* in botanical literature dates back to 1705 (Kies, 1951). The major commercially exploited species are *C. intermedia* and *C. subternata*, both of which are being developed for commercial crop production (De Nysschen *et al*., 1996). *C*. *genistoides* has also been used but on a limited scale (Du Toit & Joubert, 1998).

Honeybush tea plants have woody stems, with a relatively low leaf-to-stem ratio (Welgemoed, 1993). Leaves are trifoliate (Levyns, 1920; Marloth, 1925), while the leaf shape varies with species, from pubescent, narrow-leafed (Bond & Goldblatt, 1984) to flattened types (Kies, 1951). *C. intermedia* is a shrub with leaves which are 18-28 mm long, and 2-5 mm wide, while *C. genistoides* has smaller leaves (14-20 mm long and 1-2 mm wide) (Schutte, 1995). During flowering period, the bushes are easily recognized in the field as they bear distinctive, deep-yellow flowers which have a characteristic sweet, honey scent. Flowering is usually in spring (September to October) in most species and late autumn or early winter (May and June), e.g. *C. sessiflora* is one of the species that flowers in May and June (Du Toit, Joubert & Britz, 1998). In terms of cultivation, *C. subternata* and *C. genistoides* are the main cultivated species. *C. genistoides* grows naturally in the coastal, sandy areas from the West Coast stretching up to Mossel Bay. Plantations have been established in the Overberg and Albertinia areas. There are indications that *C. intermedia* is difficult to cultivate for commercial purposes. This species can only be harvested every second or third year, making it uneconomical to cultivate as yet. Current information indicates that too frequent harvesting does not allow plants to build up sufficient energy reserves resulting in die-back (SAHTA, 2007).

Cyclopia species grow in the fynbos botanical biome (Schutte, 1995). Much of this region is vegetated by a Mediterranean heathland characterized by sandy, acidic and low nutrient soils, with total N levels typically less than 1% (Cowling, Holmes & Rebelo, 1992). This has led to high use of chemical fertilizers which are costly to low-capital farmers and environmentally unsustainable. Part of the solution to soil productivity often lies in the use of N-fixing legumes. Honeybush tea is a symbiotic legume and a shrubby perennial endemic to the fynbos (Arnold & De Wet, 1994). To domesticate and commercialise *Cyclopia* as a tea legume would require the selection of both legume and bacterial partners (Spriggs & Dakora, 2007). Application of sufficient quantities of selected *Rhizobia* inoculant is also important for increasing N_2-fixation in legumes (Peterson & Loynachan, 1981; Peoples, Ladha & Herridge, 1995). The initial selection of a suitable strain for inoculant is usually based on its ability to fix N under bacteriologically controlled conditions in the glasshouse (Svenning, Juntilla, & Solheim, 1991; Howieson *et al*., 2000; Fenning & Danso, 2002).

Following a successful rooting of plants propagated by cuttings, the establishment and growth of such cuttings under field conditions is not always guaranteed. A number of factors such as the species in question, soil nutrient status, temperature and moisture may determine the distinction between success and failure in the establishment of the rooted *Cyclopia* spp. cuttings. As a result, a study was conducted to examine the effect of an organic fertilizer solution (Nitrosol®) with *Rhizobium* on the growth and establishment of rooted *Cyclopia* cuttings.

4.2 Materials and methods

The aim of this experiment was to study the effect of Nitrosol®, a commercial organic plant fertilizer on the establishment of rooted honeybush (*Cyclopia* spp.) cuttings. The effect of species and the origin of the cutting position on growth and establishment were also evaluated. Cuttings of two *Cyclopia* species, namely *C. intermedia* and *C. genistoides* with two different cutting positions (terminal and sub-terminal) were rooted using various root promoting hormones in a glasshouse at Welgevallen Experimental Farm at the University of Stellenbosch, Western Cape, South Africa. After eight weeks, the cuttings were examined to evaluate number of roots formed, rooting percentage and the length of roots. The rooted cuttings were then carefully transferred to pots (576 cm^3) and allowed to acclimatize under similar environmental conditions. After another eight weeks the plantlets were then treated with a natural organic fertilizer solution at two different levels with a control (without fertilizer) as a third level in a glasshouse under natural photoperiod and light intensity conditions. Day and night temperatures were regulated to ±25 and ±15°C, respectively. The pots were placed on top of the wire mesh beds 1m above ground. A mixture of washed river sand and fine pine bark at a ratio 1:1 was used as a growing substrate. Nitrosol® treatments were applied from 10 April 2007 and then every second week for a period of ten weeks. The chemical composition of Nitrosol®, irrigation water and the growing medium is reflected in Tables 1, 2, and 3 respectively.

Treatment 1

Cyclopia species have the potential to fix atmospheric nitrogen in the presence of *Rhizobium* bacteria. Since most growth media do not contain these beneficial bacteria, it is necessary to inoculate the growth media when establishing seedlings or cuttings in nurseries. *Rhizobium* bacteria strain number CI3 from the South African *Rhizobium* Collection obtainable from the Plant Protection Research Institute of the Agricultural Research Council was applied as a suspension for infecting the roots. To prepare the suspension, 50 g of the inoculant was diluted in ten litres of water as recommended by the supplier (Miss J. Bloem, ARC-Plant Protection Research Institute, Private Bag X134, Pretoria, 0001). About 50 ml of the inoculant suspension was added directly to each pot of all treatments to ensure thorough infection of the roots by the bacteria and the excess leached out of the pot. In the case of Treatment 1, which served as the control (no Nitrosol® fertilizer was applied).

Treatment 2

Nitrosol® a commercially available natural organic plant nutrient liquid is readily absorbed by plants and recommended for the cultivation of all indoor, outdoor, foliage, flowering, alkaline and acid loving plants (Envirogreen (PTY) Limited). In the case of treatment 2, Nitrosol® solution was applied directly to the medium in the pots at a concentration of 3.33 $ml.L^{-1}$. About 40 ml of the solution was applied to each pot at the beginning of the experiment. Nitrosol® liquid contains both micro and macro elements (Table 1).

Table 1 Chemical composition of full strength Nitrosol® fertilizer solution

pH	EC	Na	K	Ca	Mg	Fe	Cl	HCO^3	SO_4	B	Mn	Cu	Zn	P	NH_4-N	NO_3-N
	$mS.cm^{-1}$								$mg.l^{-1}$							
5.8	1.33	14.2	230.4	21.7	2.4	0.90	8.7	91.7	147	1.10	0.70	0.6	0.47	95.99	72.20	52.80

Table 2 Mineral analysis of irrigation water used during the study period

Date	pH	EC	Na	K	Ca	Mg	Fe	Cl	HCO^3	SO_4	B	Mn	Cu	Zn	P	NH_4-N	NO_3-N
		$mS.cm^{-1}$							$mg.l^{-1}$								
Feb.	6.9	0.09	9	1.8	7.3	1.8	0.02	18.5	16.8	9	0	0	0	0	0.3	9.2	4.6
March	7	0.10	7.3	0	7.7	1.2	0.07	17.7	38.3	7	0	0.01	0	0	0.2	0	10.6

Table 3 Chemical composition of the growing substrate used in the experiment

pH	Resist.	Bray II		Exchangeable cations				Trace elements				
		P	K	Na	K	Ca	Mg	Cu	Zn	Mn	B	C
KCl	Ohm	mg.kg^{-1}		cmol.kg^{-1}				mg.kg^{-1}				
5.1	1610	5	31	0.07	0.08	1.98	1.4	0.3	0.7	2.2	0.2	1.8

Treatment 3

Instead of using Nitrosol® at 3.33 ml.L^{-1}, a half strength Nitrosol® solution was prepared. In the case of Treatment 3, Nitrosol® was, therefore, applied at a concentration of 1.67 ml.L^{-1} and 40 ml of the solution was applied per pot.

After a period of ten weeks, the plant height, number of new shoots per plant, number of nodules per plant, average fresh and dry plant weight, and fresh and dry root weight were determined. Following fresh plant and root weight determination, the plant material was dried in an oven at 80°C for 48 hours to determine dry plant and root weight. The dried plant material was then taken to Bemlab laboratory for mineral analysis.

Statistical design

For the experiment a 2x2x3 factorial design was used and the four replications of each treatment were allocated to four blocks. The factors tested were two *Cyclopia* species (*C. intermedia* and *C. genistoides*), two cutting positions (terminal and sub-terminal) and three fertilizer treatments (*Rhizobium* with Nitrosol® fertilizer at a concentration of 3.33 ml.L^{-1}, *Rhizobium* with Nitrosol® at a concentration of 1.67 ml.L^{-1} and only *Rhizobium* inoculation as the control). Each experimental unit consisted of five rooted plantlets.

Analysis of variance was performed using GLM (General Linear Model) Procedure of SAS statistical software version 9.1 (SAS, 2000). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests.

4.3 Results and discussion

Analysis of variance

Glasshouse studies on the establishment and growth of rooted *Cyclopia* spp. cuttings indicated that plant height, number of shoots formed per plant, fresh and dry plant weight, fresh and dry root weight, and number of nodules formed per plant were significantly affected by fertilizer treatment (Table 4). The different species also had a significant effect on the above mentioned factors, but cutting position did not have any significant effects on plant height, fresh and dry weight, and number of nodules formed per plant. Significant interactions between the species and cutting position were observed with regard to plant height, and number of shoots per plant. The interactive effect of fertilizer treatment and species was significant with regard to plant height, shoot number, number of nodules per plant, fresh and dry plant weight, and dry root weight, but not significant with regard to fresh root weight. No significant interactive effects were found between the fertilizer treatments and cutting position. Interactive effects between species, fertilizer treatment and cutting position were only significant with regard to number of shoots per plant.

Table 4 Significant levels of the main factors on growth and establishment parameters of *Cyclopia* species.

	Pr>F						
Source	Plant height	Shoot no.	Fresh plant weight	Dry plant weight	Fresh Root weight	Dry root weight	Nodule no.
Fertilizer (N)	<.0001	<.0001	<.0001	<.0001	<.0001	0.001	<.0001
Species (S)	<.0001	<.0001	<.0001	<.0001	0.0015	<.0001	<.0001
Cutting Position (P)	ns	ns	ns	ns	ns	ns	ns
S x P	0.0154	0.0483	ns	ns	ns	ns	ns
N x S	<.0001	0.0018	<.0001	0.0002	ns	0.0053	0.0052
N x P	ns	ns	ns	ns	ns	ns	ns
N x S x P	ns	0.0333	ns	ns	ns	ns	ns
CV (%)	12.15	29.89	20.82	32.25	36.96	35.38	61.65

Fertilizer treatment

The results (Table 4) showed that the fertilizer treatments had a highly significant effect on growth and establishment parameters of rooted *Cyclopia* spp. cuttings. Nitrosol® applied at a concentration of 3.33 ml.L^{-1} resulted into plants with greater fresh plant weight compared to half strength (1.67 ml.L^{-1}) solutions and the control treatment (no Nitrosol® applied) (Table 5). No significant differences were found between 3.33 and 1.67 ml.L^{-1} Nitrosol® treatments with regard to dry root weight and number of nodules produced per plant. However, the number of nodules formed by the control plants where no Nitrosol® was applied was significantly lower than nodules formed by plants with Nitrosol® fertilizer. Plant growth and development were, therefore, affected by the available nutrients in the growing medium. With legumes, nodulation and N_2-fixation is dependant on an adequate supply of both macro- and micro-nutrients (Munns, 1977; Smith, 1982). These nutrients are not only essential for the symbiotic interaction but also for the host plant and microbial partner. From Table 5, it is clear that plant height was affected by Nitrosol® treatment. Plantlets fertilized with a 3.33 $ml.L^{-1}$ concentration of Nitrosol® resulted into long stemmed plants while control plants (no Nitrosol®) were the shortest. Hanley & Fenner (1997) found that nutrient deprivation significantly affected the mean dry mass of *Anthyllis vulneraria* (L), *Hippocrepis unisiliquosa* (L), *Cistus cresticus* and *Pinus brutia* (Ten.) seedlings. Plants of all the four species deprived of all essential nutrients exhibited reduced growth rates in comparison with plants grown under full nutrient conditions. Rieckermann *et al*. (1999) also reported that differences in the growth of roots and shoots were observed as a result of weekly application of varying nitrogen (N) fertilization (0, 25, 50, 100 or 200 $mg.l^{-1}$ N). Spriggs & Dakora (2007) reported that *Cyclopia* plants grew well and produced high crop yields; possibly due to an adequate supply of soil N in Kanetberg which was one of their study sites.

Table 5 Effect of Nitrosol® fertilizer on growth and establishment of rooted cuttings of *Cyclopia* species

	$g.plant^{-1}$				mm	Per plant	
Nitrosol® ($ml.L^{-1}$)	Fresh weight	Dry weight	Fresh root weight	Dry root weight	Plant height	Number of nodules	Number of shoots
3.33	1.42a	0.35a	1.93a	0.27a	119.34a	4.19a	3.21a
1.67	1.00b	0.26b	1.49b	0.23a	91.91b	3.22a	2.20b
0	0.51c	0.12c	0.91c	0.16b	65.59c	0.81b	0.40c

Means followed by the same letter are not significantly different (LSD=0.05)

Species

The results indicated that *C. genistoides* performed significantly better compared to *C. intermedia* in all the studied parameters. About 1.2 and 0.31 g.plant^{-1} were obtained as average fresh and dry plant weight from *C. genistoides* respectively, while *C. intermedia* produced significantly lower (0.75 and 0.18 g.plant^{-1}) fresh and dry weights. Similarly, *C. genistoides* produced significantly greater fresh and dry root weights per plant compared to *C. intermedia*. After ten weeks, the average height per plant of *C. genistoides* was 114.7 mm compared to 69.86 mm of *C. intermedia*. This may be due to differences between the two species in terms of their growth behavior or the utilization of available growth resources. Berry (2006) stated that although plants of the same species respond similarly to nutrient stress, plants of similar species will often show significant differences in growth rate, root distribution, phase of development and efficiency of nutrient uptake and utilization. He concluded that in any given location, plants from one species may become nutrient deficient, while those from another species growing under the same environment may not show any deficiency owing to their differences in nutrient uptake and utilization. Relative to number of nodules per plant, *C. genistoides* formed on average 4.62 nodules per plant, which was significantly greater to 0.87 nodules per plant formed in *C. intermedia* (Table 6).

Table 6 Effect of species on growth and establishment of rooted cuttings of C*yclopia* species

	g.plant^{-1}				mm	Per plant	
Species	Fresh weight	Dry weight	Fresh root weight	Dry root weight	Plant height	Number of nodules	Number of shoots
C. genistoides	1.20a	0.31a	1.71a	0.30a	114.70a	4.62a	2.79a
C. intermedia	0.75b	0.18b	1.18b	0.14b	69.86b	0.87b	1.07b

Means followed by the same letter are not significantly different (LSD=0.05)

Cutting position

The cutting position had no significant effect on the studied growth and establishment parameters of rooted *Cyclopia* cuttings. Although results obtained in Chapter 3 showed that cutting position significantly affected rooting of these *Cyclopia* species, after the formation of roots in the cuttings, cutting position did not affect the establishment of rooted *Cyclopia* spp. plantlets.

Interactive effects

Significant species x cutting position interaction was found with regard to plant height and number of shoots per plant (Table 4). No significant differences were found between the two cutting positions of *C. genistoides* in terms of number of shoots per plant (Table 7). Similarly, no significant differences were shown in *C. intermedia* with regard to plant height. Plantlets originating from terminal cuttings of *C. genistoides* were significantly better than sub-terminal cuttings of the same species and better than both cutting positions of *C. intermedia* in terms of plant height. Species x cutting position interaction revealed that terminal cuttings of *C. intermedia* were significantly better than sub-terminal cuttings of the same species regarding the number of shoots per established plant (Table 7).

Table 7 Interactive effects of species and cutting position (T – terminal and ST – sub-terminal) on growth and establishment of rooted cuttings of *Cyclopia* species

		g.plant^{-1}				mm	Per plant	
Species	Cutting Position	Fresh plant weight	Dry plant weight	Fresh root weight	Dry root weight	Plant height	Number of nodules	Number of shoots
C. genistoides	T	1.26	0.31	1.86	0.31	122.00a	4.72	2.88 a
C. genistoides	ST	1.13	0.30	1.56	0.29	107.40b	4.52	2.70 a
C. intermedia	T	0.69	0.14	1.20	0.13	68.89c	0.73	0.83 c
C. intermedia	ST	0.82	0.21	1.15	0.15	70.83c	1.00	1.33 b

Means followed by the same letter are not significantly different (LSD=0.05)

Significant species x Nitrosol® interactions were observed in terms of plant height, fresh plant weight, dry plant weight, dry root weight, number of nodules and number of shoots per plant (Table 4). Table 8 clearly indicates that *C. genistoides* showed larger responses to increasing Nitrosol® application rates compared to *C. intermedia* in the case of dry root weight and the number of nodules per plant. The control treatments for these parameters did not differ significantly and *C. intermedia* did not show differences due to the rate of Nitrosol® applied, while *C. genistoides* showed significant increases in weight and nodule number with increased Nitrosol® application rates. Although *C. intermedia* showed significant increases in fresh plant weight, dry plant weight, plant height and number of shoots with increasing Nitrosol® rates, *C. genistoides* showed larger responses with increasing Nitrosol® rates. This resulted in significant larger weights and heights in the case of *C. genistoides* plantlets which were treated with Nitrosol® compared to *C. intermedia* plantlets.

Table 8 Interactive effect of species and Nitrosol® fertilizer on growth and establishment of rooted cuttings of *Cyclopia* species

		g.plant^{-1}				mm	Per plant	
Species	Nitrosol® (ml.L^{-1})	Fresh plant weight	Dry plant weight	Fresh root weight	Dry root weight	Plant height	Number of nodules	Number of shoots
C.genistoides	3.33	1.83a	0.47a	2.35	0.40a	149.30a	6.95a	4.23 a
C.genistoides	1.67	1.28b	0.34b	1.79	0.30b	121.15b	5.38a	3.35b
C.genistoides	0	0.48e	0.12d	0.98	0.19c	73.65d	1.53b	0.80d
C. intermedia	3.33	1.00c	0.23c	1.51	0.14c	89.38c	1.08b	2.19c
C. intermedia	1.67	0.71d	0.18cd	1.19	0.16c	62.68de	1.43b	1.04d
C. intermedia	0	0.54de	0.11d	0.83	0.13c	57.53e	0.10b	0.00e

Means followed by the same letter are not significantly different (LSD=0.05)

Although the interactions between Nitrosol® treatment, species and cutting position only had a significant effect on the number of new shoots per plant, all responses are summarized in Table 9. *C. genistoides* on average produced more shoots per plant compared to *C. intermedia* and responded significantly better than *C. intermedia* to Nitrosol® application irrespective of the cutting position.

Table 9 Interactive effect of species, cutting position (T- terminal, ST – sub-terminal) and Nitrosol® on growth and establishment of rooted cuttings of *Cyclopia* species

		Nitrosol®	g.plant^{-1}				mm	per plant	
Species	Cutting position	(ml.L^{-1})	Fresh plant weight	Dry plant weight	Fresh root weight	Dry root weight	Plant Height	Number of shoots	Number of nodules
C. genistoides	T	3.33	1.94	0.48	2.73	0.41	160.15	4.40a	5.3
C. genistoides	T	1.67	1.33	0.34	1.82	0.31	127.1	3.75ab	6.95
C. genistoides	T	0	0.53	0.13d	1.03	0.2	78.75	0.50gh	1.9
C. genistoides	ST	3.33	1.72	0.46	1.98	0.4	138.45	4.05a	5.45
C. genistoides	ST	1.67	1.24	0.34	1.82	0.28	115.2	2.95bc	6.95
C. genistoides	ST	0	0.44	0.1	0.94	0.19	68.55	1.10efg	1.15
C. intermedia	T	3.33	0.93	0.2	1.51	0.12	91.62	1.78de	0.15
C. intermedia	T	1.67	0.62	0.14	1.13	0.14	59.05	0.70fgh	1.75
C. intermedia	T	0	0.51	0.1	0.98	0.14	56	0.00h	0.15
C. intermedia	ST	3.33	1.08	0.26	1.51	0.16	87.15	2.60cd	1.85
C.intermedia	ST	1.67	0.81	0.22	1.25	0.17	66.3	1.39ef	1.1
C. intermedia	ST	0	0.58	0.15	0.69	0.12	59.05	0.00h	0.05

Means followed by the same letter are not significantly different (LSD=0.05)

Plant mineral analysis

Due to insufficient dry plant material, different replications had to be pooled before being analyzed for plant minerals. For this reason data could not be analyzed statistically and the discussion will only focus on trends with regard to mean values of the main factors (species, cutting position and fertilizer treatments). To make comparison easier, individual treatment means are showed as a percentage of the factor means (Tables 10 and 11).

Macro elements

<u>*Nitrogen (N)*</u>

From Table 10, plantlets of *C. genistoides* (1.74%) had a relatively high N content in the dry matter compared to *C. intermedia* (1.16%). The values of N content in terms of species also correlate with the number of nodules produced by each species, where *C. genistoides* formed significantly more nodules per plant than *C. intermedia* (Table 6). The amount of N fixed per plant could have been more on *C. genistoides* than *C. intermedia* due to the ability of this plant to fix N in association with the *Rhizobium*. Plantlets from sub-terminal cuttings of *C. intermedia* showed the highest N content (1.33%), compared to plantlets from terminal cuttings (1.00%). Plantlets originating from *C. genistoides* terminal cuttings (1.80%) however yielded higher N content compared to those from sub-terminal cuttings (1.69%) (Table 10).

Generally, plants grown in the pots fertilized with 3.33 $ml.L^{-1}$ Nitrosol® appeared visually more vigorous with a darker green colour compared to plantlets grown with either 1.67 $ml.L^{-1}$ Nitrosol® or no Nitrosol® (control). An increase in N content of plant dry matter with increasing Nitrosol® application rates is indicated (Figure 1). From Table 10 it is clear that this increase in N content with increased Nitrosol® application rates applies for all species x cutting position combinations with the exception of plantlets from terminal cuttings of *C. intermedia.* Silber, Ganmore-Neumann & Ben-Jaacov (1998) found that the N content in plant organs of *Leucadendron* ‘Safari Sunset’ also increased with increased levels of nutrient supply.

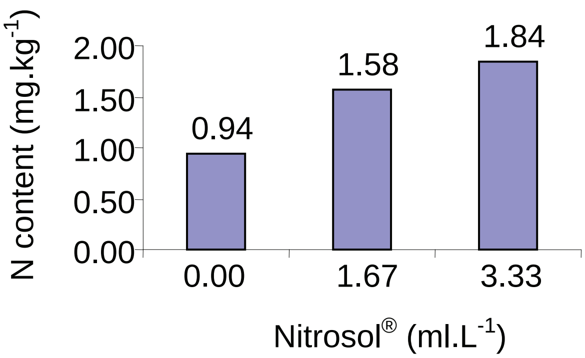

Figure 1 Effect of Nitrosol® fertilizer treatments on the nitrogen content of rooted cuttings of *Cyclopia* species

<u>Phosphorus (P)</u>

Table 10 indicates that 0.2% P was found in dry matter of plantlets from *C. genistoides* compared to 0.18% P measured in the dry matter of plantlets from *C. intermedia*. This could be an indication that *C. genistoides* had a better phosphate uptake than *C. intermedia*. The capability for active uptake of phosphate differs between plant species and even differs between cultivars of the same species. Barber & Thomas (1972) found considerable differences in the rate of uptake of phosphate by various maize cultivars and concluded that this uptake is fixed genetically. In terms of cutting position, mean values of 0.18 and 0.19 % P were obtained from plantlets grown from terminal and sub-terminal cuttings of *C. intermedia* respectively indicating no difference due to cutting positions in *C. intermedia*. In contrast to this, a P content of 0.21% was found in plant dry matter of plantlets grown from terminal cuttings compared 0.19% P in sub-terminal cuttings of *C. genistoides*.

The P content of plant dry matter increased with increasing Nitrosol® application rates (Figure 2) from 0.05% P for the control to 0.16 and 0.36% P with applications of 1.67 and 3.33 $ml.L^{-1}$ Nitrosol®, respectively. Johnston, Gikaara & Edwards (2006) found that P concentration in the shoots and roots of *Caustis blakei* increased with the rate of P application. Joubert *et al.* (2007) found that the mineral composition of different *Cyclopia* species shoots was affected by the amount of the applied P. Silber *et al*. (1998) also reported that P concentrations in the leaves and the roots increased as a result of increasing P levels in the irrigation water.Table 10 also shows that the increase in P content as a result of increased Nitrosol® application rates was true for all the species x cutting position x Nitrosol® fertilizer interactions.

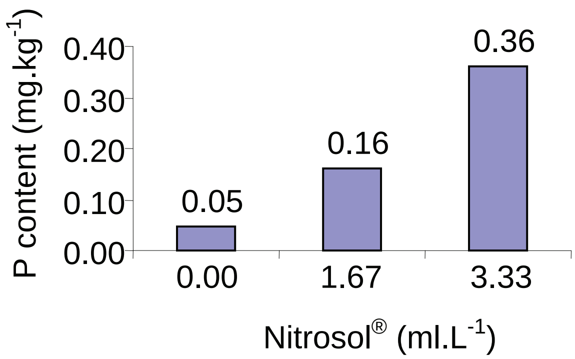

Figure 2 Effect of Nitrosol® fertilizer treatments on the phosphorus content of rooted cuttings of *Cyclopia* species

<u>Potassium (K)</u>

The results showed that *C. genistoides* (0.85% K) on average had a higher (46.6%) content of K in the dry matter compared to *C. intermedia* (0.58% K). On average, plantlets grown from sub-terminal cuttings of *C. intermedia* contained 0.62% K compared to 0.54% K in plantlets from terminal cuttings. In contrast, a K content of 0.89% on average was found in plantlets from terminal cuttings compare to 0.80% K from sub-terminal cuttings of *C. genistoides*.

On average, K content of plantlets grown from rooted cuttings of *Cyclopia* species increased from 0.34% for the control (no Nitrosol®) to 0.66% and 1.15%

with application rates of 1.67 and 3.33 $ml.L^{-1}$ Nitrosol®, respectively. Maier, Darlberg & Williams (1994) found that increasing rates of applied K significantly increased K concentrations in potato leaves. From Table 10 it is clear that an increase in K content with increased Nitrosol® application rates applied to all species x cutting position combinations.

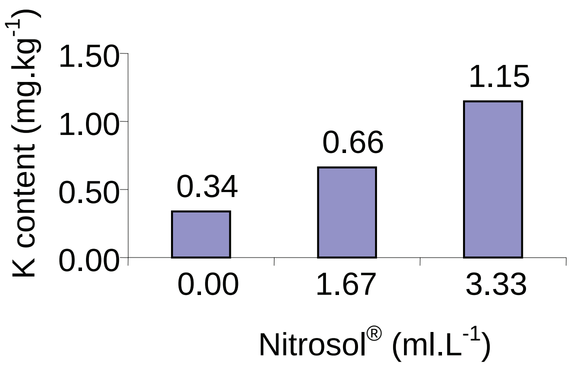

Figure 3 Effect of Nitrosol® fertilizer treatments on the potassium content of rooted cuttings of *Cyclopia* species

<u>*Calcium (Ca)*</u>

The results showed that dry matter of plantlets grown from rooted *C. genistoides* cuttings on average contained a 0.51% Ca content compared to 0.35% of *C. intermedia* (Table 10). Large differences in the uptake of macro-nutrients between different cultivars have been reported, therefore such results are not unexpected (Montarone, 2001).

On average the dry matter of plantlets grown from *C. intermedia* terminal cuttings contained 0.36% Ca compared to 0.33% of sub-terminal cuttings, while dry matter of plantlets grown from rooted *C. genistoides* sub-terminal cuttings, yielded 0.52% Ca compared to 0.50% Ca obtained from plantlets grown from rooted terminal cuttings. Plantlets grown without any added Nitrosol® (control) on average contained 0.44% Ca compared to 0.42% Ca obtained from plantlets fertilized with Nitrosol® at concentrations of 1.67 $ml.L^{-1}$ and 3.33 $ml.L^{-1}$ (Figure 4), but Table 10 clearly shows that Ca content in dry plant matter of individual

Cyclopia species tested did not correlate with the applied concentration rates of Nitrosol® fertilizer.

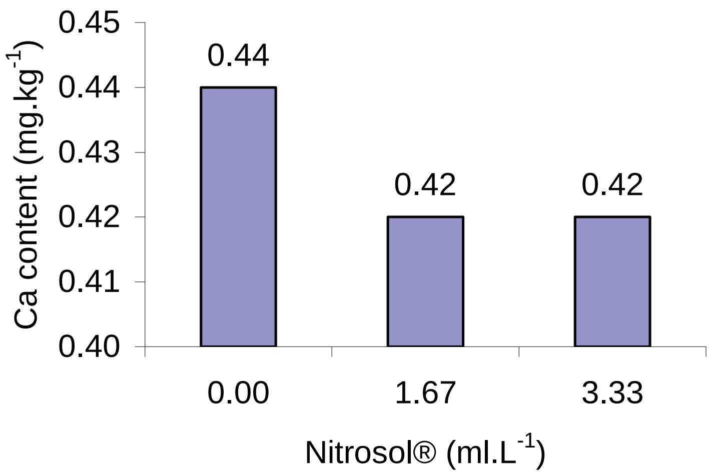

Figure 4 Effect of Nitrosol® fertilizer treatments on the calcium content of rooted cuttings of *Cyclopia* species

<u>*Magnesium (Mg)*</u>

On average the dry matter of *C*. *genistoides* plantlets contained 0.51% Mg compared to 0.14% Mg in dry matter of C*. intermedia* plantlets (Table 10). Plantlets grown from rooted *C. intermedia* terminal cuttings contained 0.15% Mg compared to 0.13% dry matter of plantlets from sub-terminal cuttings. In *C*. *genistoides*, however, Mg contents of 0.51% and 0.19% were measured in plantlets grown from sub-terminal and terminal cuttings, respectively.

Figure 5 illustrates the effect of Nitrosol® application rates on Mg content in dry matter grown from rooted cuttings with mean Mg contents of 0.17% , 0.16% and 0.42% for plantlets fertilized with 0 (control) , 1.67 and 3.33 ml.L^{-1} Nitrosol®, respectively. There was also no clear trend with regard to Nitrosol® treatment and K content for individual species or cutting positions.

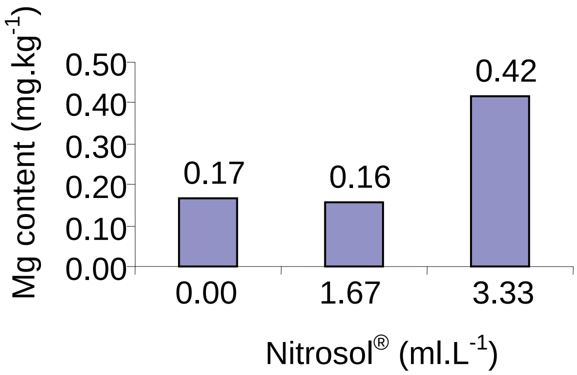

Figure 5 Effect of Nitrosol® fertilizer treatments on the magnesium content of rooted cuttings of *Cyclopia* species

Table 10 **M**ineral analysis values of essential macro-elements in dry plant matter as affected by Nitrosol®, species and position (terminal – T, sub-terminal – ST) in *Cyclopia* species

Species	Cutting Position	Nitrosol®	N		P		K		Ca		Mg	
		(ml.L^{-1})	(%)	(%mean)	(%)	(%mean)	(%)	(%mean)	(%)	(%mean)	(%)	(%mean)
C. genistoides	T	0.00	0.96	53.33	0.06	28.57	0.35	39.33	0.53	106.00	0.21	110.53
C. genistoides	T	1.67	1.98	110.00	0.21	100	0.94	105.62	0.47	94.00	0.18	94.74
C. genistoides	T	3.33	2.45	136.11	0.36	171.43	1.38	155.06	0.49	98.00	0.19	100.00
	Mean		1.80	100.00	0.21	100.00	0.89	100.00	0.50	100.00	0.19	100.00
C. genistoides	ST	0.00	1.00	59.17	0.06	31.58	0.33	41.25	0.57	109.62	0.19	37.25
C. genistoides	ST	1.67	1.80	106.51	0.17	89.47	0.87	108.75	0.47	90.38	0.16	31.37
C. genistoides	ST	3.33	2.26	133.73	0.33	173.68	1.21	151.25	0.53	101.92	1.18	231.37
	Mean		1.69	100.00	0.19	100.00	0.80	100.00	0.52	100.00	0.51	100.00
Mean			1.74	100.00	0.20	100.00	0.85	100.00	0.51	100.00	0.35	100.00
C. intermedia	T	0.00	0.97	97.00	0.04	28.57	0.28	51.85	0.36	100.00	0.15	100.00
C. intermedia	T	1.67	1.27	127.00	0.13	72.22	0.35	64.81	0.39	108.33	0.15	100.00
C. intermedia	T	3.33	0.76	76.00	0.36	200.00	1.01	187.04	0.33	91.67	0.15	100.00
	Mean		1.00	100.00	0.18	100	0.54	100.00	0.36	100	0.15	100.00

(Table 10 continued)

Species	Cutting Position	Nitrosol® (ml.L^{-1})	N (%)	N (%mean)	P (%)	P (%mean)	K (%)	K (%mean)	Ca (%)	Ca (%mean)	Mg (%)	Mg (%mean)
C. intermedia	ST	0.00	0.83	62.41	0.03	15.79	0.39	62.9	0.31	93.94	0.11	84.62
C. intermedia	ST	1.67	1.25	93.98	0.13	68.42	0.49	79.03	0.34	103.03	0.13	100.00
C. intermedia	ST	3.33	1.9	142.86	0.4	210.53	0.98	158.06	0.34	103.03	0.14	107.69
	Mean		1.33	100.00	0.19	100.00	0.62	100.00	0.33	100.00	0.13	100.00
Mean			1.16	100.00	0.18	100.00	0.58	100.00	0.35	100.00	0.14	100.00

Micro elements

Sodium (Na)

Sodium is not generally required by plants. Nevertheless, it does play important roles in many situations other than deleterious ones (Epstein & Bloom, 2005). The results showed that dry matter of plantlets grown from rooted *C. genistoides* cuttings on average contained 1016 $mg.kg^{-1}$ Na compared to 2124 $mg.kg^{-1}$ in *C. intermedia* (Table 11). On average, plantlets established from sub-terminal cuttings of *C. intermedia* contained 2144 $mg.kg^{-1}$ Na compared to 2104 $mg.kg^{-1}$ from terminal cuttings, while a Na content of 1061 $mg.kg^{-1}$ on average was found from plantlets grown from terminal cuttings compared to 970 $mg.kg^{-1}$ from sub-terminal cuttings in *C. genistoides*. The Na content in dry matter generally did not correlate with Nitrosol® application rates (Figure 6).

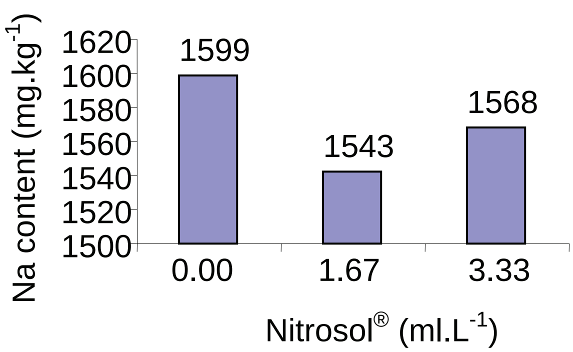

Figure 6 Effect of Nitrosol® fertilizer treatments on the sodium content of rooted cuttings of *Cyclopia* species

Manganese (Mn)

Mn is a critical element for plants and many living organisms including bacteria, for redox biochemistry, oxygen production and protection against oxidative stress (Cillier, 2001). On average, a Mn content of 91 $mg.kg^{-1}$ was found from *C. genistoides* plantlets compared to 70 $mg.kg^{-1}$ in *C. intermedia* (Table 11). Manganese content of plantlets grown from terminal cuttings of *C. intermedia* was found to be 71 $mg.kg^{-1}$ compared to 68 $mg.kg^{-1}$ from sub-terminal cuttings.

In comparison, a Mn content of 94 $mg.kg^{-1}$ was found in plantlets grown from terminal cuttings of *C. genistoides and* a content of 87 $mg.kg^{-1}$ from terminal cuttings. In general, Mn content increased with increasing Nitrosol® fertilizer rates (Figure 7). This increasing tendency was true for all species x cutting position x Nitrosol® fertilizer interactions (Table 11).

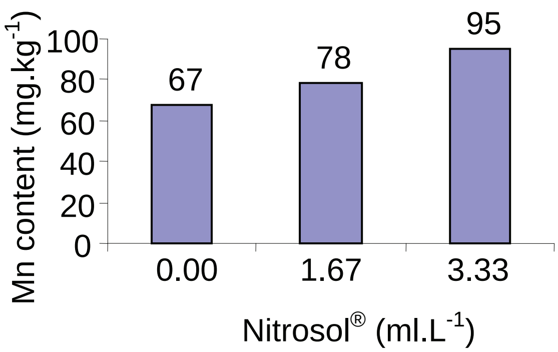

Figure 7 Effect of Nitrosol® fertilizer treatments on the manganese content of rooted cuttings of *Cyclopia* species

Iron (Fe)

Iron is a constituent of cytochromes and nonheme Fe proteins involved in photosynthesis, N_2 fixation and respiration (Taiz & Zeiger, 2006). The results indicate that the dry matter of plantlets originating from rooted cuttings of *C. genistoides* on average contained 157 $mg.kg^{-1}$ Fe content compared to 105 $mg.kg^{-1}$ of *C. intermedia*. An Fe content of 109 $mg.kg^{-1}$ was found from plantlets grown from terminal cuttings compared to 100 $mg.kg^{-1}$ from sub-terminal cuttings in *C. intermedia*. Averages of 161 and 154 $mg.kg^{-1}$ were found from plantlets grown from terminal and sub-terminal cuttings of *C. genistoides*, respectively. In control plantlets where no Nitrosol® was applied contents of 138 $mg.kg^{-1}$ Fe dry matter was found, while Fe contents of 132 and 123 $mg.kg^{-1}$ were found respectively where 1.67 and 3.33 $ml.L^{-1}$ Nitrosol® were applied, respectively (Figure 8). This was due to a decrease in Fe content with increasing application rates of Nitrosol® fertilizer in *C. genistoides*, but no clear trend in *C. intermedia* (Table 11).

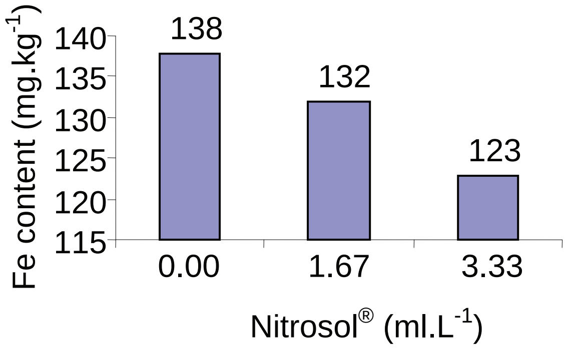

Figure 8 Effect of Nitrosol® fertilizer treatments on the iron content of rooted cuttings of *Cyclopia* species

Copper (Cu)

Copper is an essential element as it is involved in a number of physiological processes such as the photosynthetic and respiratory electron transport chains (Van Assche & Clijsters, 1990) and as a cofactor or as a part of the prosthetic growth of many key enzymes involved in different metabolic pathways, including ATP synthesis (Harrison, Jones & Dameron, 1999). The results indicate that dry matter of both *C. intermedia* and *C. genistoides* plantlets contained contents of 3 $mg.kg^{-1}$ Cu (Table 11). Plantlets established from terminal cuttings of *C. intermedia* contained 3 $mg.kg^{-1}$ Cu compared to 2 $mg.kg^{-1}$ from sub-terminal cuttings. In contrast, 3 $mg.kg^{-1}$ Cu was found from plantlets grown from sub-terminal cuttings compared to 2 $mg.kg^{-1}$ Cu dry matter from plantlets grown from terminal cuttings of *C. genistoides*. Nitrosol® fertilizer application rates did not show any correlation with Cu content found in dry plant matter (Figure 9).

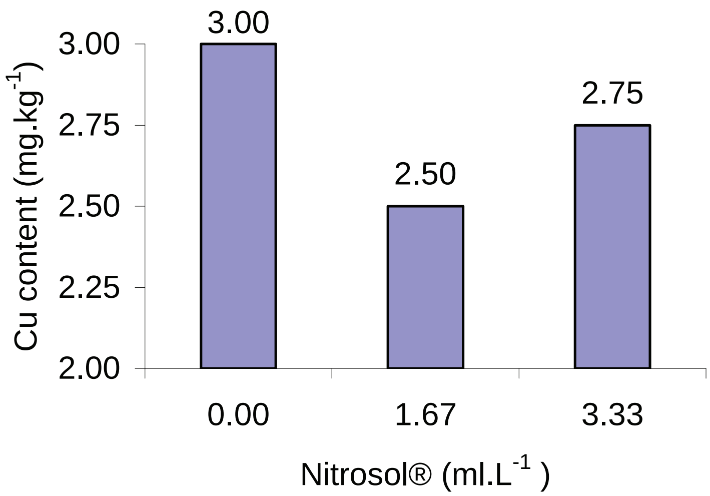

Figure 9 Effect of Nitrosol® fertilizer treatments on copper content of rooted cuttings of *Cyclopia* species

Zinc (Zn)

Zinc serves as a constituent of alcohol dehydrogenase, glutamic dehydrogenase as well carbonic anhydrase (Mengel and Kirkby, 1987). *C. genistoides* contained on average 28 $mg.kg^{-1}$ Zn compared to a Zn content of 27 $mg.kg^{-1}$ in *C. intermedia* (Table 11). Plantlets grown from both terminal and sub-terminal cuttings of *C. intermedia* had Zn contents of 27 $mg.kg^{-1}$, while plants originating from sub-terminal cuttings of *C. genistoides* contained 31 $mg.kg^{-1}$ Zn content compared to 25 $mg.kg^{-1}$ from terminal cuttings (Table 11). Figure 10 indicates that no clear relationship was found between the application rates of Nitrosol® and Zn content in dry matter of the two species on average or for plantlets grown from sub-terminal or terminal cuttings.

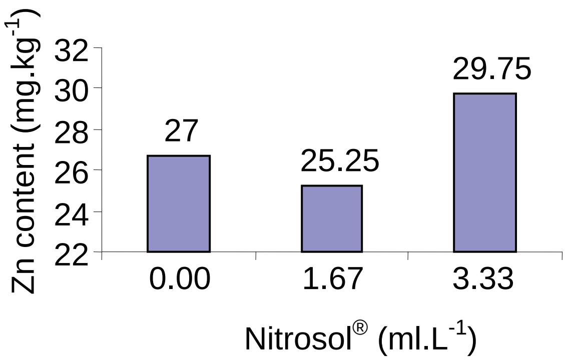

Figure 10 Effect of Nitrosol® fertilizer treatments on zinc content of rooted cuttings of *Cyclopia* species

Table 11 Mineral analysis values of essential micro-elements in dry plant matter as affected by Nitrosol®, species and position (terminal – T, sub-terminal – ST) in *Cyclopia* species

Species	Cutting position	Nitrosol®	Na		Mn		Fe		Cu		Zn	
		($ml.L^{-1}$)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)
C. genistoides	T	0.00	1073	101	68	78	171	106	2	100	21	84
C. genistoides	T	1.67	1024	97	82	94	170	106	3	150	24	96
C. genistoides	T	3.33	1086	102	111	128	141	88	2	100	29	116
	Mean		1061	100	87	100	161	100	2	117	25	99
C. genistoides	ST	0.00	941	97	82	87	175	114	4	133	38	123
C. genistoides	ST	1.67	932	96	84	89	146	95	3	100	24	77
C. genistoides	ST	3.33	1037	107	116	123	140	91	3	100	31	100
	Mean		970	100	94	100	154	100	3	111	31	100
Mean			1016	100	91	100	157	100	3	111	28	100
C. intermedia	T	0.00	2324	110	61	86	113	104	3	100	25	93
C. intermedia	T	1.67	2158	103	75	106	106	97	2	67	28	104
C. intermedia	T	3.33	1831	87	78	110	109	100	3	100	27	100

(Table 11 continued)

Species	Cutting position	Nitrosol®	Na		Mn		Fe		Cu		Zn	
		($ml.L^{-1}$)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)	($mg.kg^{-1}$)	(%mean)
	Mean		2104	100	71	100	109	100	3	89	27	99
C. intermedia	ST	0.00	2056	96	58	85	92	92	2	100	23	85
C. intermedia	ST	1.67	2057	96	72	106	106	106	2	100	25	93
C. intermedia	ST	3.33	2319	108	74	109	101	101	3	150	32	119
	Mean		2144	100	68	100	100	100	2	117	27	99
Mean			2124	100	70	100	105	100	3	117	27	99

4.4 Conclusions

On average *C. genistoides* produced greater fresh and dry plant weights as well as greater root weights, plant heights, number of nodules per plant and number of shoots compared to *C. intermedia.* The original cutting position of the rooted cuttings of *Cyclopia* species had no significant influence on the measured growth parameters in both species. Nitrosol® fertilizer significantly affected establishment and growth of rooted cuttings of *Cyclopia* species with larger responses in *C. genistoides* compared to *C. intermedia*. Plantlets fertilized with rates of 3.33 $ml.L^{-1}$ Nitrosol® appeared greener and stronger compared to plantlets fertilized with the lower rates (1.67 $ml.L^{-1}$) or the control (no fertilizer). Plant mineral analysis indicated that, generally, the levels of macro elements increased with an increase in the available amount of fertilizer due to increased Nitrosol® applications, but micro elements did not show any clear trends.

4.5 REFERENCES

ARNOLD, T. & DE WET, B.C., 1994. Plants of Southern Africa. Memoirs of the Botanical Society of South Africa 62. National Botanical Institute, Pretoria, South Africa.

BARBER, W.D. & THOMAS, W.I., 1972. Evaluation of the genetics of relative phosphorus accumulation by corn (*Zea mays* L.). *Crop Sci*. 12, 755-758.

BERRY, W., 2006. Symptoms of defieciency in essential minerals. *In*: Plant Physiology, 4th Edition, Taiz, L. & Zeiger, E. Sinauer Associates, Australia.

BOND, P. & GOLDBLATT, P., 1984. Plants of the Cape Flora. A descriptive Catalogue. pp. 285-286. CPT Book Printers, Cape Town.

CILLIER, M., 2001. Bacterial genes controlling manganese accumulation. *In*: Microbial Transport Systems. Günther, W., (ed.). Wiley - VCH, USA.

COWLING, R.M., HOLMES, P.M. & REBELO, A.G., 1992. Plant diversity and endemism. *In*: The ecology of fynbos: nutrients, fire and diversity, Cowling, R.D. (ed.). Oxford University Press, UK.

DE NYSSCHEN, A.M., VAN WYK, B.E., VAN HEERDEN., F. & SCHUTTE, A.L., 1996. The major phenolic compounds in the leaves of *Cyclopia* species (Honeybush tea*). Biochemical Syst. Ecol.* 24, 234-246.

DU TOIT, J. & JOUBERT, E., 1998. The effect of pre-treatment on the fermentation of honeybush tea (*Cyclopia maculate*). *J. Sc. Food & Agric*. 76, 537-545.

DU TOIT, J., JOUBERT, E. & BRITZ, T.J., 1998. Honeybush tea – A rediscovered South African herbal tea. *J. Sust. Agric*. 12 (2-3), 67-84.

EPSTEIN, E. & BLOOM, A.J., 2005. Mineral nutrition of plants: Principles and perspectives, 2nd Ed., Sinauer Associates, Inc., USA.

FENNING, J.O. & DANSO, S.K.A., 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Appl. Soil Ecol.* 21, 23-29.

HANLEY, M.E. & FENNER, M., 1997. Seedling growth of four fire-following Mediterranean plant species deprived of single mineral nutrients. *Functional Ecol*. 11, 398-405.

HARRISON, M.D, JONES, C.E. & DAMERON C.T., 1999. Copper chaperones: function, structure and copper-binding properties. *J. Biol. Inorg. Chem.* 4(2), 145-153.

HOWIESON, J.G., MALDEN, J., YATES, R.J. & O'HARA, G.W., 2000. Techniques for the selection and development of elite inoculant strains of *Rhizobium leguminosarum* in southern Australia. *Symbiosis* 28, 33-48.

JOHNSTON, M.E., GIKAARA, D.M. & EDWARDS, D.E., 2006. Phosphorus nutrition of *Caustis blakei* grown with phosphorus sources of different solubility in two soils of differing phosphorus adsorption capacity. *Sci. Hort*. 110, 298-304.

JOUBERT, M.E., BOTMA, P.S., KOTZÉ, W.A.G., & WOOLDRIDGE, J., 2007. Honeybush (*Cyclopia* spp.) response to phosphorus fertilisation and mulching. *S. Afr. J. Plant Soil* 24(3), 176-177.

KIES, P., 1951. Revision of the genus *Cyclopia* and notes on some other sources of bush tea. *Bothalia* 6, 161-176.

LEVYNS, M.R., 1920. A Guide to the Flora of the Cape Peninsula. p. 147. Juta & Co (Ltd), Cape Town.

MAIER, N.A., DARLBERG, N.A. & WILLIAMS, C.M.J., 1994. Effect of nitrogen, phosphorus, and potassium on yield and petiolar nutrient concentrations of potato (*Solanum tuberosum* L.) *cvv. Kennebec* and *Atlantic. Austr. J. Exp. Agric*. 34, 825-834.

MARLOTH, R., 1925. The Flora of South Africa with Synoptical Tables of the Genera of the Higher Plants. pp. 69-72. Darter Bros & Co, Cape Town.

MENGEL, K. & KIRKBY, E.A., 1987. Principles of plant nutrition. International Potsash Institute, Worblaufern-Bern, Switzerland.

MONTARONE, M., 2001. Update on the cultivation of protea. *Acta Hort*. 545, 127-133.

MUNNS, DN., 1977. Mineral nutrition and the legume symbioses. *In*: A Treatise on Dinitrogen Fixation. Section IV, Hardy, R.W.F. & Gibson, A.H. (eds). John Wiley and Sons, New York.

PEOPLES, M.B, LADHA, J.K. & HERRIDGE, D.F., 1995. Enhancing legume N_2-fixation through plant and soil management. *Plant & Soil* 174, 83-101.

PETERSON, H.L. & LOYNACHAN, T.E., 1981. The significance and application of *Rhizobium* nitrogen fixation by perennial legumes. *International Review of Cytology* 13, 311-331.

RIECKERMANN, H, GOLDFARB, B, CUNNINGHAM, W. & KELLISON, R.C., 1999. Influence of nitrogen (N), cutting type, and clone on root and shoot development of rooted stem cuttings of sweet gum. *New Forests* 18 (3), 231-244.

SAHTA, 2007. South African Honeybush Tea Association. Notes compiled for Honeybush Farmers' Day, March 2007, Kanetberg, Riversdale, South Africa.

SAS, 2000. SAS/STAT Users Guide, Version 9.1, First Edition, Volume 2. SAS Institute Inc., Cary, NC, USA.

SCHUTTE, A.L., 1995. A taxonomic study of the tribes *Podalyrieae* and *Liparieae* (Fabaceae). Ph.D. Dissertation, Rand Afrikaans University, Johannesburg, South Africa.

SHAPIRO, S.S. & WILK, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611.

SILBER, A., GANMORE-NEUMANN, R. & BEN-JAACOV, J., 1998. Effects of nutrient addition on growth and rhizosphere pH of *Leucadendron* ('Safari Sunset'). Plant & Soil 199(2), 205-211.

SMITH, F.W., 1982. Mineral nutrition of legumes. *In*: Nitrogen fixation in legumes. Vincent, J.M. (ed.). Academic Press, Sydney.

SPRIGGS, A.C. & DAKORA, F.D., 2007. Competitive ability of selected *Cyclopia* Vent. rhizobia under glasshouse and field conditions. *Soil Biol. & Biochem*. 39, 58-67.

SVENNING, M.M., JUNTILLA, O. & SOLHEIM, B., 1991. Symbiotic growth of indigenous white clover (*Trifolium repens*) with local *Rhizobium legumosarum* biovarr t*rifolli*. *Physiol. Planta.* 83, 381-389.

TAIZ, L. & ZEIGER, E., 2006. Plant physiology, 4th Ed. Sinauer, USA.

VAN ASSCHE, F. & CLIJSTERS, H., 1990. Effects of metals on enzyme activity in plants*. Plant Cell Environ*. 13, 195-206.

WELGEMOED, Z., 1993. Heuningtee straks kommersieel verbou. Landbouweekblad 792, 52-55.

CHAPTER 5

EFFECT OF DIFFERENT SEED TREATMENTS ON GERMINATION OF *CYCLOPIA* SPP. SEEDS

Abstract

A study was conducted over a period of 18 days to determine the effect of various seed treatments on germination of six different sources of seed of *Cyclopia* species. The seed sources varied in age in combination with different species of *Cyclopia*. Seeds were subjected to 10 different pre-sowing treatments including the control. Seeds were soaked in sulphuric acid (95%) for 10, 15, 20 or 25 minutes; dipped in hot water (100°C) for 15, 30, 45 or 60 seconds; soaked in water with smoked paper disks for 24 hours or in demineralized water (control) for 24 hours. The study showed that all the treatments had a significant effect on germination with the exception of eight year old seeds obtained from *C. subternata* (seed source two) after an 18 day germination period. Sulphuric acid treatments at various exposure times gave variable results regarding percentage germination. Although hot water treatment improved germination compared to smoked paper disks and the control, seeds treated with hot water degenerated rapidly compared to seeds treated with other treatments. A higher overall germination was obtained from seeds of seed source one (one year old) compared to other seed sources (older than one year). Smoked paper disk treatment generally did not improve germination compared to the control except in one year old seeds. In terms of germination rate, germination generally started after two to four days in most treatments.

Keywords: *Cyclopia* spp., germination, seed source, smoke

5.1 Introduction

Cyclopia species are leguminous shrubs belonging to the Cape fynbos biome and grow in the coastal districts of the Western and Eastern Cape Provinces of South Africa (Schutte, 1995). The foliage, fine stems and flowers of the *Cyclopia* species are used to make a beverage and a honey-like flavoured herbal infusion known as honeybush tea (Du Toit, Joubert & Brits, 1998). The tea has become internationally recognized as a substitute for ordinary tea like *Camellia sinensis* due to its health benefits (Dharmananda, 2004). In the Cape Fynbos Biome, which resembles the Mediterranean climate (winter rainfall), the environment is characterized by a number of stress factors such as summer drought, low soil fertility and periodic fires (Cowling, Holmes & Rebelo, 1992). Seeds of both the resprouter (*C. intermedia*) and the seeder type (*C. subternata*) are dormant and will not germinate under normal conditions (Sutcliffe & Whitehead, 1994). However, in the seeder, this dormancy is entirely coat imposed and scarification resulted into high percentage germination, while scarification only resulted in the partial breaking of dormancy in the resprouter. Wild fires can modify the seed germination response via both physical and chemical cues involved in the germination process (Brown, Jamieson & Botha, 1994).

Heat from flames may fracture the impermeable coat of hard-shelled seed (e.g. Fabaceae) resulting in the coats becoming permeable to water. De Lange & Boucher (1990) reported that seed germination in many fynbos species is stimulated by smoke derived from burning vegetation. Dixon, Roche & Pate (1995) found that exposure of dormant seeds to cold smoke derived from burnt native vegetation had a positive effect on germination in 45 out of 94 species of native Western Australian plants that are normally hard to germinate. Dry heat may also break dormancy by providing a heat-pulse which stimulates the embryo directly, resulting into germination. Studies indicate that fires also provide chemical cues such as the gases of ethylene and ammonia, which stimulate germination in some species of *Erica* (De Lange & Boucher, 1990; Brown *et al*., 1994).

Ethylene, a major constituent of smoke has been shown to stimulate seed germination in many species (Walker *et al*., 1989; Whitehead & Nelson, 1992). Methods developed to break down, or increase permeability of the legume seed coat included scarification with sand paper (Hopkins, 1923), soaking in sulphuric acid, and in hot water (McNair, 1917) or exposing seed to heat (Lute, 1927; Rincker, 1954). Effects of sulphuric acid and hot water on germination of many hard coated seeds are well known. Muhamad & Amusa (2003) found highest germination in seeds of *Tamarindus indica* L. treated with hot water and 50% sulphuric acid concentration at all soaking periods. The highest germination percentage was recorded when seeds were treated with 50% sulphuric acid for 60 minutes. In terms of hot water treatment, Muhamad & Amusa (2003) revealed that seed germination increased with increasing water temperature and soaking period. Duguma, Kang & Okali (1988) reported high percentage germination in seeds *of Leucaecinia leucacephala* and *Acacia nilotica* with increasing ratio of seed weight to hot water. Pela, Gerasopoulos & Maloupa (2000) found that soaking seeds in boiling water for 35 seconds or one minute showed the highest rates of germination (96% or 74%) within four days respectively in seeds of *Cistus creticus*, suggesting that hot water can be effective in inducing germination in certain species. Teketay (1996) reported that boiling water significantly improved germination in *Senna* species (Fabaceae) *S. didymobotrya*, *S. occidentalis* and *S. septemtrionalis*, but had very little effect on *S. multiglandulosa* and reduced germination in *S. bicapsularis*. He suggested that *Senna* species differ in their tolerance to higher temperatures. Injurious effects of hot water coupled with exposure time, however, have been reported by Aliero (2004) in seeds of *Parkia biglobosa.* The author reported that germination decreased when seeds were allowed in water for more than four seconds due to the destruction of the embryo on contact with boiling water for a prolonged period.

Propagation of fynbos plants by seed is often difficult, as seeds of many species possess a deep dormancy when shed, hence require very specific environmental or chemical cues for germination (Brown & Botha, 2004). As part of fynbos plants, seeds of honeybush (*Cyclopia* spp.) exhibit a seed coat and/or embryo imposed dormancy and will not germinate unless scarified (Sutcliffe & Whitehead, 1995). Previous work on *C. intermedia* and *C. subternata* indicated that smoke enhanced germination of these species. Species of the genus *Cyclopia* are divided into two groups based on the manner with which their seed germinate, namely, resprouters such as *C. intermedia* that do not germinate easily under normal conditions and seeders, e.g. *C. subternata* which germinate readily under normal conditions (Sutcliffe & Whitehead, 1995). However, research on the germination requirements and response of different *Cyclopia* spp. to different seed pre-treatments is still in its infancy. Since fire and other germination cues have been identified as effective seed germination stimulants in many fynbos species, a study was conducted to evaluate the effects of a smoke paper disk, sulphuric acid (95%) scarification pre-treatment, and a hot water pre-treatment on germination of different seed sources obtained from three honeybush (*Cyclopia*) species (*C. intermedia*, *C. genistoides*, and *C. subternata*).

5.2 Materials and methods

Mature seeds of *Cyclopia genistoides* were harvested in the Table Mountain area of the Western Cape, while seeds of *C. intermedia* and *C. subternata* were harvested in the Langkloof areas of the Eastern Cape Province, South Africa. Seeds of *C. intermedia* consisted of two seed sources (seven year old seeds and seeds with unknown age). *Cyclopia subternata* comprised of three seed sources (one year old seeds, eight year old seeds and seeds with unknown age), while seeds of *C. genistoides* were of an unknown age. Because a limited quantity of seed was available, pre-testing to determine the vigor and quality of the seed could not be done. Seeds were categorized as different seed sources of different *Cyclopia* species varying in age. Because of differences in seed age and unknown but assumable differences in seed vigor which could result in the occurrence of unexplainable interactions, it was decided to conduct six similar experiments on the six seed sources to evaluate their germination response to different seed treatments and not combine all sources in one experiment. For germination experiments, 9-cm Petri dishes were used with two porous cellulose filter papers as substrate.

Following the International Seed Testing Association rules (International Seed Testing Association, 1999) 25 seeds were used per dish and three replicates were applied. After treatment application, seeds were incubated at 22°C in the dark in a growth chamber.

Acid treatment

To render hard seed coats permeable to water, seeds were treated with concentrated sulphuric acid (95%) for 10, 15, 20 and 25 minutes. After the treatments, seeds were thoroughly rinsed with running tap water. Seeds were then incubated at 22°C in the dark.

Hot water treatment

To test the effect of hot water on the seed coats, seeds were immersed in boiling water (100°C). Seventy five seeds of each seed source were divided into three replicates of 25 seeds each. Each replicate of seeds was enclosed in a paper coffee filter, folded and fastened with paper clips to prevent seed loss. The seeds were then immersed in boiling water for 15, 30, 45 and 60 seconds. They were removed immediately after boiling and rinsed in running tap water and allowed to cool before being placed in Petri dishes for germination at 22°C in the dark.

Smoke paper disk

Smoke paper disk primer is an absorbent paper impregnated with fynbos-smoke-saturated water. The solution of smoke-saturated water contains a combination of natural substances that have been found to overcome dormancy and stimulate seed germination. The degree of response varies with the species, but on average, treated seeds give better germination results when compared to untreated seeds. The disk is recommended for treating seeds of proteas, leucospermums, leucadendrons, Ericas and many other fynbos species (South African National Biodiversity, Kirstenbosch NBG, Private Bag X7, Cape Town, 7735).

To examine the effect of smoke paper disk, 50 ml of distilled water were added on each disk to prepare a smoke-water solution and seeds were soaked in this solution for 24 hours. Immediately after 24 hours, seeds were removed and allowed to air dry and then placed between paper filter disks in 9 cm Petri dishes with distilled water added to keep the filter paper moist.

Control

In the control treatment, seeds were soaked in distilled water for 24 hours at 22°C. After 24 hours, seeds were removed from the water and allowed to air dry, placed in Petri dishes and immediately placed in a growth chamber for germination at 22° in the dark.

During the course of the experiment, the seeds were held in an environment saturated with distilled water. A seed was considered germinated when a radicle could be seen with the naked eye (Valbuena & Vera, 2002). The germinated seeds were counted every second day until the end of the experiment, when the increase in germination was less than 1% (Rivas, Reyes & Casal, 2006).

The experimental design was completely random with 10 treatment combinations (control, smoked paper disk immersed in water, four sulphuric acid treatments and four hot water treatments). Acid treatments (soaked in acid for 10, 15, 20 and 25 minutes) and four hot water treatments (dipped in boiling water for 15, 30, 45 and 60 seconds), smoked paper disk and distilled water (control) replicated three times at random. An experimental unit consisted of 25 seeds. Treatment effects were tested on seed with unknown quality obtained from three different species of *Cyclopia* (*C. genistoides* - unknown age, *C. intermedia* - seven year old, unknown age), *C. subternata* - eight year old, one year old, unknown age). Germination was assessed every second day for 18 days to determine the effect of seed treatments on germination rate.

A separate one-way analysis of variance for each seed source was performed at 18 days, using GLM (General Linear Model) Procedure of SAS statistical software version 9.1 (SAS, 2000). Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means. A probability level of 5% was considered significant for all significance tests.

5.3 Results and discussion

Analysis of variance

Results revealed that seed treatments had a highly significant effect on germination percentage on the majority of different seed sources of *Cyclopia* species after a germination period of 18 days (Table 1). However, no significant effect of the seed treatment was found in seeds obtained from seed source two (eight year old seeds of *C. subternata*) after 18 days of germination. All the treatments applied on seeds of *Cyclopia* species therefore improved germination of the hard coated seeds of *Cyclopia* species although the effect varied depending on the treatment and seed source.

Table 1 ANOVA analysis for germination percentage of different sources after 18 days of incubation

Source	Pr>F					
	S_1	S_2	S_3	S_4	S_5	S_6
Treatment	<.0001	ns	<.0001	0.0004	0.0007	0.0003
CV (%)	11.94	37.46	28.44	22.14	28.56	43.10

Key for seed source

S_1 – *Cyclopia subternata* (one year old seeds)

S_2 – *Cyclopia subternata* (eight year old seeds)

S_3 – *Cyclopia subternata* (unknown age)

S_4 – *Cyclopia intermedia* (seven year old seeds)

S_5 – *Cyclopia intermedia* (unknown age)

S_6 – *Cyclopia genistoides* (unknown age)

Seed source one - (*C. subternata* - one year old)

One year old seed of *C. subternata* started to germinate after an incubation period of only two days in the case of treatments which had a positive effect on germination percentages, but only after 10 -12 days of incubation in the case of treatments that resulted in poor germination (Figure 1). After 18 days of incubation, compared to other treatments, sulphuric acid significantly improved germination of one year old *C. subternata* seeds (Table 2). Treating seeds with sulphuric acid for either 10 or 15 minutes resulted into the highest percentage germination of 77.33% compared to 72.00% and 74.00% germination obtained with 20 and 25 minutes sulphuric acid soaking, respectively. With hot water treatment, germination decreased with an increase in soaking time, as hot water is probably injurious to the young seeds. Extending the exposure time in hot water was, therefore, injurious to the one year old seeds of seed source one. Dipping seeds in hot water for 45 and 60 seconds was not effective in improving germination. Germination after applying these two treatments was significantly lower compared to the 48.00% germination of the control treatment.

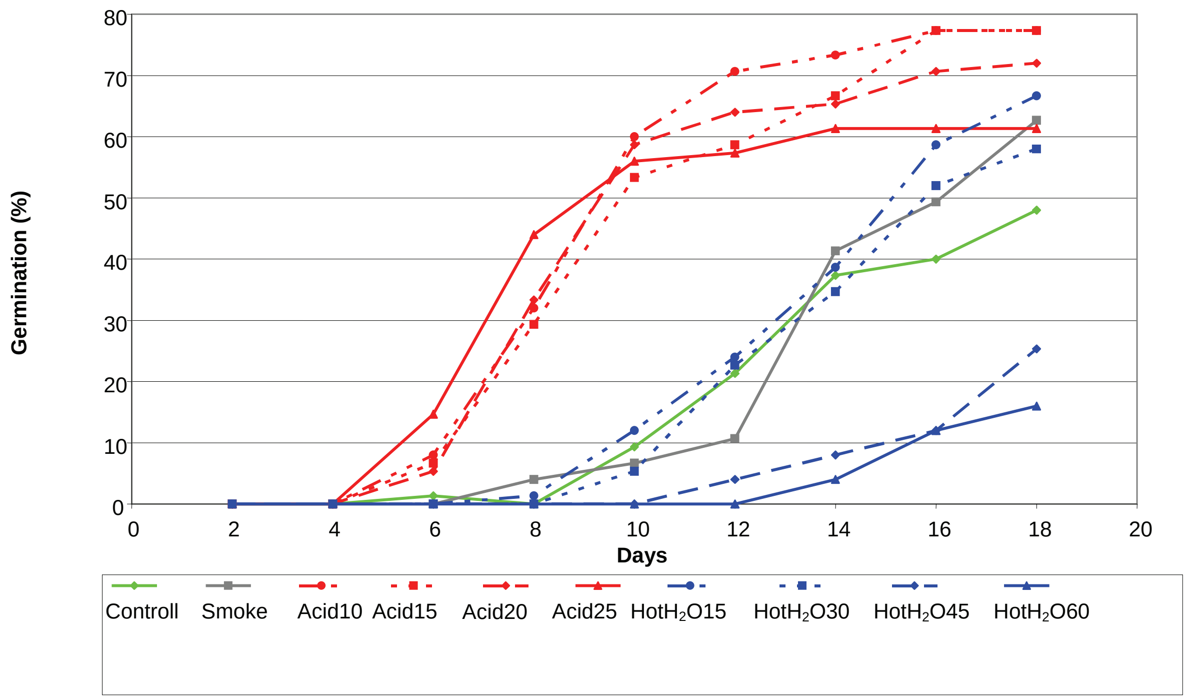

Figure 1 Effect of different treatments on germination of one year old seeds of *C. subternata* (seed source one) seeds after 18 days

Seed source two - *(C. subternata* - eight year old seeds)

For eight year old seeds of *C. subternata* (seed source two), germination of the best treatments also started after an incubation period of four days compared to 12 days of the treatment that resulted in the lowest germination (Figure 2). The germination trends indicated that treating seeds with sulphuric acid for 10 minutes (32.00%) improved germination compared to the control (16.00%), smoked paper disk (16.00%) and hot water (100°C) at 60 seconds (13.33%). Best results obtained with this seed source was, therefore, considerably lower compared to the nearly 80% (Figure 1) germination obtained with seed source one (*C. subternata* - one year old seed). Exposure of seeds to hot water treatment for 60 seconds seemed to be detrimental to the seeds while results obtained with the smoke water treatment were similar to that of the control treatment.

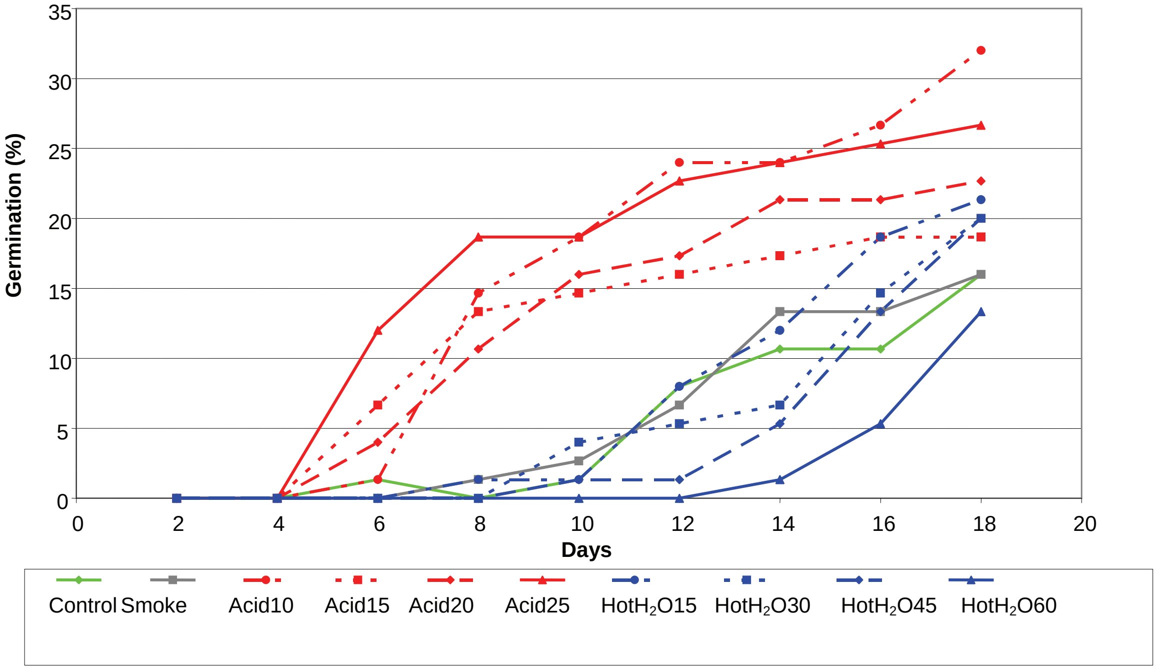

Figure 2 Effect of different seed pre-treatments on cumulative germination of eight year old seeds of *C. subternata* (seed source two) after 18 days of germination

Seed source three – (*C. subternata* – unknown age)

Although germination of seed from this source of unknown age also started after an incubation period of four to six days, germination was with the exception of the 45 seconds hot water treatment, as also found with eight year old *C. subternata* seed, generally poor and less than 42.00% after 18 days (Figure 3). A treatment of 45 seconds with hot water, however, increased germination to 62.67% compared to 13.33% for the control (Table 2). In contrast to seed sources one and two, best germination results with *C. subternata* seed of an unknown age were thus obtained with 45 seconds hot water treatment.

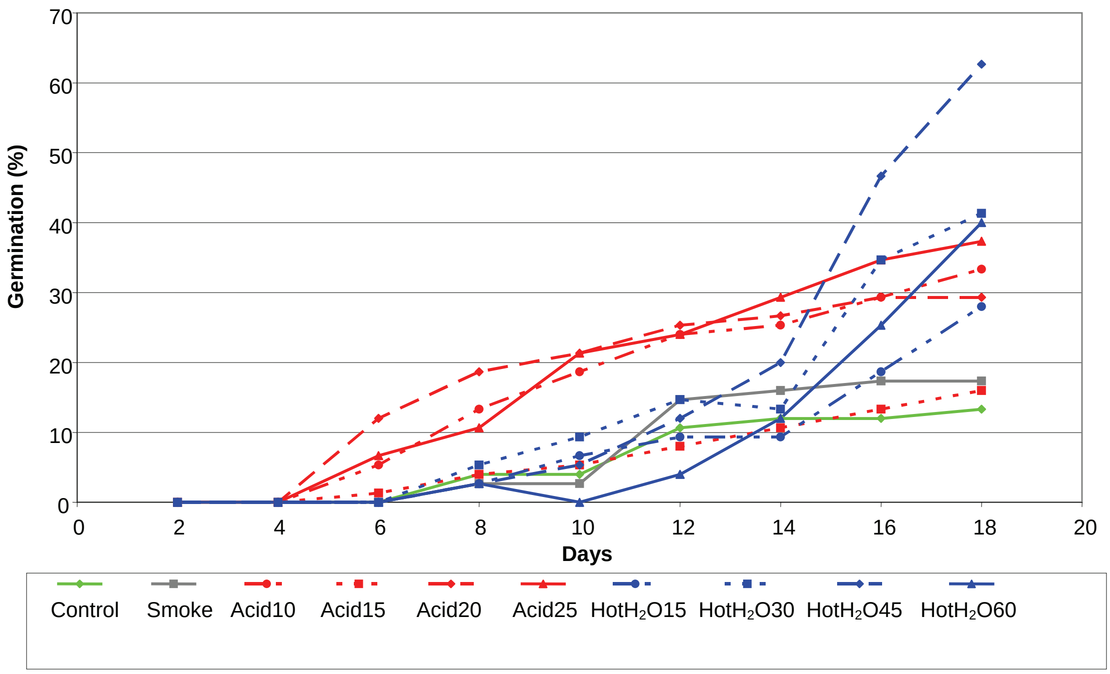

Figure 3 Effect of different seed pre-treatments on cumulative germination of *C. subternata* (seed source three) with unknown seed age after 18 days

Seed source four – (*C. intermedia* – seven year old seeds)

Germination of seeds from this source started after an incubation period of four to eight days and germination percentages after 18 days varied between 21.33% and 57.33% (Figure 4). Compared to the other treatments, germination was significantly improved by a 15 minutes soaking in concentrated sulphuric acid and 57.33% germination was obtained after 18 days (Table 2). Sulphuric acid treatments are well known as a method to break the dormancy of hard coated seeds (Muhamad & Amusa, 2003). No significant differences were found between untreated seeds (control) and seeds treated with hot water for 30 (33.33%), 45 (21.33%) and 60 seconds (28.00%) or smoked paper disks (21.33%).

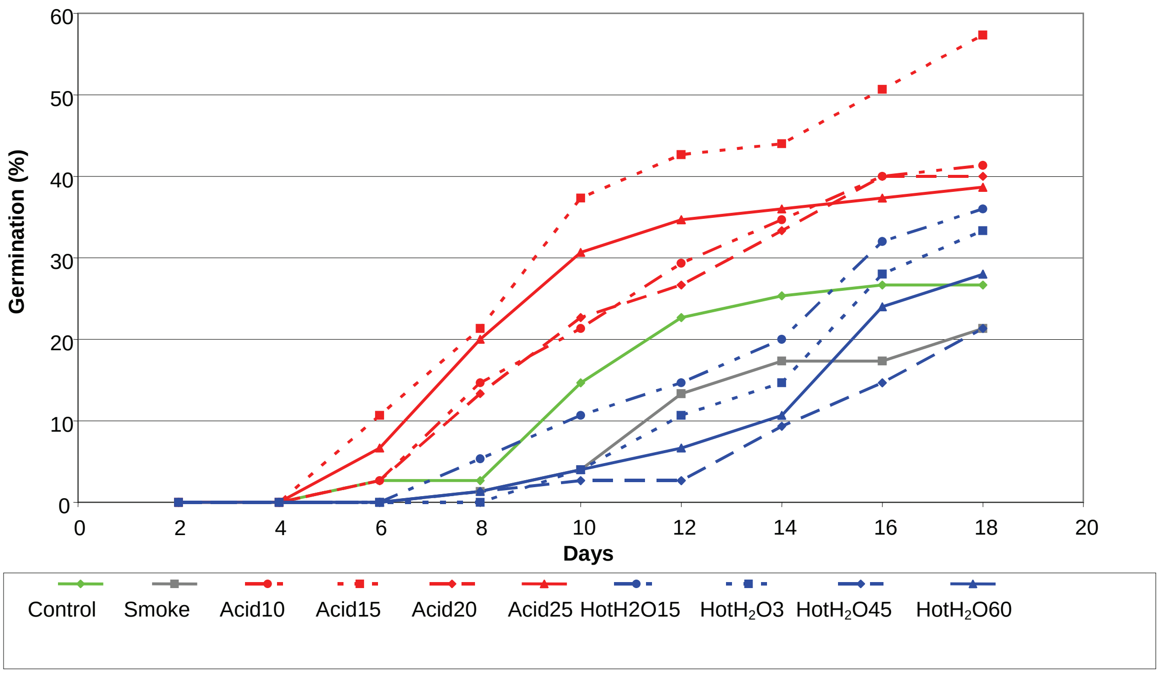

Figure 4 Effect of different seed pre-treatments on cumulative germination of seven year seeds of *C. intermedia* (seed source four) after 18 days of germination

<u>Seed source five</u> - (*C. intermedia* – unknown age)

Germination of seeds obtained from seed source five was improved by sulphuric acid treatment at 15 and 20 minute soaking period compared to other treatments. Treating seeds with sulphuric acid for 15 and 20 minutes resulted in 50.67 and 44.00% germination, respectively compared to 16.00% of the control (Table 2). The general trends illustrated in Figure 5 indicate that sulphuric acid treatment generally improved germination compared to the hot water, smoked disk paper and the control treatment.

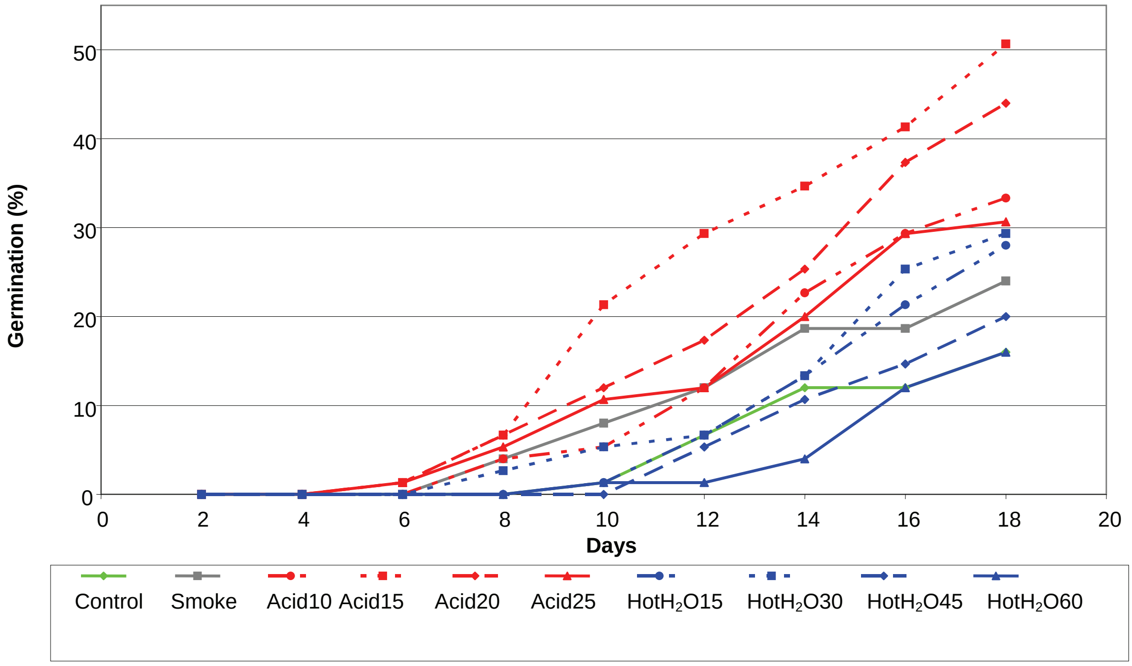

Figure 5 Effect of different pre-seed treatment on cumulative germination of seeds of *C. intermedia* (seed source five) with unknown age after 18 days of germination

<u>Seed source six</u> - (*C. genistoides* – unknown age*)*

As also found with other seed sources, germination of seeds generally started after four to six days of incubation compared to 8-12 days in the case of treatments that resulted in low germination percentages (Figure 6). It is thus clear that treatments that enhanced germination increased both the rate of germination as well as the total germination percentage. Control and smoked paper disk treatments resulted in germination percentages of only 4 and 2.67% respectively, after 18 days when *C. genistoides* seed of an unknown age was used (Table 2). In comparison, a sulphuric acid treatment for 25 minutes and hot water treatment for 45 seconds resulted in germination percentage of 40% (Table 2). As was found when *C. subternata* seeds of an unknown age (Figure 3) were used, *C. genistoides* seeds of an unknown age (Figure 6) also showed a good germination response to the different hot water treatments.

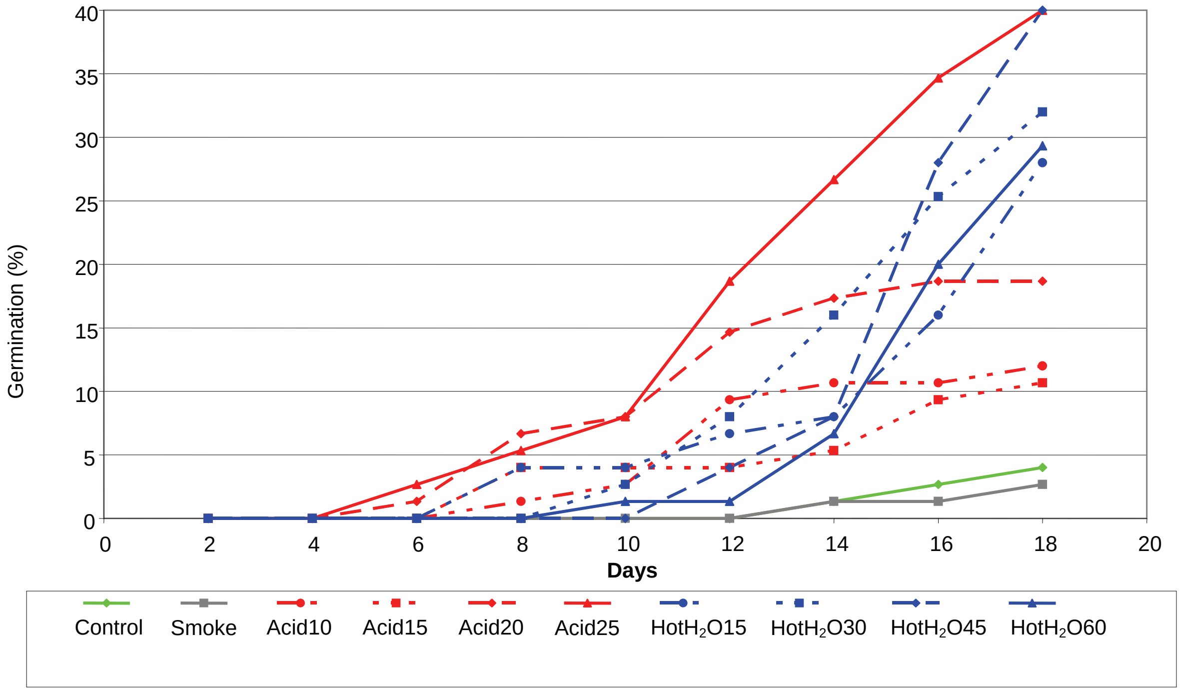

Figure 6 Effect of different seed pre-treatments on cumulative germination of *C. genistoides* (seed source six) seeds with unknown age after 18 days of germination

Ranking of germination

The ranking of germination of different seed sources (Table 2) after 18 days shows that smoked paper disk treatment generally did not improve germination of seeds because this treatment ranked between 6 and 10 for all seed sources tested. Both hot water and sulphuric acid resulted in increased germination as rankings were higher than that of the control for all seed sources tested. Sulphuric acid treatments on average provided better results than hot water (Table 2) when tested on seed sources S_1 *(C. subternata* - one year old), S_2 *(C. subternata* - eight year old seeds), S_4 (*C. intermedia* – seven year old seeds) and S_5 (*C. intermedia* – unknown age). Although the response to different sulphuric acid treatments varied between seed sources, sulphuric acid applied at ten and 15 minutes soaking seemed to give more consistent results.

Table 2 Effect of different seed treatments on the percentage germination of different seed sources after 18 days

Treatment	Exposure time	S_1 %	S_1 Ranking	S_2 %	S_2 Ranking	S_3 %	S_3 Ranking	S_4 %	S_4 Ranking	S_5 %	S_5 Ranking	S_6 %	S_6 Ranking
Sulphuric acid	10 min	77.33a	1	32.00	1	33.33b	5	41.33b	2	33.33bcde	3	12.00 cd	7
Sulphuric acid	15 min	77.33a	2	18.67	7	16.00cd	9	57.33a	1	50.67a	1	10.67d	8
Sulphuric acid	20 min	72.00ab	4	22.67	3	29.33bc	6	40.00bc	3	44.00ab	2	18.67bcd	6
Sulphuric acid	25 min	74.00ab	3	26.67	2	37.33b	4	38.67bcd	4	30.67bcd	4	40.00a	1
Hot water	15 sec	66.67abc	5	21.33	4	28.00bcd	7	36.00bcd	5	28.00cd	6	28.00abc	5
Hot water	30 sec	58.00cd	7	20.00	5	41.33b	2	33.33bcde	6	29.33bcd	5	32.00ab	3
Hot water	45 sec	25.33e	9	20.00	6	62.67a	1	21.33e	9	20.00cd	7	40.00a	2
Hot water	60 sec	16.00e	10	13.33	10	40.00b	3	28.00cde	7	16.00d	9	29.33ab	4
Smoked paper	24 hrs	62.67bc	6	16.00	9	17.33cd	8	21.33e	10	18.00d	8	2.67d	10
Control	24 hrs	48.00d	8	16.00	8	13.33d	10	26.67de	8	16.00d	10	4.00d	9

Means followed by the same letter are not significantly different (LSD=0.05)

Key

S_1 – *Cyclopia subternata* (one year old seeds)

S_2 – *Cyclopia subternata* (eight year old seeds)

S_3 – *Cyclopia subternata* (unknown age)

S_4 – *Cyclopia intermedia* (seven year old seeds)

S_5 – *Cyclopia intermedia* (unknown age)

S_6 – *Cyclopia genistoides* (unknown age)

5.4 Conclusions

All the treatments applied on seeds obtained from different seed sources of *Cyclopia* species significantly affected germination with the exception of eight year old seeds obtained from *C. subternata* (seed source two). Relative to sulphuric acid treatment, the effect of exposure time on germination varied depending on the age of the seed. The highest overall percentage germination (77.33%) was found with ten and 15 minute sulphuric acid treatment for one year old seeds of *C. subternata*. Although hot water treatment significantly improved seed germination of different *Cyclopia* species compared to smoked paper disk and the control, seeds treated with hot water degenerated rapidly compared to other treatments especially at a dipping period of 60 seconds. One year old seeds obtained from seed source one germinated better than eight year old seeds and seeds with unknown age from the same species (*C. subternata*). Smoked paper disk treatment was either equal or less than the control regarding percentage germination in seeds older than one year regardless of the species. In seed source one, smoked paper disk was significantly better than the control suggesting that seed age might be an important factor in germination of these seeds. Seed germination generally started after two to four days of seed placement in most treatments.

5.5 REFERENCES

ALIERO, B.L., 2004. Effects of sulphuric acid, mechanical scarification and wet heat treatments on germination of seeds of African locust bean tree, *Parkia biglobosa*. *Afr. J. Biotech*. 3(3), 179-181.

BROWN, N.A.C. & BOTHA, P.A., 2004. Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region. *SA. J. Bot.* 70(4), 559-581.

BROWN, N.A.C., JAMIESON, H. & BOTHA, P.A., 1994. Stimulation of germination in South African species of Restionaceae by plant-derived smoke. *Pl. Growth Regul*. 15, 93-100.

COWLING, R.M., HOLMES, P.M. & REBELO, A.G., 1992. Plant diversity and endemism. *In*: R.M. Cowling (ed.). The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxford University Press, UK.

DE LANGE, J.H. & BOUCHER, C., 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. S*A. J. Bot*. 56, 700-703.

DHARMANANDA, S., 2004. Honeybush: Healthful beverage tea from South Africa. Institute for traditional medicine, Portland, Oregon, USA.

DIXON, K.W., ROCHE, S. & PATE, J.S., 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* 101(2), 185-192.

DUGUMA, B, KANG, B.T. & OKALI, D.U.U., 1988. Factors affecting germination of *Leucaena leucaceophala*. *Seed Sci. Tech*. 16, 489-500.

DU TOIT, J., JOUBERT, E. & BRITS, T.J., 1998. Honeybush tea - a rediscovered indigenous South African herbal tea. *J. Sust. Agric*. 12, 67-84.

HOPKINS, B.F., 1923. The behavior of hard seeds of certain legumes when

subjected to conditions favourabe for germination. *Proc. Assoc. Anal. North Am.* 1923, 46-48.

INTERNATIONAL SEED TESTING ASSOCIATION, 1999. *Seed Sci. & Technol*., 27. Supplement.

LUTE, A.M., 1927. Alfalfa seed made permeable by heat. *Science* 65, 166.

MC NAIR, A.D., 1917. Boiling buffalo seed. *Science* 45, 220-221.

MUHAMAD, S. & AMUSA, N.A., 2003. Effects of sulphuric acid and hot water treatments on germination of tamarind (*Tamarindus indica* L.). *Afr. J. Biotech*. 2 (9), 276-279.

PELA, Z., GERASOPOULOS, E. & MALOUPA, E., 2000. The effects of heat pre-treatments and incubation temperature on germination of *Cistus creticus* seeds. *Acta Hort*. 541, 365-372.

RINCKER, C.M., 1954. Effect of heat on impermeable seeds of alfalfa, sweet clover and red clover. *Agron. J.* 46, 247-250.

RIVAS, M., REYES, O. & CASAL, M., 2006. Influence of heat and smoke treatments on the germination of six leguminous shrubby species. *International Journal of Wildfire* 15, 73-80.

SAS, 2000. SAS/STAT Users Guide, Version 9.1, First Edition, Volume 2. SAS Institute Inc., Cary, NC, USA.

SCHUTTE, A.L., 1995. A taxanomic study of the tribes Podalyrieae and Liparieae (Fabaceae). Ph.D. Dissertation, Rand Afrikaans University, Johannesburg, South Africa.

SHAPIRO, S.S. & WILK, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591-611.

SUTCLIFFE, M.A. & WHITEHEAD, C.S., 1995. Role of ethylene and short-saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. *J. Plant Physiol*. 145, 271-276.

TEKETAY, D., 1996. The effects of different pre-sowing seed treatments, temperature and light on the germination of five *Senna* species from Ethiopia. *New Forests* 11, 155-171.

VALBUENA, L., & VERA, M.L., 2002. The effects of thermal scarification and seed storage on germination of four heathland species. *Plant Ecol*. 161, 137-144.

WALKER, M.A., ROBERTS, D.R., WAITE, J.L. & DUMBROFF, E.B., 1989. Relationships among cytokinin, ethylene and polyamines during the stratification-germination process in seeds of *Acer saccharum*. *Physiol. Plant* 76, 326-332.

WHITEHEAD, C.S. & NELSON, R.M., 1992. Ethylene sensitivity in germinating peanut seeds: The effect of short-chain saturated fatty acids. *J. Plant Physiol*. 139, 479-483.

WHITEHEAD, C.S. & SUTCLIFFE, M.A., 1994. Effect of low temperatures and different growth regulators on seed germination in *Cyclopia* spp. *Plant Physiol*. 147, 107-112.

CHAPTER 6

GENERAL CONCLUSIONS

Cyclopia is a very distinct genus of the Podalyrieae and is classified as a member of the Fabaceae family. The species belong to the Cape fynbos biome and grow in the coastal districts of the Western and Eastern Cape Provinces of South Africa, from Darling to Port Elizabeth, being bounded in the north by the Cederberg, Koue Bokkeveld, Klein Swartberg, Groot Swartberg and Kouga mountain ranges. More than 20 species of *Cyclopia* have been described and the species are found to be fairly localized. The foliage, fine stems and flowers of the *Cyclopia* are used to manufacture a beverage and a honey-like flavoured infusion known as honeybush tea. Honeybush tea has become internationally recognised as a substitute for ordinary tea like *Camellia sinensis* due to its health benefits. There has been a dramatic growth in the use of honeybush tea over the last few years and the industry has a huge potential in the herbal tea category as it has little competition from other countries. The industry also shows the potential to position the tea within the major food trends as an organic specialty health product and value-added products such as ice tea, green tea and baby products.

To date, there's very little information published on agronomic or production practices of the crop. Previous research and funding was directed more towards chemical and medicinal properties of the tea. The main objective of this study was to evaluate the effect of species (*Cyclopia intermedia* and *C. genistoides*), cutting position (terminal or sub-terminal) and rooting hormones for three seasons (summer, winter and spring) on rooting of *Cyclopia* cuttings, the effect of an organic plant fertilizer on growth and establishment of rooted cuttings of *Cyclopia* species, as well as the influence of different seed pre-treatments on germination of seeds of *Cyclopia* species.

Cuttings of two *Cyclopia* species (*C. intermedia* and *C. genistoides*) with two cutting positions (terminal and sub-terminal) treated with different rooting hormones were rooted under glasshouse conditions where night and day temperature of the glasshouse was controlled. Intermittent mist was used as means of moisture supply to the cuttings for 45-60 seconds every 30 minutes daily. The results revealed that *C. genistoides* rooted significantly better compared to *C. intermedia* as measured by rooting percentage, number of roots per cutting, length of longest root and mean root length during the summer season. Cutting position had a significant effect on rooting of the cuttings in summer compared to winter and spring season. Cuttings taken in summer rooted better than cuttings taken either in winter or spring irrespective of the species. The interactive effect of species, treatment and cutting position resulted into 86.00% of rooting in summer from the terminal cuttings of *C. genistoides*, while only 4.00% was recorded as the highest rooting percentage in both winter and spring seasons. In terms of number of roots per cutting, 2 and 4 g L^{-1} IBA resulted into the highest number of roots (3.28 and 3.64) respectively from terminal cuttings of *C. genistoides* and were not significantly different to each other. Similarly, the longest length of root per cutting (29.80 and 29.96 mm) was obtained from terminal cuttings of *C. genistoides* treated with 2 and 4 g L^{-1} IBA respectively.

The effect of an organic plant fertilizer (Nitrosol®) and cutting position on growth and establishment of rooted cuttings of *Cyclopia* species was studied in a glasshouse under controlled environment. Rooted terminal and sub-terminal cuttings from two species of *Cyclopia* (*C. intermedia and C. genistoides*) were transferred to pots (576 cm^3) and treated with Nitrosol® fertilizer at application rates of 3.33 $ml.L^{-1}$, 1.67 $ml.L^{-1}$ and 0 $ml.L^{-1}$ (control). *Cyclopia* cuttings were uniformly inoculated once with a symbiotic *Rhizobium* bacteria to enhance the formation of nodules. An organic plant fertilizer (Nitrosol®) at 3.33 $ml.L^{-1}$ significantly affected fresh and dry plant weight, fresh and dry root weight, number of shoots and nodules per plant compared to either 1.67 $ml.L^{-1}$ or the control. Nitrosol® at an application rate of 1.67 $ml.L^{-1}$ significantly improved the above growth parameters in comparison with the control. Relative to species, *C.*

genistoides performed better in terms of fresh and dry plant weight, fresh and dry root weight, and number of shoots and nodules per plant compared to *C. intermedia*. The origin of the cutting position did not significantly affect the above mentioned parameters. Plant mineral analysis revealed that most of the essential elements increased with increasing Nitrosol® application rates. Comparing the two species of *Cyclopia*, *C. genistoides* had higher levels of mineral elements than *C. intermedia*. This could be an indication of the differences between the two species in terms of nutrient uptake, utilization and distribution within the plant tissues.

In the germination studies, seeds obtained from different seed sources of *Cyclopia* species were subjected to different pre-sowing treatments. Seed treatments were sulphuric acid (95.00%), hot water (100°C), water with smoke paper disk, and demineralised water (control). The study revealed that all the treatments had a significant effect on germination with the exception of eight year old seeds obtained from *C. subternata* (seed source two). Although hot water treatment improved germination compared to smoked paper disk and the control, seeds treated with hot water degenerated rapidly. Sulphuric acid treatments at various exposure times gave variable results regarding percentage germination. Although the response to sulphuric acid treatments of between 10 and 25 minutes varied between different seed sources, sulphuric acid treatments in general seem to be more effective than both hot water and smoked paper disk treatments, but needs to be further verified. The effect of exposure time with sulphuric acid treatment on germination, varied depending on the age of the seeds. In one year old *C. subternata* seeds the highest overall percentage germination (77.33%) was found with ten and 15 minute sulphuric acid treatment, while eight year old seeds also indicated a good germination response. Smoked paper disk generally did not improve the rate of germination of honeybush seed, compared to the control, but in one year old seeds from seed source one, this treatment greatly influenced germination. This could suggest that seed age might have an influence on germination of seeds when applying smoked paper disk. In terms of germination rate, germination generally started after two to four days in most treatments.

Stem cuttings of *C. intermedia* and *C. genistoides* taken in summer rooted more easily than during winter or spring. Due to very poor rooting results with winter and spring cuttings these seasons are not recommended for the preparation of cuttings for these two *Cyclopia* species. A follow-up study on rooting of cuttings should thus evaluate rooting of cuttings taken in autumn. The use of terminal cuttings can be recommended over sub-terminal cuttings for *C. genistoides* as the former gave better rooting results than the latter. However, for *C. intermedia*, cutting position had no significant effect on rooting, suggesting that either cutting position can be used for rooting of this species. In terms of the rooting hormone treatments, 2 g L^{-1} IBA can be recommended for rooting of cuttings of *C. genistoides* as the highest overall rooting percentage (86.00%) was obtained from this treatment. For *C. intermedia*, it remains questionable whether propagation by cuttings is the best plant multiplication method as 46.00% was recorded as the highest rooting percentage from Seradix®.

In terms of the establishment of the rooted cuttings, Nitrosol® fertilizer at an application rate of 3.33 $ml.L^{-1}$ gave the best results relative to fresh and dry plant weight, fresh and dry root weight, plant height and number shoots and nodules per plant on either species. This treatment (3.33 $ml.L^{-1}$) can, therefore, be recommended for the establishment and growth of rooted cuttings compared to 1.67 $ml.L^{-1}$ and the control.

For germination studies, the results indicated that a higher overall percentage germination (77.33%) was found from seeds obtained from seed source one (one year old) compared to other seed sources (older than one year) when treated with sulphuric acid for either 10 or 15 minutes. This suggests that further studies on germination should be conducted using fresh seed material (one year old or less) for all the species evaluated.

In conclusion, propagation of *Cyclopia* species is feasible and with the expected development of selections and cultivars in the honeybush industry, plant multiplication using cuttings will remain valuable and a useful technique.