

**Sustainable harvesting of wild populations of *Cyclopia intermedia* in Kouga, Eastern Cape, South Africa.**

By

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# Declaration

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## Abstract

*Cyclopia intermedia* E. Mey is harvested from the wild to produce honeybush tea. The presence of a lignotuber and its slow growth seems to hinder the cultivation of this species and wild harvesting is likely to continue. Species distribution modelling indicated that *C. intermedia* has a climate envelope that spans from the Eastern Cape to the Western Cape along the Cape Fold mountains. This regional distribution is threatened under future climate change scenarios with a range loss and shift identified for climate conditions predicted for 2050. More specifically, its niche was identified as south-facing slopes with sandy to loamy sand soils and water holding capacity that is higher than the surrounding areas. Some keystone Fynbos species were found to share this niche: *Leucadendron salignum*, *Leucospermum cuneiforme*, *Protea neriifolia*, *Protea repens*, *Elegia filacea* and *Rhodocoma fruticosa*. *Cyclopia intermedia* grows throughout the summer with flowering buds developing in autumn. These enlarge during winter with the flowers opening in early spring. Pods develop in November and seed set occurs during December. Harvesting plants increases their fecundity with cut plants producing more than twice the number of pods and seeds compared to their non-harvested counterparts. This is because resprouting plants produce more flowering stems than uncut plants. Controlled harvesting or localised short-cycle burning could be considered as a management option to improve fecundity of declining *Cyclopia intermedia* populations in protected areas. *C. intermedia* harvesting could occur every third year and stem colour was identified as a measure of harvest maturity. Where at least 30% of the population has orange (RHS colour 163A) stems the population is suitable for harvesting.

Keywords: Honeybush, *Cyclopia intermedia*, harvest maturity, phenology, niche.

## Chapter 1: Introduction

Honeybush tea, a growing name in the herbal tea market, has a long history in South Africa (Joubert et al. 2011; Kies 1951). Species of *Cyclopia* Vent. (Fabaceae) were traditionally harvested from the wild for home use, but in recent times, honeybush tea has developed into a commercial product that has grown in demand worldwide (Du toit et al. 1998, Van der Walt 2000, Mbangcolo 2008). The industry, although not as large as that of rooibos, has grown considerably since the mid-1990s with this tea being sold in 25 countries (Joubert et al. 2011). Rooibos is the most well-known South African herbal tea and is produced from *Aspalathus linearis* (Burm.f.) R.Dahlgren, a resprouting plant species that is endemic to the Cederberg fynbos region (Louw 2006). Both the leaves and shoots of these plants are harvested to produce rooibos that has received much praise for its health properties (Louw 2006).

The dependence of the honeybush industry on wild populations that are prone to fire, and to some degree illegal harvesting, are some of the aspects that hamper the growth of the industry (Joubert et al. 2011). This problem has increased the urgency for research into cultivation opportunities and propagation methods for the various *Cyclopia* Vent. species from which tea is made (Mbangcolo 2008).

In 2010, cultivation consisted of about 200 ha of *Cyclopia genistoides* (L.) R.Br. and *Cyclopia subternata* Vogel, but as this alone is not enough to supply the demand, wild populations of *Cyclopia intermedia* E. Mey are used to supplement and contribute a large part of the annual commercial production (Joubert et al. 2011). As a result of this method of utilisation, the populations of these sprouters that are slow growing are under threat because of the unsustainable harvesting practices such as high frequency of harvesting. Various research projects, including this one, have been launched to investigate issues that will be useful in determining sustainable harvesting practises.

At the moment there are six processing facilities used within the industry. Most of the tea produced is sold in bulk and shipped overseas, there is however a recognition within the industry to produce products ready for the local market (Joubert et al. 2011). Most of the honeybush products sold are either mixtures of different *Cyclopia*

species or a mixture of honeybush and rooibos. Some selected brands (e.g. Melmont Honeybush tea) are produced from a single species, in this case only *C. intermedia* is used.

The demand for honeybush tea outstrips the current supply. The modern trend of health consciousness has increased its popularity, and the demand at local and international markets has grown (Du Toit et al. 1998). In response, local landowners have harvested the different types of honeybush tea from the Fynbos on their properties. The international demand far exceeds the supply from wild populations. In the absence of management guidelines for this species, the demand will lead to overexploitation of the natural populations and, if unchecked, localised extinction.

Honeybush tea is an indigenous South African herbal tea product that is produced from various *Cyclopia* species (Kies 1951, Du Toit et al. 1998). The name reflects the sweet honey-like smell of the plants used to produce this tea. The stems, leaves and flowers of the shrubs are used in tea production (Du Toit et al. 1998). According to Joubert et al. (2011) “the name ‘cyclopia’ is derived from the Greek word ‘cyclops’ meaning ‘round-eyed’, which refers to the circle-shaped depression or sunken area in the base of the calyx where the pedicel is attached to the yellow flower”.

The genus *Cyclopia* consists of 23 species that are all endemic to the winter rainfall region Fynbos biome (Schutte et al. 1995; Schutte 1995; Joubert et al. 2011). Many of the species in this genus are habitat specialists found in montane habitats and are restricted to mountain slopes and high peaks (Schutte 1995). According to Kies (1951): “The genus occurs in the coastal districts of the Cape Province, from Clanwilliam to Port Elizabeth, being bounded in the north by the mountain ranges – Koue Bokkeveld Mountains, Klein Swartberg, Groot Swartberg and Kouga Mountains, and the species seem to be fairly localised”. The concentration of *Cyclopia* spp. does however decline rapidly to the north of the Caledon district (Schutte 1995). Being part of the Fynbos biome, the seeds of *Cyclopia* spp. are predominantly dispersed by ants no more than five metres from the source (Slingsby and Bond 1981).

The genus consists of long lived perennials that may be sprouters or non-sprouters with a low leaf to stem ratio (Schutte 1997, Schutte and Van Wyk 1998) but the

number of sprouters in this genus decreases from west to east along its distribution (Schutte et al. 1995).

The Fynbos biome where *Cyclopia* is found is also known as the Cape Floristic Region and is classified as a biodiversity hotspot (Schutte 1995, Pierce and Mader 2006). The Fynbos biome occupies the southern tip of South Africa and is characterised by sclerophyllous leaves and open shrubland (Manning 2007, Paterson-Jones and Manning 2007). The distribution of different plant communities found in Fynbos is influenced by the proportion of rainfall, intensity of summer drought and the fertility of the soil (Manning 2007). Fynbos is typically found in areas with nutrient poor sandy and calcareous soil (Cowling & Holmes 1992; Manning 2007; Paterson-Jones & Manning 2007) and is described as a fire dependent vegetation type. As a result it is adapted to regenerate after fire (Manders and Cunliffe 1987, Holmes et al. 2000, Fourie 2005, Crosti et al. 2006, Manning 2007, Paterson-Jones and Manning 2007).

Fynbos is widely recognised as a Mediterranean ecosystem which is dependent on periodic fire for optimum survival (Schutte et al. 1995). Given the fire dependant or fire prone nature of Fynbos, members of the genus *Cyclopia*, like most other Fynbos species, adopted certain fire survival strategies (Schutte 1995, Manning 2007, Paterson-Jones and Manning 2007). Some of the strategies plants use to regenerate after a fire include sprouting from underground storage organs or fire-cued germination from seed; whichever strategy is used it would take approximately 4 years, depending on the type of vegetation, for the vegetation to reach the size it was before the fire (Manders and Cunliffe 1987, Manning 2007).

Manning (2007) and Van Wilgen (1987) found that most Fynbos species germinate from seed after a fire and very few species actually resprout from buried organs, but those that do are able to resprout, grow and re-establish faster. Manders and Cunliffe (1987) stated that frequent low intensity fires favour species that resprout and long fire intervals favour species that germinate from seeds. According to Cowling (1987) an understanding of how dependant non-sprouting species are on seed banks after a fire can provide insight into post-fire regeneration patterns.

Plants that regenerate from a lignotuber or a similar storage organ after a disturbance are termed sprouters or resprouters. The fire survival strategies largely

influence the life forms of the species in the genus *Cyclopia* (Schutte and Van Wyk 1998). Those species that cannot regenerate and are obligated to regrow from seed are termed non-sprouters. Resprouter species have underground lignotubers from which sprouting occurs after disturbance, often resulting in a multi-stemmed appearance (Schutte et al. 1995). Schutte et al. (1995) also noted a higher number of resprouters compared to non-sprouters in this genus.

Whether a species is a resprouter or not also affects other ecological adaptations such as population density, habitat specificity, relative regional abundance and seedling production (Schutte 1995). Non-sprouters have a higher population density than resprouters as they tend to occur in dense stands after a fire or disturbance in areas with high precipitation (Schutte et al. 1995). Resprouters on the other hand appear spread out in between other Fynbos vegetation in more arid environments (Schutte et al. 1995).

The seed of non-sprouting species has been shown to germinate faster than that of resprouting species (Schutte et al. 1995). The non-sprouting *Cyclopia subternata*'s initial seed germination occurred four days before the germination of the sprouting *C. intermedia* seeds. According to Joubert et al. (2011) and Schutte (1995) the seed coats of the species in this genus are hardened and require some sort of scarification (e.g. a natural fire) to allow germination. This is valuable information that must be considered when designing a management plan that includes manually restocking wild populations.

Some of the species in this genus might also resemble each other as herbarium specimens, but may have different growth forms as they have different fire-survival strategies (Schutte 1995). Morphological changes in a species occur on both spatial and temporal scales (Schutte 1995). This means that characteristics such as leaf shape, plant height and volume might differ between individuals of the same species depending on the locality where it grows.

Schutte et al. (1995) suggested that species with a non-sprouting life history are more likely to have a higher extinction risk when compared to their resprouting counterparts. The reason for this assumption is that, although the seeds of non-sprouters germinate at a rate considerably faster than that of resprouters; the resprouters have the advantage of storing photosynthates by nutrient allocation to



the storage organ, resulting in a longer lived population than when compared to the short lifespan of the non-sprouters.

*Cyclopia intermedia* is a strictly montane species and, as such, is unique within this genus (Schutte 1995). A generalist resprouter with a fairly prominent lignotuber, *C. intermedia* has a multi-stemmed shrub-like growth habit (Schutte 1995, Schutte et al. 1995). The leaves of *C. intermedia* are digitately trifoliolate and those produced from actively growing shoots are larger than the more mature leaves (Schutte 1995). When leaves are dead or drying they turn black in colour (Schutte and Van Wyk 1998).

The yellow flowers have a distinctly keeled lower calyx lobe (Plate. 1a) that is longer and often larger than the other petals (Schutte and Van Wyk 1998). The standard petal of the flowers of this species, as in the rest of the genus, has very distinct grooves (Plate. 1b) that Schutte & Van Wyk (1998) proposed would act as nectar guides. Being from the Fabaceae family the fruits of *Cyclopia* spp. are pods, which in the case of this genus ripen and burst open to disperse the seeds. Schutte (1995) reported a considerable variation in the morphology of the calyx and the bracts of *C. intermedia* between different populations across the distribution range.



Plate 1: a) The keeled calyx lobe and b) the distinctive grooves in the standard petal of the genus *Cyclopia*.

Two of the most popular species used for the production of honeybush tea are *C. intermedia* and *C. subternata*. Upon first inspection these species look identical. Upon further investigation there are a few subtle differences used to distinguish between them. The most obvious difference is that *C. intermedia* is a resprouter and *C. subternata* is a non-sprouter. Bearing this in mind, the location where the plant is found provides a useful clue as to its identity. *C. subternata* (also known as vleitee) is found in moist areas such as valleys or floodplains. *C. intermedia* (also known as bergtee), with its lignotuber, is found in much more arid conditions to protect the tuber from rotting. The other difference is in the morphology of the bracts. The bracts of *C. subternata* are pointy and in a rough V-shape (Plate. 2a), whereas the bracts of *C. intermedia* are more rounded and overlapped, forming a 'boat' shape (Plate. 2b).

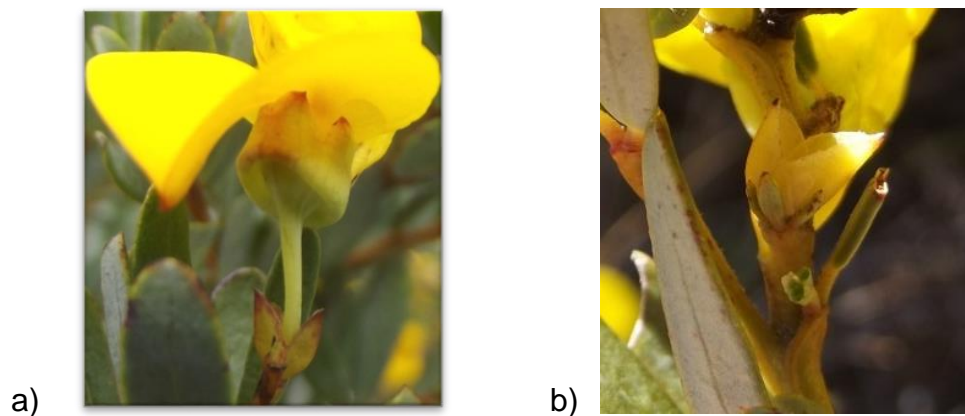


Plate 2: a) Bracts of *Cyclopia subternata*; b) Bracts of *Cyclopia intermedia*.

The sprouting and non-sprouting nature of some Fynbos species affects the harvest practices and intensities. Resprouters for example will continue to regenerate and resprout provided that the meristematic tissue has not been too badly damaged or harvesting has not occurred too frequently (Louw 2006). If resprouting plants are harvested too frequently the nutrients required by the storage organ will not be given sufficient time to be replenished. This leads to depletion of resources within the lignotuber (in the case of *C. intermedia*) and will result in increased mortality.

The slow growing nature of some *Cyclopia* spp. (longer than one year cycles) affects the time between the establishment of a plantation and the first time the plants can be harvested. This time delay along with the costs associated with the creation of a plantation is some of the aspects that hinder the cultivation of honeybush (Joubert et

al. 2011). Some cultivation efforts have been made. According to Joubert et al. (2011) the communities of Haarlem and Ericaville in the Western Cape have been engaged in cultivation and the establishment of a nursery in Genadendal. *C. maculata* and *C. subternata* have also been planted and a nursery established. These species have been selected for their relatively fast growing trait and the low selectiveness with regards to growth requirements. Joubert et al. (2011) suggest that “the honeybush industry has the potential to emulate the success of the rooibos industry”. However, if this is going to happen particular attention should be given to the current wild population and its utilisation.

Sustainable harvesting faces many challenges in the general scope of agricultural practices, even in the tea industry. In the case of both honeybush and rooibos tea there is an increased market demand for the product. This raises the concern that the pressure to supply this demand will lead to overharvesting and/or illegal harvesting of the resource (Louw 2006). The problem is that for both these products, overharvesting (both in frequency and in amount of foliage harvested) can cause a drastic decrease in the population size of wild populations and negatively influence their growth and survival. Because of this problem there is a need for studies to define the extent of the impacts of harvesting and by implication, set parameters for sustainable harvesting of the resource.

According to Louw (2006), the pressure to overharvest in South Africa is not only fuelled by the global market for the product but also by the reliance of people in rural areas on the resources for everyday sustenance. In some instances, the species in high demand have been cultivated to release much of the harvesting pressure but some markets or individuals prefer the wild populations to cultivated ones (Louw 2006).

Along with rare and endangered plant species the Fynbos biome is often victim of harvesting expeditions. The reason for this is that the unique environmental conditions of the Fynbos biome also produce high degrees of endemism and diversity. There are now several species in this biome that are commercially harvested. These include: Buchu (*Agathosma crenulata* (L.) Pillans), Honeybush tea (*C. genistoides*, *C. maculata*, *C. subternata* and *C. intermedia*), Rooibos tea (*Aspalathus linearis*) and various wild flowers (Proteas, Ericas and Pincushions) from

different species in different plant families, to name a few (Louw 2006). Even though some of these species have been successfully cultivated the supply is still in some cases supplemented with product procured through harvesting wild populations.

## 1.1 Objectives of the study

To date very little information is available about the ecology of *C. intermedia*. Valuable information is shared between farmers about agricultural practices but no scientific study has been done. The literature on *Cyclopia* spp. is predominately focused on the biochemical properties of the species used to produce the honeybush beverage. A baseline study is needed to, at the very least, identify areas where more research is required but also provide some initial findings as to what the ecology of *C. intermedia* entails across the distribution range from east to west.

### Research questions

Key questions investigated in this study are:

- Where does *Cyclopia intermedia* occur now (2000) and in the future (2050)?
  - a. Ecosystems
  - b. Habitats
  - c. Niches
- What is the status of the *Cyclopia intermedia* populations?
  - a. Abundance
  - b. Regeneration
- When do the important life history events of *C. intermedia* occur?
  - a. Phenology
- When should *Cyclopia intermedia* be harvested?
  - a. Harvest Maturity

## Research objectives

Using empirical field data the objectives of this study are to:

- Develop an ensemble species distribution map of *Cyclopia intermedia* under present (2000) and future (2050) climate conditions.
- Describe the ecosystems, habitats and niches where *Cyclopia intermedia* occur. This is done in terms of species composition and abiotic features.
- Produce harvest maturity indices that can be used to aid in sustainable harvesting of *C. intermedia*.
- Determine periods of importance in the life history of *Cyclopia intermedia*, e.g. flowering and seed set, growth, and dormancy. Also determine productivity of both harvested and non-harvested populations.
- To provide preliminary guidelines outlining the ecology and implications of this on the agricultural practices involved in the honeybush sector.

This study focuses on the little studied wild populations of *Cyclopia intermedia* and would ideally provide a contribution to the knowledge of their ecology and potential sustainable harvesting of *C. intermedia*.

## **Chapter 2: Literature review**

### **2.1 Species distribution modelling**

According to Franklin (1995) predictive mapping of species is defined as the prediction of the distribution of specific species across a specified area using environmental variables. This approach is often used in the field of biology (Robertson et al. 2003), where species distribution models (SDMs) are used to produce potential distribution maps of species (Fouquet et al. 2010). According to Elith & Franklin (2013) SDMs are empirical models that focus on the relationships between a species and its environment, species location and environmental data are used to produce these models. For the purpose of this study the term SDM will be used to refer to these models but the range of names by which SDMs are also known include: environmental models, predictive models, environmental niche modelling, and distribution models.

#### **Interfaces used for modelling**

The practice of SDM assumes that the extent of the distribution of a species can be predicted by using the known occurrence of a species and predictor variables that are associated with these occurrences (Elith et al. 2006, Elith and Franklin 2013, Hijmans and Elith 2013). According to Hijmans & Elith (2013) the process follows the following general steps: 1) known localities of a species are collected, 2) environmental variables are extracted from spatial databases for these localities, 3) the environmental variables are used to 'train' a model to predict the possibility of occurrence of the target species, 4) the model is projected onto a specified geographic extent (for example the Cape Floristic region).

#### **Data**

The locality data used for SDM are usually acquired from herbarium records or other sources where the data was not necessarily collected for the purpose of mapping (Elith et al. 2006). Many herbarium records are collected according to accessibility (Reese et al. 2005) and the most accessible areas for collection are usually next to roads. This presents a problem as roadside populations might not accurately reflect

the conditions under which natural populations occur (Reese et al. 2005). There may be a bias in the sampling records frequently used for SDM.

The locality data that is used in the modelling process can be either only 'presence' data or be both 'presence' and 'absence' data. Different algorithms use different data, some models might only use 'presence' data (BIOCLIM) whereas others might require both 'presence' and 'absence' data (GAM and GLM) to make a prediction (Elith et al. 2006, Hijmans and Elith 2013). The array of scripts written for SDM in R provide an opportunity to apply several different kinds of models, including those that use presence data and those that require presence and absence data. In the event of using herbarium records (only presence data), sites can be recorded as being absence records to complement the dataset. This is done by generating background points (pseudo-absences), which according to Hijmans & Elith (2013), are used to set up the area of interest and the presence sites are used to identify areas where the species has a higher probability to occur.

The first step is to enter predictor variables in raster format. Predictor variables can range from various climatic factors to soil characteristics or vegetation types. Most algorithms require rasters to be of the same format with regards to: extent, resolution, origin and projection (Hijmans and Elith 2013). Various functions are available in R to manipulate data to fit this format. The data is then split into a testing dataset and a training dataset, this is done with K-folding. K-folding involves the application of the testing dataset the model in an iterative approach (Hijmans and Elith 2013).

Once a model is fitted to the training data and tested using the testing dataset, it must be evaluated in order to determine how well the model can predict presence. Appropriate statistics can be generated to evaluate the model: AUC, Kappa statistics,  $D^2$ , ROC and cross-validation are some examples. If the AUC (area under the curve) statistic delivers a high value (close to 1) that this is indicative of a model where sites that have a predicted suitability closely match the known presence ones (Hijmans and Elith 2013).

A selection of 40 papers dealing with SDM (1998-2012) was reviewed for information regarding areas where SDM studies are done, models used, and how models were

evaluated. These papers were selected as they dealt with botanical and species specific mapping.

The most popular and widely used of these methods is the evaluation of the model using AUC values (Fig. 1). Very few predictive maps are evaluated by ground truthing (Elith et al. 2006). The reason for this is possibly that the scope and area of most predictive distribution studies is so large that is not financially feasible to physically ground truth a predicted area and such an exercise will take too long.

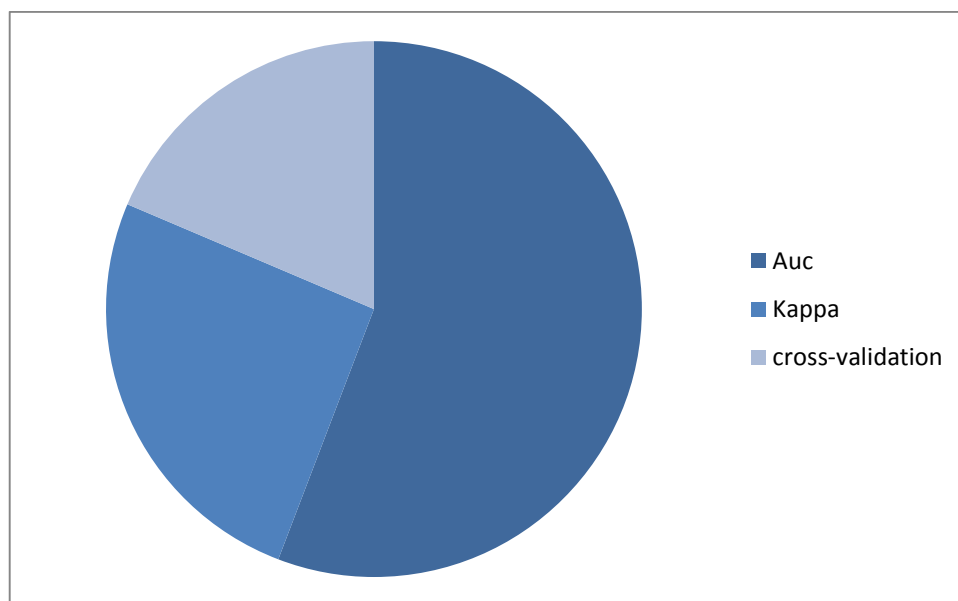


Figure 1: A summary of model evaluation methods extracted from 40 SDM papers focussing on botanical studies between 1998 and 2012 by determining the percentage of papers using the three top methods.

## Models

The latest models are composites of several approaches which mean that more information is used to produce a more detailed model. Most models use presence and pseudo-absence data together with predictor variables. Geographic models, by contrast, use locality records alone, without predictor variables to model distributions (Hijmans and Elith 2013).



Some of the more popular models / algorithms in recent publications include: BIOCLIM, Generalized Additive Models (GAM's), Maxent and RandomForests (Table 1).

Table 1: Some of the most popular algorithms used in recent SDM publications (the same ones as referred to in Figure 1), including the type of model and data used.

<b>Algorithm</b>	<b>Type of model</b>	<b>Data</b>
BIOCLIM	Climate envelope model	Presence
Generalized Additive Model (GAM)	Regression	Presence & Absence
Generalized Linear Model (GLM)	Regression	Presence & Absence
Maxent	Machine Learning	Presence & Absence
RandomForests	Machine Learning	Presence & Absence

The inventory of SDM publications (previously mentioned) also showed that the most popular models used are GAM and GLM approaches (Fig. 2). Other algorithms commonly used, in no particular order are Maxent, RandomForests, BIOCLIM, Logistic Regression and Classification Trees.

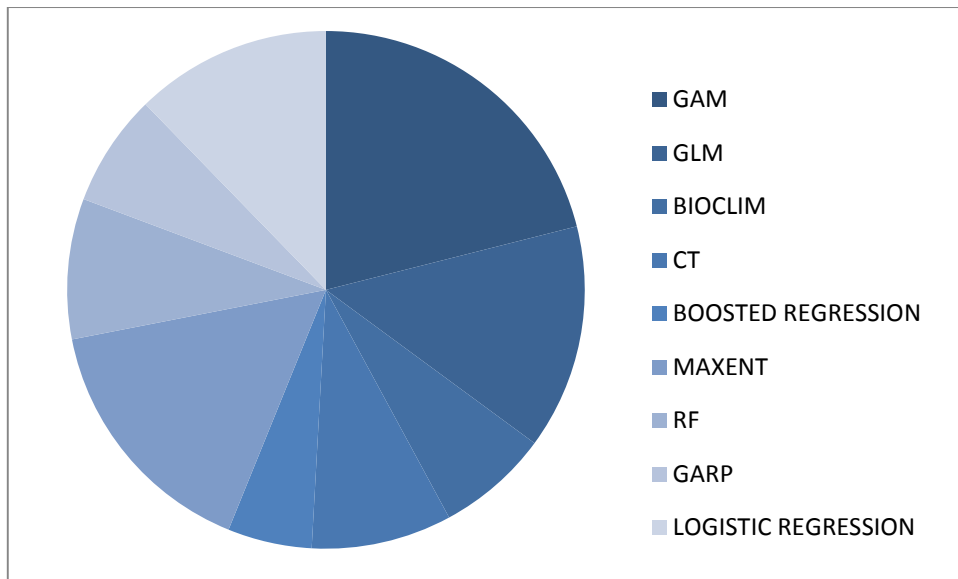


Figure 2: A summary of algorithms extracted from 40 SDM papers focussing on botanical studies between 1998 and 2012 by determining the percentage of papers that use each algorithm.

These models can all be used to predict landscape processes or outcomes over time with different variables that may influence the landscape. This can be done by determining the outcome of one area after some influence and then extrapolating it. Alternatively, a hypothetical outcome may be produced. SDM is often applied in the biological sciences as predictions may allow for management decisions to be made based both on areas that may host new populations or range shifts under future conditions.

The type of model that is used in the mapping of a species affects the quality of the projection of species distribution. Araújo & New (2006) noted that different models produce projections so variable that no definitive management decisions can be made if a multitude of models are attempted.

### Ensemble

The problem of variable projections from different models influences both present and future climate predictions and raises the question whether a single model is sufficient (Araújo and New 2006, Potts et al. 2013). A solution to this problem is a

proposed 'ensemble' approach (Araújo and New 2006), where different models are used and model uncertainty is evaluated. The idea is that a combination model would result in smaller errors and more consistency than a single model. The long-standing approach has been to pick models that statistically deliver the best prediction for the known data.

The approach proposed by Araújo & New (2006) is that no single model is selected but rather the range of predictions from different models are analysed. The method of consensus forecasting is used to determine the central tendency of the various models that constitute the ensemble (Araújo and New 2006). Consensus forecasting has been found to produce a result that is more accurate than any single model (McNees 1992). Another type of ensemble approach is that of probabilistic forecasts where the probability of occurrence is predicted. Araújo & New (2006) concluded that conservation decisions based on consensus or probabilistic forecasting are less likely to result in wasted resources by focussing on projections that provide a better understanding of results compared to decisions made from single forecasts.

### **Biodiversity modelling**

The loss of biodiversity on local and global scale has become a great concern in the modern study of science. Various factors influence the loss of biodiversity such as vegetation destruction, disturbances and climate change (Busby 2002, Prach and Walker 2011). These factors influence natural resources such as *Cyclopia intermedia* and the loss of biodiversity that is associated with this species would have detrimental effects on the industry. As these impacts increase, more legislation is developed to try to protect the remaining biodiversity. The mapping and modelling of biodiversity pattern contributes substantively to the success of the implementation of these laws (Busby 2002). The information produced can also be used by governments, local communities and individuals for management decision-making at various scales.

An evaluation of an area for conservation or, in the context of this study, harvesting of wild resources can be made by mapping attributes that form the basis of analysis of distribution data. SDM can then be done by manipulating the attributes that contribute to the current distribution pattern (Busby 2002; Skidmore 2002a; Woodcock et al. 2002).

Escudero, Iriondo, & Torres (2003) show that even genetic diversity may be expressed at different spatial scales and can be related to environmental influences. Knowledge of how factors affect biological systems is required to produce appropriate conservation targets and management plans. These factors include gene dispersal, pollinators and habitat availability amongst others (Escudero et al. 2003, Elith et al. 2006, Hijmans and Elith 2013). According to Escudero et al. (2003): "There is an urgent need to integrate the knowledge derived from genetic, demographic and ecological approaches to species conservation in order to be able to formulate management strategies that take into account all the different considerations".

Considering that the climate change paradigm is now accepted by the scientific fraternity, increasing emphasis is being placed on how natural systems will be affected. It is generally accepted that the distribution ranges of species or populations might change slowly over a long period of time, but will be required to change rapidly when directly affected by human actions (Araújo and Rahbek 2006). One of the major concerns regarding sessile organisms such as plants, is that they might not be able to adapt at a rate fast enough to allow for the survival of entire populations. The theory is that with a shift in the climate regions there will be a shift in the geographical distribution or ranges of species (Foden et al. 2007) and the populations or individuals on the edge of the distribution might suffer extinctions (Davis and Shaw 2001). Jordan & Nobel (1979) suggested that these extinctions will be more noticeable in extreme habitats or climatic conditions such as deserts or small spatial-scale niches, such as are common on the Cape Fold mountains (Cowling and Pressey 2000). Assuming range shifts, we must also deduce that there will be areas where extinction might occur and there may be areas where expansion of the population might be possible. There is however the real possibility that the rate of extinction might be faster than that of expansion. The velocity of change was found to be slower in mountainous areas and faster in grassland biomes which are at lower altitudes (Loarie et al. 2009). This could mean that the mountainous habitat of *C. intermedia* would not be extremely affected by a changing climate.

Midgley et al. (2003) addressed the issue that climate change is possibly influencing the distribution of species and by implication, our conservation efforts will have to change. More evidence suggests that human-induced climate change results in

changes in individual species distributions rather than changes occurring at a biome scale (Midgley et al. 2003; Foden et al. 2007). SDM is a valuable tool that can be used to inform management decisions and conservation efforts on various spatial scales (Midgley et al. 2003). Modelling of distributions under different climatic conditions can aid in the understanding possible range shifts or amendments to future habitats of species (Midgley et al. 2003; Martinez-Meyer 2005).

Following reports of population die-back, Foden et al. (2007) used historical and recent *Aloe dichotoma* distributions to describe the impact of regional climate change on the species. A detailed census of localities of *A. dichotoma* across its geographical range as well as predicted expansion extent was done. These localities were used to model the future distribution of this charismatic and keystone species. A general additive model (GAM) was used to produce a bioclimatic model for both present and future climates; the latter was predicted at a spatial resolution of 10 arc-min intervals. The results of this study showed that the population die-back is more likely to occur in the north of the species range, and at lower altitudes.

Midgley et al. (2003) modelled both biome and species level reactions to climate change and possible range shifts of the Fynbos-important Proteaceae family. Climate parameters that are thought to influence physiological function of Fynbos species were identified and used to construct climatic envelopes that could be used to build the models. The parameters used by Midgley et al. (2003) were: coldest month, heat units, potential evaporation and winter soil moisture days. Along with climatic parameters, Midgley et al. (2003) used three soil variables, namely fertility, sand and clay content. They identified these variables to be sufficient to address soil nutrient and water availability. Using these, they predicted that the Fynbos biome could lose large areas in the northern limits of the biome (Midgley et al. 2003). This implies a potential retraction of the biome to the south and a possible restriction to the Cape Fold mountains. The problem associated with this prediction is that the unique niches along the plains and slopes of the lower altitudes might not be suitable for all species in the biome, resulting in species extinction. However, the model suggest that these sensitive areas should be classified as areas of conservation concern (Midgley et al. 2003; Foden et al. 2007). Midgley et al. (2003) claimed that any future predictions made for the CFR will be influenced by plant water stress as the higher temperatures are expected to cause drier conditions.

Midgley et al. (2003) modelled 28 species in an area of predicted biome loss to identify the greatest risks that species would face. The major risks were identified to be range elimination, reduction and shift. Range elimination affects the species that have no suitable habitat under the predicted climate. However, Midgley et al. (2003) suggest that a loss of geographic range might not necessarily mean immediate extinction. Possible survival could be incorrectly predicted as a result of the risk factor being determined by sensitivity of seedlings. Adult plants might still be able to survive even though the suitable habitat has shifted. Plants with different life history strategies might be affected differently, where seeders might die out while resprouters survive (Midgley et al. 2003). A range gain was predicted for 39% of the species modelled, even though most species are predicted to suffer a range loss. This shows that depending on the species, future climatic condition might have either positive or negative effects. It was also suggested that the coarseness of the soil data sets could have led to optimistic values with regard to range predictions.

Areas that show overlapping distribution ranges for models using current and future climates can be considered as habitats that are at least risk for the future (Midgley et al. 2003; Foden et al. 2007). The lowlands of west coast Fynbos have been identified as habitat with the greatest risk under future climatic conditions (Midgley et al. 2003). They recommend that distributions montane species using future conditions are most important to determine the level of risk these species face. This will aid in the conservation planning as species-level focus might be significant in identifying the effects of climate change.

If the possible range restriction for species under future climates is known, protected areas can be focussed on the areas with the lowest risk and harvesting could be allowed in areas of high risk (Midgley et al. 2003).

## **2.2 Phytosociology**

As people are continuing to change natural environments to fit their needs, ecosystems are destroyed and species are lost (Brown and Bezuidenhout 2005). The only way to effectively protect any environment is to know what it is being protected. In the case of biology, what plant and animal species are found in a

specific area and their conservation status are the most important. Phytosociological studies provide the opportunities to compile species lists of specific areas that can be used to properly manage an area of importance.

Phytosociological studies are useful in identifying the community structure of a certain area (Cowling and Campbell 1983). According to Brown & Bezuidenhout (2005) an inventory of flora will aid in the management and also the detection of changes in a certain area. The reason for this is the fact that any changes in environmental conditions can be seen in the changes in vegetation. In most phytosociological studies the general community structure and the environmental factor influencing them are the key focus. The communities are usually named after the dominant species within that community.

McKenzie et al. (1977) identified different types of species specific Fynbos in Orange Kloof but the dominant Fynbos families of *Ericaceae*, *Restionaceae*, and *Proteaceae* were all present and the presence of nutrient poor soils confirms typical Fynbos conditions. In the Borakalalo nature reserve species from one specific class were found to characterise the entire area. According to Brown & Bredenkamp (1994) these species can be considered to be the common species of the area. Brown & Bezuidenhout (2005) identified diagnostic/specific species; these are species that are only found in a single community.

The methodology used in phytosociological studies is fairly uniform with small differences in quadrat size depending on the type of vegetation that was studied. The size of the quadrats are chosen to enable description of both floristic and habitat homogeneity. For Fynbos vegetation the quadrat size ranged between 10 x 10 m (McKenzie et al. 1977), 40 x 10 m (Brown and Bezuidenhout 2005), 10 x 20 m (Brown and Bredenkamp 1994) and 6 x 5 m (Brand et al. 2008). This collection of floristic data has been found to be more effective than structural surveys when the aim is to understand relationships between the vegetation and the environment (McDonald et al. 1996).

The Braun-Blanquet technique has been found to be very successful (McKenzie et al. 1977, Brown and Bredenkamp 1994, Brown and Bezuidenhout 2005, Brand et al. 2008) for the classification of dominance. McDonald et al. (1996) warns about the influence of fire on vegetation structure, where the structure will be more affected

than the floristics. As the focus is largely community identification techniques such as TWINSpan (Brown and Bredenkamp 1994, Brown and Bezuidenhout 2005, Brand et al. 2008) has also used to aid the Braun-Blanquet technique. Brown & Bredenkamp (1994) reported very similar community delineation using TWINSpan and Braun-Blanquet.

The general environmental data used in phytosociological studies include: slope, aspect, soil depth, rockiness, and notes on geology (McKenzie et al. 1977, Brown and Bredenkamp 1994, Brown and Bezuidenhout 2005). A complete set of environmental data can also be used (McDonald et al. 1996) involving various non-soil abiotic and soil variables.

A common problem encountered in these types of studies is that all species collected might not be identified to species level. The reason for this is that most phytosociological studies consist of once off sampling and there are some species that will not be flowering. This makes for very difficult identification and often samples are only identified to family or genus level (McKenzie et al. 1977, Brown and Bredenkamp 1994, Brown and Bezuidenhout 2005).

According to McKenzie et al. (1977) the dominant environmental variables that seem to affect the distribution of forest, Fynbos and shrub communities are the degree of protection against fire, altitude and geology. Fynbos communities are most affected by geology and aspect (McKenzie et al. 1977) but McDonald et al. (1996) identified percentage carbon and soil texture as influential variables.

## **Soil**

According to Winter (1974) "soil is a complex mixture of inorganic and organic material usually containing a rich variety of living and dead organisms including bacteria, fungi, both uni- and multicellular nematodes, molluscs, insects, annelids and higher animals".

Soils are typically classified using characteristics such as series, texture, horizons, colour, and soil water potential to name a few (Ward 1962, Winter 1974, Rose 2004, Van Huyssteen et al. 2005). Soil characteristics have a pronounced influence on the vegetation that is associated with it (Paudel and Sah 2003). The soil series refers to the composition and the geological origin of the soil (Winter 1974, Rose 2004). Soil



thickness is related to the environment in which it is found. Areas of high elevation or slope will have shallow soil compared to low lying areas such as swamps (Winter 1974). Soil texture refers to the size of the particles or components that make up the soil and is one of the most important characteristics used to classify soils (Winter 1974). The size ranges and names of the soil components vary slightly depending on which literature is consulted. The most common particle size classes used throughout literature are presented in Table 2.

Table 2: Most common particle names and sizes for the classification of soil texture (Winter 1974, Rose 2004).

<b>Name</b>	<b>Size in mm</b>
Clay	< 0.002
Silt	0.002 - 0.02
Fine sand	0.02 - 0.2
Coarse sand	0.2 - 2.0
Gravel	> 2.0

Permanent wilting point and field capacity are related features of soil water. Permanent wilting point refers to the stage at which the water content in the soil is so low that the tension exerted by the water molecules to stay attached to the soil particles, exceeds the absorption forces of the vegetation (Rose 2004) and water cannot flow between the soil and the roots. This means the water loss from transpiration will exceed the water uptake, which leads to the wilting of the vegetation (Winter 1974).

Field capacity can be described as the upper limit of available water and is the water that is available once the suction from gravity and lower unsaturated zones has extracted the excess water (Strangeways 2003, Rose 2004). The reason for this suction is the fact that saturated soils are unstable and all the layers in a soil profile will probably not have the same level of saturation, thus excess water is distributed between soil layers (Winter 1974). According to Winter (1974) this movement of water is achieved by the hydrostatic negative pressure that is found between the saturated layer and the other less saturated layers/zones. The water will continue to

drain from the saturated layer, replacing the pore spaces with air where the water has been removed (Strangeways 2003). This process will continue until the drainage seems to stop or be reduced, this is referred to as field capacity (Winter 1974). Winter (1974) mentions that drainage never really stops and thus the measure of field capacity is meaningless.

In the light of the above mentioned field capacity and permanent wilting point it can be deduced that the difference between these two extremes produce the available water capacity of the soil (Winter 1974, Strangeways 2003). This is the soil water that is available to the vegetation. The composition of the soil will naturally affect its water holding capacity. Soils with higher percentages of silt and clay will have a higher field capacity than soil composed mostly of sand (Winter 1974). This water might not be as easily extracted as in sandy soils and the plants might not grow as fast but the water source will last longer (Winter 1974).

The available water and field capacity of a certain soil type is of great importance in the field of agriculture where it directly links to the irrigation of crops (Winter 1974, Strangeways 2003, Rose 2004). The specific variables for each type of crop plant and soil type are known to aid in irrigation practices. This is however not the case for economically viable species harvested from the wild. The moisture requirements of wild plants are not known with as great a degree, if at all, in the case of *Cyclopia intermedia*. This results in the need for botanical studies to include soil analyses to help in the understanding of vegetation-environment interactions.

If extensive testing is required a larger sample might be required. The samples might be collected with a range of different equipment of which an auger or a hand shovel is most common. McDonald et al. (1996) reported shallow soils which resulted in soil samples only being collected in the A-horizon the depth of the samples varied between a few centimetres and 0.3 m.

### **2.3 Phenology**

The phenology of a plant is the external expression of resource use (Pierce 1984), encompassing the seasonal changes in resource use and allocation within a plant

(Rathcke and Lacey 1985, Spano et al. 2003). The different phenophases of a species are influenced both by the genetics of the species and the surrounding environmental conditions (Vachun 2003).

Phenological studies in ecosystems with varying climates such as the Mediterranean climate of the Fynbos biome are important and are used to identify the differences within the different areas (Berjano et al. 2006). Historically phenological studies were used to identify life history stages and develop methods to produce more stable crop yields with greater quality (Ruml and Vulic 2005).

### **Phenology and management**

From a management point of view, information on the phenology of a species is particularly useful as it documents important life cycle events such as: growth, flowering and fruiting (Pierce 1984, Louw 2006). Phenophases can be separated according to the end result. Growth phenophases, for instance, include leaf growth, leaf elongation and new leaf production, whereas reproductive phenophases include flowering, fruiting, and seed set (Louw 2006).

Although this is useful, information on phenophases alone is not enough for management procedures and the accompanying environmental data linked to each phenophase is also required (Pierce 1984, Vachun 2003, Litschmann et al. 2008). Environmental conditions could, along with timing and duration of phenophases, influence the number of flowers and fruits that are produced (Mcgregor 1981). The life history of a species should also be taken into account as resprouters might produce certain phenophases at a different time compared to seeders, and the intensity of the reproductive phases could also be affected (Louw 2006).

The use of phenology in both the horticulture and agriculture sectors has many values. In agriculture, for example, knowledge on the timing of specific phenophases can be vital for crop management and might be used to improve both yields and product quality (Chemielewski 2003, Litschmann et al. 2008).

### **Lignotuber phenology**

Many Fynbos species show a growth peak during summer and autumn (Pierce 1984). In a study on *Aspalathus linearis*, a plant with a similar life history strategy to *Cyclopia intermedia*, Louw (2006) reported flower production for both heavy and

moderately harvested wild and cultivated populations between October and February. The populations on the southernmost site produced flowers one month later than their northern counterparts. During October and November immature fruits were recorded on moderately harvested individuals, this was repeated in January and February the following year.

Populations of *A. linearis* showed increased growth in January and February. Louw (2006) reported the plant volume to stay unchanged for the first five months after the initial harvest, but found a volume increase between the months of August and December. Rainfall, although it had an influence on regrowth had no direct effect on the plant volume. Leaf loss was found for both heavy and moderately harvested individuals between April and July, with the moderately harvested population showing signs of another abscission period in November and December.

This information has been used to inform decisions regarding harvest height and timing in *A. linearis* (Louw 2006) so that good quality plant material could be provided for the production of the desired product and also the continuity of the population.

### **Measurements of phenology**

Phenophases can be studied in either a qualitative or quantitative manner (Pierce 1984). Simple phenograms showing which phenophase is recorded in each month is an example of the qualitative representation. Quantitative observations can be presented as bar graphs showing percentages of individuals in a population undergoing a certain phenophase. This quantitative approach provides more information about variation within a population.

According to Pierce (1984), various factors can be measured when focussing on the phenology of a species. This includes actual growth, which is expressed as leaf area per shoot, but comparing this characteristic between individuals would be difficult if the number of shoots differ between individuals (Spano et al. 2003). This is especially important in the case of *Cyclopia intermedia* where the number of stems differ dramatically from one individual to the next. The measuring of shoot elongation is also a problematic characteristic as it may be difficult to distinguish the shoot tip from apical buds (Pierce 1984). It is for this reason that new growth was simply

reported and not measured. Whichever characteristics are used to study the phenology it should be kept in mind that the growth of any species is variable and depends on the rainfall and environmental conditions of the specific year. It is this variability that resulted in the suggestion (Pierce 1984) that between 200 and 2000 shoots or individuals should be sampled to produce an acceptable confidence level for the data. The sampling of this amount of plants is only possible if the wild populations are large enough.

According to Pierce (1984) different phenophases such as shoot elongation, bud development, seed formation and maturation are sometimes complicated to interpret in the field. Because of this it is recommended that phenology studies should be done over a period of two years. This is to ensure that knowledge of a species' seasonality is gathered and to guarantee that the single growth flush characteristic of some Fynbos species is not missed (Pierce 1984).

The accuracy of growth measurements also depends on the individuals measured. To accurately compare measurements the same individuals should be tagged and measured every month. But death and predation make this a very difficult task, and the same individuals are rarely measured. According to Pierce, (1984) there is no standard method for the data collection with regards to the phenology of Fynbos species as the growth of species in this biome is so varied.

The timing at which a specific phenophase occurs depends on various environmental factors such as temperature, rainfall, soil moisture and texture, biological components including pollinators and predation and competition for resources (Pierce 1984, Aasa et al. 2004). According to Pierce (1984), the following environmental characteristics were found to be controlling factors of phenophases in some Fynbos genera: "soil moisture depletion, minimum air temperature, favourable soil temperature, low soil and air temperature, diurnal temperature, insulation, relative humidity and lowered starch reserves". Spano, Snyder, & Cesaraccio, (2003) stated that the climates in Mediterranean regions vary a lot from one year to the next.

In agriculture specific scales are used for phenological observations, depending on what commodity is being observed (Chemielewski 2003). According to Chemielewski (2003) the first widely used phenological scale used was the Feekes scale (Feekes

1941, in Chemielewski 2003), which was developed for wheat. This scale was modified over the years to be used in other crops and commodities. The general scale used today is the intricate BBCH scale developed by Strauss et al. (1994, in Chemielewski 2003), and can be modified to suit a specific species.

### **Phenology and harvest time**

Fynbos, like all other Mediterranean ecosystems, is adapted to periodic fire events. This adaptation includes different life history strategies such as seeders and resprouters (Bond and Van Wilgen 1996). Studies done on *Aspalathus linearis* (Louw 2006) and other species of Mediterranean ecosystems (Canadell et al. 1991, Canadell and Lopez-Soria 1998) highlights the importance of the role of the lignotuber in phenophase development.

The phenophases of a plant represent the morphological expression of available resources. As a result, this information can be used to manipulate the plant to encourage growth. Knowledge of phenology can also be used to make informed decisions on when the best time is to irrigate, harvest, and burn or fertilise if necessary (Chemielewski 2003).

## **2.4 Harvest maturity**

Maturity is the product of the development process that any plant goes through during its life cycle. In plants two types of maturity can be identified: physiological maturity and horticultural maturity. The physiological maturity is the stage in a plant's life cycle where the plant part (fruit) will continue to develop even after being detached (Kader 1999). Horticultural maturity is the stage at which the plant part has reached the optimum phase required for utilisation and commercial use.

Lee & Young (1983) suggested that horticultural maturity is related to physiological maturity, as the completion of general plant growth results in the production and maturation of fruit and other commodities used in the agricultural sector.

The level of maturity of a crop at harvest is of great importance as it will affect the storage-life of the commodity as well as the end quality of the product (Kader 1999).

In the case of *Cyclopia intermedia* the level of maturity will not only affect the quality of the tea produced but also the survival of the population. The lignotuber that is characteristic of this species is dependent on the translocation of photosynthates from the shoots and stems to the storage organ. Enough time should be allowed for this process to ensure sufficient reserves have been accumulated for the survival of the plants after harvesting.

The initial qualities of the commodities very much depend on the end use. Fruits, vegetables and other plant products intended to be sold as fresh may generally be of a higher quality and may be picked at an earlier age than those intended for processed products (Kader 1999). From a marketing point of view, consumers judge fresh produce and products by various standards. The initial impression, e.g. firmness and freshness, determines whether the product is bought, whereas the quality and taste of the product determines whether the consumer will buy the product again (Kader 1999). The quality and taste of the honeybush tea will be influenced by the plant material, the processing and its fermentation. The age of the plant at harvest is believed to affect the flavour of the tea (Nortje, pers comm., 2012), much like letting fruit ripen on a tree.

A simple, reliable method to determine maturity in crops has been the focus of many horticultural studies (Crisosto 1994, Kalt et al. 1995, Bourland et al. 2001, Muchui et al. 2010, Lysiak 2012, Olasoji et al. 2012). As properly mature commodities are the aim for agricultural practices and economic industry, there is a need to develop methods to determine the maturity of specific crops (Crisosto 1994). This is done by either by developing new parameters or using already developed maturity indices and applying it to different crops.

The realistic scenario of maturity indices is the fact that they must describe a maturity state that is a compromise between best eating quality and the most desirable state that would allow for timely marketing and transportation (Kader 1999). According to Crisosto (1994) “for maturity measurements to be carried out by producers, handlers, and quality control personnel they must be simple readily performed in the field or inspection point, and should require relatively inexpensive equipment”. These tests must also be an empirical measure rather than an objective evaluation to assure proper and uniform management (Crisosto 1994).

Crisosto (1994) stated that maturity indices should meet certain requirements for all parties that might use or rely on them. These include that the minimum consumption quality is insured and that the products will have a long enough storage life after harvesting. In the case of *C. intermedia* tea the requirements are a good quality product and the ensured survival of the resprouting populations.

Maturity in some fruit crops is indicated by changes in surface colour from green to which ever colour is expected of the fruit, e.g. red in the case of tomatoes or yellow in lemons. This progression in colour might also be expressed in the colour change of the stems of *C. intermedia*. Younger stems have been observed to be a different colour than the stems experienced farmers deem fit for harvest.

In the South African thatching reed industry two species of *Thamnochortus* are used: *T. insignis*, a reseeded, and *T. erectus*, a resprouter (Campbell 2006) with a similar growth form as *C. intermedia*. According to Campbell (2006) cycles of both fire and harvesting are important disturbances in the life cycles of these thatching species. *Thamnochortus* spp. are harvested once the nodes on the culms have hardened and this occurs after seed release. The plants are cut in a manner that will not damage the new culms (< 5 cm) that are present at time of harvesting. According to Linder (1990) the plants can be harvested seven years after germination and regenerate from a disturbance after a short amount of time, but cutting intervals have been suggested to be three to five years (Long 1978, Campbell 2006).

*Aspalathus linearis*, used in the production of rooibos tea, is another commercial species with similar growth characteristics to *C. intermedia*. The harvesting practice for this species involves cutting the whole bush a few centimetres from the ground during the summer and partially into the autumn (Joubert & Schulz 2006). As most of the *A. linearis* is harvested from established cultivations, the age of the plants are known and thus plants can be harvested at an age of 18 months with no other physical characteristics required to determine harvest maturity. With wild *C. intermedia*, the age of the plant might not always be known (regrowth from the lignotuber might carry on for years if managed properly) thus some physical morphological characteristics are needed to determine age and maturity.



## **Visual indices**

Visual indices are the easiest of characteristics to use as a measurement of maturity. This is because pictures or descriptions of the physical appearance of commodities can be used by any person dealing with the harvesting of produce.

### **Fruit size**

Fruit size is regularly used as a maturity index but this feature cannot be used on its own as the fruit size can be influenced by varying climatic conditions and cultural practices (Crisosto 1994). A single maturity index might, in most cases, not be enough to determine the level of maturity as this might be affected by climate or farming practices. Thus it is suggested that a combination of characteristics be used as maturity indicators (Kader 1999).

Kalt et al. (1995) suggested that when working within a cultivar the diameter of a berry shows a stronger correlation with sugar content than surface colour, thus diameter is more useful in determining ripeness than surface colour. Wild blueberries also showed weaker correlations than cultivated cultivars. This index might be used for *C. intermedia* but would be repurposed as plant size and shape to fit the requirements of the species.

### **Surface colour**

In the fruit industry fruit colour is a readily used indicator of fruit ripeness (Crisosto 1994, Kalt et al. 1995, Kader 1999, Lysiak 2012). Base/ground colour (back ground colour other than brown blemish or age marks) is used for most stone fruit such as: apricots (De Kock 2011), peaches and plums as well as apples (Lysiak 2012), strawberries and raspberries amongst others (Kader 1999). Colour is used because fruit ripeness is coupled with changes in pigmentation in the skin of the fruit as certain sugars and acids develop (Kalt et al. 1995). Crisosto (1994) suggested that the ground colour of the fruit is less affected by sunlight than overall colour and is thus a better measure of ripeness. This could be the case in *C. intermedia* stems, where the chemical compositions of the stems change with age resulting in different coloured stems. This could also result in older stems producing a tea that is more pronounced in flavour.

The changes in surface colour were used to determine the optimum harvest date for apples that have to survive storage of up to 4 months (Lysiak 2012). Fruits were picked every 5 days starting weeks before the estimated optimum harvest date. The characteristics that were measured included: fruit firmness (measured with a penetrometer), fruit colour on opposite sides of the fruit (measured with a reflectance colorimeter), and titratable acidity (Lysiak 2012). Colour changes from green to yellow hues were considered a good indicator of maturity as this character correlated well with other maturity indices. Lysiak (2012) reported difficulties in the measurement of colour as the blush of the apples in some cases almost covered the entire fruit, making it hard to find spots of true ground colour.

Kalt et al. (1995) suggested that electronic colour sorting would speed up and increase the quality of a product but might not always be the case as the surface colour does not necessarily reflect the composition of the chemicals within the fruit. The reason for this is that different cultivars might exhibit different relationships between the surface colour and actual ripeness of the fruit.

In South Africa the surface colour of apricots are determined by using an industry colour chart (De Kock 2011) but colour can also be measured with a reflectance colorimeter (Kalt et al. 1995).

Colour is not only used in the fruit industry but can be extended to other crops such as the cut flower industry and sunflowers that are used in the production of seeds. Sunflowers are harvested when at least 80% of the heads are brown, as to minimise feeding from birds (Rondanini et al. 2007, DAFF 2009). Colour change is also recorded in the leaves (green to yellow) and the back of the head that changes from green to yellow with browning bracts (DAFF 2009).

### **Physical indices**

Physical indices are the indicators that have to be physically measured using specific equipment. Flesh firmness of fruits and vegetables are the most common physical index used for maturity.

### **Flesh firmness**

According to De Kock (2011) flesh firmness is an indicator of physiological maturity in many stone fruit varieties. Firmness alone, as with size, might not be enough to determine maturity but this index is used in South Africa for plums, peaches, nectarines (De Kock 2011) and pears (Carmichael 2011). The firmness of the flesh is normally used in conjunction with colour as is the case with apples (Lysiak 2012) and apricots (Visagie 1985, De Kock 2011).

Flesh firmness is measured using a pentameter (Lysiak 2012) or a penetrometer (De kock 2011) which are variations of the same type of probe. This equipment determines the firmness or toughness of the fruit surface by measuring the force required to puncture the surface.

### **Chemical composition**

Various chemicals are tested and used as indicators of maturity of crops. The fruit industry heavily rely on measurements of titratable acidity in pears (Carmichael 2011) and apples (Lysiak 2012), starch also in apples and total soluble solids in bananas (Muchui et al. 2010).

In the sugarcane industry the sucrose content of the crops are used as an indicator of harvest maturity (Gilbert et al. 2004). The sucrose content differs within the varieties of sugarcane thus maturity curves are produced for individual varieties. According to Gilbert et al. (2004) some varieties of sugarcane must be harvested before the plants have matured and reached maximum sucrose levels.

These harvest maturity indices, specifically colour, could possibly be used to determine guidelines for the optimum harvesting time of *Cyclopia intermedia*.

## **Chapter 3: Species distribution modeling of *Cyclopia intermedia*, identifying distribution to aid the management of this economic resource.**

### **3.1 Introduction**

Presently, speedy management decisions are needed to protect the rich biodiversity of our country (Reese et al. 2005). One of the most fundamental aids that inform management decisions are potential distribution models of vegetation types or species (Franklin 1995, Midgley et al. 2003, Elith et al. 2006, Foden et al. 2007, Elith and Franklin 2013). These models are generated using sampling data of presence and either true absence or pseudo-absence records to model the potential distribution of a species or vegetation type (Reese et al. 2005). Pseudo-absences are absence record generated from background localities when no true absence records are available. If only presence data or only a small data set of presence and absence records are available, a species distribution model can be used to predict the distribution of a species (Reese et al. 2005).

According to Woodcock et al. (2002), vegetation is influenced by a combination of factors related to climate, soils, history, fire and human influences. There is an option in the modern field of mapping to either map the actual vegetation or species (this is done for small areas such as nature reserves) or to extrapolate via Species Distribution Mapping (SDM) from known records what the extent of the distribution of the vegetation or species could be (for example predictive mapping or projection under future climates) (Woodcock et al. 2002).

Current trends in SDM focus mostly on testing and comparing different models to establish the best ways to model species (Van Etten 1998, Zimmermann and Kienast 1999, Chang et al. 2004, Engler et al. 2004, Coudun et al. 2006, Elith et al. 2006, Carnaval and Moritz 2008, Syphard and Franklin 2009, Zare Chahouki and Zare Chahouki 2010, De la Estrella et al. 2012). The literature is dominated by models that focus on predicting the occurrence of invasive species in Australia (Apan et al. 2008), Europe (Chytry et al. 2009), the USA (Williams et al. 2009, Bradley et al.

2009, Stohlgren et al. 2010, Jones et al. 2010) and South Africa (Maitre et al. 2008, Jarosik et al. 2011, Thompson et al. 2011).

In South Africa distribution modelling of invasive species has used this approach at both country (Rouget et al. 2004, Thuiller et al. 2006, Maitre et al. 2008), regional, and local scale (Jarosik et al. 2011). Various mapping efforts have also been applied to the Cape Floristic Region (Midgley et al. 2002, 2003, Pearson et al. 2006), where the focus has been on the predicted impact of future climates on the distribution and range of the biodiversity hotspot.

Due to the economic importance of *Cyclopia intermedia* the knowledge of the potential distribution of the species occurrence is fundamental to the development of a management plan for this resource. This will be particularly useful in the light of the recent increase in illegal harvesting from government and privately owned land.

This chapter aims to develop a potential distribution map of *C. intermedia* using an ensemble species distribution modelling approach (Araújo and New 2006). The potential distributional shifts of this theoretical niche under future climate are also projected to deliver possible insights to the fast growing honeybush tea industry with regards to expansion in the future.

## **3.2 Materials and Methods**

### **Species data**

Locality records were collected for *Cyclopia intermedia* from various sources. Some of these presence records were recorded while other field work was being done, but most were extracted from herbarium records: Ria Olivier (PEU), National Herbarium (PRE), Bolus (BOL), Compton (NGB & SAM), and Grahamstown (GRA). Very few of the herbarium records had GPS coordinates, thus all the records were georeferenced using the descriptions on the herbarium collection sheets. Where records were not specifically identified to one species, e.g. *Cyclopia intermedia* / *subternata*, the data was omitted for the purpose of modelling the species.

## **Study area**

*Cyclopia intermedia* has been reported to occur on various mountain ranges in both the Eastern and Western Cape of South Africa and the species is considered to be endemic to the Fynbos region (Schutte 1995, Schutte and Van Wyk 1998). It is for this reason that the Cape Floristic Region (CFR) was used as the geographical extent of this study. A mask of the CFR was used to sample background data from the environmental layers as pseudo-absences (give the extent of the mask in terms of degrees south and east). This mask represented both the Eastern (non seasonal rainfall) and Western Cape (winter rainfall) regions of the CFR.

## **Environmental Predictors**

Environmental climate predictors were used for both the current and future (2050) distribution predictions. The current and future climate data was obtained from the WorldClim database (Hijmans et al. 2005, <http://www.worldclim.org>). Both the current and future climate data had a resolution of 2.5 arc-minutes and was chosen for the SRES A2A scenario. The models are cccma\_cgcm3 and for both predictions 19 bioclimatic variables were used (Appendix A).

The climate variables were grouped according to how correlated they are to one another following the method of Potts et al. (2013). The predictor variables were divided into six sets of seven random selections from the bioclimatic variables. This was done to minimize the chances of highly inter-correlated variables being selected to train the same model. This is important as inter-correlated variables will produce a model biased to these models. Within each set, each variable was randomly selected. The correlation between each variable was calculated and when a variable had a correlation of  $r^2 > 0.7$  with the previously selected variable the last selected variable was replaced by another variable with a lower correlation. These four sets of variables were applied to each of the algorithms individually. This together with the 4 fold cross-validation resulted in 16 models for each algorithm. All these models were stacked to produce an ensemble of 64 models.

## **Species Distribution Climate Models**

Given the uncertainty of SDM's that use a single model for distribution prediction, in this study ensembles were produced (two ensembles: current and future). The use of

an ensemble forecast provides a representation of distribution of a species as it incorporates the predictions of different models. The same ensemble approach used by Potts et al. (2013) was used here, where six model algorithms (produced using the same group of predictors) were summed and stacked to produce an ensemble map for the distribution of the target species.

The model algorithms used were: Maxent, Generalized additive model (GAM), BIOCLIM, and RandomForests. These models were chosen as they have been widely used in species predictions and provide a good representation of different types of models (bioclimatic, regression and machine learning). A 4-fold cross-validation approach was applied to all the algorithms and predictors resulting in four models of each of the algorithms. To standardise the output of the different models a threshold criterion (maximum sensitivity plus specificity) was applied (Potts et al. 2013). Each combination of predictors and localities had equal importance in the ensemble. The settings for the models are provided in Appendix A. For all the algorithms, with the exception of BIOCLIM, 5000 pseudo-absence localities were generated.

Performances of the models were evaluated using AUC values. This method is readily used in SDM and is a standard measure of predictive ability of a model. The AUC statistic ranges from 0 to 1, where a model with an AUC value of 0.1 is worse than random, 0.5 is no better than any random selection and a model with an AUC value of 1 predicts distribution perfectly.

The effects of future climatic variables on the distribution of *C. intermedia* were determined by calculating the gain, loss and turnover in range. These statistics were calculated in relation to current climatic prediction of the distribution. The overall predicted range gain or loss (*RGL*) of the climate envelope was estimated as a percentage using the formula  $RGL = 100(RG - RL) / CR$ , where *RG* is the range gain, *RL* is the range loss and *CR* is the current predicted range. A negative *RGL* value shows a loss in overall range, whereas a positive value shows an increase. The range turnover (*RT*) of the predicted range was estimated using  $RT = 100(RL + RG) / (CR + RG)$ . An *RT* value of 0 indicates no shift in range and a value of 100 indicates a complete range shift when compared with the predicted range under current conditions.

### 3.3 Results

The 4-fold cross validation of the four different models resulted in a total of 64 models. The majority of the AUC values (Fig. 3) were  $> 0.70$ . From the 64 different models used, the 16 GAM models showed the best performance with a mean AUC value of 0.86 (S.D = 0.003). Maxent models produced the second best models with a mean AUC value of 0.82 (S.D = 0.06). The BIOCLIM and RandomForest models showed similar results with mean AUC values of 0.72 (S.D = 0.07) and 0.77 (S.D = 0.01) respectively.

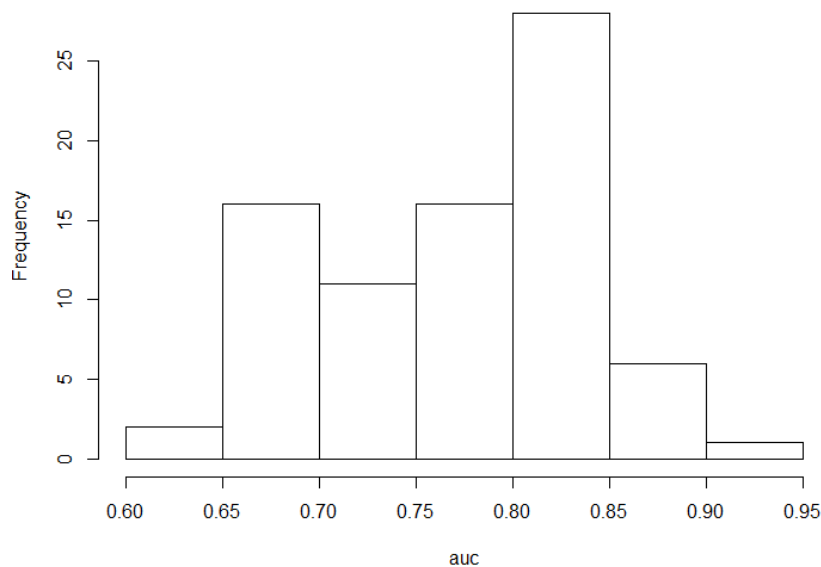


Figure 3: A histogram of the AUC values consisting of 16 GAM, 16 Maxent, 16 BIOCLIM and 16 RandomForest models.

These were compiled into an ensemble of models that performed well with a mean AUC value of 0.79 (S.D = 0.06). The modelled distribution under current climate conditions was concentrated around the Cape Fold mountain ranges (Fig. 4). The distribution stretches from roughly  $20^{\circ}$  to  $25^{\circ}$  E and  $33^{\circ}$  to  $34^{\circ}$  S within the Cape Floristic Region.



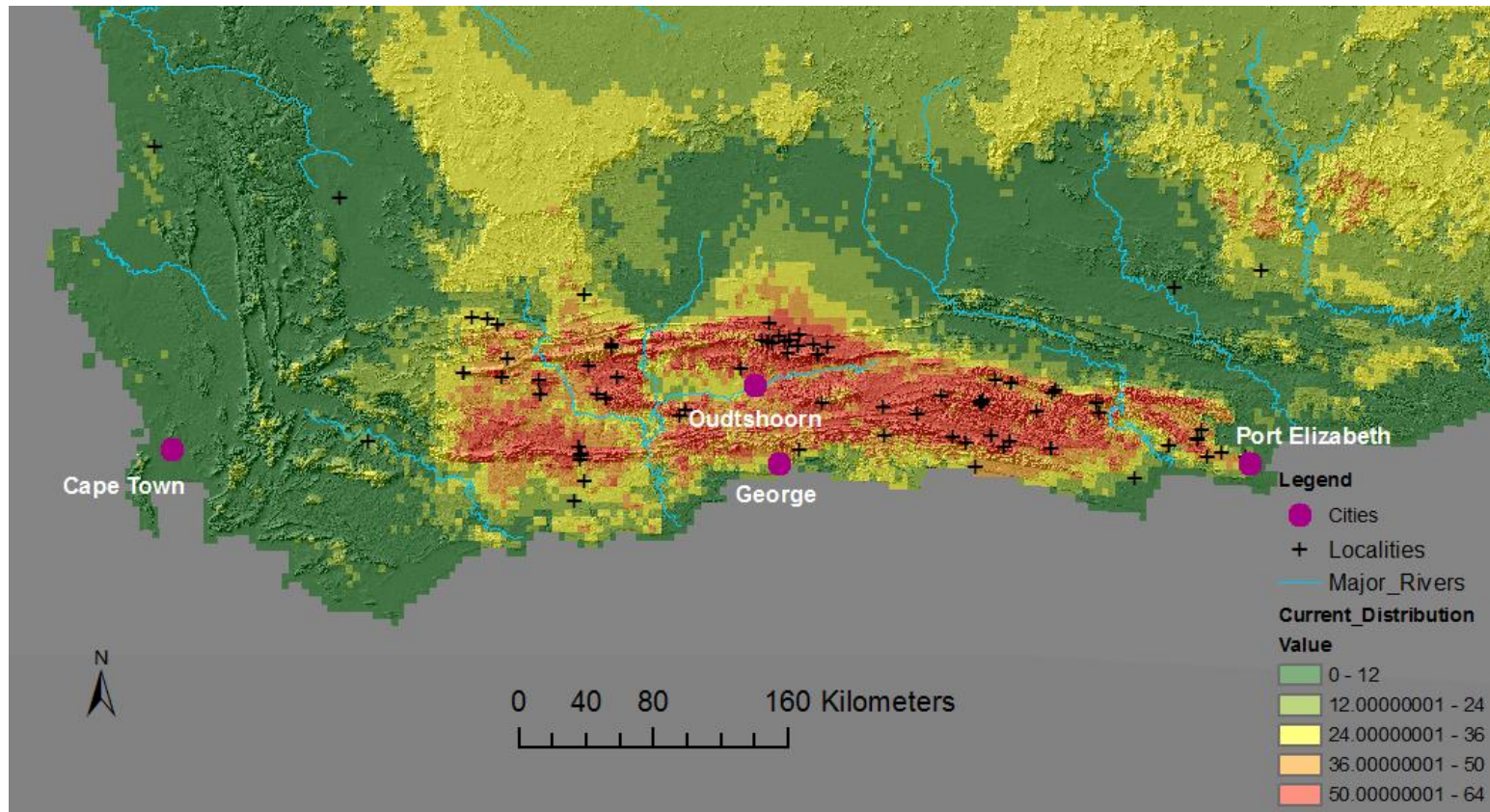


Figure 4: The modelled distribution of *Cyclopia intermedia* in South Africa under current climate conditions. The prediction was produced from an ensemble map comprising of 16 BIOCLIM, 16 GAM, 16 Maxent, and 16 RandomForests models. Red = certainty of presence, green = certainty of absence, yellow= model uncertainty.

Under future climatic conditions, the distribution range of *Cyclopia intermedia* is predicted to experience a decline and a range change under future climatic conditions (Fig. 5) with a mean RGL of -63% (SD = 27.34) and a mean RT of 75% (SD = 27.34).

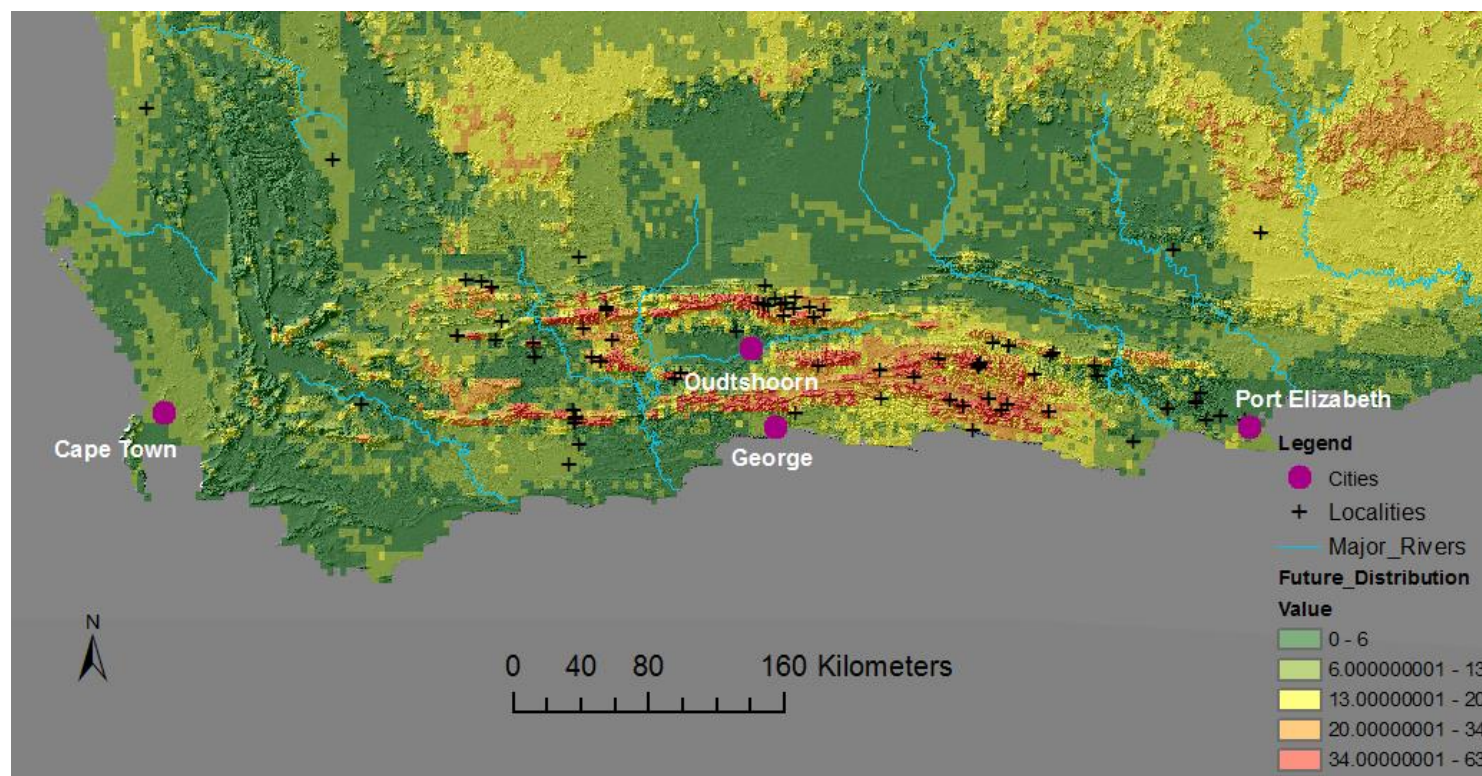


Figure 5: The predicted future (2050) distribution of *Cyclopia intermedia* in South Africa for the SRES A2A scenario. The prediction was produced from an ensemble map comprising of 16 BIOCLIM, 16 GAM, 16 Maxent, and 16 RandomForests models. Red = certainty of presence, green = certainty of absence, yellow= model uncertainty.

### 3.4 Discussion

The predicted current distribution of *Cyclopia intermedia* follows the mountain ranges of the Cape Fold mountains. This supports the findings of Kies (1951) and Schutte (1995) who stated that the *Cyclopia* genus is found in the coastal districts of the Cape provinces stretching from Clanwilliam in the West to Port Elizabeth in the East. Species from this genus were found on various mountain ranges that together comprise the Cape Fold Belt: Koue Bokkeveld Mountains, Klein and Groot Swartberg, Tsitsikamma Mountains and the Kouga Mountains. The one area within the fold belt where no *Cyclopia intermedia* is predicted is the little Karoo basin which is an intermontane basin (Cowling et al. 2009, Reyers et al. 2009). This is to be expected as *C. intermedia* is a strictly montane species. More research is required to determine what specific characteristics restrict the distribution, whether it is the specific soil properties, temperatures or solar influx radiation associated with south facing mountain slopes.

The result of the contraction of the distribution of this Fynbos species in the future climate scenario is consistent with the general dire outlook of future distribution predictions for Fynbos species in 2050 (Midgley et al. 2002, 2003). Although not a Fynbos species, the observation by Foden et al. (2007) that *Aloe dicotoma* found on lower altitudes are more susceptible to die-back in 2050, could be applied to *C. intermedia* as the distribution seems to retract to the higher altitudes of the Cape fold mountains (Midgley et al. 2003). Midgley et al. (2002) reported an average Fynbos biome loss of 58% in 2050 climatic conditions.

An estimate of the range gain and loss (RGL) of the future predicted distribution shows that an average of 63% of the current distribution will be lost in 2050. This coupled with the range turnover (RT) suggests that even though some range will be gained the distribution will undergo a significant shift (75%). This could affect the honeybush tea industry if it is to remain reliant on wild harvesting in 2050.

It should be kept in mind that this distribution is predicted using only climatic data. This does not take into account soil characteristics, elevated atmospheric CO<sub>2</sub> concentrations or land-cover change. Changes in plant water stress should also be

considered as the predicted higher temperatures will compound with the expected drier conditions of the region (Midgley et al. 2002).

Given the general restriction of *Cyclopia intermedia* to the southern slopes of the mountains, the effect of slope orientation on microclimate must be taken into account. Knight et al. (1999) found that the amount of solar radiation exposure on north versus south facing slopes is one of the most important factors in small scale distribution of species. In the Northern hemisphere south slopes are characterised by higher solar radiation resulting in higher soil and air temperature (Auslander et al. 2003). The same is true for north facing slopes in the Southern hemisphere, which means the south slopes are the cooler, more protected slopes. These cooler conditions coupled with higher soil moisture, and lower soil and air temperatures are very important in the distribution of *C. intermedia*. These small scale climate changes are not reflected in the distribution prediction at this resolution (2.5 arc-minutes), which confirms the need for finer scale modelling, an approach that is not possible in the absence of fine-scale environmental data.

The climate change predictions indicate a pessimistic future for *Cyclopia intermedia*. The range turnover together with range loss is predicted to severely minimise the resource availability both for the honeybush tea industry and for the survival of the species. It is possible that the model resolution is too coarse to allow for detection of small-scale habitats such as those found within a valley or kloof. With a large-scale integration of environmental data, it is likely that the model predicts some areas as range loss where the species could quite possibly still survive in refugia caused by the high level of small-scale variability typical of the Cape Fold mountains. Despite the limitations of this model and the resolution of the parameters used, it is clear that this species will be under some threat in the future.

It can be deduced from the results that this scale (2.5 min resolution) and modelling approach is not entirely useful for a species that is so strongly dependent on the aspect and slope that controls distribution. Midgley et al. (2002) stated that a large scale approach to climate influence on distribution underestimates the threats faced at finer scales. It appears that the approach also overestimates the threats considering that the model is not sensitive to appropriate local or fine scale niches that may represent refugia despite the surrounding hostile environment. To resolve

this, fine-scale assessments will have to be done for an accurate prediction of survival under future climate change scenarios. It would be best to include factors specific to south slopes and more detailed occurrence records targeted to these. A detailed micro-scale elevation model appears to be the fundamental component required for such an investigation.

### **3.5 Conclusion**

In conclusion the bioclimatic distribution ensemble of *Cyclopia intermedia* under current conditions, corresponds well with the distribution information already known for the *Cyclopia* genus (Kies 1951). The predicted future distribution (2050) shows a worrying forecast for the species with nearly 63% of the distribution lost and shifted to new areas (RT= 75%). This shift and contraction of the resource will have major implications for the honeybush tea industry if it is to still rely on wild populations in the future. The main shortcomings of this mapping approach should be considered and include: too coarse resolution to determine environmental differences on north and south facing slopes, and a limited number of presence records that were not specifically collected for a mapping purpose. It is recommended that a finer scale assessment be done, targeting the south-slope specific environmental factors that presently influence the distribution of *Cyclopia intermedia*.

## **Chapter 4: Phytosociology of *Cyclopia intermedia*, identifying associated species and environmental variables that might affect the distribution.**

### **4.1 Introduction**

Honeybush tea is an indigenous South African herbal tea product that is produced from various *Cyclopia* species (Kies 1951, Du Toit et al. 1998). Honeybush tea has a long history of utilisation in South Africa (Joubert et al. 2011; Kies 1951). The industry, although not as large as that of rooibos, has grown from the mid-1990s with this tea being sold in 25 countries (Joubert et al. 2011). The dependence of the honeybush industry on wild populations that are prone to fire, and to some degree illegal harvesting, are some of the aspects that hamper the growth of the industry (Joubert et al. 2011).

In the case of both honeybush and rooibos tea there is increasing market demand for the product. This raises the concern that the pressure to supply this demand will lead to over harvesting or illegal harvesting of the resource (Louw 2006). The problem is that for both these products, over harvesting (both in frequency and in amount of foliage harvested) can cause a drastic decrease in the population size of wild populations and negatively influence the growth and survival of these plants. Because of this problem there is a need for studies to identify the effect of harvesting and by extension design a protocol for sustainable harvesting of resources.

Currently cultivation consists of about 200 ha of *C. genistoides* and *C. subternata*, but as this alone is not enough to supply the demand and wild populations of *C. intermedia* are used to supplement and form a large part of the annual production (Joubert et al. 2011). As a result of this method of utilisation, the populations of, in particular, sprouters that are slow growing are under threat because of the unsustainable harvesting practices. Being the species with the immediate pressure of over harvesting *Cyclopia intermedia* is the focus species in this study.

To date very little information is available about the ecology of *C. intermedia*. Valuable information is shared between farmers about agricultural practices but very

little scientific study has been done. The literature on *Cyclopia* spp. is predominately focused on the biochemical properties of the species used to produce the honeybush beverage. If we understand the ecology of *C. intermedia*, the efforts toward its sustainable harvesting will be more informed and have a greater likelihood of success.

Phytosociological studies provide the opportunities to compile species lists of specific areas that can be used to properly manage an area of importance. According to Brown & Bezuidenhout (2005) an inventory of flora will aid in the management and also the detection of changes in a certain area. The reason for this is the fact that any changes in environmental conditions can be seen in the changes in vegetation.

In the case of *Cyclopia intermedia*, other possible useful species or species associated with *C. intermedia* can be identified to assist in the formulation of a management and conservation plan for the species. Detailed knowledge of the plant communities that include *C. intermedia* and those that do not, are essential in the understanding of factors that contribute to the current state of the communities (Eloff 2010). A change in the composition of vegetation might indicate a change in environmental variables such as a gradient in soil conditions or elevation (Campbell 1986). Niche description will be useful for the identification of specific environmental variables that might be key to the changes in species composition in Fynbos areas (Campbell 1986). The different variables may include: a moisture gradient (both from North to South and East to West), a nutrient gradient, a gradient in soil conditions, and a temperature gradient (Campbell 1986).

In the previous chapter regional distribution patterns associated with climate at a course scale was investigated. Here the aim was to describe the ecosystems and habitats where *Cyclopia intermedia* occur. This was done in terms of species composition and abiotic features, as the only way to effectively protect any environment is to know what it is being protected or what is need of protection. The hypothesis to be tested is that the species composition of areas where *Cyclopia intermedia* occurs is different from those where it does not occur. This is done through the identification of species associated with *C. intermedia*.

## 4.2 Materials and Methods

Three sites were selected across the distribution range (Eastern and Western Cape) of *Cyclopia intermedia* (Fig. 6). Two phytosociological surveys were done at each of the sites along the East-West distribution range. The sites are (from east to west): Nooitgedaght, Swartberg, and Barrydale. At each site, the two surveys covered an area with *Cyclopia intermedia* present (presence) and an area where *C. intermedia* is absent (absence).

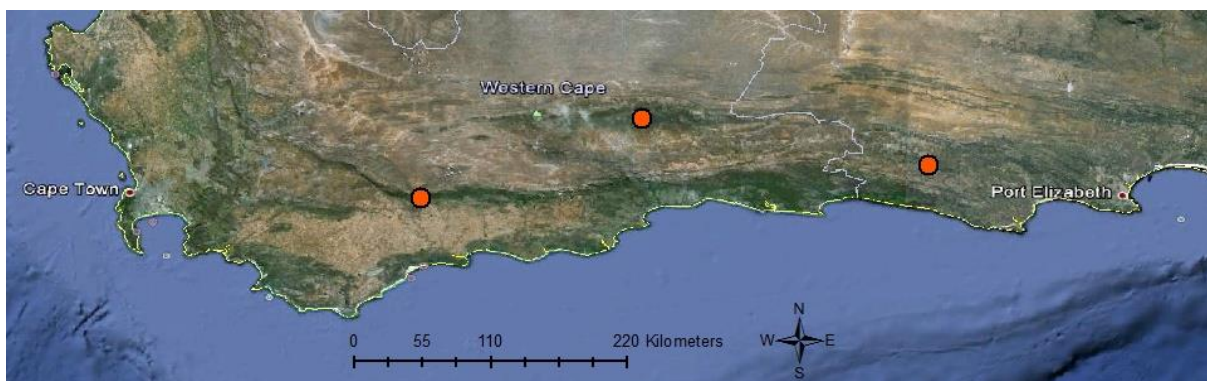


Figure 6: The sampling sites used in a phytosociological study across the distribution range of *Cyclopia intermedia*. The sites are, from the East: Kouga, Swartberg, and Barrydale.

The Tweeling camp on Nooitgedaght farm in the Kouga Mountains was used for the presence and absence sites. The Swartberg site was situated roughly 2.2 km into the pass and the Barrydale site was situated on a farm 28 km outside the town.

A stratified sampling design was used to identify the species composition of the sites. Five quadrats (replicates) were laid out at each site to cover the stretch of the slope (Fig. 7). Each quadrat was 10 x 5 m resulting in a quadrat of 50 m<sup>2</sup> (slightly larger than required for montane Fynbos: (Whittaker 1980; Du Preez 1991; Malan 1998). All vascular plant species in each quadrat were recorded and assigned a Braun-Blanquet score. Temperature (HOBO data loggers placed at each site), GPS coordinates, and soil characteristics were recorded at each site. The same temperature data was used in the phenology chapter of this study.



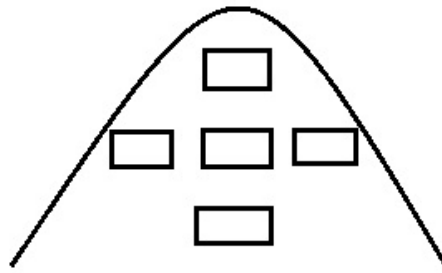


Figure 7: Stratified sampling design of the placement of phytosociological quadrats on slopes where *Cyclopia intermedia* is either present or absent.

Soil samples and soil depth measurements were taken from the four corners of each quadrat. The four samples within a quadrat were combined to form one representative sample of the soil in the quadrat. This resulted in five reps for each slope analysed. The soil characteristics measured included: soil field water capacity, soil particle size analysis, and soil bulk density.

The field water capacity was measured following Bashour & Sayegh (2007) with a few adjustments: Soil was placed in an aluminium tin with small holes in the bottom. The soil was saturated with tap water and as soon as the soil reached a point where no water was leaching out the tin was weighed. The soil was then placed in a drying oven at 105°C for 48 hours until the soil was completely dry. The tin was weighed again. The field capacity of the soil was calculated as a percentage of the dry mass.

The particle size analysis was done by using the Bouyoucos method adapted by Bashour & Sayegh (2007). The soil used in the field capacity analysis was used in this method. The dried soil sample was sifted through a 2 mm sieve to remove coarse particles. The fine particles and the large particles were stored separately. A solution of sodium hexametaphosphate was used to make a slurry with the soil. The slurry was placed on a shaker for 3 minutes to separate the soil particles. The rest of the analysis followed methods used by Bashour & Sayegh (2007). The texture of the soil was determined using a texture triangle (Fig. 8).

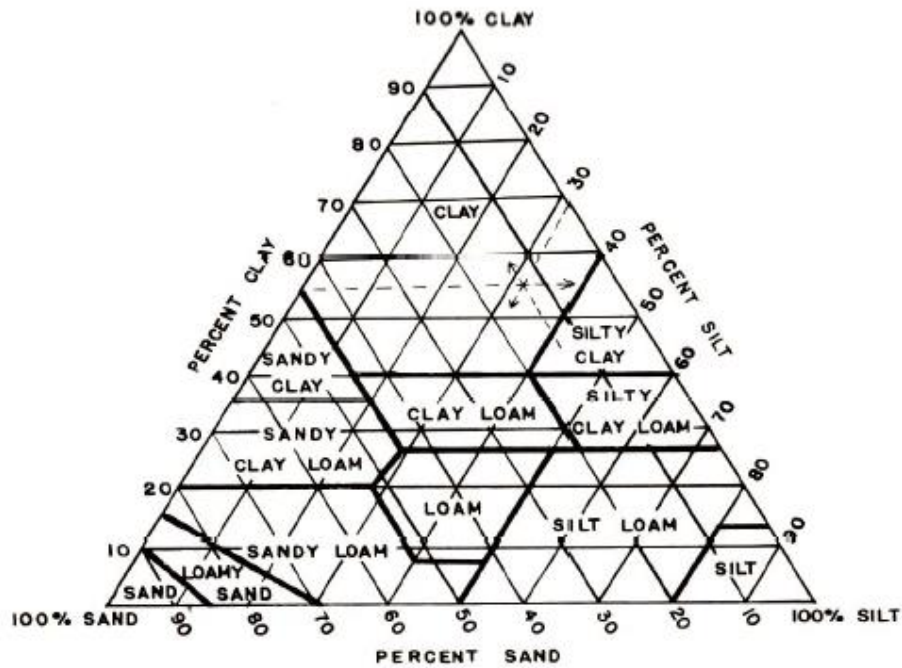


Figure 8: A textural triangle showing percentages of sand, silt, and clay from Bashour & Sayegh (2007).

The coarse particles removed in the soil particle analysis was added to the rest of the fine particles. The soil was thoroughly mixed and the bulk density was calculated with the original particle size of the soil according to Bashour & Sayegh (2007).

Species were identified using the Ria Olivier herbarium and additional literature (Germishuizen et al. 2006) to the lowest taxonomic rank possible. The cover abundance of species in samples were analysed using a detrended correspondence analyses in CANOCO (Ter Braak 1987). Species with a Braun-Blanquet score 'r' were allocated a value of 0.2 and '+' a value of 0.5. This was done to enable analysis using CANOCO. Species richness, diversity and dominance were calculated using Margalef (1958), Shannon-Weaver (1963), and Simpson (1949) indices respectively. The soil characteristics across the sample sites were analysed using Student's t-tests.

### 4.3 Results

The soils of Barrydale and Kouga where *C. intermedia* is present, differed in the sand and silt composition (Fig. 9). Barrydale had a higher percentage of sand than Kouga ( $p = 0.004$ ,  $t = 3.42$ ) and less silt ( $p = 0.002$ ,  $t = -3.97$ ). The percentage of clay particles from Swartberg was significantly less than that found in Barrydale ( $p = 0.00$ ,  $t = 4.60$ ) and Kouga ( $p = 0.008$ ,  $t = -3.15$ ). The soils of Barrydale and Kouga were therefore classified as loamy sand and Swartberg was sandy.

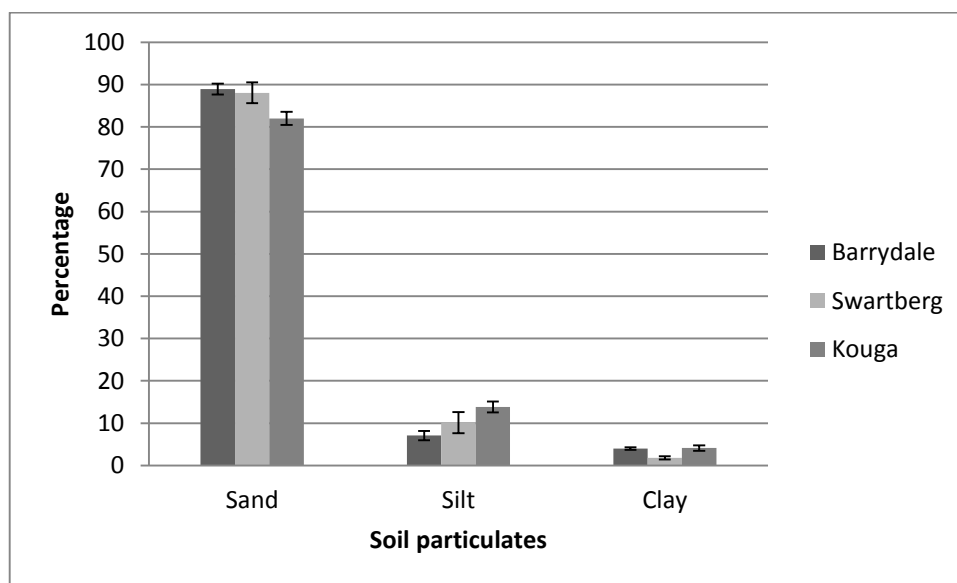


Figure 9: Percentages of different soil particulates from Barrydale, Swartberg and Kouga Mountains where *Cyclopia intermedia* is present. Vertical bars represent standard error.

The soils from the sites where no *Cyclopia intermedia* was found has lower soil moisture ( $p = 0.02$ ,  $t = -2.13$ ) and higher bulk density ( $p = 0.02$ ,  $t = 2.18$ ) than those where *Cyclopia intermedia* was found (Fig. 10). The Swartberg site was significantly more sandy than the other sites (Barrydale:  $p < 0.001$ ,  $t = -15.47$ ; Kouga:  $p < 0.001$ ,  $t = -10.65$ ) and the percentage silt in soil was lower than other sites (Barrydale:  $p < 0.001$ ,  $t = -6.62$ ; Kouga:  $p < 0.001$ ,  $t = -7.80$ ). This same result was recorded for clay in Swartberg soil (Barrydale:  $p < 0.001$ ,  $t = -4.86$ ; and Kouga:  $p = 0.01$ ,  $t = -2.57$ ).

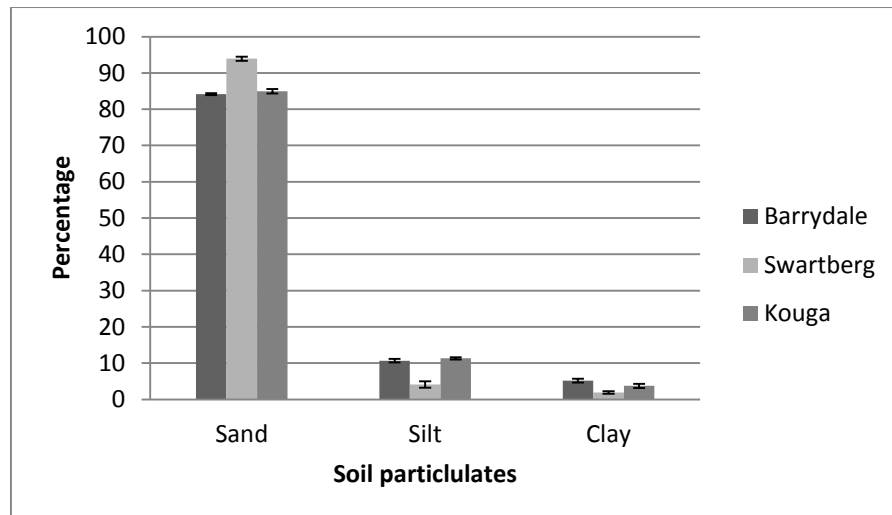


Figure 10: Percentages of different soil particulates from Barrydale, Swartberg and Kouga Mountains where *Cyclopia intermedia* is absent. Vertical bars represent standard error.

The sites where *Cyclopia intermedia* occurred had higher field capacity at both Barrydale ( $p = 0.004$ ,  $t = 2.82$ ) and Swartberg ( $p = 0.005$ ,  $t = 2.74$ ) compared to those where plants were not found (Fig. 11).

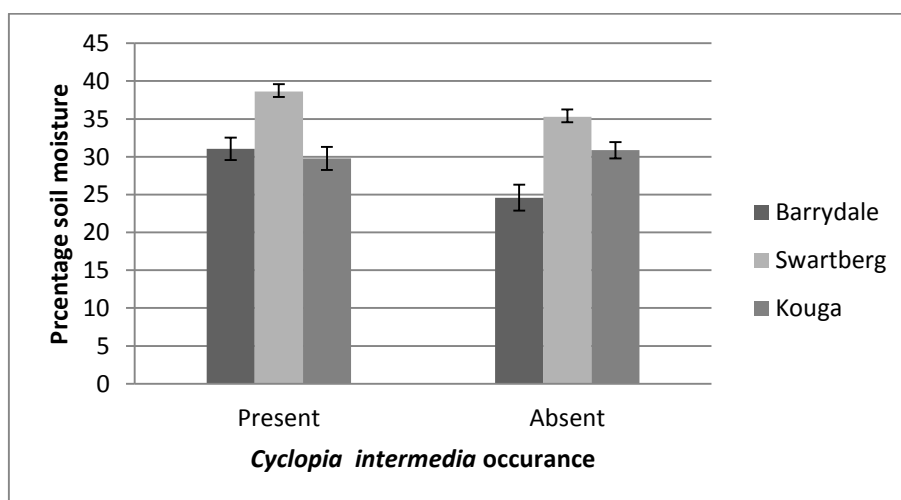


Figure 11: Percentage soil moisture at field capacity of areas where *Cyclopia intermedia* is either present or absent from Barrydale, Swartberg (Western Cape) and Kouga mountains (Eastern Cape). Vertical bars represent standard error.

The bulk density (Fig. 12) showed the opposite trend, with the absence sites at both Barrydale ( $p = 0.003$ ,  $t = -2.93$ ) and Swartberg ( $p < 0.001$ ,  $t = -3.69$ ) having higher bulk density compared to that found where plants were present. The soils from the Swartberg sites had significantly higher soil field capacity (Barrydale:  $p < 0.001$ ,  $t = -6.33$ ; Kouga:  $p < 0.001$ ,  $t = -5.75$ ) and lower bulk density (Barrydale:  $p < 0.001$ ,  $t = 7.82$ ; Kouga:  $p < 0.001$ ,  $t = 10.72$ ) compared to the other sites.

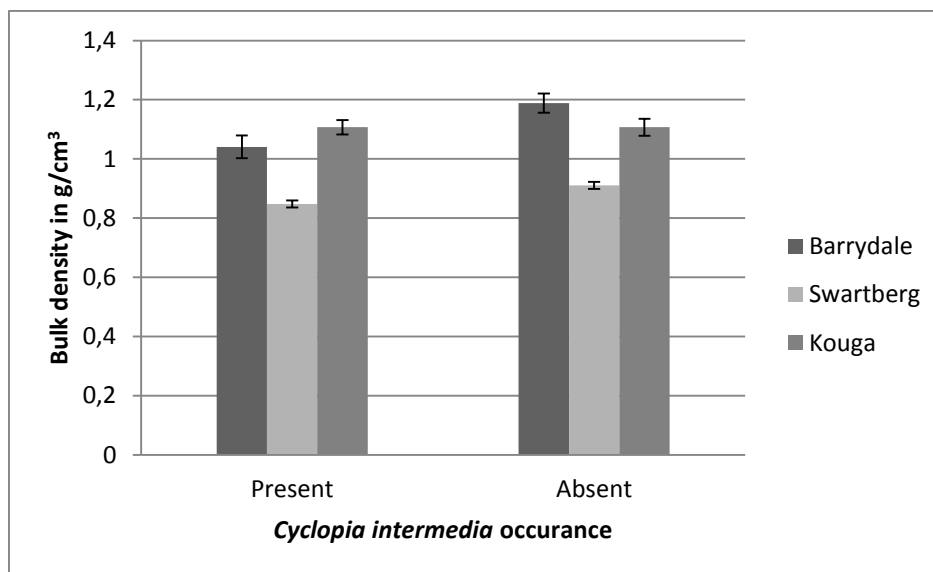


Figure 12: Soil bulk density of areas where *Cyclopia intermedia* is either present or absent from Barrydale, Swartberg (Western Cape) and Kouga mountains (Eastern Cape). Vertical bars represent standard error.

Mean monthly temperatures (Fig. 18, chapter 5) followed seasonal trends as expected in all sites. Some months showed temperatures significantly different when compared to others (Table 4, chapter 5), but no clear pattern can be identified between sites or between the Eastern Cape and Western Cape regions.

The once-off sampling technique used resulted in some plants that had no flowers or inflorescences (in the case of most grasses) and could thus not be completely identified. Some species could not even be classified to genus level with a great degree of certainty and was thus identified by family name.

The three study sites had different plant communities (Fig. 13 & 14) with two plant communities recorded at Swartberg. *Cyclopia intermedia* formed part of one of the Swartberg plant communities. This difference was not found at Barrydale and Kouga. Complete species list of each site can be found in Appendix B.

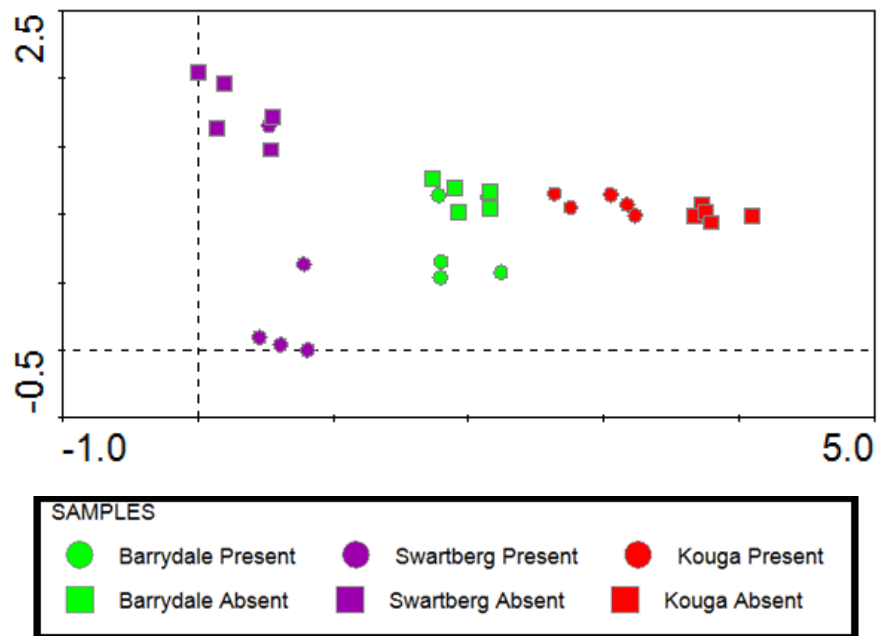


Figure 13: A Detrended Correspondence Analysis of sites sampled with or without *Cyclopia intermedia* at Barrydale, Swartberg and Kouga. The sum of all the eigenvalues is 3.760 and the cumulative variance of species data is 21.4%.

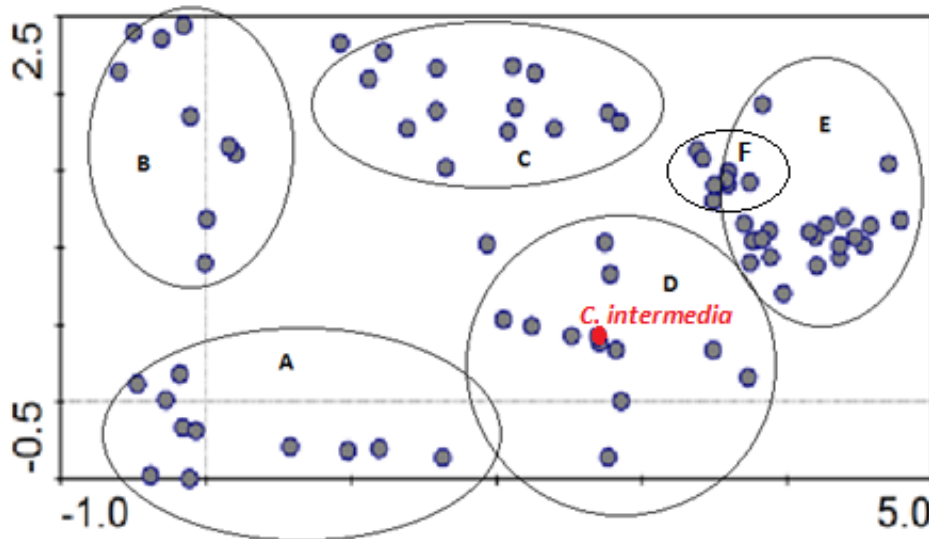


Figure 14: A Detrended Correspondence Analysis of species sampled with or without *Cyclopia intermedia* at Barrydale, Swartberg and Kouga. The sum of all the eigenvalues is 3.760 and the cumulative variance of species data is 21.4%. A - F communities identified.

Community A represents species recorded at the Swartberg present site, and is dominated by *Relhania calycina*, *Restio sejunctus*, and *Phyllica willdenowiana*. Other species within this community include: *Tritoniopsis* sp., *Watsonia* sp., *Holothrix* sp., *Helichrysum teretifolium*, *Protea neriifolia*, and *Berkheya* sp.

Community B consists of species recorded at the Swartberg absent site, and is dominated by *Syncarpha argentea*, *Metalasia densa*, *Hypodiscus argenteus*, and *Thamnochortus lucens*. Other species within this community include: *Gladiolus* sp., *Erica* sp. 3, *Anthospermum* sp., *Helichrysum cymosum*, and *Rhodocoma fruticosa*.

Community C represents the presence and absence sites at Barrydale, and is dominated by *Seriphium plumosum*, *Dicerotheramnus rhinoceratis*, and *Elegia filacea*. Other species within this community include: *Brunia noduliflora*, *Erica plukenetii*, *Erica vestita*, *Erica* sp. 1, *Erica* sp. 2, *Syncarpha* sp., *Thamnochortus* sp., *Pinus* sp., and *Ischyrolepis* sp. 4.

Community D is made up of species found at both Barrydale and Kouga present sites, and is dominated by *Cliffortia ruscifolia*, *Ischyrolepis* sp. 3, *Cyclopia intermedia*, *Protea repens*, and *Leucadendron salignum*. Other species within this

community include: *Metalasia muricata*, *Ischyrolepsis* sp. 2, *Anthospermum* sp., Poaceae, *Helichrysum* sp., *Agathosma* sp. and *Phylica* sp..

Community E consists of species recorded at both the present and absent sites at Kouga, and is dominated by *Gongyloglossa tortilis*, *Helichrysum felinum*, and *Pelargonium scabrum*. Other species within this community include: *Selago* sp., *Pelargonium* sp. 2, *Euryops* sp., *Cliffortia* sp., *Thesium* sp., *Anthospermum aethiopicum*, *Linum* sp., *Pelargonium* sp. 1, *Themeda triandra*, *Sebaea elongate*, *Lobelia* sp., *Aspalathus* sp., *Erepsia* sp., *Erica* sp. 4, *Helichrysum rosum*, *Hermannia hyssopifolia*, *Struthiola* sp., and *Knowltonia* sp.

Community F consists of species recorded at the present in Kouga, and is dominated by *Leucospermum cuneiforme*, *Grubbia* sp., and *Erica passerinae*. Other species within this community include: *Lobostemon echioides*, *Helichrysum anomalum*, *Gazania krebsiana*, and *Restio* sp..

Communities A, D and F are considered to contain species associated with *Cyclopia intermedia*. Sites where *C. intermedia* occur had higher species richness and diversity when compared to the sites without *C. intermedia* (Table 3). Growth form composition (Fig. 15), showed a dominance in shrubs in all sites and succulents only occurred where *C. intermedia* is present.

Table 3: Mean (n=3) species richness (R), diversity (H'), dominance ( $\lambda$ ) of the vascular plants of study sites with or without *Cyclopia intermedia*. BP = Barrydale Present; BA = Barrydale Absent; SP = Swartberg Present; SA = Swartberg Absent; KP = Kouga Present; KA = Kouga Absent.

	R	H'	$\lambda$
BA	15.60	3.29	0.40
SA	15.10	3.28	0.41
KA	13.09	3.08	0.43
mean	14.60	3.22	0.41
BP	17.11	3.41	0.39
SP	25.67	3.80	0.33
KP	22.15	3.66	0.35
mean	21.64	3.62	0.35



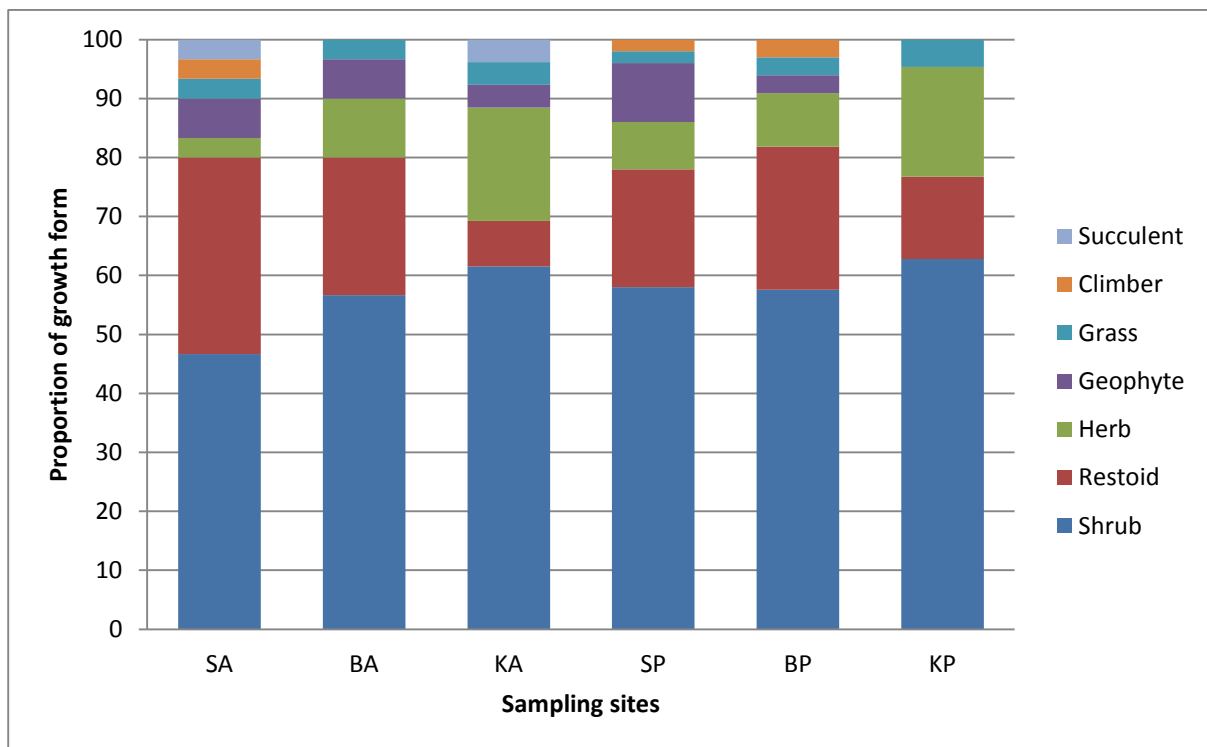


Figure 15: Relative growth form composition of sites sampled with or without *Cyclopia intermedia*. SA = Swartberg Absent; BA = Barrydale Absent; KA = Kouga Absent; SP = Swartberg Present; BP = Barrydale Present; KP = Kouga Present.

#### 4.4 Discussion

The classification of soils where *Cyclopia intermedia* occurs as loamy sand (Kouga and Barrydale) and sandy (Swartberg) was expected (Schutte 1997, Joubert et al. 2011).

There was little difference between the regional sites, with Swartberg having higher soil moisture and a lower bulk density than both Barrydale and Kouga. This relationship is expected as soils with high field capacity generally have a low bulk density (Rae et al. 2006). Despite the difference from Swartberg no difference was recorded between Barrydale and Kouga.

Overall the soils where *C. intermedia* are found have a higher soil moisture field capacity and lower bulk density than soils where no *C. intermedia* occurs. This is

supported by the fact that South slopes (where *C. intermedia* occurs) are notoriously cooler and wetter than dry and warm North slopes (McDonald et al. 1996, Auslander et al. 2003). Influence of microclimate on soil moisture in the field should be noted and it should be considered to record soil moisture in the field. The air temperature recorded showed no clear difference between the sites. Once again unique microclimates could be the driving factor.

The area on Swartberg where *C. intermedia* was not present was characterised by many more restoids than the presence site. This led to the identification of a community that is different from of the presence site. A reason for this could be that the absent site was on a more level plane and a little less slanting than the presence site. This orientation could influence the amount of sun focused on this area which could affect the microclimate. The lower soil moisture as well as soil chemistry (Campbell 1986) could also have contributed to the conditions that favoured restoids.

The species composition of the presence and absence sites sampled in Barrydale and Kouga did not show variation and were thus classified into a single community for each regional site and one shared community. The one community that was identified and contained species from both Kouga and Barrydale shows similarities between these sites and more importantly, highlights the uniqueness of the Swartberg site. The higher species richness and diversity in the sites where *Cyclopia intermedia* is present is expected as South slopes have higher species percentages than North slopes (Auslander et al. 2003). The dominance of shrubs at all sites is expected as broad leaved ericoid species and shrubs are characteristic of mountain Fynbos (Spano et al. 2003).

The effects of fire on species composition should not be overlooked (McDonald et al. 1996). The Kouga present site undergoes controlled burning every 10 years as a measure to control veld height and stimulate resprouting of *C. intermedia* after harvest. This fire cycle could influence the succession of the fynbos species. The absence site only burns when a natural fire occurs, which has not been the case for more than 10 years. Similar data is not available for the other sites but this could explain the species composition of the different sites.

Perhaps the chemistry of the soil should be tested to determine the nutrient level that is required for *Cyclopia intermedia*. It could be possible that different levels of various nutrients influence the vegetation (Campbell 1986, Paudel and Sah 2003).

#### 4.5 Conclusion

In conclusion soil properties and species composition were investigated to provide a preliminary description of the ecosystems where *Cyclopia intermedia* can be found. The soil properties included soil moisture at field capacity, soil bulk density and particle size analysis. Higher soil moisture at field capacity and lower bulk density soils characterise sites where *C. intermedia* is found when compared to areas where it is not. It is recommended that soil moisture be measured in the field to capture the influence of microclimate and solar influx radiation.

The species identified from the three regional sites across the distribution range were incomplete. The once-off sampling led to several species that could only be identified to family or genus level as no inflorescences were present. The only presence community that differed from where *C. intermedia* is absent, is in Swartberg where restios dominated the absent site. The hypothesis stated in the beginning of this study is partially rejected as only the absence and presence sites sampled in Swartberg were identified to be different communities. The samples in both Barrydale and Kouga did not show enough variation to be classified into different communities.

Using information gathered in this study, the ecosystems where *Cyclopia intermedia* are likely to occur have sandy to loamy sand soil, an average field capacity of roughly 33%, a bulk density of 0.99 g/cm<sup>3</sup>, and are generally south facing slopes which will include the following species: *Leucospermum cuneiforme*, *Elegia filacea*, *Leucadendron salignum*, *Protea neriifolia*, *Protea repens*, *Rhodocoma fruticosa*, *Grubbia* sp., *Erica passerinae*, *Helichrysum teretifolium*, and *Seriphium plumosum*. Further investigation into microclimates and soil moisture, amongst others, are required to fully describe the ecosystems where *Cyclopia intermedia* can be expected.

## **Chapter 5: Phenology of *Cyclopia intermedia*, identifying the important life history stages and which environmental factors influence the expression of these stages.**

### **5.1 Introduction**

The harvesting of *Cyclopia intermedia* for use in the honeybush industry places strain on the wild populations. Pressure is being placed on authorities to grant permits for both harvesting of biomass and seeds from wild populations. Sustainable harvesting is not possible without an understanding of the ecology of the species.

For cultivation purposes information is also required on the life history stages of the species. This information could be used to determine best times to plant, irrigate, harvest and burn prospective *C. intermedia* plantations, although this species is not favoured for cultivation (Du toit et al. 1998) given its slow growing nature.

According to the Agricultural Research Council (2010) *Cyclopia* species flower in the spring months of September and October and pods ripen in early summer (late November, early December). However, different species use resources in different ways and do not necessarily have the same phenophase at the same time.

The aim of this chapter was to describe the seasonality of the phenophases of *C. intermedia* across its distribution range. The hypothesis to be tested is that flowering occurs during spring and pods will develop in summer.

### **5.2 Materials and Methods**

#### **Study sites**

Three sites were selected across the distribution range (Eastern and Western Cape) of *Cyclopia intermedia* (Fig. 16). The sites are (from east to west): Kouga, Swartberg, and Barrydale.

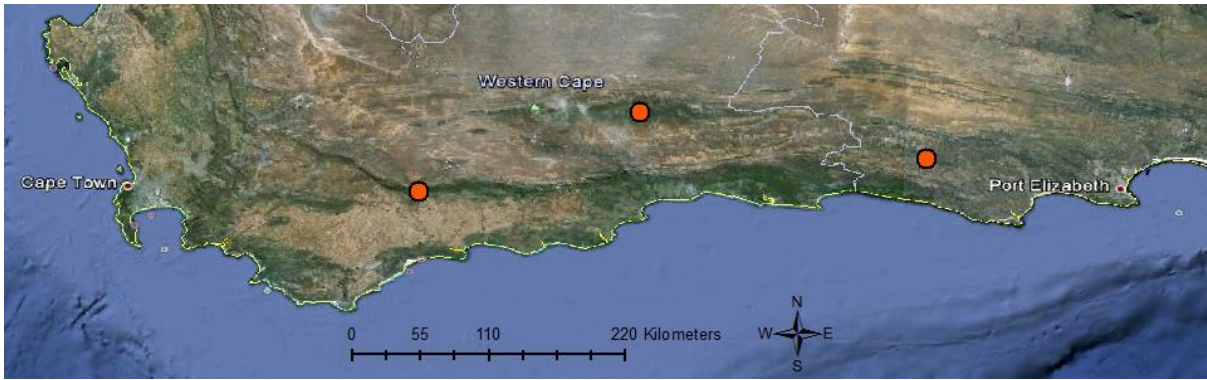


Figure 16: The sampling sites used in a phenology study across the distribution range of *Cyclopia intermedia*. The sites are, from the East: Kouga, Swartberg, and Barrydale.

### **Swartberg**

A population of 30 previously un-harvested *Cyclopia intermedia* individuals were located approximately 2 km into the Swartberg pass outside of Oudtshoorn in the Western Cape, using herbarium locality records.

### **Barrydale**

A very large population with approximately 3 years of regrowth after harvesting was used and located on a farm 25 km outside of Barrydale in the Western Cape. 125 individuals were monitored during the study.

### **Kouga**

125 Individuals were monitored on a farm in the Kouga Mountains. The farm is located 60 km from the Langkloof road and the age of the population was 3 years after harvest.

The sample size of 200 to 2000 individuals as suggested by (Pierce 1984) was considered but had to be modified due to the size of the populations found. For example the easily accessible plants in the Swartberg only consists of a population of about 35 individuals in one area, whereas the population found in the Kouga Mountains contained more than 500 individuals. It is for this reason that it was decided to record phenophases for 125 individuals. There is still a large discrepancy

between the number of species observed in Swartberg (35) compared to Kouga (125) and Barrydale (125), but it is smaller gap than there would have been if more individuals were measured at Kouga and Barrydale.

## **Field analysis**

### **Phenology**

All individuals were monitored and checked for morphological changes like new leaves, flowers, and pods for a period of a year. Due to the distance between sites this monitoring could only be done every second month. The results were extrapolated to the skipped months to produce the phenology of every month of the year. The different phenophases were also documented in photos to be used as a guideline for farmers or future researchers.

Average monthly rainfall data was obtained from the South African Weather Service from weather stations in Barrydale, Oudtshoorn and Joubertina. Temperature data was recorded using HOBO data loggers placed at each site and recording throughout the study period at intervals of two hours. To protect the logger from weather, water and animals the logger was placed in a frame created by securing metal strapping in an X-shape to a 1.5 m metal dropper. The logger was fastened to the dropper with cable ties using the X as a frame. This frame was covered with a 5 l plastic ice-cream tub. This size container ensured more airflow across the logger than a smaller cover as well as provides sufficient protection. The cover was sprayed green to camouflage the logger.

### **Fecundity**

During the months of flower production the number of flowers on two stems of each individual was counted and the average number of flowers per stem was calculated. The total number of stems of each individual was also recorded and the average number of flowers per plant was calculated by multiplying the total number of stems by the number of flowers per stem. This same procedure was followed to estimate the number of pods produced per plant.

## **Statistical analysis**

The differences between the site temperatures, the number of stems, flowers and pods of the plants from the different sites were all calculated by using simple Student's t-tests and a probability threshold of 0.05.

## **5.3 Results**

### **Phenology**

Five phenophase were identified for *Cyclopia intermedia* (Fig. 17): bud formation, bud enlargement, flowering, growth, and pod development. Growth (Plate 3) occurred in the summer from December to March. Flowering buds (Plate 4) developed in April and May during autumn and enlargement occurred from June to August in the winter. Flowering (Plate 5) was recorded in the spring from September to November with pods (Plate 6) developing in November and seed set (Plate 7) occurring during December. In the Eastern Cape phenophases were delayed an average of 2 weeks at the Kouga site, resulting in flowering only occurring in October.

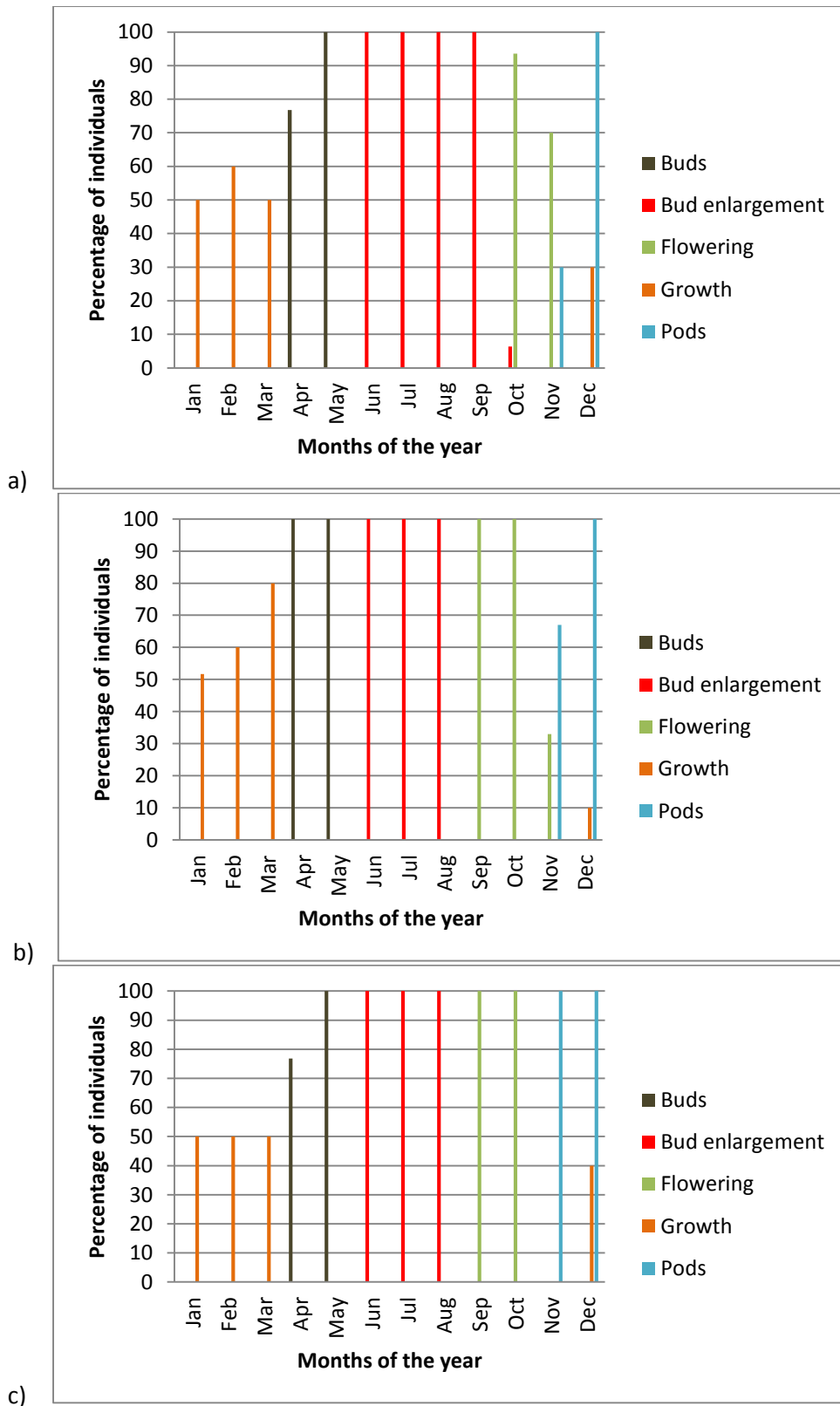


Figure 17: Phenophases of *Cyclopia intermedia* recorded in a) Kouga in the Eastern Cape, b) Swartberg and, c) Barrydale in the Western Cape.



The process of bud enlargement occurred over such a long time that some individuals might have been dormant within these months, but could not have been morphologically differentiated from actively growing buds.



Plate 3: Bright green leaves observed as new growth in *Cyclopia intermedia* during the summer months from December to March.



Plate 4: Two stages of flowering bud formation observed in *Cyclopiia intermedia* a) bud formation in autumn (April and May); and b) bud enlargement in winter (July to August).



Plate 5: a) Early flowering and b) Flowers of *Cyclopiia intermedia* in spring (Sept-Nov).

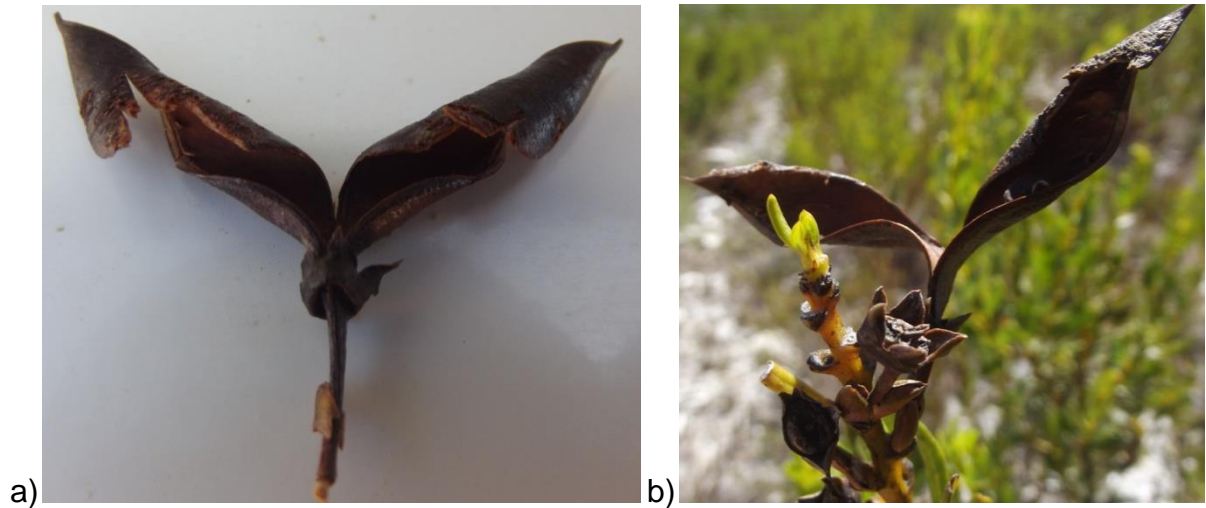


Plate 6: a) Open pod following seed release and b) new growth observed in *Cyclopia intermedia* populations during summer (Nov and Dec).



Plate 7: Different stages of pods and seeds of *Cyclopia intermedia* observed in Nov and Dec.

Mean monthly temperatures (Fig. 18) followed seasonal trends as expected in all sites. Some months showed temperatures at certain sites that were significantly different when compared to others (Table 4), but no clear difference can be identified between sites or between the Eastern Cape and Western Cape regions. Rainfall data (Fig. 19) shows a decreased amount of rainfall recorded in August 2013 when compared to August 2012 at Kouga. Both Swartberg and Barrydale received similar, or more in the case of Barrydale, amounts of rainfall in the month before flowering (August in both 2012 and 2013).

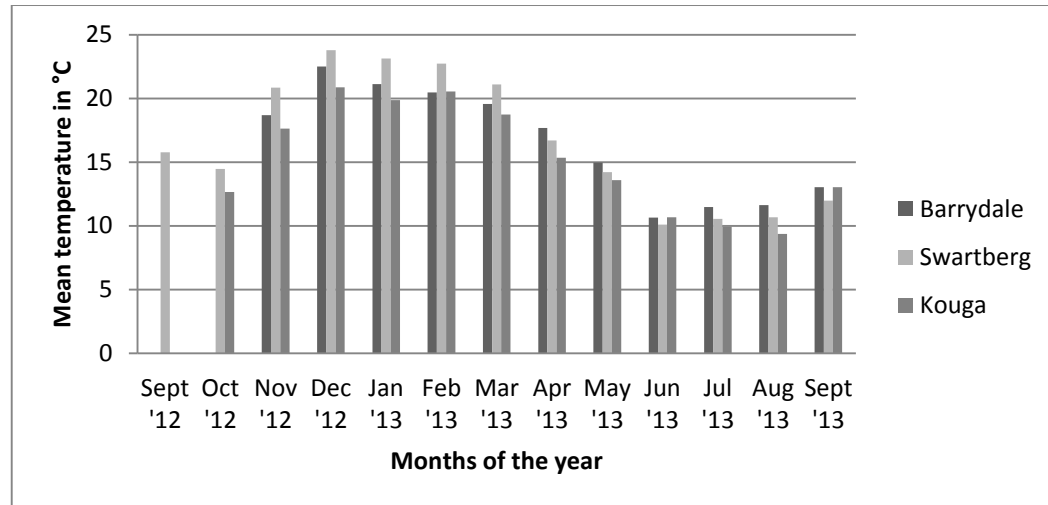


Figure 18: Mean monthly temperatures recorded at three stands of *Cyclopia intermedia* along its distribution.

Table 4: The statistical comparison between the temperatures recorded in *Cyclopia intermedia* stands at three sites along its distribution: Swartberg (S), Kouga (K) and Barrydale (B). Significant differences ( $p < 0.05$ ) are indicated by an asterisk \*.

	Sept '12	Oct '12	Nov '12	Dec '12	Jan '13	Feb '13	Mar '13	Apr '13	May '13	Jun '13	Jul '13	Aug '13
S vs K (p - value)	0.087	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *	0.074	0.087	0.076	<0.05 *
S vs K (t stat)	-1.362	3.381	4.425	4.298	4.660	2.932	3.832	2.477	1.451	-1.359	1.437	2.545
S vs B (p - value)			<0.05 *	0.070	<0.05 *	<0.05 *	<0.05 *	0.089	0.112	0.158	<0.05 *	0.060
S vs B (t stat)			2.788	1.815	2.791	3.217	2.217	-1.346	-1.217	-1.005	-1.717	-1.553
K vs B (p - value)			0.147	<0.05 *	<0.05 *	0.448	0.082	<0.05 *	<0.05 *	0.497	<0.05 *	<0.05 *
K vs B (t stat)			-1.451	-2.538	-1.948	0.131	-1.390	-3.391	-2.274	0.007	-2.780	-3.466

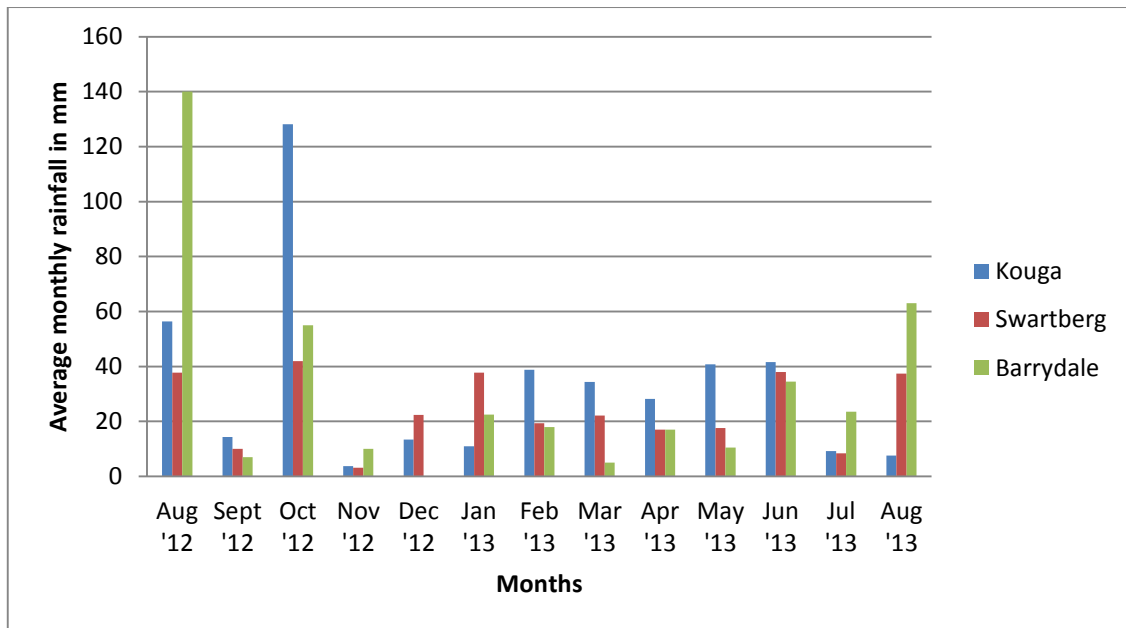


Figure 19: Average monthly rainfall data recorded at three stands of *Cyclopia intermedia* along its distribution.

### Fecundity

The plants at the Kouga site had significantly more stems (Fig. 20) than both those at Barrydale ( $p < 0.001$ ,  $t = -21.4$ ) and Swartberg ( $p < 0.001$ ,  $t = 21.063$ ). The plants at Barrydale in turn had more stems than those in Swartberg ( $p = 0.006$ ,  $t = 2.6136$ ).

It is clear the number of stems of a plant affects the number of flowers the plant would be able to produce. This is evident as the average number of flowers per plant follows the same pattern as that of the stems, with the plants at the Kouga site producing significantly more flowers (Fig. 21) than both those at Barrydale ( $p < 0.001$ ,  $t = 5.3855$ ) and Swartberg ( $p < 0.001$ ,  $t = 9.687$ ). The plants at Barrydale in turn had more flowers than those in Swartberg ( $p < 0.001$ ,  $t = -7.283$ ).

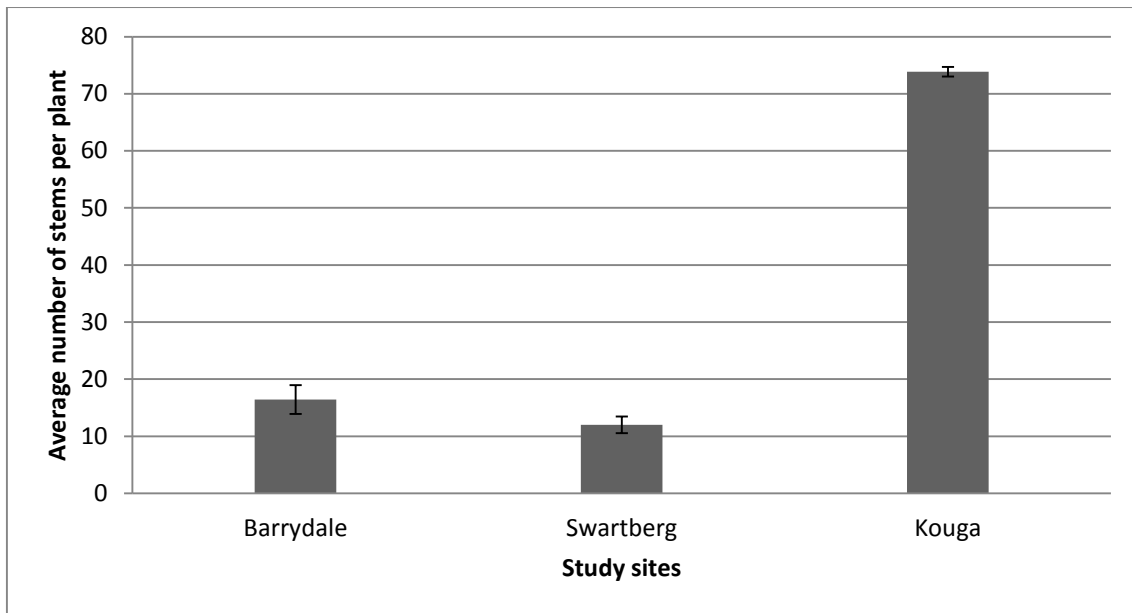


Figure 20: Average number of stems per plant recorded for *Cyclopia intermedia* at three sites across its distribution range. Vertical bars represent standard error.

The number of flowers per plant is significantly more than the number of pods produced for both Barrydale ( $p < 0.001$ ,  $t = 10.17766$ ) and Swartberg ( $p < 0.001$ ,  $t = 3.93$ ) with only 1/3 of flowers developing into pods. Overall the fecundity values for Swartberg (non-harvested plants) are significantly lower than the harvested plants of both Kouga and Barrydale. Seed counts were not done but most pods had 5 seeds in them. An estimation of seed production, using 5 seeds on average, resulted in harvested plants at Barrydale having more seeds (Mean = 1013, SE = 100) than the non-harvested plants in Swartberg (Mean = 268, SE = 118).

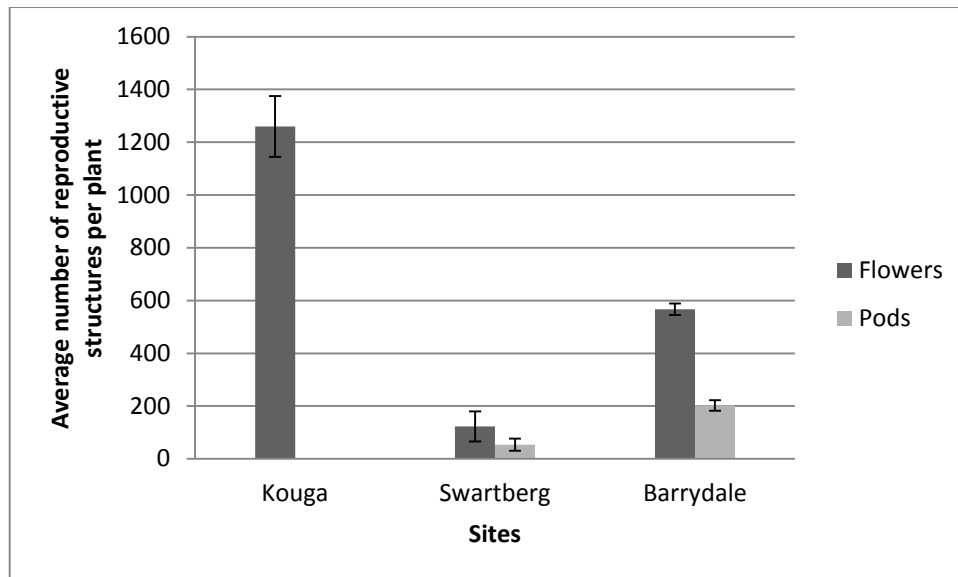


Figure 21: Average number of flowers and pods per plant recorded for *Cyclopia intermedia* at three sites across its distribution range. Vertical bars represent standard error.

## 5.4 Discussion

The simultaneous growth and reproductive stages found in *Cyclopia intermedia* is not unknown in Fynbos (Le Maitre, pers. comm, 1982 in Pierce, 1984) and have been recorded in some Proteaceae species

The early bud development and delayed flowering in *C. intermedia*, where the buds develop in April / May and flowering only occurs in September / October is a characteristic that is shared with some other Fynbos species (Pierce 1984). The developmental stages of the flowering buds could possibly be divided into different growth stages, but buds would most likely need to be dissected or chemically tested to determine small scale changes. It is possible that dormancy also occurs during this time (June – August) but the distinction could not be made with only field observations. Again, dissection could be used to determine both whether growth is taking place and if distinct stages can be identified.

In *C. intermedia* the Western Cape populations enter phenophases approximately 2-3 weeks earlier than their Eastern Cape counterparts. A delay in flowering and other phenophase development from the Western Cape to the Eastern Cape has also

been reported in *Leucodendron* (Pierce, 1984) and in montane species in Europe (Chemielewski 2003).

The temperature data shows no clear difference between the Eastern Cape site and the Western Cape sites, and can thus not be used to explain this delay in phenophase development in the Eastern Cape. The reduced amount of rain recorded in Kouga the month before flowering is expected to occur (Aug), can contribute to late flowering and less flower production (Lubbers and Christensen 1986, Aasa et al. 2004). It is a viable concern as water is required during expensive energy and resource events such as flowering, pod production and seed set. Drought was found to be an influence on fruit and seed abortion which would affect the fecundity and reproductive competition of a species (Kramer et al. 2000, Berjano et al. 2006, Richardson et al. 2013).

The low flower to fruit turnover (only 1/3 of flowers transition into pods) recorded in *Cyclopia intermedia*, is very common in most plants and has been the focus of many studies (Mcgregor 1981, Lubbers and Christensen 1986, Hirayama et al. 2005, Berjano et al. 2006). Berjano et al. (2006) as well as Lubbers & Christensen (1986) contributed the low fruit development to inadequate pollination and limiting resources. It was suggested that either the pollen is not viable or the pollinators are influenced in some way or both these factors can be limiting, which would result in low fruit production. The high number of stems recorded in Kouga could be the result of older plants with larger, more developed lignotubers, as a relationship might exist between tuber size and numbers of flowers, fruits and seeds (Berjano et al. 2006). The fire regime would also influence the resprouting potential of the lignotuber with more vigorous resprouting occurring after fire as opposed to harvesting (Nortje personal communication, 2013). An estimate of seed production shows that harvested plants with more stems have a greater competitive advantage and will produce more seed than non-harvested plants. The estimates of seed numbers made in this study are however an overestimate as some seeds would be aborted, in the same manner as the pods. For this reason not all seeds will reach maturity or full development. Predation on *Cyclopia intermedia* pods and seeds from both insects and baboons should also be taken into account. This information is important when there is an interest in harvesting seeds which could be used for cultivation or



replenishment of population stocks. Again the number of stems will affect the number of pods produced which in turn will affect the number of seeds produced.

The non-harvested Swartberg population is clearly less viable with regards to flower and seed production than the harvested population of the other sites. This is the result of the low stem count that can easily be increased through either harvesting or burning. Keeping in mind that Swartberg is a nature reserve it is not suggested that harvesting be allowed as a monetary incentive, but should be used as a management strategy. Depending on the age of the population it might be beneficial to apply localised burning to the lignotuber in an attempt to encourage resprouting and larger stem numbers. This would most definitely increase stem numbers and in return flower and seed production, if the fire intensity is properly controlled as to not inflict lasting damage to the lignotuber.

## **5.5 Conclusion**

In conclusion, the phenophases of *Cyclopia intermedia* and the time of these phases were identified. Growth occurred in the summer and flowering buds developed in during autumn and underwent enlargement in the winter. Flowering was recorded in the spring with pods developing in November and seed set occurring during December. The hypothesis stated in the beginning of the study is thus accepted. Although the temperature between the sites offered no explanation for the delay in phenophases between the Eastern and Western Cape, rainfall has shown to be an influencing factor.

The number of stems on a plant was shown to be a determining factor in the number of flowers and pods produced. More stems provided the opportunity for more flowers, of which only a small percentage survive the transition to pods. This pattern is expected to continue in the development of seed. Roughly 5 seeds were observed per pod, of which not all were fully developed, but specific counts were not done.

With regards to sustainable harvesting of seeds, the amount of seeds that could be harvested would depend on the specific characteristics of the plants within a given population. From a sustainable perspective not all seeds can be harvested if the

populations are expected to recover and produce new individuals. Measures can however be taken to increase the opportunity for seed production by increasing number of stems.

This study sheds light on the variation in seed production between plants and factors that influence this. This information can be used to inform further research with regard to how viable the produced seeds are and how much can be harvested without harming population dynamics.

## **Chapter 6: Harvest Maturity of *Cyclopia intermedia*, determining the recommended condition of a plant suitable for harvesting.**

### **6.1 Introduction**

The rapid growth of the honeybush tea industry in recent years is placing pressure on farmers and communities to supply the growing demand for *Cyclopia* spp. material used in the production of this healthy beverage. With this pressure growing demand, many efforts are focussed on developing plantations or areas where various *Cyclopia* species can be cultivated. To date only two species have been successfully cultivated and this only at small scale (Joubert et al. 2011). *Cyclopia intermedia* has not yet been cultivated as this species cannot be harvested annually like other species, thus making it less economical to plant. The *C. intermedia* that is used in the industry is sourced entirely from wild populations.

Many farmers own land where this species grows naturally and harvest from this source. Some farmers are granted permission to harvest from farms where the owners are not focussed on tea production while others retrieve their plant material from government land once a harvesting permit has been issued. The harvesting practices with regards to *C. intermedia* differ between different farmers. The cycles at which a farmer harvests the plants follow the same structure that he/she has been taught either by a previous generation family member or have found to be a popular harvest technique within the farming community.

The general consensus on the harvesting of *C. intermedia* is that this species can be harvested every two years at the earliest (Du Toit et al. 1998). According to Joubert et al. (2011), honeybush species (not specifying which) are harvested in the summer to late autumn before flowering so as to minimise the stress on the plant. The entire above ground foliage of the plant is either brush cut or harvested with a sickle at a height of roughly 5 to 10 cm above ground. The time at which this harvesting occurs is up to the farmer as there are presently no requirements or set guidelines for the harvesting of *C. intermedia*.

Although the industry has been in operation for several years and harvesting probably took place many years before that, the effect of harvesting on the ecology of the species has not yet been established. This is an area of great importance as *C. intermedia*, a resprouter, relies heavily on the resources stored in the lignotuber to regenerate after a disturbance such as fire or harvesting (Canadell et al. 1991, Canadell and Lopez-Soria 1998). Incorrect harvesting practices, for example harvesting too frequently, will severely diminish the resources available for regeneration and survival.

It is for this reason that it has become necessary to determine how the morphology of the populations changes with age. This can be used to develop maturity indices that might be useful to facilitate sustainable harvesting of *C. intermedia*.

The aim of this chapter is to study the morphology of populations at different ages to determine whether there are any characteristics that might be useful as maturity indices and therefore signal that harvesting may commence. The hypothesis of this chapter is that populations aged from two to four years will be similar in both size and shape, but will be distinguished by the orange colour of the stems with four year old stems having a deeper orange colour.

## **6.2 Materials and Methods**

### **Study area**

The study sites were situated on a farm in the Kouga mountain range in the Eastern Cape. Four areas were selected based on the age of the *C. intermedia* stands and accessibility to the site. Characteristics of 75 individuals in each of the four different age stands were measured. The ages of stands are reported as years of regrowth after harvest: these are plants with 1, 2, 4 and 5 years of regrowth.

### **Field sampling**

Following Louw (2006), the characteristics measured included plant height, length and breadth using a wooden dowel marked at 10 cm intervals. The height of the plant was measured by placing the dowel in the centre of the plant against the main

stem and perpendicular to the ground and recording the measurement on the dowel at the top of the plant (from the soil surface to the highest point of the canopy). Length and breadth were measured in the middle of the bush with length being the longest axis of the plant and breadth was measured perpendicular to length. The circumference at the top of the plant was also measured using a material tape measure.

These characteristics were recorded to compare the morphological differences between population stands of different ages. Following Phillips & Macmahon (1981) the volume of each individual was calculated as for an ellipsoid spheroid

$$V = (\pi a^2 * b) / 6$$

Where: V = Volume

a = the average of length and breadth, or height, whichever is shorter

b = the average of length and breadth, or height, whichever is longer

Stem colour was measured using Royal Horticultural Society Colour Charts. The stem colour was recorded by comparing the ground colour of the stem (colour behind the bark over growth) 10 cm from the base of the plant with the RHC colour chart. On each plant 3 stems were selected at random and the colour measured from which the representative colour for each plant was recorded.

### **Statistical analysis**

The differences between the volume, circumference and heights of the plants from different age stands were all calculated by using Student's t-tests with a 5% probability significance cut-off. The difference in stem colour from different age stands were also tested using a Student's t-test. Linear regressions were done to determine if there were relationships between age and the growth characteristics. Correlation analyses were done to determine whether a correlation exists between the number of branches and the circumference of the plants.

### 6.3 Results

The growth characteristics of *Cyclopia intermedia* seem to follow an increasing–then–decreasing trend with age. Both the height and the circumference of the plants were higher in the first year of regrowth after harvest compared to the second year. There is however no relationship between the age and the height of the plants ( $F= 0.70$ ;  $p = 0.55$ ; d.f. = 3). Similarly no relationship is found between the age and the circumference of the plants ( $F= 0.04$ ;  $p = 0.98$ ; d.f. = 3).

Even though there was no relationship between the ages and both height and circumference of the plants, the four year population stand showed an average plant height (Fig. 22) that was greater than any of the other age stands tested (Table 5). The four year stand also showed the largest average circumference when compared to all but one of the age classes. The five year stand had an average circumference that was 2 cm larger than that of the four year population.

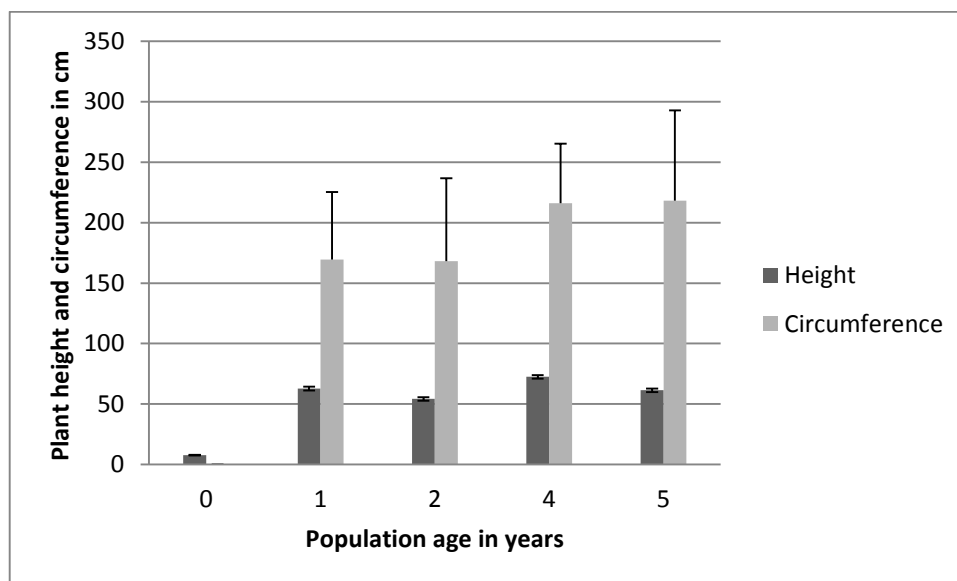


Figure 22: The height and circumference of *Cyclopia intermedia* plants in populations of different ages. Vertical bars represent standard error.

Table 5: The comparison between the height and circumference of different aged populations of *Cyclopiia intermedia*. Significant differences ( $p < 0.05$ ) are indicated by an asterisk \*.

Comparison	Height		Circumference	
	P value	t-stat	P value	t-stat
1 vs 2	< 0.001*	3.90	0.89	0.13
1 vs 4	< 0.001*	-4.43	< 0.001*	-5.40
1 vs 5	0.51	0.66	< 0.001*	-4.54
2 vs 4	< 0.001*	-8.83	< 0.001*	-4.90
2 vs 5	< 0.001*	-3.56	< 0.001*	-4.28
4 vs 5	< 0.001*	5.53	0.83	-0.22

Neither the volume (Fig. 23) ( $F = 0.35$ ,  $p = 0.78$ , d.f. = 3) nor the number of stems (Fig. 24) ( $F = 1.36$ ,  $p = 0.26$ , d.f. = 3) showed any relationship between the age and the specific characteristic. With regards to volume the four year stand was significantly larger than the 1 year ( $p < 0.001$ ,  $t = -5.30$ ), 2 year ( $p < 0.001$ ,  $t = -7.44$ ), and 5 year stands ( $p < 0.001$ ,  $t = 3.233$ ).

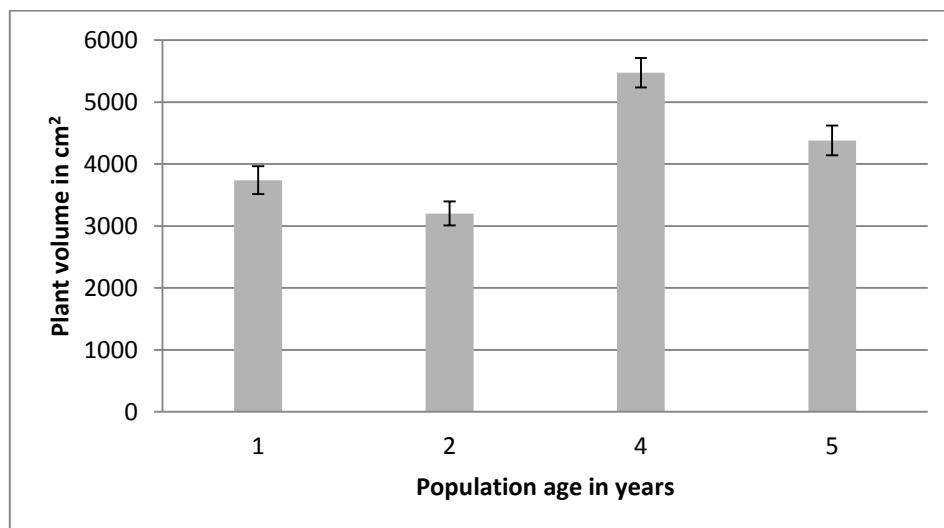


Figure 23: The average plant volume of *Cyclopiia intermedia* plants in populations of different ages. Vertical bars represent standard error.

There is a positive correlation in all the age stands between the volume of the plants and the number of branches. The highest correlations were observed in the 2 year ( $r = 0.78$ ) and 5 year stands ( $r = 0.75$ ). The 1 year population showed a slightly lower correlation ( $r = 0.68$ ), with the 4 year stand ( $r = 0.54$ ) having the lowest correlation between the number of branches and the volume of the plants.

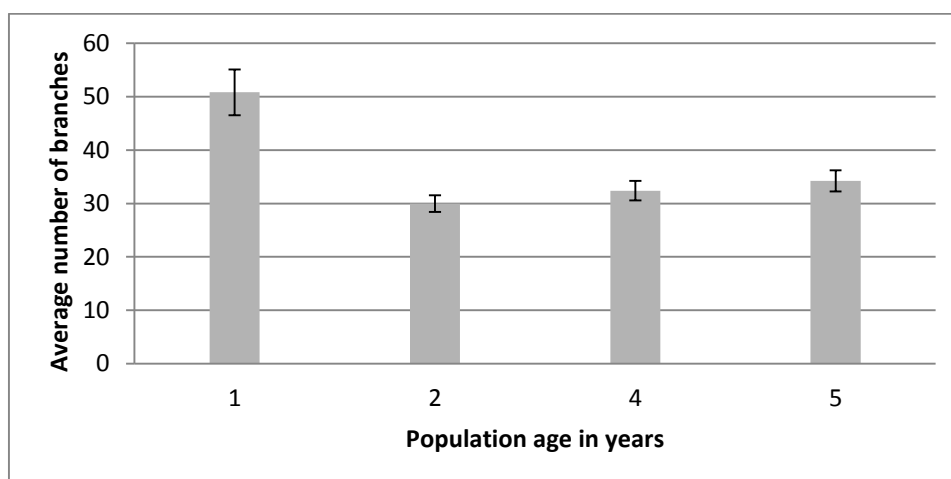


Figure 24: The average number of branches at the base of *Cyclopa intermedia* plants in populations of different ages. Vertical bars represent standard error.

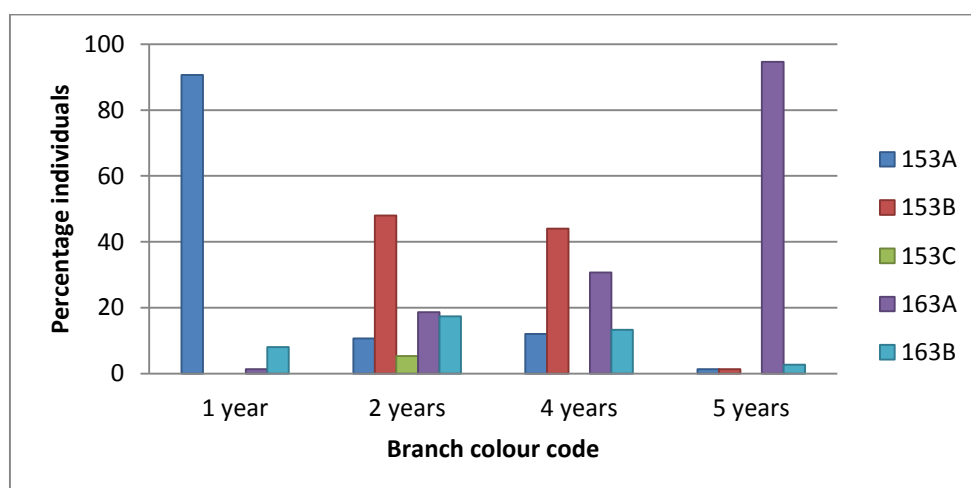


Figure 25: Classification of branch colour of individuals from different age groups using colour codes from the Royal Horticultural Society (RHS) classification system.



Significant differences between the combinations of colours (Fig. 25) were identified between all age ranges except between 2 and 4 years (Table 6). A progression in the percentage of plants with more mature orange coloured branches (163 A) can be seen from 2 years with highest percentage in 5 years.

Table 6: The comparison between combinations of stem colours of different aged populations of *Cyclopia intermedia*. Significant differences ( $p < 0.05$ ) are indicated by an asterisk \*.

Comparison	P value	t-stat
1 vs 2	< 0.001*	-7.30
1 vs 4	< 0.001*	-7.59
1 vs 5	< 0.001*	-18.45
2 vs 4	0.75	-0.31
2 vs 5	< 0.001*	-7.05
4 vs 5	< 0.001*	-6.58

## 6.4 Discussion

The variation observed in all parameters (number of stems, height, circumference, and by extension, volume) between the different age stands is the result of microclimate variation between sites. Very favourable conditions followed the harvest of the now one year stand, as it was harvested just before heavy rainfalls that broke a drought. These conditions could explain the higher averages in this stand when compared to other ages.

It is very important to note that the populations used in this study were different populations and not a single population that was monitored for several years. The slight differences in the parameters measured might also, to some degree, be affected by the unique microhabitat in which each of the populations is found. This is minimised by using one farm as a base site, but the possibilities must be noted and the influence of microhabitat on plant growth must be kept in mind.

It can be said that the initial resprouting produces: all the height of the plant, roughly three quarters of the diameter, by extension also three quarters of the volume, and

all the main branches. The rest of the diameter and volume is presumably gained at later stages when the lateral branching occurs.

A study on lignotubers found that after harvesting the entire biomass from two species of Mediterranean shrubs (Canadell et al. 1991), a decrease in the starch content of the lignotuber was recorded for both species. The levels of starch together with all nutrients was still somewhat depleted after the first growing season, with the exception of phosphorus (Canadell and Lopez-Soria 1998). According to this study, no statistical difference was found between the nutrient concentration of the lignotubers of plants that have been harvested and non-harvested plants after two years of the harvest. This presents the possibility that the nutrients lost from the lignotuber during the resprouting could be replenished after two years.

Canadell & Lopez-Soria (1998) stated that death of the plants will be imminent if disturbances that required regrowth from the lignotuber (harvesting or fire) occur on a more frequent basis than two year intervals. This is because continuous regrowth from the lignotuber will eventually result in the depletion of the resources stored in the tuber. Studies on the translocation of photosynthates to the lignotuber and nutrient fluxes within the lignotuber should still be done for *Cyclopia intermedia* to definitively say when would be the best time to harvest with regards to nutrient storage and resprouting potential.

The highest average volume was recorded in the five year stand but this is not particularly useful for harvesting purposes. At this stage the individual stems of the plant are too thick for the industrial blades used to chop the material into the correct size for tea. If plants were harvested at 5 years the equipment cost of replacing the blades would be a draw-back, and farmers would not happily agree to this.

From an economic point of view, the high volume recorded for the four year stand might be a favourable result for farmers that want to produce a high volume of tea. There is of course the other option of simply harvesting every two years and producing the same amount of tea. The trade off would come in the quality, could the younger plants have the same flavour as the older plants?

When using colour as a maturity index the problem arises where the colour required for commodities to survive storage and transportation and still be ripe and fresh,

might not be the optimum colour needed for immediate consumption. De Kock (2011) found that apricots picked with a more advanced surface colour exhibited more internal diseases after storage than the fruits picked at a less mature age. In the case of processed products such as jams, juices and teas (*Cyclopia intermedia*, *C.subternata* and *Aspalathus linearis*) this problem is however overcome as the suitable surface colour would be those of already ripe or mature produce, instead of a colour that would still require some ripening after harvest.

The gradual increase in the orange stem colour (163 A) coincided with the proposed maturation and translocation of photosynthates to the lignotuber. Stem colour can thus be used as a valuable maturity index as it can produce a visual representation of what a three or four year old plant looks like. The problem reported by Łysiak (2012), where the blush of the apples covered the colour was the same problem encountered while measuring the stem surface colour of *C. intermedia* as the thin bark cover over the surface had to be ignored and the green or orange base colour measured.

In *Aspalathus linearis* a specific harvest height is treated as the benchmark for harvesting as to ensure no lasting detrimental effect on the plants (Louw 2006). In *C. intermedia* the age of the plants, rather than the height should be the regulation as these plants reach a large size at two years of age. The height and circumference could be used as auxiliary indices but these characteristics are dependent on environmental factors like rainfall, soil properties and competition for space from surrounding vegetation. Even though the plant has reached a seemingly large size the resources may not yet have been translocated to the lignotuber to allow for sustainable management and survival after harvesting.

With regards to other possible maturity indices, although the chemical composition of *C. intermedia* would be useful for the biochemical analysis of the tea and development of taste, these characteristics would not be particularly valuable in the determination of maturity. Unless the chemical compositions of different age stands are tested to determine the optimum stage at which the best taste develops. This information would then have to be combined with the ecological requirements of the species to ensure survival after harvest. Some farmers believe that with *C. intermedia*, as with fruit, the plants left to mature naturally deliver the best flavour.

## 6.5 Conclusion

Although the height, circumference and volume can be used as secondary indices, in this study it proved more useful as it led us to a closer understanding of at which age to harvest. This provides an answer to one of the biggest questions that challenge harvesting management for *Cyclopia intermedia*.

Populations with at least 30% orange (163 A) stems that fit the codes from the RHS colour chart, can be estimated to be roughly 3 years of age and is thus suitable for harvesting. Stem colour would be most useful when the age of the population is not known. This index can still be used if some link can be found between the flavours of tea produced from the different coloured stems.

The hypothesis stated in the beginning of the study is thus partially rejected as plants of four years of age are significantly larger in height, circumference and volume than the two year stands. It is also partially accepted, even though the combination of stem colours of the two and four year stands were not significantly different a clear gradient in change from green (153 A) to orange (163 A) can be seen and can be used to determine age and maturity.

In the case where the age of the population is known, populations can be harvested on three year cycles following Canadell and Lopez-Soria (1998). Four years would ecologically be better as this allows the plant another flowering season to produce seed, but the pressure of industry should also be considered.

It is important in these transitional areas, where the ecology and the agriculture meet, to find a balance where both parties have to sacrifice a little to maintain the species. This would result in farmers having to wait at least three years to harvest the tea and the ecologically having to sacrifice a flowering event.

Further studies in the link between the chemical composition and flavour of the tea in relation to the stem colour would be useful to determine if more mature plants have a more pronounced flavour. This could also influence the decision on when to harvest as the quality of the product is essential to the industry. Studies on the nutrient fluxes within the lignotuber would also help narrow down the most important time in the life cycle of *Cyclopia intermedia* that must be respected.

## Chapter 7: Conclusions and recommendations

The distribution of *Cyclopia intermedia*, produced with predictive modelling, extends along all the mountain ranges in the Cape Fold mountains from the Eastern Cape to the Western Cape. This regional distribution is expected to undergo a shift and lose up to 63% of the current range under future (2050) climate conditions. This range shift and contraction will have implications for the honeybush tea industry if, at that stage, there is still a reliance on wild populations.

Locally, areas with *Cyclopia intermedia* were associated with south-facing slopes with sandy to loamy sand soils and higher water holding capacity than surrounding areas. *Cyclopia intermedia* generally occurs with Proteaceae, *Leucadendron salignum*, *Protea neriifolia* and *Protea repens* as well as Restionaceae *Elegia filacea* and *Rhodocoma fruticosa*, and Asteraceae *Seriphium plumosum*.

The stem colour of a plant has been identified as a possible harvest maturity index where at least 30% of the population has orange stems (163A on the Royal Horticultural colour chart) indicate an individual of roughly 4 years of age. This index would be most useful when the age of the population is not known. If however the age is known it is suggested that harvesting should occur in three year cycles (every third year). It is important in these transitional areas, where the ecology and the agriculture meet, to find a balance where both parties have to sacrifice a little to maintain the species. This would result in farmers having to wait at least three years to harvest the tea and the ecologically having to sacrifice a flowering event. There should however be no sacrifice that would put the species at risk.

The timing of important life cycle events of *C. intermedia* was also identified. Growth occurred in the summer and flowering buds developed in during autumn and underwent enlargement in the winter. Flowering was recorded in the spring months with pods developing in November and seed set occurring during December. The number of stems on a plant was shown to be a determining factor in the number of flowers and pods produced. Harvested individuals had more stems than non-harvested plants and thus produced more seed. More stems provided the opportunity for more flowers, of which only a small percentage survive the transition to pods. The difference between harvested and non-harvested fecundity variables is

valuable information for the management of the species. Controlled harvesting or localised short-cycle burning should be investigated for management purposes to encourage greater stem numbers and consequently seed production in declining protected populations.

Detailed small scale modelling using soil characteristics will be required to identify the microclimate of the south-facing slopes on the distribution of *C. intermedia*. More presence and true absence records suited for the purposes of predictive mapping should also be collected.

With regards to harvest maturity, further studies in the link between the chemical composition and flavour of the tea in relation to the stem colour would be useful to determine if older stems have a more desirable flavour. This could also influence the decision on when to harvest as the quality of the tea is essential to the industry. Studies on the time of basipetal translocation to the lignotuber will also be required to inform harvesting frequency.

It is recommended that wild populations be burned as a management procedure to increase the number of stems and thereby fecundity. This aspect should be investigated as a conservation measure to increase seed production and support population rejuvenation. Specific seed counts should also be done to determine guidelines on how much seed can be harvested without damaging population dynamics.

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## Appendix A: Settings for species distribution modelling

The clustering and selection of BIOCLIM variables and the settings used for model fitting following the layout in Potts et al. (2013).

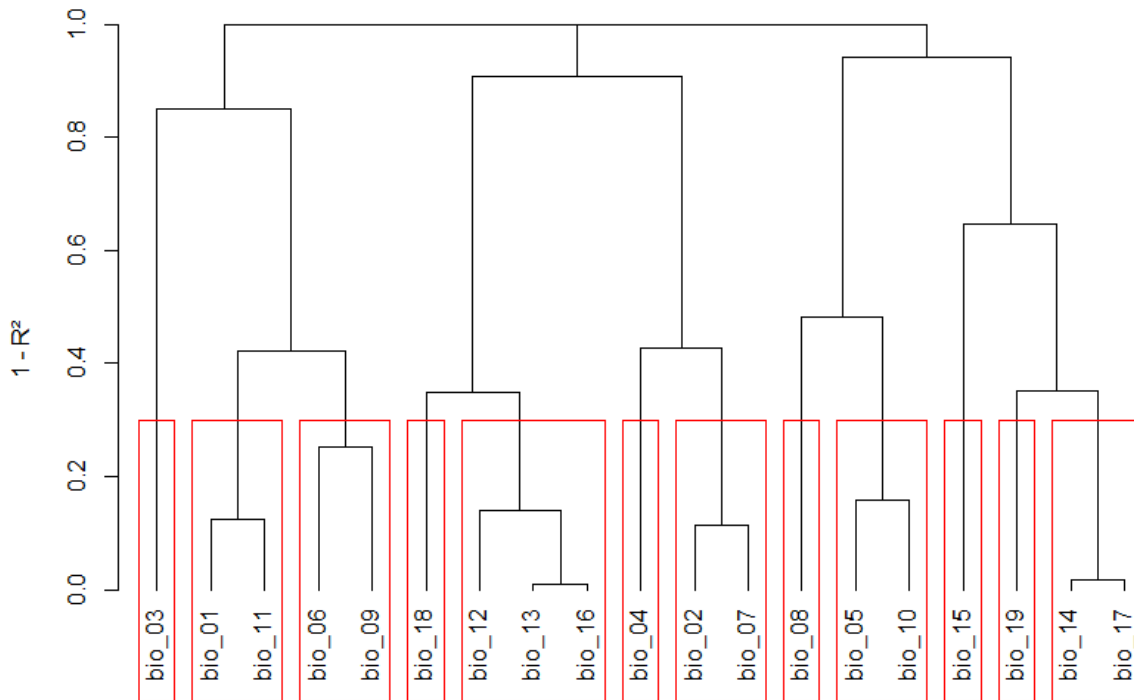


Figure A1: The hierarchical clustering of the 19 bioclimatic variables using a dissimilarity dendrogram. The clusters shown in red boxes are highly intercorrelated ( $r^2 > 0.7$ ).

Table A1: A description of the clustering and selection of the 19 bioclimatic variables (Busby 1991). The selected variables were used in the species distribution modelling of *Cyclopia intermedia* under current and future climates.

Climate variables	BIOCLIM code	Climate variable set					
		1	2	3	4	5	6
<b>Cluster 1</b>							
Annual precipitation (mm)	BIO_12				X	X	
Precipitation in the wettest month (mm)	BIO_13		X	X			
Precipitation in the wettest quarter (mm)	BIO_16	X					
<b>Cluster 2</b>							
Annual mean temperature	BIO_01		X	X	X	X	X
Mean temperature of the coldest quarter (°C)	BIO_11		X	X			
<b>Cluster 3</b>							
Mean diurnal range (mean of monthly maximum temperature minus minimum temperature)	BIO_02					X	
Temperature annual range	BIO_07				X		X
<b>Cluster 4</b>							
Maximum temperature of the warmest month (°C)	BIO_05	X		X			X
Mean temperature in the warmest quarter (°C)	BIO_10		X			X	
<b>Cluster 5</b>							
Precipitation in the driest month (mm)	BIO_14			X	X		
Precipitation in the driest quarter (mm)	BIO_17						X
<b>Cluster 6</b>							
Minimum temperature of the coldest month (°C)	BIO_06	X			X	X	
Mean temperature in the driest quarter (°C)	BIO_09		X				X
<b>Unclustered</b>							
Isothermality	BIO_03				X		X
Precipitation in the warmest quarter (mm)	BIO_18						X
Temperature seasonality (standard deviation of annual mean temperature × 100)	BIO_04	X	X	X			
Mean temperature of the wettest quarter (°C)	BIO_08	X	X				
Precipitation seasonality (standard deviation of monthly precipitation values)	BIO_15	X		X	X	X	
Precipitation in the coldest quarter (mm)	BIO_19	X				X	

Table A2: The settings used for the model fitting in the production of ensemble models of current and future distributions of *Cyclopia intermedia* (Potts et al. (2013)).

Model class	Method	Name for model	Settings and notes
Profile	Bioclim (Nix 1986)	BC	Used R <sup>1</sup> and function <i>bioclim</i> from the DISMO library.
Regression	Generalized additive models (Hastie and Tibshirani 1990)	GAM	Used R <sup>1</sup> and function <i>gam</i> from the MGCV library. The smoothing parameter estimation was solved using generalized cross validation and best models were selected using null space penalization.
Machine learning	Random forests (Breiman 2001)	RF	Used R <sup>1</sup> and function <i>randomForests</i> from the RANDOMFORESTS library to build an ensemble of classification trees.
	Maximum entropy (Phillips et al. 2006)	MX	Used version 3.3.3e from within R <sup>1</sup> with the <i>maxent</i> function in the DISMO library. All settings were the defaults except that the background samples were the sampled 5000 absence points combined with presence points.

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R Development Core Team (2011).



## Appendix B : Phytosociology species lists

<b>Threatened</b>	<b>CE</b>	<b>Critically endangered</b>	(Raimondo et al. 2009)
	<b>E</b>	<b>Endangered</b>	
	<b>V</b>	<b>Vulnerable</b>	
	<b>D</b>	<b>Declining</b>	
	<b>DD</b>	<b>Data deficient</b>	
	<b>NT</b>	<b>Near-threatened</b>	
	<b>R</b>	<b>Rare</b>	
<b>Protected</b>	<b>EC</b>	Eastern Cape Environmental Conservation Bill as introduced 23 November 2003	

### Barrydale species list

Division	Family	Species	Status	Uses
Magnoliopsida	Asteraceae	<i>Athanasia</i> sp. L.		
Magnoliopsida	Asteraceae	<i>Seriphium plumosum</i> L.		Ornamental
Magnoliopsida	Asteraceae	<i>Syncarpha</i> sp. DC.		
Magnoliopsida	Asteraceae	<i>Dicerthamnus rhinocerotis</i> (L.f.) Koekemoer		Indigestion & Ornamental
Magnoliopsida	Asteraceae	<i>Euryops rehmannii</i> Compton		
Magnoliopsida	Asteraceae	<i>Helichrysum</i> sp. Mill.		
Magnoliopsida	Asteraceae	<i>Metalasia densa</i> (Lam.) P.O. Karis		
Magnoliopsida	Asteraceae	<i>Metalasia muricata</i> (L.) D. Don		Herbal tea & management of dune erosion
Magnoliopsida	Ericaceae	<i>Erica plukenetii</i> L.	EC	Ornamental
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 1 L.	EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 2 L.	EC	
Magnoliopsida	Ericaceae	<i>Erica vestita</i> Thunb.	EC	

Magnoliopsida	Fabaceae	<i>Cyclopia intermedia</i> E. Mey.	D, EC	Honeybush tea
Magnoliopsida	Grubiaceae	<i>Grubbia</i> sp. P.J. Bergius		
Magnoliopsida	Lauraceae	<i>Cassytha ciliolata</i> Nees		
Magnoliopsida	Lobeliaceae	<i>Lobelia</i> sp. L.		Ornamental
Magnoliopsida	Mesembryanthemaceae	<i>Erepsia</i> sp. N.E.Br.	EC	
Magnoliopsida	Proteaceae	<i>Protea neriifolia</i> R.Br.	EC	Ornamental & Wild flower trade
Magnoliopsida	Proteaceae	<i>Protea repens</i> (L.) L	EC	Ornamental & Wild flower trade
Magnoliopsida	Proteaceae	<i>Leucadendron salignum</i> P.J. Bergius	EC	Ornamental
Magnoliopsida	Proteaceae	<i>Leucospermum cuneiforme</i> (Burm.f.) Rourke	EC	Ornamental
Magnoliopsida	Rhamnaceae	<i>Phyllica</i> sp. L.		
Magnoliopsida	Rosaceae	<i>Cliffortia ruscifolia</i> L.		
Magnoliopsida	Rubiaceae	<i>Anthospermum</i> sp. L.		
Magnoliopsida	Rutaceae	<i>Agathosma</i> sp. Willd.	EC	
Magnoliopsida	Thymelaeaceae	<i>Passerina</i> sp. 2 L.		
Liliopsida	Iridaceae	<i>Tritoniopsis</i> sp. L. Bolus	EC	
Liliopsida	Iridaceae	<i>Watsonia</i> sp. Mill.	EC	
Liliopsida	Poaceae	Poaceae		
Liliopsida	Restionaceae	<i>Restio dispar</i> Mast		Ornamental & Thatching
Liliopsida	Restionaceae	<i>Restio</i> sp. Rottb.		
Liliopsida	Restionaceae	<i>Rhodocoma fruticosa</i> (Thunb.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Thamnochortus</i> sp. P.J. Bergius		
Liliopsida	Restionaceae	<i>Elegia filacea</i> Mast		Ornamental
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 1 Steud.		
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 2 Steud.		
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 3 Steud.		
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 4 Steud.		

### Swartberg species list

Division	Family	Species	Status	Uses
Magnoliopsida	Asteraceae	<i>Berkheya</i> sp. Ehrh.		
Magnoliopsida	Asteraceae	<i>Dicerotheramnus rhinocerotis</i> (L.f.) Koekemoer		Indigestion & Ornamental
Magnoliopsida	Asteraceae	<i>Seriphium plumosum</i> L.		Ornamental
Magnoliopsida	Asteraceae	<i>Syncarpha argentea</i> (Thunb.) B. Nord.		Ornamental
Magnoliopsida	Asteraceae	<i>Euryops rehmannii</i> Compton		
Magnoliopsida	Asteraceae	<i>Gongyloglossa tortilis</i> (DC.) Koekemoer		
Magnoliopsida	Asteraceae	<i>Helichrysum cymosum</i> (L.) D. Don		Herbal tea & Ornamental
Magnoliopsida	Asteraceae	<i>Helichrysum teretifolium</i> (L.) D. Don		
Magnoliopsida	Asteraceae	<i>Metalasia densa</i> (Lam.) P.O. Karis		
Magnoliopsida	Asteraceae	<i>Metalasia muricata</i> (L.) D. Don		Herbal tea & management of dune erosion
Magnoliopsida	Asteraceae	<i>Osteospermum junceum</i> P.J. Bergius		
Magnoliopsida	Asteraceae	<i>Relhania calycina</i> (L.f.) L' Hér		
Magnoliopsida	Boraginaceae	<i>Lobostemon fruticosus</i> (L.) H. Buek		Herbal tea
Magnoliopsida	Bruniaceae	<i>Brunia noduliflora</i> Goldblatt & J.C. Manning		Ornamental & Wild flower trade
Magnoliopsida	Campanulaceae	<i>Wahlenbergia</i> sp. Schred. Ex Roth		
Magnoliopsida	Celastraceae	<i>Maytenus oleoides</i> (Lam.) Loes		
Magnoliopsida	Ericaceae	<i>Erica inamoena</i> Dulfer	R, EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 1 L.	EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 3 L.	EC	
Magnoliopsida	Fabaceae	<i>Cyclopia intermedia</i> E. Mey.	D, EC	Honeybush tea
Magnoliopsida	Fabaceae	<i>Psoralea oligophylla</i> Eckl. & Zeyh		
Magnoliopsida	Geraniaceae	<i>Pelargonium</i> sp. 3 L' Hér		
Magnoliopsida	Lauraceae	<i>Cassytha ciliolata</i> Nees		
Magnoliopsida	Mesembryanthemaceae	<i>Ruschia intricata</i> (N.E.Br.) H.E.K. Hartmann & Stüber	EC	

Magnoliopsida	Proteaceae	<i>Leucadendron salignum</i> P.J. Bergius	EC	Ornamental
Magnoliopsida	Proteaceae	<i>Protea neriifolia</i> R.Br.	EC	Ornamental & Wild flower trade
Magnoliopsida	Proteaceae	<i>Protea repens</i> (L.) L	EC	Ornamental & Wild flower trade
Magnoliopsida	Rhamnaceae	<i>Phylica willdenowiana</i> Eckl & Zeyh		
Magnoliopsida	Rubiaceae	<i>Anthospermum aethiopicum</i> L.		
Magnoliopsida	Rubiaceae	<i>Anthospermum</i> sp. L.		
Magnoliopsida	Rutaceae	<i>Agathosma cerefolium</i> (Vent) Bartl & H.L. Wendl.	EC	Ornamental
Magnoliopsida	Thymelaeaceae	<i>Passerina</i> sp. 1 L.		
Liliopsida	Iridaceae	<i>Ixia orientalis</i> L. Bolus	EC	
Liliopsida	Iridaceae	<i>Tritoniopsis</i> sp. L. Bolus	EC	
Liliopsida	Iridaceae	<i>Gladiolus</i> sp. L.	EC	
Liliopsida	Iridaceae	<i>Watsonia</i> sp. Mill.	EC	
Liliopsida	Iridaceae	<i>Nivenia binata</i> Klatt	EC	
Liliopsida	Orchidaceae	<i>Holothrix</i> sp. Rich. Ex Lindl.	EC	
Liliopsida	Poaceae	Poaceae		
Liliopsida	Restionaceae	<i>Restio dispar</i> Mast		Ornamental & Thatching
Liliopsida	Restionaceae	<i>Restio sejunctus</i> Mast		
Liliopsida	Restionaceae	<i>Rhodocoma fruticosa</i> (Thunb.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Hypodiscus albo-aristatus</i> (Nees) Mast		
Liliopsida	Restionaceae	<i>Hypodiscus argenteus</i> Mast		
Liliopsida	Restionaceae	<i>Thamnochortus lucens</i> (Poir.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Thamnochortus</i> sp. P.J. Bergius		
Liliopsida	Restionaceae	<i>Elegia filacea</i> Mast		Ornamental
Liliopsida	Restionaceae	<i>Elegia racemosa</i> (Poir.) Pers		

### Kouga species list

Division	Family	Species	Status	Uses
Magnoliopsida	Asteraceae	<i>Euryops</i> sp. (Cass.) Cass		
Magnoliopsida	Asteraceae	<i>Gazania krebsiana</i> Less.		Ornamental & Grazing
Magnoliopsida	Asteraceae	<i>Gongyloglossa tortilis</i> (DC.) Koekemoer		
Magnoliopsida	Asteraceae	<i>Helichrysum anomalum</i> Less.		
Magnoliopsida	Asteraceae	<i>Helichrysum felinum</i> Less.		
Magnoliopsida	Asteraceae	<i>Helichrysum rosam</i> (P.J. Bergius) Less.		
Magnoliopsida	Asteraceae	<i>Seriphium plumosum</i> L.		Ornamental
Magnoliopsida	Boraginaceae	<i>Lobostemon echioides</i> Lehm.		
Magnoliopsida	Bruniaceae	<i>Brunia noduliflora</i> Goldblatt & J.C. Manning		Ornamental & Wild flower trade
Magnoliopsida	Ericaceae	<i>Erica passerinae</i> Montin	EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 1 L.	EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 2 L.	EC	
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 4 L.	EC	
Magnoliopsida	Fabaceae	<i>Aspalathus</i> sp. L.		
Magnoliopsida	Fabaceae	<i>Cyclopia intermedia</i> E. Mey.	D, EC	Honeybush tea
Magnoliopsida	Gentianaceae	<i>Sebaea elongata</i> E. Mey		
Magnoliopsida	Geraniaceae	<i>Pelargonium scabrum</i> (Burm.f.) L' Hér		Ornamental
Magnoliopsida	Geraniaceae	<i>Pelargonium</i> sp. 1 L' Hér		
Magnoliopsida	Geraniaceae	<i>Pelargonium</i> sp. 2 L' Hér		
Magnoliopsida	Grubiaceae	<i>Grubbia</i> sp. P.J. Bergius		
Magnoliopsida	Linaceae	<i>Linum</i> sp. L.		
Magnoliopsida	Lobeliaceae	<i>Lobelia</i> sp. L.		Ornamental
Magnoliopsida	Malvaceae	<i>Hermannia hyssopifolia</i> L.		Herbal tea & Ornamental

Magnoliopsida	Mesembryanthemaceae	<i>Carpobrotus</i> sp. N.E.Br.	EC	
Magnoliopsida	Mesembryanthemaceae	<i>Erepsia</i> sp. N.E.Br.	EC	
Magnoliopsida	Proteaceae	<i>Leucadendron salignum</i> P.J. Bergius	EC	Ornamental
Magnoliopsida	Proteaceae	<i>Leucospermum cuneiforme</i> (Burm.f.) Rourke	EC	Ornamental
Magnoliopsida	Proteaceae	<i>Protea neriifolia</i> R.Br.	EC	Ornamental & Wild flower trade
Magnoliopsida	Proteaceae	<i>Protea repens</i> (L.) L	EC	Ornamental & Wild flower trade
Magnoliopsida	Ranunculaceae	<i>Knowltonia</i> sp. Salisb.		
Magnoliopsida	Rhamnaceae	<i>Phyllica</i> sp. L.		
Magnoliopsida	Rosaceae	<i>Cliffortia</i> sp. L.		
Magnoliopsida	Rubiaceae	<i>Anthospermum</i> sp. L.		
Magnoliopsida	Santalaceae	<i>Thesium</i> sp. L.		
Magnoliopsida	Scrophulariaceae	<i>Selago</i> sp. L.		
Magnoliopsida	Thymelaeaceae	<i>Passerina</i> sp. 2 L.		
Magnoliopsida	Thymelaeaceae	<i>Struthiola</i> sp. L.		
Liliopsida	Iridaceae	<i>Tritoniopsis</i> sp. L. Bolus	EC	
Liliopsida	Poaceae	Poaceae		
Liliopsida	Poaceae	<i>Themeda triandra</i> Forssk.		Grazing
Liliopsida	Restionaceae	<i>Restio</i> sp. Rottb.		
Liliopsida	Restionaceae	<i>Rhodocoma fruticosa</i> (Thunb.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Thamnochortus lucens</i> (Poir.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Thamnochortus</i> sp. P.J. Bergius		
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 3 Steud.		
Liliopsida	Restionaceae	<i>Elegia filacea</i> Mast		Ornamental

### Shared species between presence and absence sites of all regional sites

Division	Family	Species	Status	Uses
Magnoliopsida	Asteraceae	<i>Dicerotheramnus rhinocerotis</i> (L.f.) Koekemoer		Indigestion & Ornamental
Magnoliopsida	Asteraceae	<i>Seriphium plumosum</i> L.		Ornamental
Magnoliopsida	Ericaceae	<i>Erica</i> sp. 1 L.	EC	
Magnoliopsida	Grubiaceae	<i>Grubbia</i> sp. P.J. Bergius		
Magnoliopsida	Lauraceae	<i>Cassytha ciliolata</i> Nees		
Magnoliopsida	Lobeliaceae	<i>Lobelia</i> sp. L.		
Magnoliopsida	Proteaceae	<i>Leucadendron salignum</i> P.J. Bergius	EC	Ornamental
Magnoliopsida	Proteaceae	<i>Protea neriifolia</i> R.Br.	EC	Ornamental & Wild flower trade
Magnoliopsida	Proteaceae	<i>Protea repens</i> (L.) L	EC	Ornamental & Wild flower trade
Magnoliopsida	Rubiaceae	<i>Anthospermum aethiopicum</i> L.		
Magnoliopsida	Rubiaceae	<i>Anthospermum</i> sp. L.		
Magnoliopsida	Rutaceae	<i>Agathosma</i> sp. Willd.	EC	
Liliopsida	Iridaceae	<i>Tritoniopsis</i> sp. L. Bolus	EC	
Liliopsida	Poaceae	Poaceae		
Liliopsida	Restionaceae	<i>Restio dispar</i> Mast		Ornamental & Thatching
Liliopsida	Restionaceae	<i>Rhodocoma fruticosa</i> (Thunb.) H.P. Linder		Ornamental
Liliopsida	Restionaceae	<i>Thamnochortus</i> sp. P.J. Bergius		
Liliopsida	Restionaceae	<i>Elegia filacea</i> Mast		Ornamental
Liliopsida	Restionaceae	<i>Ischyrolepsis</i> sp. 3 Steud.		