

DIVERSITY, GENE FLOW AND CONSERVATION OF THE SERPENTINE FLORA ON THE WITWATERSRAND

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A thesis submitted to the Faculty of Science, University of the Witwatersrand,
Johannesburg, in partial fulfilment of the requirements for the degree of Doctor of
Philosophy.

Johannesburg, 05 October 2010.

Diversity, gene flow and conservation of the serpentine flora on the Witwatersrand

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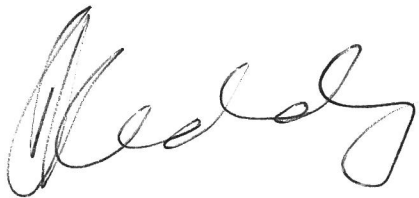
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Abstract:

Serpentine soils and their floras are extremely variable in soil composition, age, elevation and climate and thus make generalizations difficult. The stressful and highly selective nature of the serpentine habitat, a consequence of the interplay of physical, chemical and biotic factors, defines the serpentine syndrome. Soils derived from serpentinites (serpentine soils) usually have very high magnesium to calcium ratios and high levels of toxic trace metals such as nickel and chromium. Regions containing serpentine soils are often considered to be living laboratories where the processes of evolution can be studied *in situ* because plants respond in various ways to elevated levels of toxic metals and the lowered nutrient status of serpentine-rich soils and often have characteristic vegetation growing on them, usually with few species or with stunted plants. In some species ecotypic races have evolved and survive on these less than optimum areas. Other species are endemic to these soils, yet others appear to be indifferent to these toxic soils (*bodenvag*) and are found growing equally well on and off serpentine soils. Botanists have been aware of Greenstone outcrops on the Witwatersrand, but, until now, little work had been undertaken on the Witwatersrand. The aim of this study was to gain an understanding of the relationship between the serpentine flora and its associated soils, between the serpentine flora and the adjacent non serpentine flora on the Witwatersrand, to investigate life histories, breeding biology and gene flow patterns in selected *bodenvag* taxa at specific sites and thus observe any possible processes involved in the formation of ecotypic races and to determine the implications of the results of this study for the conservation of serpentine flora on the Witwatersrand. This study found that the serpentine areas on the Witwatersrand were not very toxic and did not support a unique flora and possessed no endemic taxa. Species richness and diversity between the serpentine and non serpentine sites was also not significantly different indicating that soil factors, such as the concentrations of nickel, magnesium and iron, do not significantly influence diversity. Reproductive and life history data did not separate the study taxa into ecotypes implying that the *bodenvag* serpentine taxa investigated were either pre-adapted for the serpentine condition or, because the serpentine conditions are similar to that of the Highveld grasslands, there was no selection pressure for the formation of ecotypes. The AFLP results indicated that gene flow occurred amongst the sub populations growing on and off serpentine soils and that these sub populations were genetically similar. It can thus be concluded that other factors, such as the harsh climatic conditions in the form of severe Highveld winters, being part of a fire dependent grassland and occurring at a high elevation, exert a stronger influence on these plants than does a relatively mild serpentine condition thus resulting in a flora that is similar to the non serpentine flora on the Witwatersrand. Currently three of the nine study sites receive some protection as municipal parks or nature reserves and one as a conservancy; but none are nationally proclaimed parks, so the future of these sites is not assured. However, if the ridge development policy guidelines are strictly implemented, then the rocky serpentine outcrops may remain safe from development and contribute towards the richness of the flora of the Witwatersrand as a whole and act as corridors and refuges of local biodiversity.

DECLARATION

I, Ren   Ansuria Reddy, declare that this dissertation is my own work. The published and unpublished articles are the result of my own, original, work and writing. Guidance and assistance were received from my supervisors to the extent usually and reasonably expected. The dissertation is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at another university.

A handwritten signature in black ink, appearing to read 'Reddy', with a large, stylized initial 'R'.

5th day of October 2010.

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Chapter 1: General Introduction.

General Introduction

Serpentine is the common name for a suite of rock types that contain at least 70% ferromagnesian minerals and other magnesium-rich minerals of composition close to $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$. Serpentine is just one of those minerals and the igneous or metamorphic rocks containing them are better designated as ultramafic (Coleman & Jove, 1992; Proctor 1999; Brady *et al.*, 2005). However, the term serpentine has been cemented in the field (Brooks 1987) and it is this term that shall be used in this study.

The stressful and highly selective nature of the serpentine habitat is a consequence of the interplay of physical, chemical and biotic factors (Kruckeberg, 1984; Oberhuber *et al.*, 1997). The interplay of these factors defines the serpentine syndrome (Kruckeberg, 1984). Abiotically stressed environments serve as refuges from competition (Stadt *et al.*, 1994) and as refuges for relict species (Kruckeberg, 1991). Soils derived from ultramafic parent materials or serpentinites have characteristic vegetation growing on them, often with few species or with stunted plants. This is because serpentinite soils usually have very high magnesium to calcium ratios and high levels of toxic trace metals such as nickel and chromium (Wild, 1974; Westerbergh, 1994). The floras of serpentine regions are so characteristic that plant species have often been used to pinpoint and assess mineral concentrations of commercial value, i.e. geobotanical prospecting (Kruckeberg, 1984; Brooks, 1998).

Plants respond in various ways to elevated levels of toxic metals and the lowered nutrient status of serpentine-rich soils. In some species ecotypic races have evolved and survive on these less than optimum areas. Other species are endemic to these soils; yet others appear to be indifferent to these toxic soils (*bodenvag*) and are found growing equally well on and off serpentine soils (Kruckeberg, 1984).

Regions containing serpentine soils are often considered to be living laboratories where the processes of evolution can be studied *in situ* (Kruckeberg, 1984). Most

geobotanical work undertaken on serpentinite-rich areas has been done in the northern hemisphere. In southern Africa, the vegetation of the Great Dyke in Zimbabwe is well documented (Wild, 1974; Proctor & Craig, 1978; Proctor *et al.*, 1980). In South Africa, geobotanical investigations in serpentine areas are more recent, with most of the work thus far being in the Barberton Greenstone Belt (Morrey *et al.*, 1989, 1992; Balkwill *et al.*, 1997; Williamson *et al.*, 1997; Changwe & Balkwill, 2003; Williamson & Balkwill, 2006; McCallum, 2007; Siebert, 2001). Botanists have been aware of Greenstone outcrops on the Witwatersrand for more than fifty years (Mogg, 1961), but little work had been undertaken in these areas. Mogg (1961) investigated the woody vegetation of the Witwatersrand region with respect to the geology and physiography and made only brief mention of areas underlain by serpentinites.

Most serpentinite masses are derived from igneous and volcanic ultramafic rocks tectonically emplaced along continental margins or in ancient Greenstone Belts. Ultramafic rocks are igneous rocks that have >70% of minerals containing magnesium and iron and have a relatively low content of silica (Brooks, 1987). There are several types of ultramafic rocks, including igneous (dunite or harzburgite) and volcanic (komatiites and peridotites). Olivine and pyroxene are minerals that have their origins from within the earth's mantle or from magmas derived from the mantle. Serpentine soils are the hydrated products of rocks containing olivine and pyroxene (Anhaeusser 1973; Coleman & Jove, 1992). These soils consist mainly of chrysotile, lizardite and antigorite. The Great Dyke in Zimbabwe and the Barberton Greenstone Belt in Mpumalanga, South Africa, are examples of ultramafic intrusions that are olivine rich (Coleman & Jove, 1992; Morrey *et al.*, 1992).

Weathering results in changes in mineral composition and ionic status of the soil. Most soils derived from serpentinites are residual or colluvial and are formed in place over the parent rocks. The soils are therefore shallow, stony, highly erodible and usually of poor fertility (Kruckeberg, 1984; Morrey *et al.*, 1989). Different types of ultramafic rocks give rise to soils of greatly different chemical compositions.

While strictly speaking the term “serpentine” should be applied only to magnesium-rich minerals of sheet-like structure such as antigorite and/or chrysotile, it is often used to describe serpentinites i.e. the rocks composed of serpentine minerals. It is in the latter context that the word “serpentine” will be used in this study.

Despite differences in concentration, the main chemical criteria of serpentine soils are the elevated levels of siderophilous trace elements, e.g. cobalt, chromium, iron and nickel, and the lower concentrations of essential plant nutrients such as calcium, magnesium, nitrogen, phosphorous and potassium (Brooks, 1987; Kruckeberg, 1991). There are chemical similarities between the Barberton Greenstone Belt and the Great Dyke, but the chemical and physical characteristics of the sites studied within the Barberton Greenstone Belt area are different due to elevation, climate and disturbance regimes (Morrey *et al.*, 1989; Changwe & Balkwill, 2003; McCallum, 2007). The weathered products of ultramafic rocks such as serpentinites and peridotites are responsible for drastic changes in the vegetation and species composition of vascular plant communities nearly everywhere that they occur (Kruckeberg, 1984).

Most of the regions containing serpentinites in southern Africa are of Archaean age, i.e. they are between 2.5 – 3.5 billion years old and therefore constitute some of the most primitive assemblages on earth (Anhaeusser, 1973). It is therefore probable that the African serpentine flora has had a very long period to evolve (Brooks, 1987).

On the Witwatersrand some of the oldest rocks, which are of the Archaean Greenstone type, are serpentinised (Anhaeusser, 1973). Around 3.2 billion years ago, this Archaean terrain was intruded by a granite dome, known as the Johannesburg Dome, which has an area of about 700 km² (Anhaeusser, 1973). It forms part of a fundamental basement framework upon which the stratigraphic successions, ranging in age from Archaean to Phanerozoic times, were laid down (Anhaeusser, 1973). The successive stages of sedimentation and volcanism over widespread regions resulted in the development of the Witwatersrand, Ventersdorp

and Transvaal sequences and during this time, the granite dome continued to rise (Anhaeusser, 1973). Today, the serpentinised regions are small patches only present around the western, south-western and south-eastern margins of the granite dome (Chapter 2, Figure 1, pg 487).

The aims of this study were to:

- gain an understanding of the relationship between serpentine flora and its associated soils,
- compare the diversity between the serpentine flora and the adjacent non serpentine flora on the Witwatersrand and to compare these relationships with those of the serpentine floras of the Barberton Greenstone Belt,
- investigate whether life histories, breeding biology of selected, apparently *bodenvag*, taxa could indicate whether ecotype formation is taking place or has taken place and
- to investigate the gene flow patterns in selected, apparently *bodenvag*, taxa at specific sites in order to observe whether ecotypic races are forming.

Relevance of this Research:

This study attempts to provide an in depth investigation into the interactions of systematics, ecology and genetics at some of the serpentine sites on the Witwatersrand.

Floristic analysis will indicate the affinities of the serpentine flora and identify any rare and endangered species. Distributions of taxa on the serpentine checklists, on the whole of the Witwatersrand will show whether or not there are any serpentine endemic taxa. The results of this study will also indicate whether the serpentine condition overrides the effects of climate and topography to support a savanna biome where a grassland biome could have been expected.

Correlations between soil types and characteristics such as species richness, diversity, particular growth forms, hyperaccumulation and endemic taxa of the serpentine flora will indicate the extent to which soil chemistry and soil composition influence the flora. Comparisons between the Witwatersrand serpentine flora and the serpentine flora of the Barberton Greenstone Belt will indicate how similar or different these two South African serpentine areas are.

A study of the breeding biology and life histories of the selected taxa will enable a better understanding of the different strategies, if any, that may evolve to survive in this specialised environment. An investigation of the breeding biology and life histories will also indicate whether or not ecotype formation has occurred amongst the selected study taxa.

An outcrossing population with low gene flow and small local effective size has the same genetic consequences and evolutionary dynamics as a predominantly selfing population (Templeton, 1989). In addition, small populations are likely to be less attractive to biotic pollinators; this leads to a decrease in seed production (Ägren, 1996; Fritz & Nilsson, 1994). These results will also contribute towards the debate on whether or not sympatric speciation occurs in organisms other than insects. Lynch (1989) and Mayr (1992) oppose the view that sympatric speciation also occurs in organisms other than insects, but Endler (1977), White (1978), and Tauber & Tauber (1989) view sympatric speciation as a valid mode of speciation for other organisms as well.

Gene flow studies will help to determine whether gene flow still occurs between a selected *bodenvag* taxon at the interface of serpentine and non serpentine sites. Should there be any significant differences in the gene flow rates of the selected *bodenvag* taxon, then these observations could mean that a portion of the population is moving towards ecotypic race formation. Gene flow studies may show the first steps in the processes involved in what may eventually be a sympatric speciation event.

Serpentine areas can be regarded as edaphic islands as they act as refuges for rare and endemic species of conservation value (Morrey *et al.*, 1989; Jaffré, 1992). Species richness of a community is dependent upon area of the community (Wilson & Shmida, 1984; Bond, 1991; Cowling *et al.*, 1991), thus the species richness in the smaller, urban parks is expected to be less than the larger sites. Urbanization is the major contributor of habitat loss. An increase in land use and resource requirements associated with a growing human population places biodiversity at an increasing risk through transformation as well as landscape fragmentation (Reyers *et al.*, 2007). Habitat fragmentation often curtails gene flow which leads to smaller, isolated neighbourhoods. Isolation can lead to increased inbreeding and to biotype depletion (Boshier *et al.*, 1995). Beta diversity, an indication of habitat diversity (Usher, 1991), is also an important factor when deciding upon conservation measures as it indicates the degree to which the habitat has been partitioned by plant species.

The results obtained from this study will help to determine whether any of the unprotected sites warrants conservation. Should the results of the gene flow study indicate that the taxon investigated is in the process of becoming ecotypically differentiated, and may potentially speciate, then that site should be conserved.

This study is unique in that it attempts to investigate the Witwatersrand serpentine flora on a number of different levels, from a broad study of the floristics and floral diversity of the area to the level of genes and gene flow. This study will add to our knowledge of serpentine floras as the serpentine flora on the Witwatersrand has as yet not been studied in depth.

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Chapter 2: Is there a unique serpentine flora on the Witwatersrand?

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Is there a unique serpentine flora on the Witwatersrand?

R.A. Reddy^{a*}, K. Balkwill^a and T. McLellan^b

The flora found on serpentine soils on the Witwatersrand, South Africa, which is only now being studied in detail, superficially appears to be similar to that found on non-serpentine soils. The soils have magnesium to calcium ratios and acidities (pH) similar to the soils of the Barberton Greenstone Belt, but chromium levels are lower and nickel levels are very low. This study compares the Witwatersrand serpentine flora with three non-serpentine nature reserves (Melville Koppies Nature Reserve, Witwatersrand Botanical Garden and Suikerbosrand Nature Reserve). The percentage of exotic flora for each area was also noted. Rare and endangered plants that occur on the Witwatersrand serpentine study sites were recorded and the biome affinities of Witwatersrand serpentine areas were examined. The non-serpentine sites appear to be more similar to the serpentine sites than to each other. There are few exotic taxa present at any of the sites. The serpentine sites on the Witwatersrand are classified as a grassland biome rather than a savanna biome. Three of the taxa that occur on serpentine soils are listed on the Gauteng list of rare and endangered species, but these taxa also occur on non-serpentine soils on the Witwatersrand. No endemic or nickel-hyperaccumulating taxa occur on the serpentine sites on the Witwatersrand. We conclude that the serpentine areas on the Witwatersrand do not support a unique flora.

Introduction

Serpentine soils are inhospitable to plant growth¹ and botanists have long recognized the striking effects they have on some species.² The vegetation is often sparse or stunted and contains species which are rare, endemic, or both.³ For example, the flora of the Great Dyke in Zimbabwe is distinct from the surrounding flora and has many endemic plant taxa.⁴ In South Africa, the serpentine flora of the Barberton Greenstone Belt has a number of new and endemic taxa and a sparse tree cover,⁵ contrasting with the surrounding vegetation.

Ecotypic variation in plants is a response to diverse environments.⁶ Soils derived from ultramafic rocks can act as agents of ecotypic selection.⁷ In any given locality, *bodenvag* or indifferent taxa occur both on serpentine and adjacent non-serpentine soils and although taxonomically identical, may have differentiated ecotypically. The more stressful the serpentine site, the less common are *bodenvag* taxa.²

The effects of time on pedology and its influence on plant life

There is a strong correlation between the length of exposure of rock, its subsequent soil formation, the richness of the flora and the number of endemic taxa on serpentine soils. Where outcrops are exposed, rocks act as direct substrates for plants and all soils bear a chemical imprint of their parent material.⁸ Geology therefore exerts a selective force on plant life in diverse ways. The

greater the length of exposure, the longer the time frame for speciation steps leading to organic diversity.⁸ For example, geological evidence indicates that the Cuban serpentines have been exposed for long periods,^{9,10} which has led to a high diversity of endemic taxa. Thus on serpentine soils that have been exposed for long periods, prolonged evolution has led to a very high floral diversity at both generic and specific levels.^{9,11,12}

The Witwatersrand serpentine outcrops are considered to be the roots of a former, more extensive greenstone belt.¹³ Geologists are uncertain about how long these outcrops have been exposed (C.R. Anhaeuser, pers. comm.), but at some sites, the extent of current exposure is poor.¹⁴

Plant distribution in stressful environments

The environment of metal mine tailings often includes stressful factors such as nutrient deficiencies, unfavourable soil structure, excessive dryness and toxic concentrations of metals. Because of the higher mortality rate of metal mine plant populations,¹⁵ metal-tolerant populations are often small and surrounded by non-tolerant populations growing on normal soils.¹⁶ As serpentine soil environments are similar to metal mine tailings, one would expect a similar pattern of plant distribution.

Endemism

Divergence between taxa growing on and off serpentine could either be abrupt or gradual. Initially gene flow may still occur, but divergence in structural and functional traits, followed by isolation between the two populations, may result in two morphologically and genetically distinct populations.^{2,17} With time the serpentine-tolerant population could become endemic to serpentine soils. Serpentine endemics may either be neo-endemics or paleoendemics. Paleoendemics have arisen from taxa that once colonized a greater range of substrates but are now restricted by competition or climatic changes to their present sites.¹⁸ The closest relatives of paleoendemic taxa are geographically separated from the endemic taxa.^{19,20} Neo-endemic taxa have evolved recently and have restricted distributions as they have not yet had time to spread.²¹ The closest relatives of these taxa are not geographically separated from endemic taxa.²⁰

If the Witwatersrand serpentine outcrops have only been exposed recently, and should the exposure be of a sufficient length of time for serpentine endemics to occur here, then these endemics are likely to be neoendemics. It is hypothesized, however, that the Witwatersrand serpentine soils have not been exposed for a sufficiently long time for any endemic taxa to have evolved. In addition, the number of rare or endemic plants in the grassland biome in South Africa is relatively small.^{22,23} Only 28% of grassland biome taxa are endemic, whereas 43% of taxa that occur in the savanna biome are endemic.²⁴

Hyperaccumulation

High concentrations of nickel affects plant growth and functioning.^{19,25} Nickel tolerance either by hyperaccumulation or exclusion, is a common component of the serpentine syndrome.^{26,27}

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Plants are said to be hyperaccumulators of nickel or chrome if they contain $>1000 \mu\text{g g}^{-1}$ of metal in above-ground tissue on a dry weight basis.¹⁸ Worldwide there are approximately 190 nickel hyperaccumulators in more than 60 genera and 32 families;²⁶ this is approximately 1–2% of all plant species.²⁶ Five species of hyperaccumulators have been found on the Great Dyke⁵ and five on the Barberton Greenstone Belt serpentine region⁵ (Smith and Balkwill, in press).

Nickel is likely to be at non-toxic concentrations in soil solutions with a pH greater than 6.0.²⁷ The pH range of the Witwatersrand serpentine soils is 4.91–6.18; if nickel is present at high concentrations, one might expect these soils to be toxic to plants and therefore expect to find nickel-hyperaccumulating taxa.

Species richness

In the northern hemisphere, because of exposure to glaciation processes, the non-serpentine areas surrounding serpentine areas are not rich in species, providing a smaller pool of species from which distinctive serpentine taxa may evolve.²⁹ The Zimbabwean serpentine flora is not related to the anomalous serpentine floras of the northern hemisphere — the serpentine-tolerant plants have been shown to have evolved from the local flora;³⁰ it is likely that the serpentine flora of the Barberton Greenstone Belt has also been derived from the local, non-serpentine flora.³¹ One would expect a similar pattern on the Witwatersrand.

Exotic taxa

In southeastern Sweden, species richness was found to increase with disturbance in a grassland.³² In grassland communities, succession after disturbance usually involves annuals in the initial stages, followed by the establishment of perennial grasses.³³ The Witwatersrand serpentine sites, for most part, occur in highly built-up urban areas, which are subject to disturbance. Weedy taxa are mainly annual species.³⁴ Exotic taxa that colonize disturbed areas appear to increase the species diversity of the serpentine sites.

Floristics

Mogg³⁵ investigated the woody vegetation of the Witwatersrand in relation to the geology and physiography of the area. He investigated only the four serpentine sites that supported woody vegetation and provided a checklist of the woody species that occurred there. To date, on the Witwatersrand, floristic studies have only been conducted at the Suikerbosrand Nature Reserve,³⁶ Witwatersrand Botanical Garden³⁷ and Melville Koppies Nature Reserve.

The objective of this paper is to:

- Describe some of the 'remaining' serpentine patches on the Witwatersrand in terms of the physical and chemical properties of the soils.
- Determine the affinities of the flora at the level of formally recognized taxa.
- Determine the effects of length of exposure and the effect of soil nickel concentrations on the composition of the serpentine flora.
- Determine the level of endemism.
- Determine the effect of disturbance on the species composition of the Witwatersrand serpentine flora.

Materials and methods

Study sites

The serpentine areas on the Witwatersrand occur mainly around the southwestern and southeastern margins of the

granite dome found to the north of Johannesburg, and at some localities within the dome (Fig. 1).³⁸ Most of the large serpentine areas have been built over and only small relatively undisturbed patches remain. Nine sites were investigated. At Darrenwood, Diamond Four, Estherpark and Robindale, the low rocky hills are surrounded by rocky serpentine or non-serpentine grasslands. Illiondale, Ruimsig, Weltevreden and Zandspruit are grasslands with low rocky outcrops. Ndabushe consists of a large, fairly flat grassland and a few low, rocky hills. At two sites, Estherpark and Ndabushe, the hills consist of numerous large boulders, some of which reach heights of 2–3 m.

Anthropogenic disturbances

The sites are situated in a densely populated urban area, and are subject to various forms of disturbance. Four of the nine sites — Darrenwood, Robindale, Ndabushe and Ruimsig — are nature reserves. The future of the Ndabushe Reserve, the largest of the serpentine areas, is uncertain, as there are plans to develop this property for housing (J. Swart, pers. comm.). At Darrenwood, disturbances are in the form of squatting, a thoroughfare, some dumping, Zionist Church meeting places and recreation (e.g. fishing and picnicking). At the Robindale site there is squatting and some dumping. At Ruimsig people create illegal pathways through the reserve. Mining and agriculture were practiced at Ndabushe in the past and the site is now grazed by game. Diamond Four has occasional trespassing, but is well fenced and the fence is well tended so there is very little disturbance. The Illiondale site has had major excavations in the past to install power lines and at present some dumping occurs; it is also used as an informal roadside meeting place. At the Estherpark site, disturbance is in the form of squatting; dumping also occurs on the edges of the site. At the Weltevreden site, some dumping has occurred, it is also an informal meeting place and a thoroughfare. The granite areas of Zandspruit have been mined for sand in the past and the serpentine outcrops were used as informal meeting places; some dumping has occurred and the site is currently grazed by cattle.

Soils

Soil samples were collected from eight sites to depths of 5–10 cm. On each site, three samples were collected and bulked. On ridges or hills, soils were collected from either side and the top of the ridge or hill.

Samples were dried in an oven at approximately 60°C for 48 hours. These samples were sent to the Pretoria division of the Institute for Soil, Climate and Water of the Agricultural Research Council for analysis of composition, pH, %C, %N, Fe, Co, Cu, extractable Mg, Ca, Cr, Ni and exchangeable bases (K, Na). The results obtained were compared with the results from the Great Dyke³ and the Barberton Greenstone Belt⁵ (Williamson, unpublished). Because the methods used to obtain the results for the Great Dyke soils differed from those used to obtain the results for the South African serpentine soils, and because the South African serpentine soils were analysed on separate occasions, only descriptive statistics were used to compare the data.

Floristics

Nine serpentine sites were visited monthly in spring and summer and once each in autumn and winter. All fertile specimens were collected, identified and accessioned into the C.E. Moss Herbarium (J). A checklist of the serpentine flora of the Witwatersrand was compared with checklists of Melville Koppies Nature Reserve (MKNR, 'Herbarium Manager' database, C.E. Moss Herbarium, 1999), Witwatersrand Botanical Gar-

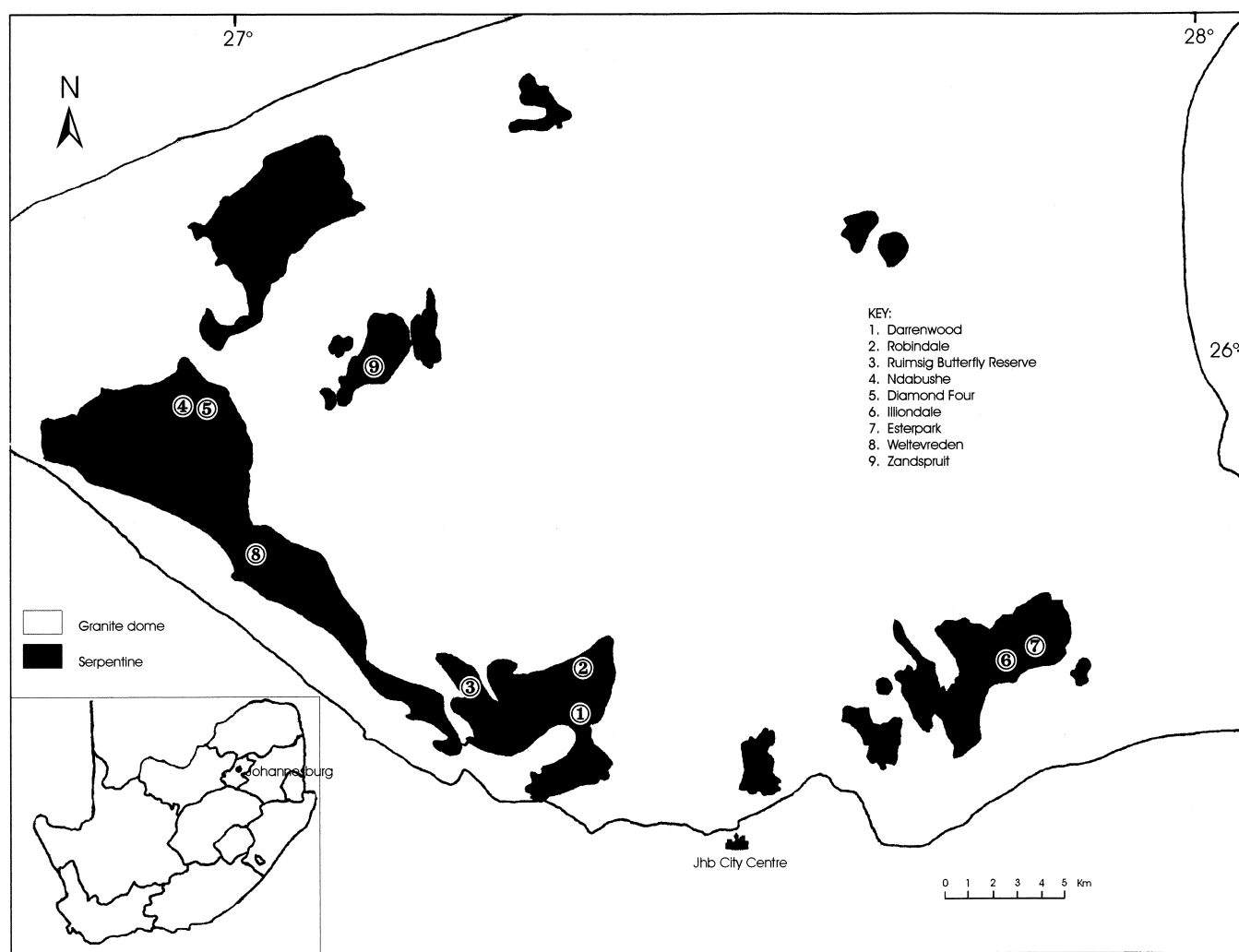


Fig. 1. Serpentine areas on the Witwatersrand and serpentine sites visited.

den, (WBG)³⁷ and Suikerbosrand Nature Reserve, (SNR).³⁶ Similarities between serpentine and non-serpentine sites were calculated using Sørensen's index of similarity. The Chi-square test was used to compare the floristics of serpentine and non-serpentine areas.

Affinities

Spearman's rank correlation was used to investigate the correlation between the major families of each serpentine site, and the savanna and grassland biomes. Spearman's rank correlation was also used to investigate the correlation between the grassy and woody serpentine sites.

Exotic taxa

Pearson's correlation coefficient was used to test for correlations between total area of site, estimated area disturbed and the number of exotic taxa present.

Results

Soils

Soil depths were shallow and ranged between 5 and 10 cm. The concentration of exchangeable nickel in serpentine soils on the Witwatersrand (WWRs) is three times higher than that of the Great Dyke (GD) but 1.5 times less than that of the Barberton Greenstone Belt (BGB) (Table 1). The Great Dyke, however, has the highest concentration of total nickel: five times higher than

WWRs and three times more than BGB. BGB has 1.5 times more total nickel than WWRs. The total chromium concentrations of BGB and GD are similar, but BGB is 12 times higher than WWRs and GD nine times greater. The Mg:Ca ratio of GD and BGB is 2–3.5 times greater than that of WWRs. The pH range of WWRs is narrower than that of BGB. The BGB serpentine soils are mainly clay soils, whereas the GD soils are loams or fine sandy loams. The WWRs serpentine soils are mainly loams, clay loams or coarse sandy clay loams.

Analyses and comparison of the serpentine flora

There is a total of 449 taxa in 258 genera and 81 families present on the serpentine areas of the Witwatersrand (Appendix 1). Of these, 409 taxa in 226 genera and 64 families are indigenous.

The total number of families is comparable to those of the non-serpentine areas (Table 2). The Witwatersrand serpentine sites have almost the same ratio of genera per family as Melville Koppies Nature Reserve, but in comparison to the non-serpentine sites, they have a lower ratio of taxa per family. The ratio of taxa per genus of the serpentine areas is similar to that of Suikerbosrand and the Witwatersrand Botanical Garden. Melville Koppies has the highest ratio of taxa per genus and is significantly different from the serpentine sites ($P \leq 0.001$, with the χ^2 -test, d.f. = 1). The number of genera per family at Suikerbosrand was significantly different from that of the Witwatersrand as a whole.

Table 1. Selected soil elements and properties of serpentine soils on the Witwatersrand (WWRS), Barberton Greenstone Belt (BGB) and the Great Dyke (GD). Means and standard deviations are given in brackets.

		WWRS (<i>n</i> = 8)	GD (<i>n</i> = 6) ³	BGB (<i>n</i> = 8) ⁵ , Williamson, unpublished
Exchange cations ^a (mg kg ⁻¹)	Fe	158.7–331.8 (226.1 ± 60.8)	0–2.7 (0.8 ± 0.97)	
	Cu	3.6–13.3 (7.4 ± 3.8)		
	Ni	16.5–173.1 (94.9 ± 58.0)	0.4–56 (31.1 ± 22.4)	14.52–258.6 (143.6 ± 79.0)
	Ca	472–1434 (831.8 ± 323.6)		
	Cr	0.23–0.62 (0.3 ± 0.1)		
Total metal conc. in soil ^b (mg kg ⁻¹)	Mg	4132–26340 (13151 ± 8235)		
	Ca	1360–2840 (1999 ± 577)		
	Ni	251–1600 (969 ± 515.1)	430–7100 (4838 ± 2583)	256.0–2604.8 (1452.2 ± 762.4)
	Co	40.6–129.3 (81.3 ± 34.6)	73.0–270.0 (210.5 ± 71.2)	
	Fe	24 740–76 300 (47 470 ± 19 889)		
	Cu	17.2–106.8 (50.6 ± 33.5)		
	Cr	64.1–403.2 (153 ± 112)	110.0–2400.0 (1382 ± 942)	
C & N, C:N	% C ^c	3.51–6.30 (5.2 ± 111.5)		
	% N ^d	0.2–1.5 (0.6 ± 0.4)		
	C:N	4.3–17.3 (18.3 ± 208.4)		
Chem. character of surface soils ^e (cmol g ⁻¹)	Na	0.01–0.1 (0.04 ± 0.03)	1.0–5.2 (2.3 ± 1.5)	
	K	0.2–1.2 (0.5 ± 0.3)	54–120 (94.0 ± 23.0)	
	Ca	2.7–8.8 (5.1 ± 2.0)	180.0–750.0 (18.3 ± 208)	
	Mg	2.0–10.9 (6.0 ± 3.1)	560.0–1300.0 (895 ± 309)	
	Mg:Ca	0.5–2.3 (1.2 ± 0.6)	0.9–3.7 (2.1 ± 1.2)	1.04–15.9 (4.3 ± 4.8)
	CEC	12.9–29.7 (23.5 ± 6.3)		
pH		4.9–6.2	6.1–6.6	5.4–7.9
Soil texture		Loam/coarse sandy clay Loam/clay loam	Loam/fine sandy loam	Clay/loam/ coarse sandy loam

Method: ^aAmmonium EDTA; ^bTotal EPA 3050; ^cNelson and Somers;⁴³ ^dAmmonium acetate or LiCl.

Major plant families on the Witwatersrand

On the Witwatersrand, MKNR, WBG, BGB and WWRS, the Asteraceae is the largest family, followed by the Poaceae and the Fabaceae. At SNR, Poaceae numbers are slightly higher (115 taxa) than Asteraceae numbers (110 taxa). The Fabaceae is the third largest family.

Major plant genera on the Witwatersrand

At all the sites that were compared, most genera were represented by only one species. *Helichrysum* and *Senecio* were the largest genera at all sites; for all the Witwatersrand serpentine sites, there are 34 taxa of *Senecio* and 28 of *Helichrysum*, at MKNR, 14 taxa in both, SNR, 19 taxa in both, WBG, 13 *Helichrysum* and 11 *Senecio* BGB, 19 *Helichrysum* and 21 *Senecio*, and WWRS, 9 taxa in both.

Woody taxa on serpentine

Most of the woody taxa are confined to the rocky serpentine hills. Mogg³⁵ listed 137 woody taxa on the Witwatersrand and 70 woody taxa on serpentine soils. The present study added three taxa to his Witwatersrand list and 14 taxa to his serpentine list.

Since the basement granite forms the longest border with the serpentine areas on the Witwatersrand, and has the highest number of woody taxa,³⁵ the woody taxa of this soil type were compared with the woody taxa found on serpentine soils. Mogg³⁵ listed 117 woody taxa on soils derived from basement granite, whereas there are 84 taxa on serpentine soils.

Affinities

A comparison of the serpentine sites with the grassland and savanna biomes

Physiognomically, six of the serpentine study sites on the Witwatersrand match the savanna biome (Fig. 3), but most vegetation on the Witwatersrand would be classified in the grassland biome. Gibbs Russell²⁴ ranked the largest families that are common to all major biomes in South Africa. In each biome, the ranking of families differs. The ranking of the largest families of the serpentine flora was compared to Gibbs Russell's rankings of families in grassland and savanna biomes²⁴ (Table 3). The five largest families on the Witwatersrand correlate exactly with those of the grassland biome. The rank of major plant families at

Table 2. Floristic comparison of serpentine and non-serpentine areas on the Witwatersrand.

	Locality		WBG	SNR
	WWR	WWRS		
Number of families	145	80	83	92
Number of genera	635	258 (350)	199 (363)	352 (433)
Number of taxa	1524	448 (598)	535 (478)	556 (845)
Ratio of genera per family	4.4	3.2	2.4	3.6
Ratio of taxa per family	10.5	5.5	6.4	5.6
Ratio of taxa per genus	2.4	1.7	2.7	1.6
Area (km ²)	435	9.9	1.6	1
Taxon density (taxa km ⁻²)	3.5	45.3	334.4	534.6

WWR = All Witwatersrand taxa, WWRS = Witwatersrand serpentine areas, MKNR = Melville Koppies Nature Reserve, WBG = Witwatersrand Botanical Gardens, SNR = Suikerbosrand Nature Reserve.

Bold values are actual values, expected values in brackets (*significant at $P \leq 0.05$ with the χ^2 -test).

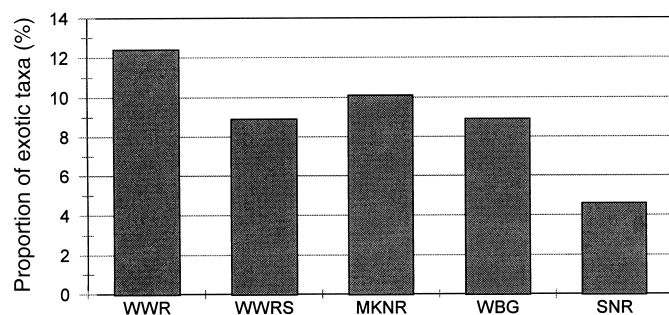


Fig. 2. Comparison of exotic taxa at different sites on the Witwatersrand with that of the entire Witwatersrand flora. WWR = all Witwatersrand taxa; WWRS = Witwatersrand serpentine areas; MKNR = Melville Koppies Nature Reserve; WBG = Witwatersrand Botanical Gardens; SNR = Suikerbosrand Nature Reserve.

Table 3. The ranking of the five largest families of the Witwatersrand serpentine flora compared to the ranking of families in grassland and savanna biomes.³⁴ Numbers in brackets refer to the number of taxa per family. The ranking of the first five families on the Witwatersrand serpentine areas correspond exactly to the ranking of the families of the grassland biome.

Grassland	Witwatersrand serpentine	Savanna
Asteraceae	Asteraceae (62)	Poaceae
Poaceae	Poaceae (54)	Fabaceae
Fabaceae	Fabaceae (35)	Asteraceae
Liliaceae <i>sensu lato</i>	Liliaceae <i>sensu lato</i> (32)	Rubiaceae
Cyperaceae	Cyperaceae (13)	Liliaceae <i>sensu lato</i>

each serpentine site was compared with the rank of the six largest families of the grassland biome (Table 4a). All but two sites, Weltevreden Park and Estherpark, conformed with the grassland biome.

Similarity between serpentine sites

The results showed that Diamond Four, the least disturbed site, was correlated with all other sites at $P = 0.5$ (Estherpark, Zandspruit, Weltevreden) and $P = 0.1$ (Ndabushe, Darrenwood, Ruimsig, Robindale, Illiondale). Both the anomalous sites show a lower correlation with Diamond Four than the other sites.

The ranks of the two anomalous sites were then compared with the ranks of the six major families of the savanna biome (Table 4b). A Spearman's rank correlation test indicated that there was no correlation between either site and the savanna biome (Wel: $r = 0.60$, $P = 0.5$; Est: $r = 0.71$, $P = 0.20$). The Estherpark site has a very variable habitat and is marshy in some places; this could account for the Poaceae being more dominant than the Asteraceae. At the Weltevreden site, the Poaceae is only

slightly more dominant than the Asteraceae. This site is a vacant lot in a suburb and is very small.

Similarities between serpentine and non-serpentine areas

All the non-serpentine areas show a greater similarity to the total serpentine flora than to each other (Table 5a, b). A comparison between serpentine and adjacent non-serpentine areas (Table 6) yields intermediate values. Higher similarity values for adjacent plots, regardless of substratum, implies that the serpentine flora is autochthonous. This also indicates that the serpentine patches are, to some extent, more isolated from one another than are the adjacent non-serpentine areas.

Endemic taxa on the Witwatersrand serpentine

Because serpentine areas are characterized by unique soil conditions, endemic taxa are often associated with them. Taxa can either be endemic to a region or biome irrespective of soil type or be restricted to serpentine soils wherever they occur. Thus the question to ask with respect to the WWRS is: Are there any WWRS taxa endemic to the WWR?

Nineteen of the Witwatersrand serpentine taxa are endemic to the former Transvaal.³⁹ Retief and Herman⁴⁰ divided the former Transvaal into five subregions: North, West, Central, South and East. The major parts of the south and central subregions fall within the borders of the Gauteng province. Two Witwatersrand serpentine taxa, *Aloe greatheadii* Schönland var. *davyana* (Schönland) Glen & D.S. Hardy and *Hypoxis interjecta* Nel, occur only in the south and central subregions.⁴⁰ It is therefore likely that these two taxa are indigenous to the Gauteng province. These taxa are not restricted to serpentine soils.

Cineraria austrotransvaalensis Cron, *C. longipes* S. Moore and *Habenaria mossii* (Williamson) J.C. Manning are considered to be near-endemics to the Witwatersrand; they are known from only a few localities, most of which are on the Witwatersrand (D.A. McCallum, pers. comm.). *Cyphia stenodonta* Wimm. and *Lepidium mossii* Thell. are known only from the type specimens that were collected on the Witwatersrand and are considered to be endemic (D.A. McCallum, pers. comm.). None of these taxa occurs on serpentine soils on the Witwatersrand. The Witwatersrand serpentine sites therefore have no endemic taxa.

Rare or endangered taxa on the Witwatersrand serpentine

According to the Red Data List,⁴¹ *Asclepias eminens* (Harv.) Schltr. is rare in the former Transvaal province and *Parapodium costatum* and *Trachyantha erythrorrhiza* are insufficiently known. On the list of 52 Red Data species for Gauteng (M. Pfab, pers. comm.), *T. erythrorrhiza* is given a priority rating of 6, *P. costatum* a

Table 4a. A comparison of the number of taxa per major family of the physiognomically savanna-like serpentine sites with the physiognomically grassland-like serpentine sites with the six major families of the grassland biome.³⁴

Six Major families occurring in grassland biomes	Number of taxa per family								
	Grassy serpentine sites			Woody serpentine sites					
	Nda	Ill	Wel [†]	Zan	Dar	D4	Rui	Rob	Est [†]
Asteraceae	61	26	14	31	32	27	25	27	24
Poaceae	59	22	15	26	27	20	22	20	31
Fabaceae	23	6	6	8	18	13	10	6	7
Liliaceae <i>sensu lato</i>	11	6	13	6	14	10	7	8	15
Cyperaceae	7	5	2	8	2	5	2	3	5
Scrophulariaceae	7	3	3	5	6	4	2	2	3
Floristic classification	Grass	Grass	?	Grass	Grass	Grass	Grass	Grass	?

Nda = Ndabushe, Ill = Illiondale, Wel = Weltevreden Park, Zan = Zandspruit,

Dar = Darrenwood, D4 = Diamond Four, Rui = Ruimsig, Rob = Robindale, Est = Estherpark. D4 correlates with Nda, Dar, Ruim, Rob and Ill at $P = 0.5$ and with Zan, Est and Wel at $P = 0.1$. Both anomalous sites show a lower correlation with D4 than the other sites.

[†]The anomalous sites.

Table 4b. Comparison of the ranks of anomalous sites with the six major families of the savanna biome²³, numbers in brackets are actual values. There is no significant correlation between either of these two sites and the savanna biome (Wel: $r = 0.6$, $P = 0.5$; Est: $r = 0.71$, $P = 0.20$).

Savanna	Wel	Est
Poaceae	1 (15)	1 (31)
Fabaceae	4 (6)	4 (7)
Asteraceae	2 (14)	2 (24)
Rubiaceae	5 (3)	5 (6)
Liliaceae <i>sensu lato</i>	3 (13)	3 (15)
Cyperaceae	4 (6)	6 (5)

rating of 32 and *A. eminens* a rating of 51. A higher rating indicates a lower conservation priority in the province.

Exotic taxa

In addition to edaphic factors, disturbance may also influence the species richness on the Witwatersrand, especially at Ndabushe, Illiondale and Estherpark (Table 7). There are 40 taxa in 32 genera and 17 families that are exotic on the Witwatersrand serpentine sites. In total, only 8.9% of the taxa found on serpentine soils are exotic as compared to 10.1% at MKNR (Fig. 2).

Illiondale, the most disturbed site, has the highest proportion of exotic taxa (10.4%). Diamond Four, the least disturbed site, has the lowest proportion of exotic taxa (0.6%). Only the correlation between the total area and the estimated area disturbed (Table 7), is significant ($P = 0.000004$, using the Pearson correlation coefficient); all other correlations were not significant.

Discussion

The soils of the Witwatersrand serpentine areas have lower concentrations of nickel than the Barberton Greenstone Belt, although they fall within the range. The possibly recent exposure of these soils may contribute to the absence of endemic, hyperaccumulating and rare taxa and the abundance of *bodenwag* taxa. It is also possible that the scarcity of rare plant taxa is not due to recent exposure or low nickel concentrations, but because the grassland biome supports relatively few rare plants when compared with the fynbos (chaparral) and savanna biomes.^{21,22}

Table 5a. Comparison of the combined flora of all serpentine sites on the Witwatersrand with various non-serpentine sites on the Witwatersrand using Sørensen's index of similarity (%).⁴⁴

Locality	WWRS	WBG	MKNR
WBG	55.2		
MKNR	60.1	16.5	
SNR	46.5	3.3	24.6

WWRS = Witwatersrand serpentine areas, MKNR = Melville Koppies Nature Reserve, WBG = Witwatersrand Botanical Gardens, SNR = Suikerbosrand Nature Reserve.

Table 5b. A comparison of the similarity between serpentine and non-serpentine and between non-serpentine sites in the Witwatersrand. The mean of the similarity of the non-serpentine areas to WWRS is significantly different from the mean of the similarity of the non-serpentine areas to each other ($P = 0.0097$).

Similarity of non serpentine areas to WWRS	Similarity of non serpentine areas to each other
55.2	16.5
60.1	33.3
46.5	24.6
53.93	24.8

Affinities and the effects of disturbance on the number of exotic taxa

Despite the savanna-like appearance of most study sites, the sequence of the first five major families of the Witwatersrand serpentine soils conforms exactly to that of the grassland biome. In South Africa, the grassland biome contains the greatest concentration of urban areas — the Witwatersrand complex has a population in excess of 2 million.³³ Urban areas are often very disturbed and we expected that the serpentine flora would have a high number of exotic taxa. However, the percentage of exotic taxa on serpentine soils on the Witwatersrand ranges from 0.6–10.4% and only 8.9% of the total serpentine flora consists of exotic taxa. This implies that while some sites are more disturbed than others, as a unit, the serpentine areas on the Witwatersrand are not very disturbed and still largely consist of indigenous taxa.

Table 6. Comparison of mean Sørensen's similarity values ($\pm$ standard deviation; sample size in brackets) within and between plots of three adjacent serpentine and non-serpentine sites. The comparison between serpentine and adjacent non-serpentine areas yields intermediate results.

Site	Similarity between serpentine plots	Similarity between serpentine and non-serpentine plots	Similarity between non-serpentine plots
Robindale	42 $\pm$ 15 (3)	46 $\pm$ 6 (4)	
Ruimsig	41 $\pm$ 12 (3)	46 $\pm$ 15 (6)	65 $\pm$ 4 (3)
Zandspruit	48 (2)	43 $\pm$ 8 (4)	58 (2)
Mean $\pm$ s.d.	43.7 $\pm$ 3.8	45 $\pm$ 1.7	61.5 $\pm$ 5.0

Table 7. Estimated area disturbed and the percentage exotic taxa per site. There is no significant correlation, except between total area and estimated proportion of area disturbed ($P = 0.000004$).

Site	Total area (km ²)	Estimated area disturbed (km ²)	Estimated area disturbed (%)	Number of exotic taxa	Percentage exotic taxa
1 Darrenwood	0.2	0.11	55	24	8.9
2 Robindale	0.8	0.12	15	8	7
3 Ruimsig	1.2	0.1	8	12	8
4 Ndabushe	6	1.5	25	7	6.6
5 Diamond Four	0.2	0.01	0.5	1	0.6
6 Illiondale	0.15	0.014	90	11	10.4
7 Estherpark	0.2	0.07	35	12	8.6
8 Weltevreden	0.02	0.001	30	4	3.3
9 Zandspruit	0.3	0.11	35	8	3.9



Fig. 3. The Ruimsig site shows the typical physiognomy of woody Witwatersrand serpentine sites. The woody hillock in the background is on serpentine-derived soils; the foreground shows a grassland on basalt-derived soils.

Conclusion

Floristically the serpentine areas are part of the grassland biome, although physiognomically they appear to be savanna. The study sites appear to be relatively undisturbed, despite occurring in urban areas. For the following reasons, the serpentine areas on the Witwatersrand cannot be said to support a unique flora. Floristic comparisons of serpentine and non-serpentine sites indicate that the total number of families and taxa on serpentine and non-serpentine areas are similar. The floras of the non-serpentine areas show a greater affinity to the serpentine areas than to each other. There are no taxa endemic to the serpentine areas.

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Appendix 1

Checklist of the serpentine flora of the Witwatersrand

Families are arranged according to the Cronquist order and taxa within each family are arranged according to Dalla Torre and Harms.⁴² * denotes exotic taxa. Site numbers are used to indicate presence of a taxon at that site.

Site numbers and (total number of taxa per site):

1 = Darrenwood (201) 2 = Robindale (119) 3 = Ruimsig (132)
4 = Ndabushe (165) 5 = Diamond Four (136) 6 = Illiondale (105)
7 = Estherpark (164) 8 = Weltevreden Road (109) 9 = Zandspruit (173)

P18 ADIANTACEAE

Cheilanthes contracta (Kunze) Mett. ex Kuhn 1
Cheilanthes hirta Swartz var. *hirta* 1
Cheilanthes viridis (Forssk.) Swartz var. *viridis* 1, 4
Pellaea calomelanos (Swartz) Link var. *calomelanos* 1, 2, 3, 4, 5

P23 ASPLENIACEAE

Ceterach cordatum (Thunb.) Desv. 4

27 RANUNCULACEAE

Clematis brachiata Thunb. 1, 5, 7
Ranunculus multifidus Forssk. 5

31 MENISPERMACEAE

Antizoma angustifolia (Burch.) Miers ex Harv 1, 2, 3, 7

46 ULMACEAE

Celtis africana Burm. f. 1, 2, 9

60 PHYTOLACCACEAE

Phytolaca octandra L. 1

63 CACTACEAE

*Opuntia ficus-indica** (L.) Mill. 4, 7

64A MESEMBRYANTHEMACEAE

Delosperma herbeum (N.E. Br.) N.E. Br. 7, 9

66 CARYOPHYLLACEAE

Silene burchelli Oth var. *angustifolia* Sond. 1, 2, 7
Dianthus mooiensis F.N. Williams ssp. *kirkii* (Burt-Davy) Hooper 2, 3, 6, 7

66 A ILLECEBRACEAE

Pollichia campestris Ait. 1, 2, 3, 4, 9

67 PORTULACACEAE

*Portulaca oleracea** L. 1
Talinum cafrum (Thunb.) Eckl. & Zeyh. 1, 7

69 CHENOPODIACEAE

*Chenopodium album** L. 1, 2, 6, 7
*Chenopodium opulifolium** Schrad. ex Koch & Ziz 6, 7
*Chenopodium schraderianum** Roem. & Schult. 1, 3

70 AMARANTHACEAE

Amaranthus hybridus L. ssp. *hybridus* var. *hybridus* 1, 2, 3, 6, 7
Cyathula uncinulata (Schrad.) Schinz 7, 9
Aerva leucura Moq. 1
Achyranthes aspera L. var. *aspera** 1, 3, 7
Achyropsis leptostachya (E.Mey. ex Meisn.) Bak. & C.B. Cl. 2
Guilleminea densa (Willd.) Moq. 4, 7
*Gomphrena celosioides** Mart. 6, 9

72 POLYGONACEAE

Rumex sagittatus Thunb. 7
Persicaria serrulata (Lag.) Webb & Moq 1

89 CLUSIACEAE/GUTTIFERAE

Hypericum aethiopicum Thunb. ssp. *sonderi* (Bred.) N.K.B. Robson 3, 5, 7

92 TILIACEAE

Corchorus asplenifolius Burch. 1
Corchorus confusus Wild 7
Grewia occidentalis L. 9

93 STERCULIACEAE

Dombeya rotundifolia (Hochst.) Planch. var. *rotundifolia* 1, 2, 3, 4, 5
Hermannia depressa N.E. Br. 1, 2, 3, 4, 5, 6, 7, 8, 9

95 MALVACEAE

Sida dregei Burt Davy 1
Sida rhombifolia L. 7
Pavonia burchellii (DC.) R.A. Dyer 2, 3, 9
Hibiscus aethiopicus L. var. *ovatus* Harv. 1, 6

Hibiscus microcarpus Garcke 3, 6, 8
Hibiscus trionum L. 6, 7

100 FLACOURTIACEAE

Kiggelaria africana L. 1, 7

120 CUCURBITACEAE

Cucumis hirsutus Sond. 8
Cucumis zeyheri Sond. 4, 9

123 CAPPARACEAE

Cleome monophylla L. 1, 2, 6, 7
Maerua cafra (DC.) Pax 1

124 BRASSICACEAE

*Lepidium bonariense** L. 7
Sisymbrium thellungii O.E. Schulz 2, 6, 7
Rorippa nudiuscula Thell. 1

135 SAPOTACEAE

Englerophytum magaliesmontanum (Sond.) T.D. Penn. 4

141 MYRSINACEAE

Myrsine africana L. 4

136 EBENACEAE

Euclea crispa (Thunb.) Guerke ssp. *crispa* 2, 4, 5, 7, 9
Diospyros lycioides Desf. ssp. *guerkei* (Kuntze) De Winter 2, 3, 4, 5, 6, 7, 8, 9

153 CRASSULACEAE

Kalanchoe rotundifolia (Haw.) Haw. 2
Kalanchoe thyrsiflora Harv. 1, 7
Crassula alba Forssk. var. *alba* 1
Crassula capitella Thunb. ssp. *nodulosa* (Schonl.) Tölken 2

159A FABACEAE/LEGUMINOCEAE — MIMOSOIDEAE

Acacia caffra (Thunb.) Willd. 1, 2, 4, 5, 7
Acacia karroo Hayne 5
Elephantorrhiza elephantina (Burch.) Skeels 3, 4, 5, 8, 9

159B FABACEAE/LEGUMINOSEAE — CAESALPINIOIDEAE

Chamaecrista biensis (Steyaert) Lock 4

159C FABACEAE/LEGUMINOSAE — PAPILIONOIDEAE

Lotononis calycina (E. Mey.) Benth. 1, 2, 4
Lotononis eriantha Benth. 1, 5, 9
Lotononis foliosa H. Bolus 4, 6
Lotononis wilmsii Dümmer 5
Pearsonia aristata (Schinz) Dümmer 3
Pearsonia cajanifolia (Harv.) Polhill ssp. *cajanifolia* 1, 2, 6, 7, 8
Pearsonia sessilifolia (Harv.) Dümmer ssp. *sessilifolia* 2, 3, 4, 5, 6, 7, 9
Dichilus lebeckioides DC. 1, 7
Medicago lupulina L. 1
*Melilotus alba** Desr. 1
Indigofera hedyantha Eckl. & Zeyh. 3, 4, 5, 7
Indigofera hilaris Eckl. & Zeyh. 2, 3, 5, 9
Indigofera obscura N.E. Br. 1, 2
Indigofera zeyheri Spreng. ex Eckl. & Zeyh. 1, 8
Indigastrium burkeanum (Benth. ex Harv.) Schrire 1, 2, 3, 4, 5
Indigastrium fastigiatum (E. Mey.) Schrire 1, 9
Tephrosia capensis (Jacq.) Pers. var. *capensis* 2, 5, 6, 7, 8
Tephrosia elongata E. Mey. var. *elongata* 5, 6
Tephrosia multijuga R.G.N. Young 1
Tephrosia semiglabra Sond. 1, 9
Alysicarpus rugosus (Willd.) DC. ssp. *perennirufus* J. Leonard 1, 8
*Vicia sativa** L. 1
Erythrina lysistemon Hutch. 4
Rhynchosia monophylla Schltr. 9
Rhynchosia nervosa Benth. & Harv. var. *nervosa* forma *nervosa* 1, 2, 5
Rhynchosia totta (Thunb.) DC. var. *totta* 1, 2, 6, 8
Rhynchosia totta (Thunb.) DC. var. *graciliflora* Bak. f. 1, 4, 5, 7
Eriosema cordatum E. Mey. 1, 6
Eriosema nutans Schinz 8, 9
Vigna vexillata (L.) A. Rich. var. *vexillata* 1, 2, 8
Dolichos angustifolius Eckl. & Zeyh. 1, 2, 3, 9

166 LYTHRACEAE

Nesaea schinzii Koehne 4, 6, 7

169 THYMELAEACEAE

Gnidia caffra (Meisn.) Gilg 3, 4, 5
Gnidia capitata L.f. 1, 3, 4, 5, 7, 8
Gnidia gymnostachya (C.A. Mey.) Gilg 1

174 ONAGRACEAE	252 GENTIANACEAE
<i>Oenothera rosea</i> * L'Hérit. ex Ait. 1, 4, 7	<i>Sebaea grandis</i> (E. Mey.) Steud. 7, 9
<i>Oenothera tetraptera</i> * Cav. 1, 4	
177 COMBRETACEAE	253 APOCYNACEAE
<i>Combretum erythrophyllum</i> (Burch.) Sond. 1	<i>Acokanthera oppositifolia</i> (Lam.) Codd 2, 4, 5, 9
<i>Combretum molle</i> R. Br. ex G. Don 4, 5	<i>Carissa bispinosa</i> (L.) Desf. ex Brenen ssp. <i>bispinosa</i> 9
179 PROTEACEAE	254 ASCLEPIADACEAE
<i>Protea caffra</i> Meisn. ssp. <i>caffra</i> 4, 5	<i>Parapodium costatum</i> E. Mey. 8
191 SANTALACEAE	<i>Aspidoglossum glabrescens</i> (Schltr.) Kupich 3
<i>Thesium deceptum</i> N.E. Br. 1, 2	<i>Gomphocarpus fruticosus</i> (L.) Aiton f. 7
<i>Thesium exile</i> N.E. Br. 1, 2, 3, 8	<i>Pachycarpus schinzianus</i> (Schltr.) N.E. Br. 5, 9
<i>Thesium inversum</i> N.E. Br. 8	<i>Asclepias adscendens</i> (Schltr.) Schltr. 2, 3, 5, 7, 8, 9
<i>Thesium rasum</i> (A.W. Hill) N.E. Br. 1, 7	<i>Asclepias eminens</i> (Harv.) Schltr. 4, 6, 7, 8
<i>Thesium scirpioides</i> A.W. Hill 8	<i>Asclepias gibba</i> (E. Mey.) Schltr. var. <i>gibba</i> 7
<i>Thesium transvaalense</i> Schltr. 1, 3, 4, 5, 9	<i>Asclepias stellifera</i> Schltr. 1, 3, 4, 5, 7, 8
<i>Thesium utile</i> A.W. Hill 3, 9	<i>Ceropegia rendallii</i> N.E. Br. 1, 7
192 LORANTHACEAE	254A PERIPLOCACEAE
<i>Tapinanthus natalitius</i> (Meisn.) Danser ssp. <i>zeyheri</i> (Harv.) Wiens 4, 5, 7, 9	<i>Raphionacme galpinii</i> Schltr. 1, 2, 3, 9
192A VISCACEAE	<i>Raphionacme hirsuta</i> (E. Mey.) R.A. Dyer ex Phill 4, 5, 9
<i>Viscum rotundifolium</i> L.f. 1, 4, 9	256 SOLANACEAE
201 CELASTRACEAE	<i>Physalis viscosa</i> * L. 1, 3
<i>Gymnosporia buxifolia</i> (L.) Szyszyl. 1, 2, 3, 4, 7, 9	<i>Solanum chenopodioides</i> Lam. 2, 6, 7
210 EUPHORBIACEAE	<i>Solanum incanum</i> L. 3, 9
<i>Phyllanthus maderaspatensis</i> L. 8, 9	<i>Solanum nigrum</i> * L. 1, 3, 7, 9
<i>Phyllanthus parvulus</i> Sond. 2, 4, 5	<i>Solanum panduriforme</i> E. Mey. 1, 2, 7, 8, 9
<i>Acalypha peduncularis</i> E. Mey. ex Meisn. 1, 2, 3, 4, 5, 6, 7, 8, 9	<i>Solanum pseudocapsicum</i> * L. 7
<i>Euphorbia clavaroides</i> Boiss. var. <i>truncata</i> (N.E. Br.) White, Dyer & Sloane 1, 9	<i>Solanum sisymbirifolium</i> * Lam. 3, 4, 6, 7, 8, 9
<i>Euphorbia striata</i> Thunb. var. <i>striata</i> 7	257 CONVULVULACEAE
<i>Euphorbia trichadenia</i> Pax 4	<i>Cuscuta campestris</i> * Yunc. 1
214 RHAMNACEAE	<i>Convolvulus sagittatus</i> Thunb. ssp. <i>grandiflorus</i> (H-f.) Meeuse var. <i>graminifolius</i> (H-f.) Bak. & C.H. Wright 4
<i>Ziziphus mucronata</i> Willd. ssp. <i>mucronata</i> 2, 3, 4, 5, 9	<i>Convolvulus sagittatus</i> Thunb. ssp. <i>sagittatus</i> var. <i>sagittatus</i> 3, 4, 5, 7, 8
<i>Ziziphus zeyheriana</i> Sond. 1, 2, 4, 5, 6, 7, 8, 9	<i>Convolvulus sagittatus</i> Thunb. ssp. <i>sagittatus</i> var. <i>phyllosepalus</i> (Hallier f.) A. Meeuse 1, 8
216 VITACEAE	<i>Convolvulus thunbergii</i> Roem. & Schult. 1, 2, 6
<i>Rhoicissus tridentata</i> (L.f.) Wild. & Drum. ssp. <i>cuneifolia</i> (Eckl. & Zeyh.) N.R. Urton 1, 9	<i>Merremia tridentata</i> (L.) Hallier f. ssp. <i>angustifolia</i> (Jacq.) van Oostr. var. <i>angustifolia</i> 1, 9
227 ANACARDIACEAE	<i>Ipomoea bathycolpos</i> Hallier f. var. <i>bathycolpos</i> 2, 8
<i>Ozoroa paniculosa</i> (Sond.) R. & A. Fernandes var. <i>paniculosa</i> 9	<i>Ipomoea crassipes</i> Hook. 6, 7, 8, 9
<i>Rhus discolor</i> E. Mey. ex Sond. 1, 8	<i>Ipomoea obscura</i> (L.) Ker-Gawl. 1, 3, 5
<i>Rhus leptodictya</i> Diels 3, 9	<i>Ipomoea obscura</i> (L.) Ker-Gawl. var. <i>obscura</i> 4, 7, 9
<i>Rhus pyroides</i> Burch. var. <i>pyroides</i> 1, 2, 4, 9	<i>Ipomoea ommaneyi</i> Rendle 7, 8
<i>Rhus rigida</i> Mill. var. <i>margaretae</i> Burt Davy ex Moffett 1, 2, 3, 4, 5, 6, 7, 8	<i>Ipomoea papilio</i> Hallier f. 1, 3, 4, 5
231 RUTACEAE	<i>Ipomoea purpurea</i> * (L.) Roth 2, 3
<i>Zanthoxylum capense</i> (Thunb.) Harv 2, 4, 5, 9	263 BORAGINACEAE
<i>Calodendrum capense</i> (L.f.) Thunb. 1	<i>Ehretia rigida</i> (Thunb.) Druce 1, 2, 3, 4, 9
232 MELIACEAE	<i>Heliotropium amplexicaule</i> * Vahl 1
<i>Melia azedarach</i> * L. 1	265 VERBENACEAE
233 ZYGOPHYLLACEAE	<i>Verbena bonariensis</i> * L. 1, 6, 8
<i>Tribulus terrestris</i> L. 1	<i>Verbena tenuisecta</i> * Briq. 1, 6
234 OXALIDACEAE	<i>Lantana camara</i> * L. 3, 9
<i>Oxalis corniculata</i> * L. 1, 3	<i>Lantana rugosa</i> Thunb. 3, 4, 9
<i>Oxalis latifolia</i> * H.B.K. 1	<i>Clerodendrum louwialbertsii</i> Herman 3, 8, 9
<i>Oxalis obliquifolia</i> Steud. ex A. Rich. 1, 2, 4, 6, 7, 8	267 LAMIACEAE/LABIATAE
235 GERANIACEAE	<i>Teucrium trifidum</i> Retz. 3
<i>Monsonia attenuata</i> Harv. 1	<i>Leonotis ocymifolia</i> (Burm. f.) Iwarsson var. <i>raineriana</i> (Visiani) Iwarsson 1, 9
<i>Monsonia burkeana</i> Planch. ex Harv. 1, 4, 6, 7, 8, 9	<i>Stachys natalensis</i> Hochst. var. <i>natalensis</i> 4, 5
242 MALPIGHIACEAE	<i>Salvia reflexa</i> * Hornem. 7
<i>Sphedamnocarpus pruriens</i> (Juss.) Szyszyl. ssp. <i>pruriens</i> 3	<i>Salvia runcinata</i> L. f. 1, 3, 6, 7
247 POLYGALACEAE	<i>Aeollanthus buchnerianus</i> Briq. 7
<i>Polygala gerrardii</i> Chod. 9	<i>Plectranthus madagascariensis</i> (Pers.) Benth. var. <i>madagascariensis</i> 4
<i>Polygala gracilentia</i> Burt Davy 3, 5	<i>Ocimum obovatum</i> E. Mey. ex Benth. ssp. <i>obovatum</i> var. <i>obovatum</i> 1, 2, 3, 4, 5, 6, 7, 8, 9
<i>Polygala hottentotta</i> Presl 1, 2, 3, 4, 5, 6, 7, 8, 9	268 PLANTAGINACEAE
<i>Polygala transvaalensis</i> Chod. var. <i>transvaalensis</i> 5, 8	<i>Plantago lanceolata</i> * L. 1, 8
250 APIACEAE/UMBELLIFERAE	270 OLEACEAE
<i>Heteromorpha arborescens</i> (Thunb.) Cham. & Schlechtd. var. <i>abyssinica</i> (A. Rich.) H. Wolff 1	<i>Olea europaea</i> L. ssp. <i>africana</i> (Mill.) P.S. Green 9
<i>Ciclospermum leptophyllum</i> * (Pers.) Eichler 1	<i>Menodora africana</i> Hook. 1, 5, 9
<i>Peucedanum magalismontanum</i> Sond. 4, 6, 7, 8, 9	271 SCROPHULARIACEAE
	<i>Nemesia fruticans</i> (Thunb.) Benth. 5, 6, 7, 9
	<i>Jamesbrittenia aurantiaca</i> (Burchell) Hilliard 1, 4, 5, 6, 7
	<i>Jamesbrittenia burkeana</i> (Benth.) Hilliard 1, 4, 5
	<i>Alectra orobanchioides</i> Benth. 1

- Graderia subintegra* Mast. 1, 3, 4, 6, 9 *Athrixia elata* Sond. 2, 3, 4, 5, 7, 9
Striga asiatica (L.) Kuntze 1, 3, 4, 8, 9 *Geigeria burkei* Harv. ssp. *burkei* var. *burkei* 4, 5
Striga bilabiata (Thunb.) Kuntze 1, 2, 7, 8, 9 *Callilepis leptophylla* Harv 1, 9
Striga elegans Benth. 2, 4, 5, 8, 9 *Zinnia peruviana** (L.) L. 9
271A SELAGINACEAE
Walafrida densiflora (Rolfe) Rolfe 1, 4, 8
274 GESNERIACEAE
Streptocarpus vandeleurii Bak. f. & S. Moore 4, 5, 7
277 ACANTHACEAE
Chaetacanthus costatus Nees 1, 2, 3, 4, 5, 6, 7, 8, 9
Chaetacanthus glandulosus Nees 5
Ruellia cordata Thunb. complex 9
Crabbea acaulis N.E. Br. 1, 3, 4, 7
Crabbea angustifolia Nees 3, 5, 9
Barleria macrostegia Nees 9
Barleria obtusa Nees 9
Blepharis integrifolia (L.f.) E. Mey. ex Schinz var. *integrifolia* 3
Blepharis squarrosa (Nees) T. Anders. 5
Hypoestes forskalii (Vahl) R. Br. form B 1, 2
Justicia anagalloides (Nees) T. Anders. 5, 7, 8
277A THUNBERGIAEAE
Thunbergia atriplicifolia E. Mey. ex Nees 8, 9
83 CAMPANULACEAE
Wahlenbergia dieterlenii (E. Phillips) Lammers 9
Wahlenbergia undulata (L.f.) A. DC. var. *undulata* 1, 4, 5, 7, 8, 9
Wahlenbergia undulata (L.f.) A. DC. var. *macrantha* Sond. 1
Wahlenbergia virgata Engl. 4, 6, 7
283A LOBELIACEAE
Lobelia erinus L. 1
Monopsis decipiens (Sond.) Thulin 1
287 RUBIACEAE
Kohautia amatymbica Eckl. & Zeyh. 1, 2, 3, 4, 5, 6, 7, 8, 9
Kohautia virgata (Willd.) Brem. 9
Pentanisia angustifolia (Hochst.) Hochst 1, 3, 4, 5, 6, 7, 8, 9
Vangueria infausta Burch. ssp. *infausta* 1, 2, 4, 5
Canthium gilfillanii (N.E. Br.) O.B. Miller 1, 2, 3, 4, 9
Canthium mundianum Cham. & Schlechtd. 9
Pavetta gardeniifolia A. Rich. var. *subtomentosa* K. Schum. 4, 6, 7
Pavetta zeyheri Sond. 3, 5
Anthospermum rigidum Eckl. & Zeyh. ssp. *pumilum* (Sond.) Puff 1, 2, 3, 4, 5, 6, 8, 9
291 DIPSACACEAE
Cephalaria zeyheriana Szabo 8
Scabiosa columbaria L. 1, 3, 4, 5, 6, 7, 8
293 ASTERACEAE/COMPOSITAE
Vernonia galpinii Klatt 1, 2, 3, 5, 6, 9
Vernonia natalensis Sch. Bip. ex Walp. 2, 3, 6, 9
Vernonia oligocephala (DC.) Sch. Bip. ex Walp. 1, 3, 4, 5, 6, 7, 8, 9
Vernonia poskeana Vatke & Hildebr. ssp. *botswana* Pope 1, 4, 5, 9
Vernonia sutherlandii Harv. 1, 2, 3, 4, 5
Aster harveyanus Kuntze 1, 2, 3, 4, 5, 6, 7, 8, 9
Felicia fascicularis DC. 1
Felicia muricata (Thunb.) Nees ssp. *muricata* 1, 2, 3, 4, 5, 6, 7, 8, 9
Nidorella anomala Steetz 5, 7, 8, 9
Nidorella hottentotica DC. 1, 2, 3, 4, 5, 6, 7, 8, 9
*Conyza bonariensis** (L.) Cronq. 4, 6
Conyza podocephala DC. 1, 2, 3, 6, 7, 8, 9
Nolletia rarifolia (Turcz.) Steetz 3, 4, 5, 9
Tarchonanthes camphoratus L. 9
Denekia capensis Thunb. 6, 7
Gnaphalium filagopsis Hilliard & Burtt 6, 7
Pseudognaphalium luteo-album (L.) Hillard & Burtt 7
Pseudognaphalium oligandrum (DC.) Hilliard & Burtt 4
Helichrysum callicomum Harv. 4
Helichrysum cephaloideum DC. 5
Helichrysum cerastioides DC. var. *cerastioides* 4
Helichrysum coriaceum Harv. 3, 4, 5, 6, 8, 9
Helichrysum lesliei Hilliard 4, 5
Helichrysum nudifolium (L.) Less. 3, 4
Helichrysum oxyphyllum DC. 4, 5
Helichrysum rugulosum Less. 1, 6, 7, 8, 9
Helichrysum setosum Harv. 1
Stoebe vulgaris Levyns 3, 4, 7, 9
311 COMMELINACEAE
Commelina africana L. var. *lancispatha* C.B. Cl. 1, 2, 3, 4, 7, 8, 9
Cyanotis speciosa (L.f.) Hassk. 1, 4, 5, 7, 8, 9
320 CYPERACEAE
Cyperus esculentus L. 7
Cyperus haematocephalus Boeck. ex C.B. Cl. 7
Cyperus obtusiflorus Vahl var. *flavissimus* Boeck. 9
Cyperus obtusiflorus Vahl var. *obtusiflorus* 3, 5, 7, 9
Cyperus semitrifidus Schrad. var. *semitrifidus* 1, 2, 4, 5, 6, 7, 9
Cyperus usitatus Burch. var. *usitatus* 6
Mariscus uitenhagensis Steud. 1, 2, 4, 6, 7, 8, 9
Fuirena coerulescens Steud. 6
Bulbostylis burchellii (Fical & Hiern.) C.B. Cl. 9
Bulbostylis contexta (Nees) Bodard 4
Bulbostylis oritrephes (Redley) C.B. Cl. ssp. *australis* B.L. Burtt 3, 4, 5, 8, 9
Abildgaardia ovata (Burm. f.) Kral 3, 6, 9
Scleria bulbifera Hochst. ex A. Rich. 5, 9
Schoenoxiphium sparteum (Wahlenb.) C.B. Cl. 5
9321 POACEAE/GRAMINAE
Urelytrum agropyroides (Hack.) Hack. 3, 4, 9
Elionurus muticus (Spreng.) Kunth 2, 3, 4, 5, 6, 7, 8, 9
Imperata cylindrica (L.) Reauschel 1
Schizachyrium sanguineum (Retz.) Alst 1, 3, 4, 5, 9
Andropogon schirensis A. Rich. 2, 3, 5, 9
Cymbopogon excavatus (Hochst.) Stapf ex Burtt Davy 1, 2, 3, 4, 5, 7, 8, 9
Cymbopogon prolixus (Stapf) Phill. 4
Cymbopogon validus (Stapf) Stapf ex Burtt Davy 1, 2, 7
Hyparrhenia anamesa Clayton 4, 8
Hyparrhenia dregeana (Nees) Stapf 1
Hyparrhenia hirta (L.) Stapf 1, 2, 3, 4, 5, 6, 7, 8, 9
Monocymbium ceresiiforme (Nees) Stapf 4, 9
Trachypogon spicatus (L.f.) Kuntze 4, 5, 6, 7, 9
Heteropogon contortus (L.) Roem. & Schult 1, 2, 3, 4, 7, 8, 9

<i>Diheteropogon amplexans</i> (Nees) Clayton	1, 3, 4, 5, 7, 9	<i>Aloe marlothii</i> Berger ssp. <i>marlothii</i>	2, 5
<i>Themeda triandra</i> Forssk.	1, 2, 3, 4, 5, 6, 7, 8, 9	<i>Chortolirion angolense</i> (Bak.) A. Berger	8
<i>Digitaria diagonalis</i> (Nees) Stapf var. <i>diagonalis</i>	3, 4, 7, 9		
<i>Digitaria eriantha</i> Steud.		340C HYACINTHACEAE	
<i>Allotriopsis semialata</i> (R. Br.) Hitchc. ssp. <i>eckloniana</i> (Nees)		<i>Schizobasis intricata</i> Bak.	2
Gibbs Russell	3, 4, 5, 6, 7, 8, 9	<i>Albuca glauca</i> Bak.	7
<i>Brachiaria serrata</i> (Thunb.) Stapf	1, 2, 3, 4, 5, 6, 7, 8, 9	<i>Albuca pachychlamys</i> Bak.	1, 2, 3, 6, 7, 8
<i>Urochloa brachyura</i> (Hack.) Stapf	6	<i>Albuca setosa</i> Jacq.	1
<i>Urochloa panicoides</i> Beauv.		<i>Urginea calcarata</i> (Bak.) Hilliard & Burtt	1, 5, 7, 8
<i>Panicum natalense</i> Hochst	1, 4	<i>Urginea depressa</i> Bak.	7
<i>Setaria lindenbergiana</i> (Nees) Stapf	3, 4	<i>Urginea multisetosa</i> Bak.	1, 3, 5, 7, 8
<i>Setaria nigrirostris</i> (Nees) Dur. & Schinz	1, 2, 3, 5, 6, 7, 8, 9	<i>Dipcadi marlothii</i> Engl.	1, 4, 5, 7, 8
<i>Setaria sphacelata</i> (Schumach.) Moss var. <i>sphacelata</i>	2, 6, 7	<i>Dipcadi viride</i> (L.) Moench	4, 5, 7, 9
<i>Setaria sphacelata</i> (Schumach.) Moss var. <i>torta</i> (Stapf) Clayton	2, 4, 5, 6, 7	<i>Eucomis autumnalis</i> (Mill.) Chitt. ssp. <i>clavata</i> (Bak.) Reyneke	8
<i>Setaria verticillata</i> (L.) Beauv.	2, 7	<i>Ornithogalum saundersiae</i> Bak.	1
<i>Melinis nerviglutinis</i> (Franch.) Zizka	1, 2, 3, 4, 5, 6, 7, 9	<i>Ornithogalum tenuifolium</i> Delaroche ssp. <i>tenuifolium</i>	7, 8, 9
<i>Melinis repens</i> (Willd.) Zizka ssp. <i>repens</i>	1, 2, 3, 4, 5, 6, 9	<i>Drimiopsis burkei</i> Bak.	1, 2, 7, 8
<i>Arundinella nepalensis</i> Trin.		<i>Ledebouria cooperi</i> (Hook. f.) Jessop	1, 3, 6, 9
<i>Tristachya leucothrix</i> Nees	1, 5, 6, 9	<i>Ledebouria graminifolia</i> (Schonl.) Jessop	1, 5
<i>Helictotrichon turgidulum</i> (Stapf) Schweick	7	<i>Ledebouria luteola</i> Jessop	1
<i>Aristida aequiglumis</i> Hack.		<i>Ledebouria marginata</i> (Bak.) Jessop	1, 3, 5, 6, 8
<i>Aristida canescens</i> Henr. ssp. <i>canescens</i>	5, 7	<i>Ledebouria ovatifolia</i> (Bak.) Jessop	1, 2, 7, 8
<i>Aristida congesta</i> Roem. & Schult. ssp. <i>barbicollis</i> (Trin. & Rupr.) De Winter	6, 9	340E ALLIACEAE	
<i>Aristida congesta</i> Roem. & Schult. ssp. <i>congesta</i>	1, 4, 9	<i>Tulbaghia acutifolia</i> Harv.	1, 5, 7, 9
<i>Aristida junciformis</i> Trin. & Rupr. ssp. <i>junciformis</i>	7	340G ASPARAGACEAE	
<i>Tragus berteronianus</i> Schult.	1, 3, 4, 6	<i>Asparagus aethiopicus</i> L.	1
<i>Sporobolus fimbriatus</i> (Trin.) Nees	1	<i>Asparagus africanus</i> Lam.	1, 3, 7, 9
<i>Sporobolus stapfianus</i> Gand.	2, 7, 9	<i>Asparagus larinus</i> Burch.	5
<i>Eragrostis capensis</i> (Thunb.) Trin.	1, 2, 3, 8, 9	<i>Asparagus suaveolens</i> Burch.	1, 2, 3, 4, 5, 8, 9
<i>Eragrostis chloromelas</i> Steud.	1, 2, 3, 4, 5, 6, 7, 8, 9	341 AMARYLLIDACEAE	
<i>Eragrostis curvula</i> (Schrud.) Nees	1, 2, 6, 7, 8, 9	<i>Boopha disticha</i> (L.f.) Herb.	5, 9
<i>Eragrostis gummiiflua</i> Nees	6, 7	341A HYPOXIDACEAE	
<i>Eragrostis racemosa</i> (Thunb.) Steud.	1, 2, 3, 4, 5, 6, 7, 8, 9	<i>Hypoxis acuminata</i> Bak.	1, 3, 6, 7, 8
<i>Microchloa caffra</i> Nees	3, 8, 9	<i>Hypoxis argentea</i> Harv. ex Bak. var. <i>argentea</i>	3, 4, 6, 7, 9
<i>Cynodon dactylon</i> (L.) Pers.	1, 2, 4, 5, 6, 7, 8	<i>Hypoxis filiformis</i> Bak.	9
<i>Harpachloa falx</i> (L.f.) Kuntze	4, 7, 9	<i>Hypoxis hemerocallidea</i> Fischer & Meyer	1, 2, 6, 8, 9
<i>Chloris pycnothrix</i> Trin.		<i>Hypoxis interjecta</i> Nel	3, 8
<i>Eustachys paspaloides</i> (Vahl) Lanza & Mattei	1, 2, 4, 7, 9	<i>Hypoxis iridifolia</i> Bak.	3, 7, 8, 9
<i>Bewsia biflora</i> (Hack.) Goossens	2, 3, 8	<i>Hypoxis multiceps</i> Buchinger ex Bak.	1
<i>Diplachne fusca</i> (L.) Beauv. ex Roem & Schult.	1, 5	<i>Hypoxis rigidula</i> Bak. var. <i>pilosissima</i> Bak.	2, 3, 4, 5, 7, 8
<i>Koeleria capensis</i> (Steud.) Nees	1, 7	<i>Hypoxis rigidula</i> Bak. var. <i>rigidula</i>	1, 4, 7, 9
340B ASPHODELACEAE		342 IRIDACEAE	
<i>Bulbine abyssinica</i> A. Rich.	4	<i>Moraea stricta</i> Bak.	4, 5, 9
<i>Bulbine capitata</i> V. Poelln.	7	<i>Babiana hypogaea</i> Burch. var. <i>hypogaea</i>	9
<i>Trachyandra asperata</i> Kunth var. <i>asperata</i>	3	<i>Gladiolus dalenii</i> Van Geel ssp. <i>delineate</i>	1, 2, 3, 4, 5, 7
<i>Trachyandra erythrorrhiza</i> (Conrath) Oberm.	7	<i>Gladiolus woodii</i> Bak.	1, 3, 4, 5, 7
<i>Trachyandra saltii</i> (Bak.) Oberm. var. <i>saltii</i>	5, 6, 7	355 ORCHIDACEAE	
<i>Chlorophytum cooperi</i> Bak.	1, 3, 5, 8	<i>Eulophia clavicornis</i> Lindl. var. <i>clavicornis</i>	8
<i>Chlorophytum fasciculatum</i> (Bak.) Kativu	2, 3, 4, 5, 6, 7, 8, 9	<i>Eulophia clavicornis</i> Lindl. var. <i>inaequalis</i> (Schltr.) A.V. Hall	1, 8
<i>Chlorophytum transvaalense</i> (Bak.) Kativu	9		
<i>Aloe greatheadii</i> Schonl. var. <i>davyana</i> (Schonl.) Glen & Hardy	1, 2, 3, 4, 6, 7, 8, 9		

Chapter 3: Plant species richness and diversity of the serpentine areas on the Witwatersrand.

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Plant species richness and diversity of the serpentine areas on the Witwatersrand

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Abstract Soil chemistry can play an important role in determining plant diversity. Serpentine soils are usually toxic to many plant taxa, which limits plant diversity compared to that on adjacent non-serpentine soils. The usually high concentrations of toxic metals in serpentine soils are considered to be the edaphic factors that cause low diversity and high endemism. This paper aimed primarily to determine whether there is a relationship between serpentine soil chemistry and species richness on the Witwatersrand and to compare species richness of the serpentine areas with that of adjacent non-serpentine areas as well as with the species richness of the serpentine areas in the Barberton Greenstone Belt. The alpha- and beta-diversity of the Witwatersrand serpentine and non-serpentine areas was also investigated. A secondary aim of this study was to determine which of the non-serpentine taxa were more common on the serpentine than off the serpentine, which taxa were more common off the serpentine than on the serpentine and which taxa were equally common on and off

serpentine soils. There was no significant difference in alpha-diversity between the serpentine and the adjacent non-serpentine areas, but beta-diversity is higher between serpentine plots than between non-serpentine plots. Although soil factors do affect species richness and diversity of plants on the Witwatersrand to a limited extent, the concentrations of soil chemicals in serpentine soils are not sufficiently different from those in non-serpentine soils to significantly influence the species richness and diversity of the serpentine soils. The high, but similar, diversity on serpentine and non-serpentine soils on the Witwatersrand indicates that soil factors do not play a significant role in determining diversity on potentially toxic soils in the area.

Keywords Alpha-diversity · Beta-diversity · Modified-Whittaker plots

Introduction

Southern Africa has the world's highest plant species density at the sub-continental level (Cowling et al. 1989). The main reasons for this high species richness are environmental heterogeneity, two different climatic regimes, i.e. summer and winter rainfall regions, recurrent climatic fluctuations since the mid-Pliocene and the number of relicts that survived in pockets along the coast (Goldblatt 1978).

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Biodiversity represents the complexity of life on earth (Wilsey et al. 2005). High species diversity means a complex community, as a greater variety of species means a greater array of interactions (Brower and Zar 1984). The stability of the ecosystem is also linked to diversity, since most stable communities have large numbers of species and an even distribution of individuals between species (Williams 1987; Tilman 1996). Humans, by overexploitation, habitat modification or deliberate destruction, reduce this influx (Leps 2004; Sodhi et al. 2004). Fragmentation, grazing, forestry and human influence are decreasing the biological diversity of earth's resources (Tilman 1996). The official estimate by the IUCN indicates that 13% of the world's plant species are under threat; however other scientists (Pitman and Jorgensen 2002) place this figure between 22% and 47%. Understanding and predicting species diversity is therefore essential in identifying areas vulnerable to species loss and thus providing a focus for conservation efforts (Cowling et al. 1989; Pearson 1996).

Three important components of species diversity are: alpha-diversity—the number of species in a homogenous community (Cowling et al. 1992); Beta-diversity—species turnover along an environmental or habitat gradient (Whittaker 1972); and gamma-diversity—species turnover for different ecosystems along geographic gradients (Cowling et al. 1992).

Alpha-diversity, in the strict sense, is species richness (Whittaker 1972). However, species richness is only one aspect of diversity (Gaston 1996) and on its own is not as important in an ecosystem as the combined effects of richness and evenness (Hooper and Vitousek 1997; Wilsey et al. 2005). Diversity indices, such as the Shannon–Weiner and Simpson indices, are therefore useful because they combine both species richness and evenness into a single value (Magurran 1988; Dörgeleh 1999) which can be used in environmental monitoring and conservation.

Beta-diversity is the extent of species replacement or biotic change along environmental gradients (community turnover) and depends on habitat diversity (Wilson and Shmida 1984). An accurate measure of beta-diversity is important because it indicates the degree to which habitats are partitioned by species, i.e. the degree of patchiness. Alpha- and beta-diversity together measure the overall diversity of an area (Peet 1974; Wilson and Shmida 1984).

Environmental factors, especially soil fertility and climate, play an important role in determining species richness (Stohlgren et al. 1999; Grace 2001). Edaphic factors account for much variation in species data and soil pH, and the Mg:Ca ratio plays an important role in species distribution patterns (Esler and Cowling 1993). In the semi-arid areas of southern Africa, soil factors play an important role in determining transitions between vegetation types and plant communities (Esler and Cowling 1993). High levels of local diversity have been considered characteristic of nutrient poor soils in the Cape (Goldblatt and Manning 2002).

Species numbers are controlled by biological interactions such as edaphic conditions and competition (Grime 1979; Bond 1983 in Cowling 1990). Serpentine soils are very diverse due to various factors such as parent material, climate, relief and biological activity (Brooks 1987). These soils generally have high concentrations of Ni, Cr, Co and Fe, high Mg:Ca ratios and low concentrations of N, P and K. All this makes for harsh environmental conditions which in turn result in a low diversity of plant species and unique, usually endemic flora (Kruckeberg 1954; Proctor et al. 1980). The serpentine soils on the Witwatersrand are not as toxic as the serpentine soils of other areas (Reddy et al. 2001). Thus it is expected that there will be a higher diversity on the serpentine soils of this area relative to other serpentine areas.

In 1934, Raunkiaer proposed a system to describe vegetation based on the position of buds or regenerating parts which he found to be related to climate (Ricklefs 1973). He proposed several life form categories the major ones being: phanerophytes—buds occur on the tips of branches, associated with tropical climates, i.e. trees and shrubs; chamaephytes—buds occur at ground level during unfavourable seasons, mainly associated with cold, dry climates, i.e. small shrubs and herbs; hemicryptophytes—dormant buds occur just beneath or at the soil surface; cryptophytes—buds are deeply buried and also store food; and therophytes—“annuals” that survive in seed form, associated mainly with deserts and some grasslands. Hemicryptophytes and cryptophytes are usually associated with cold, moist temperate areas; most dieback to ground level during winter and regenerate the following season (Ricklefs 1973). Fire plays an important role in the

maintenance of the grassland biome on the Witwatersrand (Kerfoot 1987) and thus it is expected that cryptophytes and hemicryptophytes, with their buds protected below the soil surface, will be the dominant life forms on the Witwatersrand.

The Witwatersrand region is geologically and topographically diverse (McCallum and Balkwill 1999). This provides a diversity of habitats and hence the potential for a high level of plant diversity in this region. There is no unique serpentine flora and there are no serpentine endemic taxa on the Witwatersrand (Reddy et al. 2001). On serpentine soils that have been exposed for long periods, prolonged evolution has led to a very high floral diversity at both the genus and species level (Berazain 1992; Batianoff and Specht 1992; Williamson 1995). So, with the potential high diversity of this region and the possible long exposure of the Witwatersrand serpentine soils, are the serpentine areas on the Witwatersrand as species diverse as the adjacent non-serpentine areas?

The aims of this paper are to: (1) compare the relationship between species richness of the flora and selected soil elements at serpentine sites and their adjacent non-serpentine sites, (2) determine how species rich the serpentine areas on the Witwatersrand are and how this richness compares to the adjacent, non-serpentine areas, (3) investigate the alpha- and beta-diversity of the Witwatersrand serpentine soils and the adjacent non-serpentine soils and to compare the beta-diversities of the all Witwatersrand serpentine areas with those of the sites studied in the Barberton Greenstone Belt and (4) determine which of the *bodenvag* taxa, i.e. taxa that are indifferent to edaphic conditions, are more common on the serpentine than off the serpentine, which taxa are more common off the serpentine than on the serpentine and which taxa are equally common on and off the serpentine.

The following predictions are made:

1. Because the serpentine soils on the Witwatersrand are not as toxic as the serpentine soils of other areas, e.g. the Barberton Greenstone Belt (Reddy et al. 2001), species richness of herbaceous taxa on serpentine soils on the Witwatersrand will be similar to species richness of herbaceous taxa of adjacent, non-serpentine areas,
2. Studies of other serpentine sites globally have shown that serpentine soils with high concentrations of Ni, Cr, Co and Fe, high Mg:Ca ratios and

low concentrations of N, P and K usually have a low diversity and a high degree of endemism (Kruckeberg 1954; Proctor et al. 1980). Thus, the lower concentrations of chromium and nickel or the magnesium:calcium ratio of serpentine sites would result in a higher number of tolerant species and few or no endemic taxa,

3. The similarity between the floras of the serpentine and non-serpentine soils on the Witwatersrand is greater than that between the serpentine flora of the Witwatersrand and that of the Barberton Greenstone Belt serpentines.

Study areas

Johannesburg is situated in an area known as the Highveld of South Africa. The topography of the region is flat grassland with irregular, undulating rocky hills and ridges. Altitude on the Witwatersrand ranges between 1450 and 1750 m. This region, including the Witwatersrand serpentine areas (Reddy et al. 2001), is within the grassland biome and for the most part the vegetation falls into the rocky Highveld grassland (Bredenkamp and van Rooyen 1996). This grassland type, formerly known as Bankenveld, is fire-maintained (Acocks 1988), with the vegetation adapted to fire and consisting mainly of resprouting forbs. Trees and shrubs are confined to the rocky ridges and outcrops which offer some protection from fire.

Precipitation ranges between 600 and 750 mm per annum and occurs mainly in summer. Temperatures range between 12°C (daily minimum) and 39°C (daily maximum) with an average of 16°C. Winters are very dry and severely frosty (Bredenkamp and van Rooyen 1996). Many of the trees found at the sites have high latex or resin contents in the sap (pers. obs.). This is possibly a mechanism to overcome the harsh, frosty Highveld winters and another reason why they are confined to the less fire prone, rocky areas (Balkwill and Botany II 1993, 1995). High latex or resin contents in the sap could also act as a deterrent to insect herbivory.

The serpentine areas on the Witwatersrand mainly occur around the south-western and south-eastern margins of the granite dome found to the north of Johannesburg, and at some localities within the dome. These sites are situated in a densely populated urban area and are subject to various forms of disturbance.

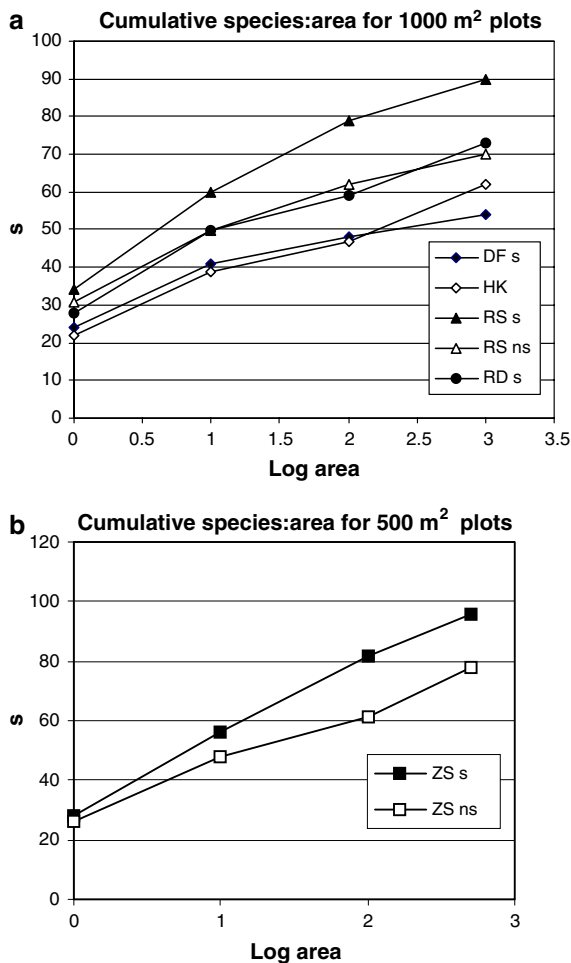


Fig. 1 (a) Cumulative species richness: log area curves for 1000 m² serpentine (s) and non-serpentine (ns) plots excluding the single non-serpentine plot at Robindale. DF: Diamond Four, HK: Honingklip, RD: Robindale, RS: Ruimsig. (b) Cumulative species richness: log area curves for 500 m² serpentine (s) and non-serpentine (ns) plots at Zandspruit (ZS)

Most of the large serpentine areas have been built upon and only small patches remain (Fig. 1 in Reddy et al. 2001).

Methods

Modified Whittaker plots (Stohlgren et al. 1995) were used to determine the species diversity of these sites. These plots usually are 0.1 ha, i.e. 1000 m² (50 m × 20 m); however, on the Witwatersrand, some serpentine bands were too narrow to accommodate full-size plots. In these cases (Zandspruit and Weltevreden) half-sized plots (50 m × 10 m) were used. Nested

within each plot (whether full- or half-sized) were ten 0.5 m × 2 m (1 m²) sub-plots systematically placed within the inside border, two 2 m × 5 m (10 m²) sub-plots in alternate corners and a 5 m × 20 m (100 m²) sub-plot in the centre (Stohlgren et al. 1999).

The number of plots per site varied depending on the size of the site. On five of the nine serpentine sites, namely Darrenwood, Diamond Four, Illiondale, Robindale and Ruimsig, three modified Whittaker plots were used. At the Dombeya site, because of its size, six plots were used; four plots were used at the Estherpark site for the same reason. Where possible, the four non-serpentine sites had the same number of plots as their adjacent or proximate serpentine sites; so Honingklip and Ruimsig had three plots and the Zandspruit site only two half-sized plots. The non-serpentine soils at Robindale consisted of only a narrow band on which only one full-sized plot could be fitted. The plot sizes on both the serpentine and the adjacent non-serpentine soils were of the same size. For sites that were on ridges, plots were randomly placed at the base and mid-slopes and top of the slope. On hills, plots were placed at the base and mid-slopes on either side of the hill.

The taxa present in each plot and its sub-plots were recorded. Soil was gathered to a depth of about 5 cm from the four corners and the centre of each plot 0.1 ha plot, and because of the cost of soil analysis, soils from all the plots of each site were bulked together, so soils analysis was done per site, not per plot. In the case of the interface sites, the serpentine and non-serpentine soils were analysed separately. Soil samples were sent to the Agricultural Research Council (ARC) for analysis of %C, %N, soil pH and exchangeable cations. Exchangeable cations were determined using the ammonium acetate method (Schollenberger and Simon 1945).

A paired *t*-test was used to compare selected soil chemicals at the sites that had both serpentine and adjacent non-serpentine soils. The Pearson's Correlation coefficient was used to determine whether or not there was a significant correlation between total species richness, at the 500 m²/1000 m² plot scale, and soil chemicals at each site.

Cover was determined using a modified line intercept method. A 50 m tape was placed lengthwise in the centre of the plot. The presence or absence and identity of taxa at every 10 cm interval were recorded

(Whittaker et al. 1979). Percentage cover was determined from the number of points along the 50 m tape at which taxa occurred.

Paired *t*-tests were used to test for species richness between serpentine and non-serpentine sites at the 1 m² (point) and 500 m²/1000 m² (plot) levels. Because of the differing sizes of the plots, the log of species richness was plotted against the log of area to give a linear graph for the serpentine and adjacent non-serpentine sites. The extrapolated species richness values were then read off at the 500 m² and 1000 m² points (Witkowski pers. comm.).

The Shannon–Weiner and the Simpson indices were used to measure alpha-diversity. The Shannon–Weiner index expressed heterogeneity whilst the Simpson index is sensitive to changes in common species and is determined by the relative concentration of dominance (Whittaker, 1972; Peet 1974; Kent and Coker 1994; Dörgeleh 1999).

The formula for the Shannon–Weiner index is given by: $H' = -\sum^s I = 1 p_i \ln p_i$, where s = number of species, p_i = the proportion of individuals or abundance of the i th species and $\ln$ = log base e and expressed heterogeneity. In this instance abundance was used. The formula for the Simpson index is: $D = 1 - (\sum [n_i (n_i - 1)] / [N_i (N_i - 1)])$, where D = Simpson's Index, n_i = number of individuals of species i and N = the total number of individuals. The closer the D value to 1, the more even the distribution of species. A paired *t*-test was used to test for significance. The Shannon–Weiner index values were then used to calculate the Evenness Index using the formula $J = H' / \ln s$ where s is the number of species present.

The mean cumulative species number was calculated per site for both the serpentine and non-serpentine plots. These results were then plotted against the log of area, using Microsoft Excel (2003). Log of area was used so that one can more easily see details for small values.

Sørensen's similarity index ($Q_s = 2c/a + b$, where c = taxa in common, a = total taxa in plot 'a' and b = total taxa in plot 'b') was used to measure similarity between plots (Kent and Coker 1994). Beta-diversity is the dissimilarity between plots. Two identical plots would have a similarity value of 1, then dissimilarity (beta-diversity) is calculated as 1–Sørensen's similarity index (Kent and Coker 1994). An unpaired *t*-test was used to test for significance

between total similarity of serpentine sites against total similarity of the non-serpentine sites. A complete linkage cluster diagram, using Statistica version 6, was then constructed to show beta-diversity for all sites excluding the smaller Weltevreden, Zandspruit and Robindale sites. Beta-diversity (dissimilarity) was plotted against the log of area.

Sørensen's similarity index was used to compare the similarities between the Barberton Greenstone Belt serpentine and non-serpentine groups and the Witwatersrand serpentine and non-serpentine groups. It was also used to compare similarities between the different growth forms at each of the serpentine/non-serpentine sites on the Witwatersrand.

The *bodenvag* taxa were determined by listing all the taxa occurring in the plots on and off serpentine soils, and then counting the number of times they occurred in serpentine and adjacent non-serpentine plots. Student's *t*-test was used to determine which *bodenvag* taxa are significantly more common on the serpentine than off the serpentine, which taxa are more common off the serpentine than on the serpentine and which taxa are equally common on and off the serpentine.

Statistical analysis

The Epistat statistical programme (Gustafson 1986) was used to perform Student's *t*-tests and Pearson correlations. Microsoft Excel (2003) was used to construct the species:area graph (Fig. 1). STATISTICA (data analysis software system) version 6 (2001) was used to construct Figs. 2 and 3.

Results

There was significantly more total Cr and soluble N in the serpentine soils than in the adjacent non-serpentine soils (Table 1a). There was no significant correlation between species richness and the various soil parameters of the serpentine soils (Table 1b). There was a significant, positive correlation between species richness and the total Cr and soluble Ni in the non-serpentine soils (Table 1c).

A total of 449 taxa and 415 species occur on the Witwatersrand serpentine areas (Reddy et al. 2001). The intensive study using the modified Whittaker

Fig. 2 Graphs showing average percentage cover based on line intercept counts for serpentine and adjacent non-serpentine areas. DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine

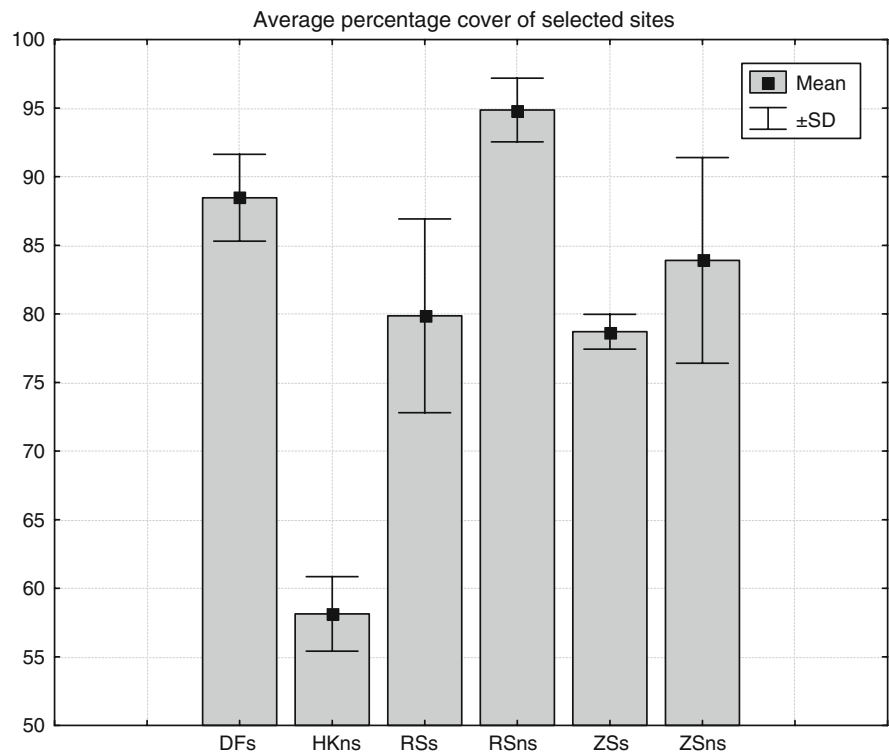
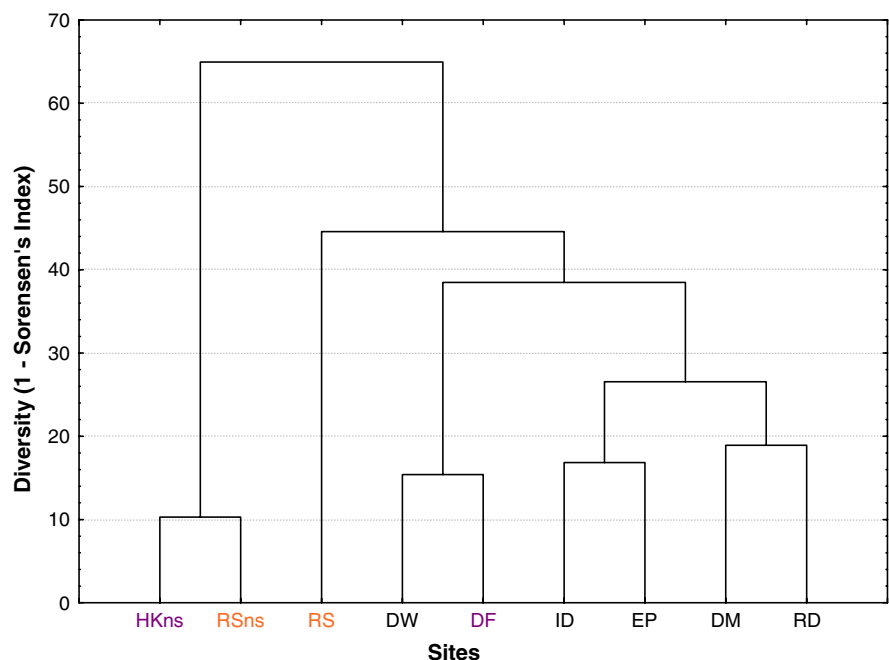


Fig. 3 Cluster diagram of beta-diversity for 9 sites. DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP: Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, s = serpentine, ns = non-serpentine



plots added a further 15 taxa. This brought the total number to 464 taxa and 413 species. According to McCallum and Balkwill (1999), there are 1478 plant species on the Witwatersrand. This means that 27.9%

of the total Witwatersrand species occur on serpentine soils.

The Robindale and Ruimsig sites showed the greatest point species richness among the serpentine

Table 1 (a) Comparison between soil chemicals for the four serpentine sites with adjacent non-serpentine areas. (b) Correlation between species richness and various soil properties for all serpentine plots combined, average total speciesrichness $\pm$ sd: 74.0 ± 17.65 , $n = 9$, $df = 7$. (c) Correlation between species richness and soil properties for all for all non-serpentine plots combined, $n = 4$, $df = 2$. Average total species richness $\pm$ sd: 82.8 ± 22.5

Soil chemical	Mean $\pm$ sd		Paired t -test (df = 3)	
	Serpentine	Non-serpentine	t	P
Panel A				
Mg:Ca	1.78 $\pm$ 0.36	1.25 $\pm$ 0.24	2.5	0.09
pH	5.88 $\pm$ 0.47	5.80 $\pm$ 0.42	0.35	0.75
Total Cr ($\mu\text{g/g}$)	1152.62 $\pm$ 120.36	721.81 $\pm$ 213.30	4.76	0.02*
Sol. Cr ($\mu\text{g/g}$)	0.45 $\pm$ 0.07	0.37 $\pm$ 0.05	2.08	0.13
Total Ni ($\mu\text{g/g}$)	752.04 $\pm$ 404.55	257.08 $\pm$ 190.04	2.37	0.1
Sol. Ni ($\mu\text{g/g}$)	16.02 $\pm$ 5.13	6.31 $\pm$ 3.23	2.88	0.06
Total Fe ($\mu\text{g/g}$)	50722.5 $\pm$ 7063.55	38312.5 $\pm$ 9667.67	1.74	0.18
Sol. Fe ($\mu\text{g/g}$)	147.6 $\pm$ 22.28	110.08 $\pm$ 26.24	2.02	0.14
Sol. Mg ($\mu\text{g/g}$)	269.54 $\pm$ 22.61	211.43 $\pm$ 163.59	0.46	0.68
Sol. N (%)	0.29 $\pm$ 0.04	0.19 $\pm$ 0.08	5.09	0.02*
Parameter	Parameter values (mean $\pm$ sd)		t	P
Panel B				
Mg:Ca	1.6 $\pm$ 0.59	−0.23	0.64	0.54
pH	6.04 $\pm$ 0.92	−0.01	0.03	0.98
Total Cr ($\mu\text{g/g}$)	1002.28 $\pm$ 293.23	0.17	0.46	0.66
Total Ni ($\mu\text{g/g}$)	685.94 $\pm$ 353.10	−0.41	1.19	0.27
Sol. Ni ($\mu\text{g/g}$)	12.45 $\pm$ 5.64	0.09	0.25	0.81
Total Fe ($\mu\text{g/g}$)	47278.9 $\pm$ 10746.2	−0.17	0.45	0.67
Sol. N (%)	0.26 $\pm$ 0.6	0.27	0.75	0.48
Panel C				
Mg:Ca	1.3 $\pm$ 0.2	0.89	2.73	0.11
pH	5.8 $\pm$ 0.4	0.62	1.11	0.38
Total Cr ($\mu\text{g/g}$)	721.8 $\pm$ 213.3	0.9998	63.7	0.0003*
Total Ni ($\mu\text{g/g}$)	257.1 $\pm$ 190.0	0.94	3.88	0.06
Sol. Ni ($\mu\text{g/g}$)	6.3 $\pm$ 3.2	0.96	5.02	0.03*
Total Fe ($\mu\text{g/g}$)	38312.5 $\pm$ 9667.7	0.08	0.12	0.92
Sol. N (%)	0.18 $\pm$ 0.08	−0.71	1.44	0.29

* = significant, sol. = soluble

sites (Table 2a). The Weltevreden site, despite being the smallest serpentine site, also had a high point species richness. There were no significant differences in species richness between the paired serpentine and non-serpentine sites.

Extrapolating adjacent serpentine and non-serpentine plots down to 500 m^2 (Table 2b), all the serpentine plots, except Diamond Four, had more taxa than the non-serpentine plots. Weltevreden and Zandspruit, the 2 small sites, had the greatest species

richness (100 and 133 respectively). Extrapolating up to 1000 m^2 shows the same trend.

The cumulative species: area curves (Fig. 1a, b) show a steady increase in species as area increases for all sites. At the Zandspruit and Ruimsig sites, the serpentine areas showed a greater increase in species as the area increased than the non-serpentine areas. The Diamond Four serpentine area and Honingklip non-serpentine area, although further apart from each other than the other serpentine/non-serpentine paired

Table 2 (a) Point species richness (i.e. average number of species recorded for 1 m² sub-plots on and off serpentine soils). ! indicates half-sized (500 m²) plots. (b) Species richness (i.e. number of species recorded per 1000 m²/500 m² plots) on and off serpentine soils, with extrapolations shown in *italics*

Sites	Serpentine mean $\pm$ sd (<i>n</i>)		Non-serpentine mean $\pm$ sd (<i>n</i>)		<i>t</i> (df)	<i>P</i>
Panel A						
DM	7.9 $\pm$ 2.9 (6)					
DW	6.2 $\pm$ 3.0 (3)					
EP	5.1 $\pm$ 1.9 (4)					
ID	6.6 $\pm$ 2.2 (3)					
!WV	9.4 $\pm$ 0.6 (2)					
RD	10.4 $\pm$ 2.7 (3)		13.3 $\pm$ 3.9 (1)			
DF/HK	8.7 $\pm$ 3.4 (3)		7.9 $\pm$ 2.3 (3)		1.04 (2)	0.41
RS	10.6 $\pm$ 2.7 (3)		11.6 $\pm$ 3.2 (3)		0.40 (2)	0.73
!ZS	7.9 $\pm$ 3.7 (2)		9.4 $\pm$ 3.7 (2)		0.28 (1)	0.83
Sites	Serpentine		Non-serpentine		<i>t</i> (df)	<i>P</i>
	Calculated mean $\pm$ sd (<i>n</i>) or <i>Extrapolated</i>		Calculated mean $\pm$ sd (<i>n</i>) or <i>Extrapolated</i>			
	1000 m ²	500 m ²	1000 m ²	500 m ²		
Panel B						
DW	79.3 $\pm$ 4.5 (3)	59.6				
DM	58.8 $\pm$ 14.2 (6)	53.1				
EP	68.3 $\pm$ 10.3 (4)	37.6				
ID	57 $\pm$ 8.0 (3)	39.8				
RD	93.3 $\pm$ 29.9 (3)	70.8	115 (1)	59.6		
!WV	100.0	73.5 $\pm$ 2.1 (2)				
DF/HK	56 $\pm$ 12.2 (3)	42.2	64.3 $\pm$ 7.2 (3)	53.1	0.75 (2)	0.53
RS	89.7 $\pm$ 10.1 (3)	84.1	70.3 $\pm$ 1.5 (3)	59.6	3.52 (2)	0.1
!ZS	133.4	96.0 $\pm$ 14.1 (2)	89.1	78 $\pm$ 9.9 (2)	1.05 (1)	0.48

DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP: Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit. ! = half-sized (500 m²) plots

sites, were very similar to each other with regard to the number of species occurring in both these areas.

The serpentine plots of the Diamond Four/Honingklip site comprise the only site which consistently shows significantly more cover ($t = 12.6$, $P = 0.0002$, 4 df) than the non-serpentine plots (Fig. 2). The cover of the non-serpentine plots at Ruimsig was significantly higher than that of the serpentine sites ($t = 3.5$, $P = 0.02$, 4 df). The Zandspruit site gave variable results for both the serpentine and non-serpentine areas.

Darrenwood and Diamond Four were the most diverse serpentine sites, with the Weltevreden site being the least diverse. There was no significant difference in diversity between the serpentine and the adjacent non-serpentine areas, for both the

Shannon–Weiner and Simpson indices (Table 3a). With regard to evenness (Table 3b), only the half plots, at Weltevreden and Zandspruit, show evenness values of less than 0.50. Diamond Four, the least disturbed site (Reddy et al. 2001), showed the most even distribution of species (0.74) of all the serpentine sites and Weltevreden showed the least evenness (0.42). Estherpark and Illiondale, despite the high degree of disturbance (Reddy et al. 2001), show high evenness values, 0.71 and 0.61, respectively. However there was no significant difference in species richness between the serpentine and adjacent non-serpentine sites. All sites show a greater dominance than evenness; Weltevreden, which shows the least evenness, has the highest D value.

Table 3 (a) Shannon–Weiner (H') and Simpson (D) Indices of serpentine plots and adjacent non-serpentine plots. (b) Species evenness indices of serpentine plots and non-serpentine plots

Site (<i>n</i>)	HN (Mean ± sd)		<i>t</i>	<i>P</i>	df	D (Mean ± sd)		<i>t</i>	<i>P</i>	df
	s	ns				s	ns			
Panel A										
DF/HK (3)	1.31 ± 0.41	1.05 ± 0.33	0.8	0.5	2	0.79 ± 0.10	0.96 ± 0.01	2.81	0.11	2
RS (3)	1.06 ± 0.17	0.97 ± 0.03	0.8	0.5	2	0.80 ± 0.05	0.90 ± 0.07	1.16	0.25	2
ZS (2)	0.95 ± 0.37	1.10 ± 0.11	0.4	0.7	1	0.94 ± 0.01	0.94 ± 0.01	1	0	1
Site (<i>n</i>)	DF s (3)		HK ns (3)	RS s (3)		RS ns (3)	ZS s (2)	ZS ns (2)		
Panel B										
Evenness index (J): (H/log s)	0.74	0.58		0.54		0.54	0.48	0.58		

DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine

The Barberton Greenstone Belt serpentines have 884 plant taxa while only 442 taxa occur on the Witwatersrand serpentine areas. There are 239 taxa that are common between these two areas. Thus there is only a 36% similarity (64% dissimilarity) between the total serpentine taxa of the Witwatersrand serpentine areas and the Barberton Greenstone Belt serpentine areas. A comparison of Sørensen's similarity values for the serpentine and non-serpentine plots of the Barberton Greenstone Belt (Williamson and Balkwill unpublished, Changwe and Balkwill 2003) with that of the Witwatersrand serpentine sites with adjacent non-serpentine plots shows a very high degree of similarity between the Witwatersrand plots (Table 4a).

Dissimilarities between serpentine plots ranged from 42 to 90% (Table 4b). Estherpark has the lowest similarity, or conversely, the greatest beta-diversity of all the serpentine sites. It has a large, very diverse site encompassing grassland, a seepage area and rocky outcrops and thus has a diverse flora compatible with the various micro-habitats within this site. Zandspruit and Weltvreden are small sites, the plots sampled are closer together, and hence they show a greater similarity. Non-serpentine scores range from 30 to 51% dissimilarity (Table 4b). Zandspruit non-serpentine plots showed the greatest beta diversity; this could perhaps be due to small sample error.

An unpaired t -test showed that there was a significant difference in the total similarity between serpentine and non-serpentine plots, with the serpentine plots being significantly more diverse than the non-serpentine plots. The dendrogram showing beta-diversity (Fig. 3) also shows this difference between the serpentine and non-serpentine sites.

There is a very high similarity between grass taxa found on and off serpentine soils, ranging from 73 to 86% using the Sørensen's Similarity Index (Table 4c). At all sites, grass and forb taxa showed the greatest similarities, the Zandspruit site having the greatest similarity. Diamond Four showed the next highest similarity to Honingklip despite these two sites not being adjacent to each other. With regard to trees and shrubs, the Robindale site was most similar and Zandspruit the least.

Hemicryptophytes are the dominant life form both on and off serpentine (Fig. 4a). There were more cryptophytes, hemicryptophytes and chamaephytes off serpentine soils and more phanerophytes and therophytes on serpentine (Fig. 4b). Using a paired t -test, there is no significant difference between life forms on and off serpentine (Table 5) with the exception of the number of phanerophytes that occur on and off serpentine at the Zandspruit site where there are significantly more phanerophytes (trees and shrubs) on serpentine than off serpentine.

Fifty-six *bodenvag* taxa were identified (Appendix 1); of these, 24 were more common on serpentine, 30 were more common on the adjacent non-serpentine areas and 2 taxa were equally common on and off serpentine. Hemicryptophytes made up 80.36% of these *bodenvag* taxa. Of these 56 taxa, only *Nidorella hottentottica* was significantly more common on serpentine soils and *Hermannia depressa* was significantly more common off serpentine.

The *bodenvag* taxa that are more common on and more common off serpentine are from eight and 12 families, respectively. Only four families, Graminae, Asteraceae, Leguminosae and Liliaceae, are common

Table 4 (a) Sørensen's similarity indices between serpentine and non-serpentine plots, from the Barberton Greenstone Belt (BGB) and the Witwatersrand serpentine and non-serpentine plots (WWRS). (b) Comparison of average Sørensen's similarity values and beta-diversity within and between plots of four adjacent serpentine and non-serpentine sites on the

Witwatersrand Results of unpaired *t*-test to compare total similarity between serpentine and non-serpentine sites: $df = 10$, $t = 2.5$, $P = 0.03$ (significant) (c) Percentage Sørensen's similarity for different growth forms of each site with serpentine and non-serpentine plots

Site	BGB								WWRS		
	* RT	* SM	* KL	* GV	* MC	* MM	* CZ	+DV	RS	DF	ZS
Panel A											
% Similarity	46	44	43	40	39	36	31	31	70	69	49
Mean $\pm$ sd	38.75 $\pm$ 5.7								68.25 $\pm$ 11.87		
Site (<i>n</i>)	Similarity between serpentine plots Mean $\pm$ sd		Beta-diversity (1 – Q _s)		Similarity between non-serpentine plots Mean $\pm$ sd		Beta-diversity (1 – Q _s)				
Panel B											
RD (3)	0.42 $\pm$ 0.07		0.58		–						
RS (3)	0.45 $\pm$ 0.16		0.55		0.70 $\pm$ 0.02		0.3				
ZSs (2)/ZS ns (2)	0.58		0.42		0.49		0.51				
DFs (3)/HKns (3)	0.52 $\pm$ 0.08		0.48		0.69 $\pm$ 0.04		0.31				
DM (6)	0.40 $\pm$ 0.12		0.6		–						
DW (3)	0.51 $\pm$ 0.08		0.49		–						
EP (4)	0.31 $\pm$ 0.11		0.9		–						
ID (3)	0.43 $\pm$ 0.05		0.57		–						
WV (2)	0.58		0.42		–						
Mean (<i>n</i>) $\pm$ sd	0.47 (9) $\pm$ 0.09		0.53 $\pm$ 0.15		0.63 (3) $\pm$ 0.12		0.37 $\pm$ 0.22				
Site (<i>n</i>)	% Similarity of growth forms between serpentine and non-serpentine plots										
	Grasses		Forbs		Trees and Shrubs						
Panel C											
DF/HK (3)	80.9		63.9		23.5						
RD (3)	76		51.2		50						
RS (3)	79.2		63.4		27.3						
ZS (2)	88.5		64.5		10.8						

* = Williamson & Balkwill (unpublished data) and + = Changwe & Balkwill (2003). RT: Rosentuin, SM: Sawmill, KL: Kalkloof, GV: Groenvaly, MC: Mundt's Concession, MM: Magnesite, CZ: Core Zone, DV: Dunbar Valley, RS: Ruimsig, DF: Diamond Four, ZS: Zandspruit

DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP, Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit

to both. Thus there is only a 40% similarity (Sørensen's Index) between families more common on and more common off serpentine.

In total, 23 of the *bodenvag* taxa belong in the Graminae (12% of the Witwatersrand total for the family), nine (3.9% of the Witwatersrand total for the family) in the Asteraceae and four (3% of the Witwatersrand total for the family) in the

Leguminosae. There are three representatives each of the Liliaceae *s.l.* (4% of the Witwatersrand total for the family) and Rubiaceae (9% of the Witwatersrand total for the family) and two each from the Euphorbiaceae (7% of the Witwatersrand total for the family) and Acanthaceae (11% of the Witwatersrand total for the family). The remainder are single representatives from 10 other families.

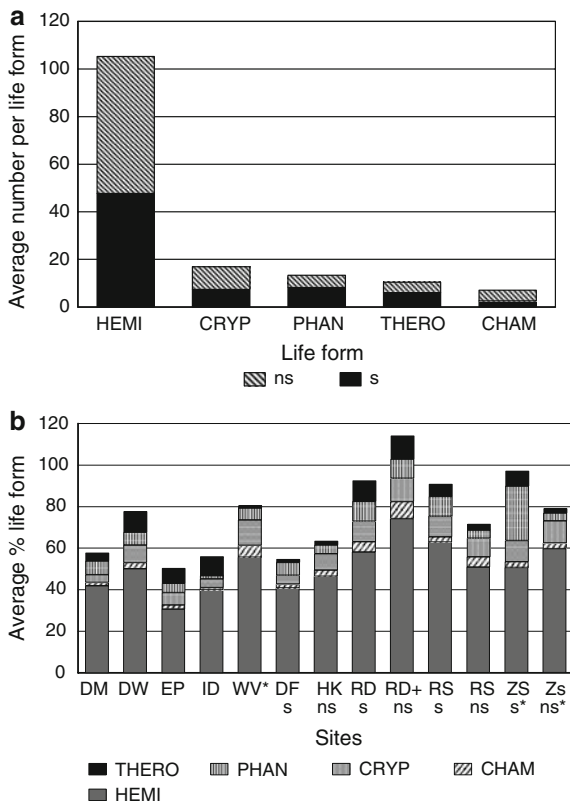


Fig. 4 (a) Average contribution of the various life forms on and off serpentine soils. HEMI = Hemicryptophyte, CHAM = Chamaephyte, CRYP = Cryptophyte, PHAN = Phanerophyte, THERO = Therophyte, s = serpentine, ns = non-serpentine. (b) Average percent of a particular Raunkiaer life form per 500 m²/1000 m² plots at each site. DF: Diamond Four, DM: Dombeya, DW: Darrenwood, EP: Estherpark, HK: Honingklip, ID: Illiondale, RD: Robindale, RS: Ruimsig, WV: Weltevreden, ZS: Zandspruit, s = serpentine, ns = non-serpentine. HEMI = Hemicryptophyte, CHAM = Chamaephyte, CRYP = Cryptophyte, PHAN = Phanerophyte, THERO = Therophyte. ! indicates half-sized, (500 m²) plots. RD⁺ ns = single plot

The Graminae are the best represented *bodenvag* taxa on and of the serpentine soils. There are more grasses common on serpentine (13) than off serpentine (11), but not significantly so.

Discussion

The grassland biome on the Witwatersrand, including the serpentine areas (Reddy et al. 2001), is under threat from urbanisation and development. The Zandspruit site currently has been fenced off and appears to be targeted for development, the

Estherpark and Darrenwood sites are now incorporated into office parks, and Ndabushe, once a private game reserve, is now being developed as a luxury housing estate; the koppies still remain, but are isolated from other natural habitats. Emma Park and Robindale are parks, but are home to vagrants. Diamond Four, a privately owned plot, and the Ruimsig Entomological Reserve appear to be safe, for now; however even the Ruimsig site is bordered by houses on two sides and by roads on the remaining two sides.

There is a significant, positive correlation between total Cr and species richness for the non-serpentine plots. Chromium concentrations in normal soils usually are in the order of 100 µg/g and range from 1000 to 25,000 µg/g on serpentiniferous soils, with an average of 5000 µg/g (Brooks 1987). So, although the concentration of chromium in the non-serpentine soils is higher than that given by Brooks (1987) for normal soils, it is still below toxic concentrations of chromium usually found in serpentine soils. Even the concentrations of chromium in the Witwatersrand serpentine soils, with an average of 1153 µg/g, are at the lower end of the range for chromium given by Brooks for serpentine soils (1987). The Cr concentrations of the Barberton Greenstone Belt serpentines range between 163 and 7329 µg/g (Balkwill et al. 1995).

However, even though chromium may be abundant in soils, it is not necessarily available to plants, as Cr forms highly insoluble complexes in the soil (Wild 1974b; Tilston and MacNair 2001). Tetyana (1998) found plants with between 50 and 10500 ppm of Cr in both above and below ground parts. It was also found that at high concentrations of chromium, the more toxic, less common, though more easily taken up Cr IV did inhibit growth (Tetyana 1998). As there is a higher than normal Cr concentration in both the serpentine and the adjacent non-serpentine areas, but no distinct floras, it is possible that the available Cr is in the less bioavailable form of Cr III. As Tetyana (1998) concluded, more work needs to be done in this area.

The positive correlation in the level of soluble N perhaps implies that there is sufficient soluble N to support that number of taxa. The converse is true for the non-serpentine plots. There is mainly a positive correlation between mean species richness and mean levels of soil chemicals.

Table 5 A comparison of life form classes between serpentine and adjacent non-serpentine sites, $n = 3$, $df = 2$ for HK/HN and RSs/RSns; $n = 2$, $df = 1$ for ZSs/ZSns

Raunkiaer life forms	Average occurrence											
	DF	HK	<i>t</i>	<i>P</i>	RS s	RS ns	<i>t</i>	<i>P</i>	ZS s	ZS ns	<i>t</i>	<i>P</i>
	Mean $\pm$ sd	Mean $\pm$ sd			Mean $\pm$ sd	Mean $\pm$ sd			Mean $\pm$ sd	Mean $\pm$ sd		
Hemicryptophytes	40.7 $\pm$ 8.6	46.3 $\pm$ 5.5	0.7	0.6	62.7 $\pm$ 13.3	50.7 $\pm$ 3.5	1.9	0.2	50.5 $\pm$ 13.4	59.5 $\pm$ 4.9	0.69	0.61
Chamaephytes	1.7 $\pm$ 0.6	2.7 $\pm$ 1.5	0.9	0.5	2.3 $\pm$ 0.6	4.7 $\pm$ 2.1	1.8	0.2	2.5 $\pm$ 2.1	2.5 $\pm$ 0.7	0	1
Cryptophytes	4.0 $\pm$ 2.6	7.7 $\pm$ 0.6	2.5	0.1	9.7 $\pm$ 5.5	8.7 $\pm$ 0.6	0.3	0.8	10.0 $\pm$ 1.4	10.5 $\pm$ 6.4	0.1	0.94
Phanerophytes	6.0 $\pm$ 4.4	4.0 $\pm$ 1	0.7	0.6	9.3 $\pm$ 4.9	3.7 $\pm$ 1.2	2.6	0.1	26.0 $\pm$ 2.8	3.5 $\pm$ 0.7	15	0.04*
Therophytes	1.3 $\pm$ 0.6	1.7 $\pm$ 0.6	1	0.4	5.7 $\pm$ 4.5	2.7 $\pm$ 1.5	0.9	0.5	7.0 $\pm$ 1.4	2.0 $\pm$ 1.4	2.5	0.24
Total taxa	53.7 $\pm$ 11.6	62.3 $\pm$ 7.2	0.8	0.5	89.7 $\pm$ 10.1	70.3 $\pm$ 1.5	3.5	0	96.0 $\pm$ 14.1	78.0 $\pm$ 9.9	1.05	0.48

DF: Diamond Four, HK: Honingklip, RS: Ruimsig, ZS: Zandspruit. s = serpentine, ns = non-serpentine. * = significant

Nickel toxicity is cited as one of the main reasons for the differences in species composition and richness between serpentine and non-serpentine soils (Wild 1974a; Morrey et al. 1989; Robertson 1992). While the soluble Ni concentrations of the serpentine soils are higher than the non-serpentine soils on the Witwatersrand (Table 1a), it is considerably lower than the serpentine soils of the Barberton Greenstone Belt where the Ni concentrations range from 85 to 314 $\mu\text{g/g}$ (Balkwill et al. 1995).

Reddy et al. (2001) reported that the concentration of total chromium was 12 times greater and that of nickel 1.5 times greater in the Barberton Greenstone Belt serpentine soils than in the Witwatersrand serpentine soils, thus indicating that the serpentine soils on the Witwatersrand are not as toxic as that of the Barberton Greenstone Belt serpentine soils.

The alpha-diversity of southern African vegetation does not differ greatly from equivalent biomes elsewhere at 1000 m^2 (Cowling et al. 1989). By global alpha-diversity standards 80–100 species per 1000 m^2 indicates a rich flora (Whittaker et al. 1984; Scholes and Walker 1993; Dögeloh 1999). South African grassland biomes have a higher average richness than Fynbos (Cowling et al. 1989). Species richness in the Cape Floristic Region ranges from 40 to 85 per 1000 m^2 (Goldblatt and Manning 2002); in the lowveld savanna Bushbuckridge area Shackleton (2000) counted 68 taxa per 1000 m^2 on conserved lands and 76 taxa per 1000 m^2 on communal lands; in the Barberton Greenstone Belt region, Changwe and Balkwill (2003) counted 31–84 taxa per 1000 m^2 on non-serpentine soils and 29–83 taxa per 1000 m^2 on serpentine soils. Cowling et al. (1989) found the South African grassland biome to contain between 58 and 98

taxa with an average of 82 taxa per 1000 m^2 . On the Witwatersrand, the serpentine and adjacent non-serpentine plots that were within the global range of a rich flora were: the serpentine soil plots at Weltevreden (100 taxa—extrapolated), Robindale (93), Ruimsig (90) and Zandspruit (133—extrapolated). The only non-serpentine site within the globally species rich range was the Zandspruit site with 89 taxa (extrapolated). In contrast the California chaparral only had 34 species per 1000 m^2 whilst in Western Australia plots were found to have only 69 species for the same area (Goldblatt and Manning 2002). Thus several serpentine sites on the Witwatersrand have rich floras according to global standards.

More trees and shrubs occur in the serpentine plots and the serpentine areas as a whole because these areas are rocky and provide some protection from fires which occur frequently in this grassland biome. The high numbers of herbs in common implies that there is very little difference between the serpentine and adjacent non-serpentine areas. A greater tree/shrub cover does not imply a lower forb/grass cover (Stohlgren et al. 1999). This is true for the Witwatersrand serpentine areas where there are conspicuous woody elements due to the rockiness of these areas (mean number of rocks/ m^2 of serpentine soil = 14; mean number of rocks/ m^2 of adjacent non-serpentine soil = 2; $n = 13$), but the number of forb and grass taxa occurring in these areas is still high.

The tree and shrub cover is very dense on some serpentine areas at the Zandspruit site. The second serpentine plot was on one of these areas; thus shading effects could account for the low cover. Abundance of herbaceous plants tends to be associated with high soil fertility (Rothstein and Zak 2001).

Lower cover at Honingklip might have been because the non-serpentine site had been more recently burnt than the serpentine area and so many of the resprouters had not yet begun to grow or reached their full extent.

Non-serpentine plots are more similar to each other than to the serpentine plots. This could be because woody vegetation occurs in the rockier serpentine plots. There is very little turnover (beta-diversity) between both serpentine and non-serpentine sites. This is either because the serpentine soils are only mildly toxic or because the harsh climatic factors on the Witwatersrand override the serpentine condition resulting in a uniform Highveld grassland vegetation. In addition, if environmental gradients are gradual, then turnover (beta-diversity) with distance is relatively low (Scholes 1997).

Green plants tend to evolve towards a scattered arrangement of distribution within habitats or geographical areas in order to escape intraspecific competition at a given locality, as long as the species are located within reasonable dispersal distances (Whittaker 1965; Grace 2001). Stohlgren et al. (1999) found that the species composition overlap between each 1000 m² study plot significantly decreased with increasing distance apart. There is a closer similarity between non-serpentine areas than between serpentine areas (Fig. 3). This indicates that the serpentine patches are, to some extent, more isolated from one another than are the adjacent non-serpentine areas. A comparison of the total Witwatersrand serpentine flora with that of the total Barberton Greenstone Belt serpentine flora, using the Sørensen's Similarity index, indicated only a 34% similarity between these two serpentine areas.

Hemicryptophytes are the dominant life form among the grassland herbs and grasses. Herbaceous taxa flush during early spring (pre-rain flora) or summer. This life strategy ensures survival not only during fires but during the harsh winters when there is bright sunshine but very little rainfall. In grasslands, moisture (due to erratic rainfall), soil properties and temperature are responsible for variations in floristic composition, functional attributes of species, vegetation dynamics and ecosystem functioning (Roux 1969; O'Connor and Bredenkamp 1997; Ellery et al. 1991). According to Roux (1969) water is stored in underground structures during the rainy season. The rise in temperature after winter stimulates flowering utilising

the water and nutrients that were stored the previous season. Above ground dieback, together with burning, facilitates long-term maintenance of high levels of species richness (Grace 2001). This is because dieback opens up spaces and reduces shading and soil insulation (Dell et al. 2005), thus allowing for seed recruitment and the flushing of hemicryptophytes. Selection for hemicryptophytes in a fire prone environment reduces the probability of population fragmentation and this also reduces the rate of turnover (Cowling et al. 1989). Thus the predominance of phanerophytes and cryptophytes on the serpentine areas is likely to be because the Highveld grassland is a fire driven ecosystem and these rocky outcrops provide protection from fires. A greater number of therophytes, most of which are weeds, occur on serpentine soils because the phanerophytes provide a shady environment unsuitable for most species indigenous to the grassland habitat and these pioneer species are therefore able to exploit the shady niche.

Grasses are dominant both on and off serpentine on the Witwatersrand, but only a few of the 62 taxa that occur on and off serpentine occur per plot. This means that richness and diversity is due to the non-grass species, especially the hemicryptophytes. Growth and flowering of grasses peak in autumn, and during winter the tall, dried grass stalks provide a microclimate that protects the hemicryptophytic herbs from frost and low temperatures provided that there is no fire.

Humans have played a major role in the fragmentation of natural habitats. South African grasslands are facing their biggest threat as the human population grows and the demand for housing increases. Currently 40% of the grassland biome has been irreversibly transformed (O'Connor and Kuyler 2005). Even the serpentine areas, rocky and with shallow soils, are not exempt. According to Leps (2004), many plant sub-populations depend on a constant influx of diaspora from their surroundings. With the serpentine sites and indeed the grasslands becoming so fragmented, this influx is constantly being hampered and could lead to losses in species diversity.

We predicted that the lower levels of chrome and nickel and the lower magnesium to calcium ratio of serpentine sites on the Witwatersrand would have led to a higher number of tolerant species. As there is no significant difference in diversity between serpentine

soils and adjacent non-serpentine soils on the Witwatersrand and there are no serpentine endemic taxa, we conclude that on the Witwatersrand, soil factors do not significantly influence diversity.

There is a greater similarity between the floras of the serpentine and non-serpentine soils on the Witwatersrand than the similarity between the serpentine floras of the Witwatersrand and the Barberton Greenstone Belt. The serpentine soils on the Witwatersrand are not as toxic as those of the Barberton Greenstone Belt area. So although there is no distinct serpentine flora on the Witwatersrand, there do appear to be distinct serpentine communities.

In addition, the extreme seasonal variations in temperature and the availability of water on the Highveld could possibly override the effects of edaphic factors of the Witwatersrand serpentine areas (Kerfoot 1987). As a result there is no significant difference in species richness or diversity on and off serpentine soils on the Witwatersrand.

Environmental factors and or climate are the reasons most often cited for high species diversity (Cowling et al. 1989; O'Connor and Bredenkamp 1997) in a region. For serpentine soils, edaphic factors, usually high concentrations of toxic metals in the soils, are the reasons given for a high level of floral diversity and endemism. The average concentration of total Fe in normal soils is 38,000 µg/g (Kamprath 2000). This is similar to the concentrations found in the non-serpentine soils. The total Fe concentration of the serpentine soils, however, is only slightly higher than the non-serpentine soils.

Climatic factors, e.g. rainfall, frost and temperature, together with fire are the reasons cited for the formation of grasslands (Ellery et al. 1991; Stock

et al. 1997; O'Connor and Bredenkamp 1997). Regarding the Witwatersrand serpentine soils, the high, but similar, diversity on serpentine and non-serpentine soils and the predominance of hemicryptophytes indicates that climate, rather than soil factors, is the main determinant of species diversity.

Conclusions

The lack of any significant difference in species richness and diversity between the serpentine and non-serpentine sites on the Witwatersrand indicates that soil factors do not significantly influence diversity. Despite this, there does appear to be a distinct serpentine community. This is possibly because the rocky serpentine ridges at the study sites, like most rocky ridges on the Witwatersrand, provide fire protection for phanerophytes. The similarities in species richness and diversity between the serpentine and non-serpentine areas on the Witwatersrand, together with the predominance of hemicryptophytes, would indicate that climate, rather than soil factors, is the determining factor in species diversity on the Witwatersrand.

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Appendix 1 Likely *bodenvag* taxa: Life forms and whether taxa are equally common or more common on/off serpentine and their levels of significance. Ch = Chamaephyte, C =

Cryptophyte, H = Hemicryptophyte, P = Phanerophyte, T = Therophyte, s = serpentine, ns = non-serpentine. 18df, $n = 20$, * = significant.

	Taxon	Life form	s mean $\pm$ sd	ns mean $\pm$ sd	<i>t</i>	<i>P</i>
More common on serpentine	<i>Indigofera hedyantha</i> Eckl. & Zeyh.	P	3.4 $\pm$ 5.7	0.4 $\pm$ 1.3	1.5	0.15
	<i>Phyllanthus maderasparensis</i> L.	H	1.2 $\pm$ 0.9	0.3 $\pm$ 1.0	0.9	0.41
	<i>Nidorella hottentotica</i> DC.	H	0.8 $\pm$ 0.6	4.9 $\pm$ 5.7	2.7	0.01*
	<i>Bewsia biflora</i> (Hack.) Goossens	H	3.6 $\pm$ 6.0	0.6 $\pm$ 1.1	1.5	0.16
	<i>Tephrosia capensis</i> (Jacq.) Pers. var. <i>capensis</i>	H	4.2 $\pm$ 7.9	0.6 $\pm$ 1.7	1.3	0.2
	<i>Ipomoea crassipes</i> Hook.	H	0.1 $\pm$ 0.3	0.6 $\pm$ 0.9	1.6	0.12

Appendix 1 continued

	Taxon	Life form	s mean $\pm$ sd	ns mean $\pm$ sd	<i>t</i>	<i>P</i>
	<i>Melinus nerviglumis</i> (Franch.) Zizka	H	29.9 $\pm$ 32.3	12.3 $\pm$ 15.4	1.5	0.15
	<i>Hypoxis rigidula</i> Bak. var. <i>pilosissima</i> Bak.	C	0.8 $\pm$ 1.5	1.0 $\pm$ 2.0	0.2	0.82
	<i>Aster harveyanus</i> Kuntze	H	1.7 $\pm$ 3.3	0.3 $\pm$ 0.7	1.3	0.23
	<i>Gazania krebsiana</i> Less. ssp. <i>serrulata</i> (DC.) Roessl.	H	0.3 $\pm$ 0.7	1.1 $\pm$ 1.6	1.6	0.13
	<i>Eragrostis chloromelas</i> Steud.	H	10.4 $\pm$ 22.1	2.8 $\pm$ 4.2	1	0.33
	<i>Cymbopogon excavatus</i> (Hochst.) Stapf ex Burt Davy	H	10.5 $\pm$ 25.6	11.9 $\pm$ 6.4	0.2	0.87
	<i>Andropogon schirensis</i> A. Rich.	H	12.2 $\pm$ 22.0	5.3 $\pm$ 8.7	0.9	0.39
	<i>Eragrostis curvula</i> (Schrud.) Nees	H	3.2 $\pm$ 7.4	1.2 $\pm$ 2.7	0.8	0.46
	<i>Elionurus muticus</i> (Spreng.) Kunth	H	17.9 $\pm$ 22.9	15.7 $\pm$ 11.9	0.3	0.79
	<i>Commelina africana</i> L. var. <i>lancispatha</i> C-B. Cl.	H	4.1 $\pm$ 7.1	2.1 $\pm$ 6.3	0.7	0.52
	<i>Acalypha peduncularis</i> E. Mey. ex Meisn.	H	11.1 $\pm$ 24.5	5.8 $\pm$ 9.0	0.6	0.55
	<i>Melinus repens</i> (Willd.) Zizka ssp. <i>repens</i>	T	4.6 $\pm$ 7.9	1.4 $\pm$ 3.0	1.1	0.28
	<i>Diheteropogon amplexans</i> (Nees) Clayton	H	10.6 $\pm$ 14.6	7.4 $\pm$ 7.8	0.6	0.56
	<i>Schizachyrium sanguineum</i> (Retz.) Alst.	H	5.9 $\pm$ 12.8	6.0 $\pm$ 15.1	0	0.99
	<i>Asparagus saueolens</i> Burch.	P	3.2 $\pm$ 6.2	1.1 $\pm$ 2.0	0.98	0.35
	<i>Cynodon dactylon</i> (L.) Pers.	Ch	2.6 $\pm$ 5.5	1.8 $\pm$ 2.9	0.4	0.68
	<i>Chlorophytum fasciculatum</i> (Bak.) Kativu	C	0.6 $\pm$ 0.8	0.4 $\pm$ 0.7	0.3	0.78
	<i>Setaria sphacelata</i> (Schumach.) Moss var. <i>sphacelata</i>	H	13.4 $\pm$ 19.2	12.1 $\pm$ 15.1	0.2	0.87
Equal	<i>Asclepias adscendens</i> (Schltr.) Schltr.	H	0.3 $\pm$ 0.7	0.3 $\pm$ 1.0	0.2	0.87
	<i>Chaetacanthus costatus</i> Nees	H	3.8 $\pm$ 4.2	4.1 $\pm$ 5.4	0.14	0.89
More common off serpentine	<i>Hermannia depressa</i> N.E. Br.	H	0.7 $\pm$ 1.7	8.7 $\pm$ 11.1	−2	0.02*
	<i>Senecio venosus</i> Harv.	H	1.0 $\pm$ 2.0	4.4 $\pm$ 5.1	−2.1	0.05
	<i>Kohautia amatymbica</i> Eckl. & Zeyh.	H	0.8 $\pm$ 1.8	1.7 $\pm$ 0.9	0	0.92
	<i>Eragrostis racemosa</i> (Thunb.) Steud.	H	1.6 $\pm$ 2.3	15.6 $\pm$ 27.2	−2	0.1
	<i>Helichrysum nudifolium</i> (L.) Less.	H	0.4 $\pm$ 0.8	3.4 $\pm$ 6.9	−2	0.2
	<i>Scabiosa columbaria</i> L.	H	0.6 $\pm$ 1.8	3.3 $\pm$ 5.8	−2	0.15
	<i>Thesium exile</i> N.E. Br.	H	0.6 $\pm$ 1.2	1.9 $\pm$ 3.8	−1	0.28
	<i>Ziziphus zeyheriana</i> Sond.	P	0.6 $\pm$ 1.5	5.3 $\pm$ 11.2	−1	0.17
	<i>Helichrysum coriaceum</i> Harv.	H	0.6 $\pm$ 1.0	1.7 $\pm$ 2.2	−2	0.15
	<i>Anthospermum rigidum</i> Eckl. & Zeyh. ssp. <i>pumilum</i> (Sond.) Puff	H	1.1 $\pm$ 2.2	2.2 $\pm$ 2.4	−1.4	0.17
	<i>Brachiaria serrata</i> (Thunb.) Stapf	H	10.9 $\pm$ 9.9	17.3 $\pm$ 13.1	−1	0.23
	<i>Panicum natalense</i> Hochst.	H	1.2 $\pm$ 1.8	2.7 $\pm$ 6.6	−1	0.48
	<i>Trachypogon spicatus</i> (L.f.) Kuntze	H	2.5 $\pm$ 6.4	6.0 $\pm$ 9.8	−1	0.34
	<i>Vernonia oligocephala</i> (DC.) Sch. Bip. ex Walp.	H	2.7 $\pm$ 3.6	4.0 $\pm$ 4.4	−1	0.48
	<i>Sonchus dregeanus</i> DC.	T	0.4 $\pm$ 0.9	1.8 $\pm$ 5.0	−1	0.36
	<i>Hyparrhenia hirta</i> (L.) Stapf	H	13.6 $\pm$ 15.1	21.3 $\pm$ 33.2	−1	0.49
	<i>Themeda triandra</i> Forssk.	H	37.8 $\pm$ 75.0	58.0 $\pm$ 27.2	−1	0.45
	<i>Pearsonia sessilifolia</i> (Harv.) Dümmer ssp. <i>sessilifolia</i>	H	0.5 $\pm$ 1.5	1.1 $\pm$ 2.0	−1	0.48
	<i>Crabbea angustifolia</i> Nees	H	0.4 $\pm$ 0.9	0.6 $\pm$ 1.1	0	0.68
	<i>Urelytrum agropyroides</i> (Hack.) Hack.	H	5.1 $\pm$ 9.8	7.6 $\pm$ 18.7	0	0.71

Appendix 1 continued

Taxon	Life form	s mean $\pm$ sd	ns mean $\pm$ sd	<i>t</i>	<i>P</i>
<i>Rhynchosia totta</i> (Thunb.) DC. var. <i>totta</i>	H	0.6 $\pm$ 1.2	0.8 $\pm$ 1.4	−0.4	0.69
<i>Pentanisia angustifolia</i> (Hochst.) Hochst.	H	3.0 $\pm$ 6.4	3.3 $\pm$ 3.2	0	0.89
<i>Heteropogon contortus</i> (L.) Roem. & Schult.	H	9.9 $\pm$ 12.5	13.0 $\pm$ 17.2	−1	0.65
<i>Setaria nigrirostris</i> (Nees) Dur. & Schinz	H	25.2 $\pm$ 69.1	39.6 $\pm$ 70.2	−1	0.65
<i>Ocimum obovatum</i> E. Mey. ex Benth. ssp. <i>obovatum</i> var. <i>obovatum</i>	H	1.8 $\pm$ 3.4	2.3 $\pm$ 2.3	0	0.7
<i>Tristachya leucothrix</i> Nees	H	3.2 $\pm$ 10.6	6.0 $\pm$ 9.8	−1	0.55
<i>Gerbera viridifolia</i> (DC.) Sch. Bip. ssp. <i>viridifolia</i>	H	1.1 $\pm$ 1.6	1.3 $\pm$ 1.7	0	0.74
<i>Aristida congesta</i> Roem. & Schult. ssp. <i>barbicollis</i> (Trin. & Rupr.) De Winter	H	1.1 $\pm$ 1.9	6.2 $\pm$ 14.3	−1	0.25
<i>Ledebouria marginata</i> (Bak.) Jessop	C	0.6 $\pm$ 1.8	0.7 $\pm$ 1.1	0	1

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Chapter 4: Are selected plant taxa differentiating into serpentine soil ecotypes on the Witwatersrand?

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Are selected plant taxa differentiating into serpentine soil ecotypes on the Witwatersrand?

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Abstract:

Serpentine soils are very diverse due to factors such as toxicity of parent material, climate, relief, and biological activity resulting in a harsh environment. This is likely to result in a unique, usually endemic, flora. However, not all plants growing on serpentine soils are endemic; some plants appear to be indifferent (bodenvag) to the serpentine condition. It is likely that these apparently bodenvag serpentine taxa may be ecotypically differentiated into serpentine tolerant and intolerant races whilst appearing morphologically indistinguishable from each other. This study investigated whether aspects of life history, such as biomass, productivity and life forms as well as breeding system characteristics, such as pollen viability, pollen/ovule ratios, pollinator exclusions and pollination syndromes, could indicate ecotype formation in selected, apparently bodenvag, serpentine taxa on the Witwatersrand. Multivariate analyses, namely correspondence analysis (CA) and principal component analysis (PCA), were used to analyse the data. The results indicate that reproductive and life history data do not separate the study taxa into ecotypes. This study concludes that the relatively mild serpentine condition on the Witwatersrand does not exert a strong influence on plants and that there is still likely to be gene flow amongst the study taxa growing on and off serpentine soils.

Keywords: serpentine soils, life history, reproductive strategies, ecotypes, *bodenvag* taxa, multivariate analyses.

Introduction:

Geology exerts a selective force on plant life in diverse ways (Kruckeberg, 1986). Serpentine outcrops tend to have insular distributions. This distribution exerts a selective force which may either promote plant diversity by enhancing opportunities for speciation or reduce diversity by increasing rates of extinction (Kruckeberg, 1986; Harrison *et al.*, 2000).

Serpentine soils are very varied due to diversity in factors such as chemical composition of parent material, climate, relief, and biological activity (Brooks, 1987). These soils generally have high concentrations of Ni, Cr, Co and Fe, high Mg:Ca ratios and low concentrations of N, P and K. This results in harsh environmental conditions (Brooks, 1987). The degree of tolerance required for plants to survive correlates with the amount of metal in the soil (Westerbergh, 1994). In addition to the chemical composition of the parent material, the length of exposure of the outcrop will determine how plant taxa behave on these soils. The striking edaphic differences between serpentine and non serpentine soils, and the marked biological discontinuity in areas where they abut, suggest strong selective pressures imposed on plants by serpentine soils (Springer, 2006).

These harsh environmental conditions, as well as strong selective pressures, are likely to result in a low diversity of plant species and a unique, usually endemic, flora (Kruckeberg, 1954; Proctor *et al.*, 1980; Kruckeberg, 1991). The distribution patterns of many of these endemic species effectively demarcate the often sharp boundaries of insular serpentine patches (Springer, 2006).

Not all plants growing on serpentine soils are endemic. Plants that are good colonisers and good persisters tend to show limited island effects and endemism (Bond, 1991). These plants appear to be tolerant of the serpentine condition while others appear to be *bodenvag* i.e. taxa that are indifferent to edaphic conditions (Kruckeberg, 1984). Divergence between taxa growing on and off serpentine soils could either be abrupt or gradual. Initially gene flow may still occur, but divergence in structural and functional traits may result in ecotypic differentiation (Kruckeberg, 1984). This may be followed by isolation between the two populations and may result in two morphologically and or genetically distinct populations (Kruckeberg, 1984; 1991) that are either endemic to or excluded from the serpentine soils. Williamson *et al.* (1997) investigated modes of evolution in *Berkheya rehmannii* Thell. var. *rehmannii*, a taxon excluded from serpentine soils, and *Berkheya rehmannii* Thell. var. *rogersiana* Thell., a serpentine endemic taxon and concluded that these taxa were genetically distinct and could be a progenitor-derivative pair.

It is likely that some apparently *bodenvag* serpentine taxa may be ecotypically differentiated into serpentine tolerant and serpentine intolerant races whilst appearing morphologically indistinguishable. Ecotypic differentiation has been demonstrated in serpentine taxa (Kruckeberg, 1984) and is most clearly seen in herbaceous perennials (Kruckeberg, 1991; Linhart & Grant, 1996). On the Californian serpentine soils, common indifferent taxa occur amongst the Compositae, Gramineae and weedy, introduced species (Kruckeberg, 1984).

Taxa can differ widely in their apparent habitat “preferences” their vulnerability to competition and other threats, and fundamental aspects of their life histories (Farnsworth, 2007); and life histories are subject to local selection pressures (Linhart & Grant, 1996). Key life-history traits such as breeding system, reproductive allocation and phenology can vary in response to the environment (Sultan, 2000).

Breeding systems, an integral part of life history, are important because they have an impact on gene flow and population divergence (Linhart & Grant, 1996). Plant breeding systems are very variable, ranging from apomixis through selfing and out-crossing to dioecy; these traits are sometimes facultative. Asexual reproduction is advantageous in stable environments to which a species is already well adapted, while production of seedlings by sexual reproduction is advantageous in relation to unstable or changing environments (Grant, 1971; Menken *et al.*, 1995).

Whether a species evolves as a cohesive unit depends critically on the dynamic balance between homogenizing gene flow among populations and potentially disruptive local adaptation (Sambatti & Rice, 2006). In both abiotic and biotic pollination, pollen dispersal distances as well as seed dispersal distances play important roles in reproductive success and gene flow distances.

Pollen is often the major contributor to gene flow (Broyles *et al.*, 1994; Boshier *et al.*, 1995). The mating systems of hermaphroditic plant populations are either obligately inbreeding (autogamous), obligately outcrossing (xenogamous) or intermediate (facultative) (Charlesworth, 2006). Generally outcrossing plants tend to produce more pollen grains per anther than do inbreeding ones. Cruden (1977) proposed that pollen/ovule (P/O) ratios gave a conservative indication of the breeding system. P/O ratios increase with the likelihood of cross pollination, thus the more efficient the transfer of pollen, the lower the P/O ratio (Cruden, 1977).

Pollination is an important ecological process for vegetation communities since it can directly affect the reproductive success of plants (Wunderlee, 1997).

Pollination usually involves abiotic (water or wind) or biotic (animal) vectors ranging from non-specialist insects to animals strictly dependent on flowers for their survival, such as bees, birds, and bats (Proctor *et al.*, 1996). For plants that

rely on biotic pollination, the ability to attract biotic vectors to their flowers can be an important component of fitness.

Flowers signal pollinators by colour, shape, size, and fragrance and variation in these characteristics may have consequences on the visitation rates and in turn, on plant reproductive success (Aragón & Ackerman, 2004). Flower colour is thus an important component of pollination syndromes, which assume that certain colour preferences are specific or innate to a pollinator or a guild of pollinators (Faegri & van der Pijl, 1979).

Plant dispersal is generally achieved through seeds (Vittoz & Engler, 2007). Long seed dispersal distances also ensure that gene flow occurs over great distances. The importance of seed dispersal depends on the characteristics of the seeds and fruit as well as the characteristics of the dispersers (Boshier *et al.*, 1995). While many seeds or fruits have adaptations that promote dispersal by wind or animals, the majority of seeds do not have obvious adaptations for dispersal (Fenner & Thompson, 2005). Bullock and Clarke (2000) recorded a dispersal distance of 80 m for the wind dispersed seeds of *Calluna vulgaris* and *Erica cinerea* while Narbona *et al.* (2005) found that two autochorous species of *Euphorbia* ranged between 130 cm and 8.0 m. Experimental projections of ballistically released seeds of *Vicia sativa* had a range of 0.2 – 9.0 m, with a mean of 3.4 m (Garrison *et al.*, 2000). Propagules that attract specialist dispersers are more likely to be effectively dispersed (Larson, 1996). Ectozoochory has the potential to disperse these propagules between 100 – 1000 m, depending on the size of the disperser (Fenner & Thompson, 2005; Corlett, 2009).

The Pine Hill area of California consists of gabbrodiorite rather than serpentinite (Wilson *et al.*, 2009). Despite the low concentrations of toxic metals such as chromium, cobalt and nickel, this area was rich in endemic species (Hunter & Horenstein, 1992). However while location, topography and substratum all

contribute to endemism, areas of higher elevations usually have few or no endemics (Hunter & Horenstein, 1992; Harrison *et al.*, 2004).

The serpentine soils on the Witwatersrand are not as toxic as the serpentine soils of other southern African ultramafic areas and occur at a higher elevation than the Barberton Greenstone Belt and Swaziland serpentine areas (Reddy *et al.*, 2001). There are no serpentine endemics on the Witwatersrand and all the taxa that occur on these serpentine soils appear to be indifferent (*bodenvag*) to the serpentine condition (Reddy *et al.*, 2001). Very little previous research has been conducted on *bodenvag* taxa on serpentine soils and most studies on ecotype formation are based on reciprocal transplant experiments in greenhouses rather than *in situ*.

This study investigates whether there are any differences in the life history and breeding system characteristics of selected *bodenvag* taxa on and off serpentine soils on the Witwatersrand which could give an indication whether gene flow is being interrupted in any way. Comparison of plants growing in different edaphic environments is useful for detecting commonalities and differences among these taxa. This enables the taxa to be categorized into functional groups on the basis of shared traits and responses (Farnsworth, 2007). To this end, multivariate analyses were used to determine whether the serpentine and non serpentine populations of the selected taxa separated in multidimensional space for each distribution category of species. Should there be differences in any of the study taxa, then this could be an indication that ecotype formation is occurring for that particular taxon.

Materials and Methods:

Study sites:

Data from four serpentine sites were collected during the growing seasons of September 2002 - June 2003. Three sites, Robindale (26° 06' S 27° 59' E, 1620 m), Ruimsig (26° 04' S 27° 51' E, 1550 m) and Zandspruit (26° 02' S 27° 55' E,

1550 m) have adjacent non serpentine soils that were used for comparison. At the fourth site, a distance of 4.7 km separated the Diamond Four site (26° 01' S 27° 48' E, 1520 m) from its comparative non serpentine site at Honingklip (26° 01' S 27° 44' E, 1540 m). The Diamond Four site was also included, despite being distant from its non serpentine site, because it had been found to be one of the most diverse of the sites investigated (Reddy *et al.*, 2009). The Honingklip site was selected because it was the closest, relatively undisturbed, non serpentine (quartzite shale) site to Diamond Four. These sites have been previously described by Reddy *et al.* (2001).

Study taxa :

Students t-tests were used to identify six taxa in each of three categories, namely, taxa most common on serpentine, taxa most common off serpentine and taxa equally common on and off the serpentine soils (Reddy *et al.*, 2009). These taxa are common elements of the Witwatersrand flora. The taxa investigated were:

1. **Most common on serpentine** – *Bewsia biflora* (Hack.) Gooss., *Indigofera hedyantha* Eckl. & Zeyh., *Ipomoea crassipes* Hook., *Nidorella hottentotta* DC., *Phyllanthus maderaspatensis* L. and *Tephrosia capensis* (Jacq.) Pers. var. *capensis*
2. **Most common off serpentine** – *Bulbostylis oritrephes* (Ridl.) C.B. Clarke, *Eragrostis racemosa* (Thunb.) Steud., *Helichrysum nudifolium* (L.) Less. var. *nudifolium*, *Kohautia amatymbica* Eckl. & Zeyh., *Ledebouria marginata* (Baker) Jessop, and *Scabiosa columbaria* L.
3. **Equally common on and off serpentine**
In the category of the taxa equally common on and off serpentine soils, only *Asclepias adscendens* (Schltr.) Schltr. and *Chaetacanthus costatus* Nees appeared to be equally common on and off serpentine soils. In order to have six taxa for this category, two taxa each, with the lowest t-test values, from both the more common on serpentine soil category (*Chlorophytum fasciculatum* (Baker) Kativu and *Setaria sphacelata*

(Schumach.) Stapf & C.E.Hubb. ex M.B.Moss var. *sphacelata*) and the more common off serpentine soil category (*Hermannia depressa* N.E. Br. and *Senecio venosus* Harv.) were added to the equally common category (Reddy *et al.*, 2009).

For these 18 taxa growing on and off serpentine soils, aspects of biomass, productivity and reproduction were investigated. Data, for each character selected, for each study taxon, were combined from all four serpentine sites as were the data from the non serpentine sites. This was done in order to have sufficient data to conduct statistical tests.

Biomass and productivity

Aerial parts were collected into brown paper bags and air dried. The leaves, stems, stem bases and inflorescences were each weighed separately to determine biomass. The number of flowering shoots, number of branches from base, number of heads per plant and the number of spikes per rachis or flowers per head were counted, separately, in order to determine productivity.

Reproductive attributes and life forms

The percentage pollen viability was determined where possible. Anthers of recently opened flowers were squashed, the pollen stained with Alexander Stain (Alexander, 1969) and the viable (red) and inviable (green) pollen grains counted.

Where possible, potential pollinator activity was observed and pollinators were collected at the various sites during October and November 2000. Pollinators were observed visiting flowers or flower heads. Visiting insects were caught, killed in jars containing fumes of ethyl acetate, identified and examined for pollen.

Unopened buds were covered with mesh bags and left to mature to test for potential autogamy where possible. These bags were opened once mature fruit

formation was observed in uncovered plants. Calipers were then used to measure the length and width of the fruit. Where possible, the fruit length:width ratio was calculated for bagged and unbagged fruit. A greater length to width ratio in the bagged inflorescences compared to the unbagged inflorescences would imply that in the absence of pollinators, the seeds of the fruit that formed were likely to be less viable.

In order to determine the P/O ratio, unopened buds were preserved in 70% ethanol and studied at a later date. The ovaries were dissected and the number of ovules per ovary counted. Pollen from a single anther of each flower was placed in a vial with 40 μ l of a mixture of wetting agent and 70% ethanol then vortexed for 30 sec to scatter the grains (adapted from Harder, 1990). Five 8 μ l drops per specimen were placed on a clean microscope slide, allowed to air dry and then counted, using the Simple PCI ver. 4.0 (2001) computer programme. The P/O ratio was then calculated. In cases where there was a discrepancy between the predicted breeding system using the P/O ratios and the outcome of the bagging of flowers to determine fruit set, the Outcrossing Index (OCI) of Cruden (1977) was used to try and resolve the differences.

The data collected were then used to make assessments of the breeding systems of these taxa based on floral morphology (Ornduff, 1969) and by the use of Cruden's (1977) P/O ratios (Table 1). Flower and fruiting times were obtained from the literature, herbarium specimens and personal observations. Flower shape was classified according to Faegri & van der Pijl (1979). Reddy *et al.* (2009) had determined the life forms of the taxa selected for study using the classification of Raunkiaer (1934).

Table 1. A summary of the range of pollen/ovule ratios that define potential breeding systems (Cruden, 1977).

Breeding System	Range of mean Pollen/Ovule ratios
Cleistogamy (C)	$\leq 5:1$
Obligate Autogamy (OA)	30:1
Facultative Autogamy (FA)	180:1
Facultative Xenogamy (FX)	800:1
Obligate Xenogamy (X)	5000:1

Multivariate Analysis

Grouping of the taxa was carried out by CA and PCA performed on the distance matrix (Ds) using the programme NTSYSpC 2.02 (Rolf, 1997). The modules “SAHN”, using the UPGMA algorithm, were used to plot the phenograms for the cluster analyses. The cophenetic correlation coefficient (r) was calculated to determine how well the phenograms fit to the data. The modules “Dcenter” and “Eigen” were used for the PCA. A model distance matrix, generated from the eigenvector matrix (using “SIMINT”), was compared to the original Ds matrix (using “Mxcomp”) to measure the goodness of fit of the PCA.

Various permutations of characters and taxa groups were tested for goodness of fit. In all cases the clusters had strong “r” values. The cluster with the best fit (highest “r” value) was for the combination of the serpentine taxa growing on serpentine soils (excluding the data for these taxa from non serpentine soils), the non serpentine taxa (excluding the data for these taxa from serpentine soils) and the data of the equally common taxa from both serpentine and non serpentine soils (termed “*bodenvag*” in the multivariate section) and therefore this data set was used for further analysis. In addition, data for the six taxa more common on serpentine soils were compared both on and off serpentine soils as were the data

for the six taxa more common on non serpentine. Eigenvalues >5 were considered to be high eigenvalues.

Statistical analysis:

The Epistat statistical program (Gustafson, 1986) was used to perform Student's t-tests on biomass and productivity data as well as the length to width ratios of bagged and unbagged fruit.

Results:

Biomass and productivity:

There was no significant difference in biomass for four of the six species that appeared to be equally common both on and off serpentine soils (Table 2A) indicating that these taxa were truly *bodenvag* taxa i.e. indifferent to the serpentine condition. *Bewisia biflora* and *Ipomoea crassipes* (taxa common on serpentine soils), *Bulbostylis oritrephes*, *Kohautia amatymbica* and *Scabiosa columbaria* (taxa common on non serpentine soils) and *Chlorophytum fasciculatum* and *Setaria sphacelata* var. *sphacelata* (taxa equally common on both soil types) showed significant differences in the leaf, stem and inflorescence biomass (Table 2A). With the exception of *S. sphacelata* var. *sphacelata*, the stem and leaf biomass of the taxa are greater on the serpentine soils than on the non serpentine soils.

Table 2A. Characters of taxa that showed significant differences in biomass per plant when growing on the two substrates. S = more common on serpentine soils, equal = equally common on both soil types, NS = more common on non serpentine soils.

Species	Where common	Character	Biomass (g) Mean $\pm$ SD (n)		t, P, df (unpaired tests)
			Serpentine	Non serpentine	
<i>Bewisia biflora</i>	S	Inflorescence	0.13 $\pm$ 0.13 (6)	0.51 $\pm$ 0.10 (3)	t = 4.48, P= 0.004, df = 7
<i>Ipomoea crassipes</i>	S	Leaf	10.25 $\pm$ 3.58 (3)	2.96 $\pm$ 1.74 (9)	t = 4.9, P = 0.0006, df = 10
		Stems	8.94 $\pm$ 0.99 (3)	1.87 $\pm$ 1.33 (9)	t = 8.37, P = 0.000008, df = 10
<i>Chlorophytum fasciculatum</i>	equal	Leaf	1.47 $\pm$ 0.10 (2)	0.29 $\pm$ 0.16 (2)	t = 8.80, P= 0.01, df= 2
<i>Setaria sphacelata</i> var. <i>sphacelata</i>	equal	Stems	0.22 $\pm$ 0.11 (6)	0.54 $\pm$ 0.31(12)	t = 2.46, P = 0.02, df = 16
<i>Kohautia amatymbica</i>	NS	Stem base	0.14 $\pm$ 0.05 (10)	0.08 $\pm$ 0.03 (4)	t = 2.3, P = 0.04, df = 12
<i>Bulbostylis oritrephes</i>	NS	Inflorescence	0.08 $\pm$ 0.02 (3)	0.02 $\pm$ 0.03 (4)	t = 3.68, P = 0.01, df = 5
<i>Scabiosa columbaria</i>	NS	Inflorescence	0.41 $\pm$ 0.27 (8)	0.19 $\pm$ 0.15 (14)	t = 2.44, P = 0.02, df = 20

Productivity of the reproductive parts (Appendix 1) appeared to be greater on serpentine soils for the taxa more common on serpentine. *N. hottentotica*, a member of the Compositae, also showed greater productivity on serpentine soils; producing more fruit. The fruits of *Nidorella*, however, are more robust in the plants growing on non serpentine soils.

For the taxa that were more common on non serpentine soils, the productivity of the flowering shoots in *B. oritrephes* was greater off serpentine. *H. nudifolium* was only found on non serpentine soils implying that this taxon, which had been previously collected on serpentine soils, is scarce on this soil type. Although *S. columbaria* produced twice as many inflorescences per plant off serpentine, the inflorescence biomass was greater in plants growing on serpentine soils. This could imply that proportionately more energy is channelled into the reproductive

effort because this is an unfavourable environment for this taxon. Generally, however, overall productivity for the “more common on serpentine” taxa appeared to be greater off serpentine.

Life forms and reproductive attributes

Hemicryptophytes contribute 72% (13) of the taxa in this study while geophytes contribute 17% (3) and therophytes 11% (2). For the taxa more common on serpentine soils 67% (4) of the taxa were hemicryptophytes while 33% (2) were therophytes (Table 3A). For the taxa equally common on and off serpentine soils, five of the six taxa (83%) were hemicryptophytes, the sixth being a geophyte (Table 3B). Four of the six taxa (67%) in the category more common on non serpentine soils were hemicryptophytes while two (33%) were geophytes (Table 3C).

One taxon common on serpentine soils, one taxon common on non serpentine soils and two taxa equally common on both soil types showed significant differences in the ratios of fruit length:width (Table 2B). The unbagged fruit of *N. hottentotica* (common on serpentine soils) showed a significantly greater fruit length:width ratio in non serpentine than the serpentine soil plants. There is a significant difference between the length:width ratios of the unbagged fruit of *S. sphacelata* (equally common on serpentine and non serpentine soils) plants growing on and off serpentine soils. The ratio for the fruit from the non serpentine soils was greater indicating that these fruit were thinner and not as well developed.

Table 2B. Pollinator exclusion treatments that showed significant differences in fruit length: width ratios when growing on the two substrates. The thinner, longer fruit indicates that these fruit are probably underdeveloped and thus inviable. S = more common on serpentine soils, equal = equally common on both soil types, NS = more common on non serpentine soils.

Species	Where common	Treatment	fruit length:width ratio Mean $\pm$ SD (n)		t, P, df	
			Serpentine	Non serpentine		
<i>Nidorella hottentotica</i>	S	Unbagged	2.03 $\pm$ 0.50 (36)	2.24 $\pm$ 0.44 (47)	t = 2.02, P= 0.05, df= 81	
		Bagged vs unbagged	2.39 $\pm$ 0.75 (36) vs 2.03 $\pm$ 0.50 (36)		t = 2.3, P = 0.03, df = 35 (paired)	
<i>Senecio venosus</i>	equal	Bagged	5.50 $\pm$ 1.76 (24)	13.66 $\pm$ 8.93 (24)	t = 4.6, P= 0.0001, df = 23 (paired)	
		bagged vs unbagged		13.66 $\pm$ 8.93 (24) vs 5.25 $\pm$ 2.09 (12)	t = 3.20, P = 0.003, df= 34 (unpaired)	
<i>Setaria sphacelata</i> var. <i>sphacelata</i>	equal	Unbagged viable fruit	2.22 $\pm$ 0.28 (12)	2.63 $\pm$ 0.23 (36)	t = 5.11, P = 0.000006, df = 46	
<i>Scabiosa columbaria</i>	NS	Bagged vs unbagged	4.11 $\pm$ 4.24 (36) vs 2.34 $\pm$ 1.11 (36)	5.32 $\pm$ 5.33 (36) vs 2.11 $\pm$ 0.89 (36)	t = 2.51, P= 0.02, df= 35 (s) (paired)	t = 3.85, P= 0.0007, df= 35 (ns) (paired)

Taxa more common on serpentine soils:

Four of the taxa in this category were long-lived hemi-cryptophytes and two were short-lived therophytes. Therophytes are often pioneers in areas that are unstable or disturbed. This is also an indication that the serpentine soil areas are perhaps not as stable as the non serpentine soil areas.

Flower colour as well as flower shape varied among the taxa common on serpentine soils (Table 3A). Flowers were mainly yellow and red or pink. Flower shape appeared to vary between the seasons; winter flowering taxa were arranged in heads, spring flowers were flag- or bell-shaped and the summer flowering taxa had small, inconspicuous flowers. Most of the taxa on serpentine soils were fertile during spring and autumn and all had long flowering (5 – 8 months) and

fruiting (5 – 8 months) times. The exception was *I. hedyantha* which had a fruiting time of only three months. *N. hottentotica* was the only taxon that began flowering during winter. Insects appeared to be the main pollinators for most of these taxa (Table 3A), the only apparently wind-pollinated taxon being *B. biflora*.

The breeding systems of the study taxa were inferred from the P/O ratios (Table 1). Each taxon showed the same type of breeding system on and off serpentine soils. The P/O ratios predicted that *I. hedyantha* and *I. crassipes* were facultatively autogamous, but when pollinators were excluded, no fruit formed. Outcrossing Index (OCI) scores (Cruden, 1977) were five for *I. hedyantha* and seven for *I. crassipes*. Thus indicating an outcrossing, sometimes self-incompatible, protandrous system that requires a pollinator (Cruden, 1977).

Where data were available, taxa either did not produce fruit or produced underdeveloped fruit when biotic pollinators were excluded, the exception being *T. capensis* var. *capensis* which formed apparently viable seeds. In *N. hottentotica* the difference in fruit length:width ratio of bagged to unbagged fruit was only significant for plants growing on serpentine soils. The ratios, however, were greater for the bagged specimens. This could indicate that the fruits were underdeveloped and possibly non viable. This in turn implies that these taxa could possibly have a preference for outcrossing. The fruit dispersal mechanism for most serpentine common study taxa appeared to be mainly autochorous, irrespective of the soil type. *B. biflora* and *I. crassipes*, however, could possibly be achorus, although *I. crassipes* may also be dispersed autochorously.

Table 3A. Summary of the life histories of the investigated taxa more common on serpentine soils. S = serpentine, NS= non serpentine, FA=Facultative Autogamy, FX= Facultative Xenogamy.

Taxon	Life form	Flower/ Inflorescence colour	Flower/ Inflorescence shape	Possible pollinator	Flower times	fruit times	Pollen/Ovule ratios		Presence/absence of fruit in exclusion bags	Possible seed dispersal mechanism
							S	NS		
<i>Bewisia biflora</i>	hemi- cryptophyte	purple-green	inconspicuous	wind	Nov – Apr	Dec – Apr	No data	No data	No data	Achory
<i>Indigofera hedyantha</i>	hemi- cryptophyte	red	flag	bee	Aug – Dec	Nov – Jan	129=FA	109=FA	absent	possibly Autochory, explosive
<i>Ipomoea crassipes</i>	hemi- cryptophyte	mauve	funnel/bell	bee	Sep – Apr	Nov–Apr	286=FA	286=FA	absent	Achory or autochory, explosive
<i>Nidorella, hottentotica</i>	therophyte	yellow	head	insect	Jul – Feb	Jan– Jun	718=FX	1079=FX	present, but underdeveloped	wind
<i>Phyllanthus maderaspatensis</i>	therophyte	green	inconspicuous	not observed, possibly insect	Oct – Feb	Nov– Jun	No data	No data	No data	possibly Autochory, explosive
<i>Tephrosia capensis</i> var. <i>capensis</i>	hemi- cryptophyte	mauve	flag	bee	Sep – Mar	Oct – Mar	191=FA	195=FA	present	possibly Autochory, explosive

Taxa equally common on serpentine and non serpentine soils:

For the taxa that were equally common on and off serpentine soils (Table 3B), most appeared to be hemicryptophytes, the exception being *C. fasciculatum* which is a geophyte. All these taxa are thus long-lived life forms.

Flower colour of the study taxa varied, with greens and whites being common. Flower shape was also variable. Most of the study taxa displayed fairly long flowering (4 – 9 months) and fruiting (5 – 9 months) periods. *A. adscendens*, however, had a very short fruiting period of only two months. Insects appeared to be the main pollinators for most of the taxa (Table 3B), the only apparently wind pollinated taxon being *S. sphacelata* var. *sphacelata*.

Where data was available, the breeding systems of three study taxa were facultatively autogamous both on and off serpentine soils. The P/O ratios predicted that *C. fasciculatum* on non serpentine soils, would be obligately autogamous, however the exclusion of pollinators did not result in fruit formation. The OCI score (Cruden, 1977) for this taxon was 6, indicating an outcrossing, sometimes self-incompatible, protandrous system that requires a pollinator (Cruden, 1977). As the pollen data collected for this taxon were insufficient, it is more likely that the OCI gives a better prediction of the breeding system of this taxon than the P/O ratio. *S. sphacelata* var. *sphacelata* appeared to be facultatively xenogamous on non serpentine soils, while *S. venosus* appeared to be facultatively autogamous when growing both on and off serpentine soils.

In the few taxa where data were available when biotic pollinators were excluded, *C. costatus* and *S. venosus* were the only two taxa that set fruit. The fruit of *S. venosus* however appeared to be underdeveloped. However, the fruit length:width ratio of bagged:unbagged fruit was only significant for the non serpentine plants. Again the ratios were greater for the bagged specimens, implying that this taxon could also have a preference for outcrossing. Only the flowers on the upper part of

the stems of *C. costatus* appeared to set fruit in the exclusion samples. Fruit dispersal in this category appeared to be mixed, with three appearing to be achorous and three autochorous. Although *C. fasciculatum* was predicted as being obligately autogamous, there was no fruit set when pollinators were excluded.

Table 3B. Summary of the life histories of the investigated taxa equally common on serpentine and non serpentine soils. S = serpentine, NS= non serpentine, OA= Obligate Autogamy, FA=Facultative Autogamy, FX= Facultative Xenogamy.

Taxon	Life form	Flower/ Inflorescence colour	Flower/ Inflorescence shape	Possible pollinator	Flow er times	fruit times	Pollen/Ovule ratio		Presence/absence of fruit in exclusion bags	Possible seed dispersal mechanism
							S	NS		
<i>Asclepias adscendens</i>	hemi– cryptophyte	green	head	insect	Aug – Dec	Nov– Dec	No data	No data	No data	wind
<i>Chaetacanthus costatus</i>	hemi– cryptophyte	white	gullet	insect	Sep – Dec	Sep – Apr	111=FA	114=FA	present on upper part of stem	Autochory, explosive
<i>Chlorophytum fasciculatum</i>	geophyte	white	dish	insect	Aug – Apr	Oct – Apr	No data	36=OA	absent	Achory
<i>Hermannia depressa</i>	hemi– cryptophyte	peach	bell	insect	Aug– Feb	Oct– Feb	87=FA	111=FA	No data	Achory
<i>Setaria sphacelata</i> var. <i>sphacelata</i>	hemi– cryptophyte	green with gold awns	inconspicuous	wind	Sep – Feb	Oct– Jun	No data	1626=FX	No data	Achory with some endozoochory
<i>Senecio venosus</i>	hemi– cryptophyte	yellow	head	insect	Sep – Feb	Jan – Jun	448=FA	417=FA	present	wind

Taxa more common on non serpentine soils:

The six study taxa that were more common off serpentine soils (Table 3C) are also mainly hemicryptophytes. *B. oritrephes* and *L. marginata* were the exceptions being geophytes. Again, all these taxa exhibit long-lived life forms.

Flower colour varied between these taxa being mainly yellow/cream/white. Flower shapes also varied. The lengths of the flowering and fruiting periods were very variable amongst the study taxa, with flowering periods ranging from 3 – 8 months and fruiting periods from 3 – 6 months. *L. marginata* and *K. amatymbica*, however, only flowered for three months. *L. marginata* also had a short, three month, fruiting period. Only two of the taxa, *B. oritrephes* and *E. racemosa*, appeared to be wind pollinated, while the rest appeared to be insect pollinated.

Most taxa were predicted to be either facultatively xenogamous or autogamous both on and off serpentine soils. From Table 1, it was predicted that *K. amatymbica* is obligately autogamous, however insects, especially moths were observed visiting these sweetly scented flowers during the early evening. Thus in this case, the breeding system results need to be treated with caution as there is a discrepancy between the predicted breeding system and the observations.

Pollinator exclusion data were available for only two of the taxa, *H. nudifolium* and *S. columbaria*. In both cases, fruit formed, but appeared to be underdeveloped. Achory appears to be the main mechanism by which seeds in this category are dispersed.

Table 3C. Summary of the life histories of the investigated taxa more common on non serpentine soils. S = serpentine, NS= non serpentine, FA=Facultative Autogamy , FX= Facultative Xenogamy, OA= Obligate Autogamy, OX= Obligate Xenogamy.

Taxon	Life form	Flower/ Inflorescence colour	Flower/ Inflorescence shape	Possible pollinator	Flower times	Fruit times	Pollen/Ovule ratio		Presence/absen ce of fruit in exclusion bags	Possible seed dispersal mechanism
							S	NS		
<i>Bulbostylis oritrephes</i>	geophyte	brown	inconspicuous	wind	Aug–Dec	Oct– Dec	1134=FX	8850=OX	No data	Achory
<i>Eragrostis racemosa</i>	hemi– cryptophyte	blackish	inconspicuous	wind	Jul – Jan	Jan– Jun	1921=FX	2785=FX	No data	Achory
<i>Helichrysum nudifolium</i>	hemi– cryptophyte	yellow	head	insect	Sep – Feb	Dec – Mar	not on serpentine	332=FA	present	wind
<i>Kohautia amatymbica</i>	hemi– cryptophyte	cream	funnel	moth	Sep– Nov	Oct– Jan	23=OA	19 = OA	No data	Achory
<i>Ledebouria marginata</i>	geophyte	greenish purple	dish	insect	Aug– Oct	Oct– Jan	257=FA	260=FA	No data	Achory
<i>Scabiosa columbaria</i>	hemi– cryptophyte	white	head/ brush	butterfly	Sep– Apr	Nov– Apr	296=FA	293=FA	Present, but underdeveloped	wind

Multivariate analysis:

Eleven qualitative characters were used to graphically determine whether there was any separation between taxa in the three categories. The breeding system characters used were percentage pollen viability, P/O ratio, and the number of ovules per ovary. Biomass characters used were for the leaves, stems and stem bases; while productivity characters were the reproductive index, and the number of fruit per plant, flower shoots per plant, the number of flowers per axil as well as the number of branches per plant.

Ordination techniques, like Principal Components Analysis (PCA), summarise large amounts of information in only a few dimensions which are then easier to interpret. Each axis accounts for the variability and the spread of the data along the axis (Pimentel, 1981; Chandler & Crisp, 1998). For the combined *bodenvag* taxa, the first three principal components respectively account for 33%, 21% and 20% of the total variation, cumulatively representing 74% of the variation. On the first principal component axis (Table 4) the number of fruit per plant, the percentage viable pollen and the reproductive index had strong eigenvectors. The number of branches per plant, the number of flowers per axil and the stem base biomass were the main contributors to the second axis while the number of ovules per ovary and the stem biomass contributed most to the third principal component axis.

Positive eigenvalues indicate high expression values while negative values indicate low expression values. On the first principal component axis (Table 4) the percentage viable pollen, leaf biomass and the stem biomass had strong positive eigenvectors, thus it is mainly vegetative characters that contribute the most towards separation of the taxa. While the percentage viable pollen shows a strong positive value on this axis, the number of fruit per plant gives a strong negative value. This could possibly indicate that although there is a high percentage of viable pollen available, few seeds are set. The number of flowers per axil, stem

base biomass and the P/O ratio are the main positive contributors on the second principal component axis. Along this axis there is a strong positive value for the number of flowers per axil and a strong negative value for the number of branches per plant. This could imply that the fewer the branches, the more flowers are produced indicating that more energy is allocated to the reproductive effort. The number of ovules per ovary, the number of flowers per axil and the % viable pollen (mainly reproductive productivity characters) are the main positive contributors on the third principal component axis.

Table 4. Eigenvectors for characters used for the *bodenvag* taxa with the high eigenvectors per axis in **bold**.

Characters	Axis 1	Axis 2	Axis 3
% viable pollen	0.97	0.22	0.3
P/O ratio	0.12	0.46	-0.47
No. of ovules per ovary	0.16	-0.37	0.86
Leaf biomass	0.42	-0.11	-0.39
Stem biomass	0.31	-0.13	-0.6
Stem base biomass	-0.19	0.58	0.19
Reproductive index	-0.85	-0.35	0.1
No. of fruit per plant	-1.04	0.1	-0.19
No. of flower shoots per plant	0.14	-0.24	-0.29
No. of flowers per axil	-0.15	0.63	0.44
No. of branches per plant	0.1	-0.79	0.06

Principal components analysis data (Figures 1 & 2) showed no distinct grouping of the majority of the taxa into the three categories, although the non serpentine components of *S. columbaria* and *E. racemosa*, the serpentine components of *B. biflora* and *I. crassipes* and both serpentine and non serpentine components of *S.*

spacelata var. *spacelata* group out of the main cluster. The phenogram (Figure 3) did not show distinct clustering of serpentine or non serpentine taxa, rather all taxa were intermingled.

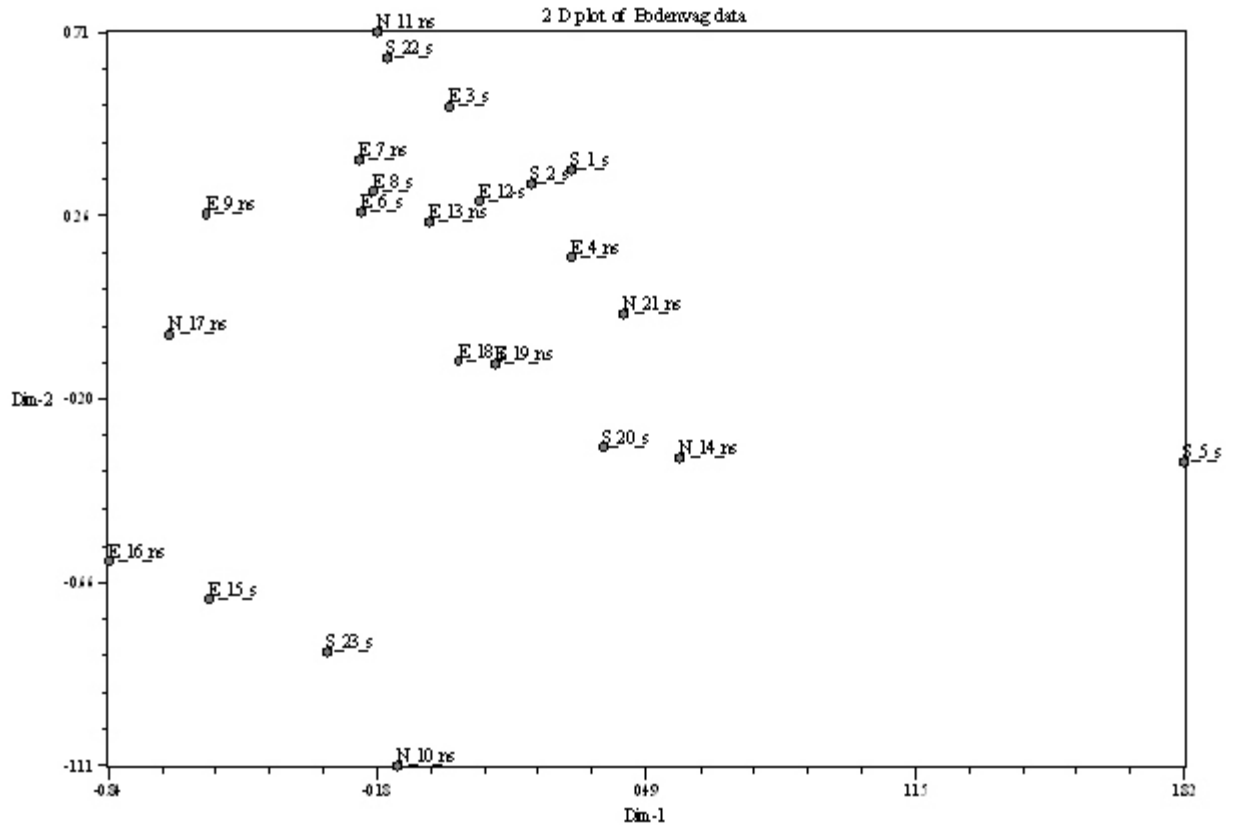


Figure 1. Two dimensional PCA plot of the first two principal component axes for the *bodenvag* taxa shows no distinct grouping of the majority of the taxa.

1 = S *Indigofera hedyantha* s, 2 = S *Tephrosia capensis* var. *capensis* s, 3 = E *Asclepias adscendens* s, 4 = E *A. adscendens* ns, 5 = S *Ipomoea crassipes* s, 6 = E *Hermannia depressa* s, 7 = E *H. depressa* ns, 8 = E *Chlorophytum fasciculatum* s, 9 = E *C. fasciculatum* ns, 10 = N *Scabiosa columbaria* ns, 11 = N *Kohautia amatymbica* ns, 12 = E *Chaetacanthus costatus* s, 13 = E *C. costatus* ns, 14 = N *Bulbostylis oritrephes* ns, 15 = E *Setaria sphacelata* var. *sphacelata* s, 16 = E *S. sphacelata* var. *sphacelata* ns, 17 = N *Eragrostis racemosa* ns, 18 = E *Senecio venosus* ns, 19 = E *S. venosus* ns, 20 = S *Nidorella hottentotica* s, 21 = N *Helichrysum nudifolium* ns, 22 = S *Phyllanthus maderaspatensis* s, 23 = S *Bewisia biflora* s. S, N and E respectively represent the categories “more common on serpentine soils”, “more common on non serpentine soils” and “equally common on both soil types”. s = serpentine, ns = non serpentine.

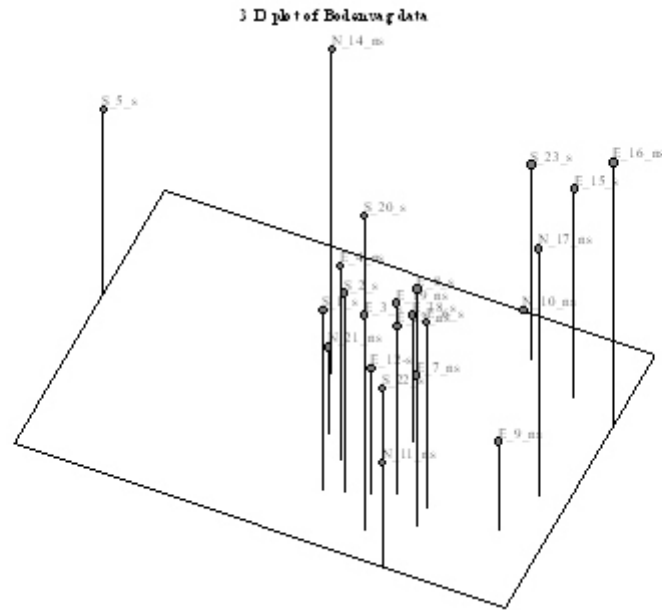


Figure 2. Three dimensional PCA plot for the first three principal component axes for the *bodenvag* taxa shows no distinct grouping of the majority of the taxa.

1 = S *Indigofera hedyantha* s, 2 = S *Tephrosia capensis* var. *capensis* s, 3 = E *Asclepias adscendens* s, 4 = E *A. adscendens* ns, 5 = S *Ipomoea crassipes* s, 6 = E *Hermannia depressa* s, 7 = E *H. depressa* ns, 8 = E *Chlorophytum fasciculatum* s, 9 = E *C. fasciculatum* ns, 10 = N *Scabiosa columbaria* ns, 11 = N *Kohautia amatymbica* ns, 12 = E *Chaetacanthus costatus* s, 13 = E *C. costatus* ns, 14 = N *Bulbostylis oritrephes* ns, 15 = E *Setaria sphacelata* var. *sphacelata* s, 16 = E *S. sphacelata* var. *sphacelata* ns, 17 = N *Eragrostis racemosa* ns, 18 = E *Senecio venosus* ns, 19 = E *S. venosus* ns, 20 = S *Nidorella hottentotica* s, 21 = N *Helichrysum nudifolium* ns, 22 = S *Phyllanthus maderaspatensis* s, 23 = S *Bewsia biflora* s. S, N and E respectively represent the categories “more common on serpentine soils”, “more common on non serpentine soils” and “equally common on both soil types”. s = serpentine, ns = non serpentine. s = serpentine, ns = non serpentine.

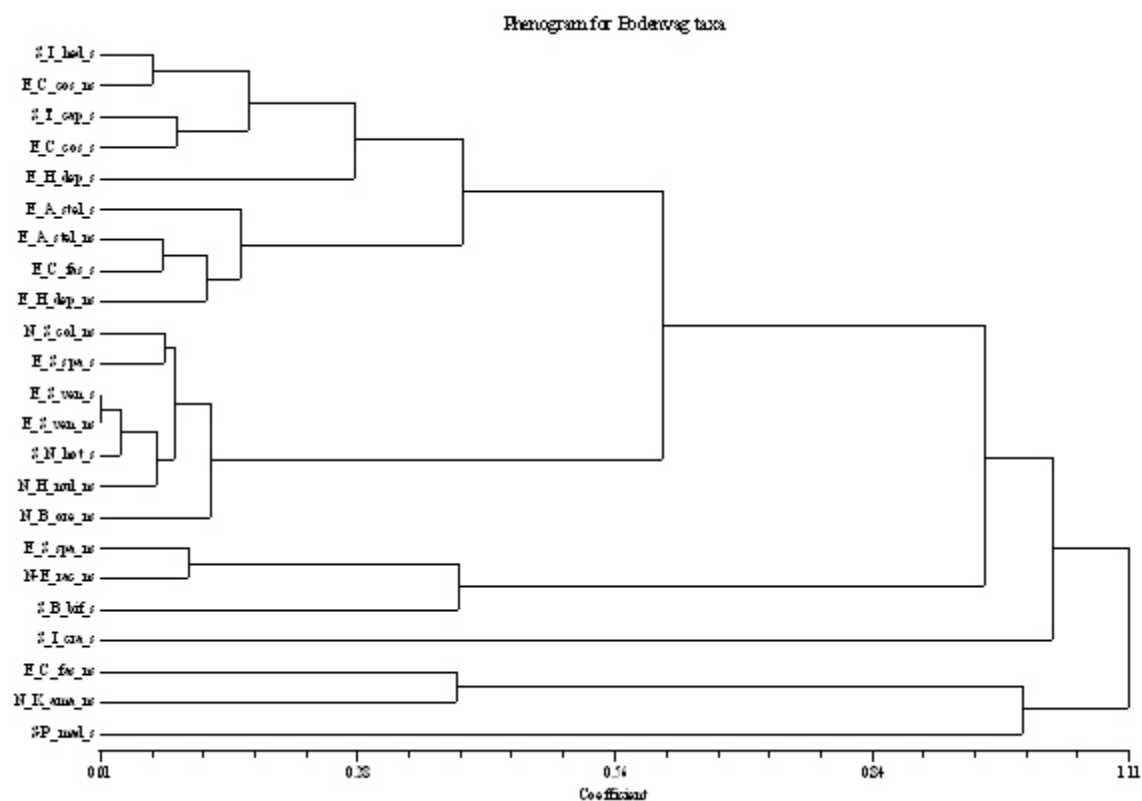


Figure 3. Phenogram of a UPGMA cluster analysis for the *bodenveg* taxa using 11 characters shows no distinct clustering of serpentine or non serpentine taxa. $r = 0.86$.

A_stel = *Asclepias adscendens*, C_cos = *Chaetacanthus costatus*, C_fas = *Chlorophytum fasciculatum*, H_dep = *Hermannia depressa*, S_ven = *Senecio venosus*, S_spa = *Setaria sphacelata* var. *sphacelata*, B_bif = *Bewsia biflora*, I_hed = *Indigofera hedyantha*, I_cra = *Ipomoea crassipes*, N_hot = *Nidorella hottentotica*, P_mad = *Phyllanthus maderaspatensis*, T_cap = *Tephrosia capensis* var. *capensis*, B_ore = *Bulbostylis oritrephes*, E_rac = *Eragrostis racemosa*, H_nud = *Helichrysum nudifolium*, K_ama = *Kohautia amatymbica*, S_col = *Scabiosa columbaria*.

S, N and E respectively represent the categories “more common on serpentine soils”, “more common on non serpentine soils” and “equally common on both soil types”. s = serpentine, ns = non serpentine.

For taxa more common off serpentine soils, the first three principal components respectively account for 39%, 35% and 18% of the total variation, cumulatively representing 92% of the variation. On the first principal component axis (Table 5), the P/O ratio was the main contributor towards separation of the taxa. On the

second principal component axis three characters namely viable pollen, the number of ovules per ovary and the number of fruit per plant contributed towards the separation of taxa. No characters contributed significantly on the third principal component axis, but cumulatively it accounts for 18% of the variation.

Table 5. Eigenvectors for characters used for taxa more common off serpentine soils with the high eigenvectors per axis in **bold**.

Characters	Axis 1	Axis 2	Axis 3
% viable pollen	0.15	0.9	0.28
P/O ratio	-2.49	-0.002	0.05
No. of ovules per ovary	0.18	0.95	-0.31
Leaf biomass	0.38	-0.2	0.09
Stem biomass	0.36	-0.26	0.1
Stem base biomass	0.38	-0.2	0.08
Reproductive index	0.38	-0.2	0.09
No. of fruit per plant	-0.31	-0.54	-0.2
No. of flower shoots per plant	0.35	-0.23	0.04
No. of flowers per axil	0.31	-0.12	-0.09
No. of branches per plant	0.33	-0.09	-0.14

Graphical representation (Figure 4) shows that most of the taxa cluster together irrespective of soil type along the first axis but are spread out along the second axis. In *K. amatymbica*, the serpentine and non serpentine taxa separate along the second axis.

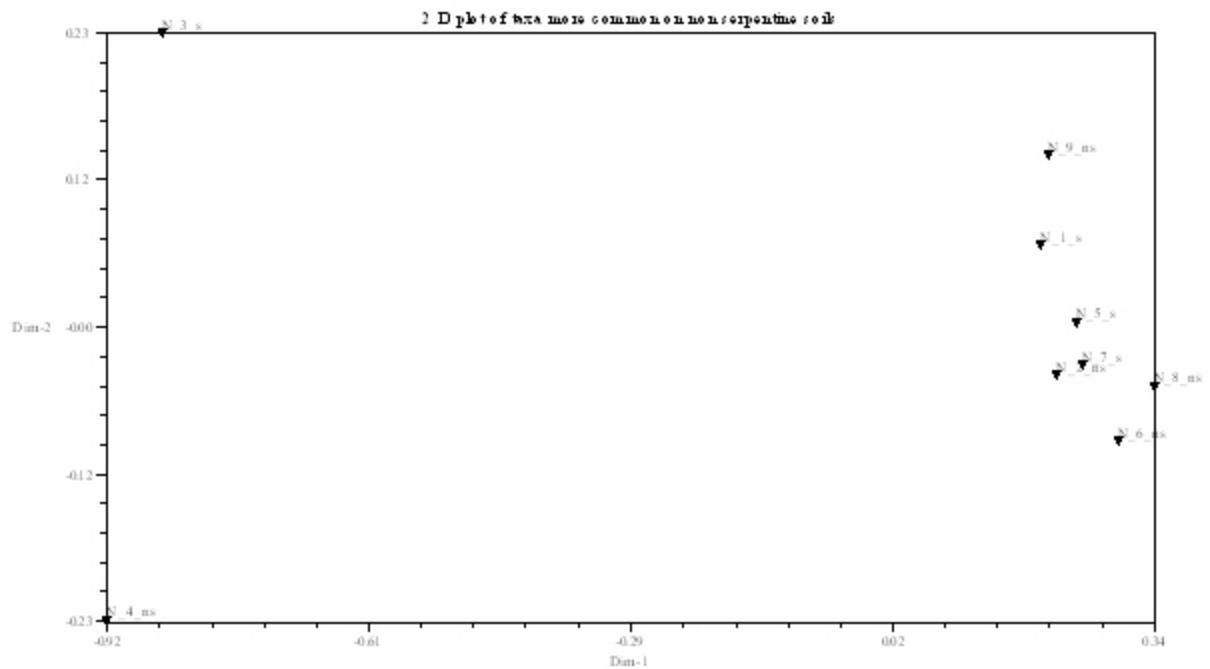


Figure 4. Two dimensional PCA plot of the first two principal component axes for taxa more common off serpentine soils shows that, with the exception of *K. amatymbica*, most of the taxa cluster together.

1 = *Scabiosa columbaria* s, 2 = *S. columbaria* ns, 3 = *Kohautia amatymbica* s, 4 = *K. amatymbica* ns, 5 = *Bulbostylis oritrephes* s, 6 = *B. oritrephes* ns, 7 = *Eragrostis racemosa* s, 8 = *E. racemosa* ns, 9 = *Helichrysum nudifolium* ns. s = serpentine, ns = non serpentine. N = the category “more common on non serpentine”

The first three principal component axes respectively account for 51%, 25% and 18% of the total variation for taxa more common on serpentine soils, cumulatively representing 93% of the variation. Seven characters contributed towards the separation of taxa on the first principal component axis (Table 6); these were the number of ovules per ovary, the number of branches per plant, the P/O ratio, the stem base biomass, the number of flowers per axil, the number of flower shoots per plant and the reproductive index. Separation on the second principal component axis was mainly due to the stem and leaf biomass, number of fruit per plant as well as the stem base biomass. On the third principal component axis, the reproductive index and the percentage viable pollen were the main contributors.

Table 6. Eigenvectors for characters used for taxa more common on serpentine soils with the high eigenvectors per axis in **bold**.

Characters	Axis 1	Axis 2	Axis 3
% viable pollen	0.03	0.19	0.65
P/O ratio	0.77	-0.14	-0.44
No. of ovules per ovary	-0.93	-0.17	-0.06
Leaf biomass	0.4	-0.73	0.21
Stem biomass	0.43	-0.78	-0.08
Stem base biomass	0.73	0.5	0.01
Reproductive index	-0.59	0.26	-0.69
No. of fruit per plant	-0.08	0.51	0.42
No. of flower shoots per plant	-0.66	0.15	-0.21
No. of flowers per axil	0.7	0.45	-0.1
No. of branches per plant	-0.79	-0.23	0.31

The main positive contributors towards separation of the taxa on the first principal component axis (Table 6) are the P/O ratio, stem base biomass and the number of flowers per axil, mainly reproductive characters. The main positive contributing characters on the second principal component axis are the number of fruit per plant, the stem biomass and the leaf biomass and the number of flowers per axil. On the third principal component axis, the percentage viable pollen the number of fruit per plant and the number of branches per plant are the main positive contributors. Graphical representation (Figure 5) showed that most of the taxa cluster together irrespective of soil type. *B. biflora* (10 and 11) differ along the first axis while *I. crassipes* (5) and *P. maderaspatensis* (9) differ from the other taxa along the second axis.

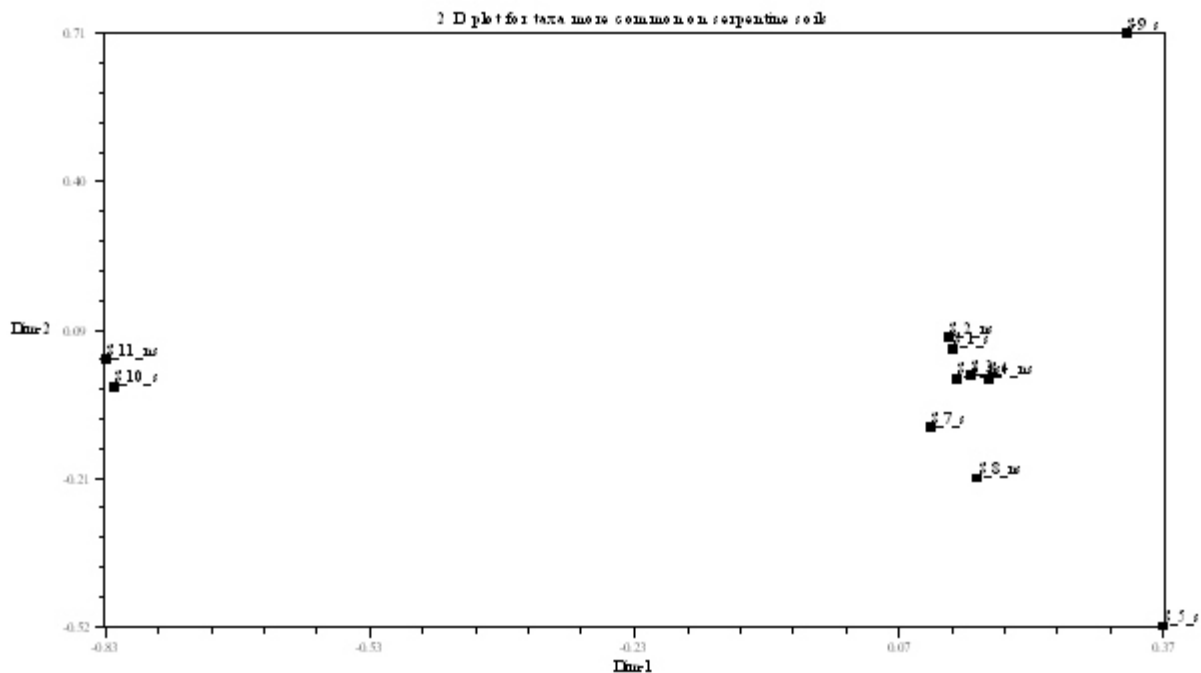


Figure 5. Two dimensional PCA plot of the first two principal component axes for taxa more common on serpentine soils shows that most of the taxa cluster together indicating that the taxa are similar on and off serpentine soils.

1 = *Indigofera hedyantha* s, 2 = *I. hedyantha* ns, 3 = *Tephrosia capensis* var *capensis* s, 4 = *T. capensis* var *capensis* ns, 5 = *Ipomoea crassipes* s, 6 = *I. crassipes* ns, 7 = *Nidorella, hottentotica* s, 8 = *N. hottentotica* ns, 9 = *Phyllanthus maderaspatensis* s, 10 = *Bewsia biflora* s, 11 = *B. biflora* ns. s = serpentine, ns = non serpentine. S = the category “more common on serpentine”.

Discussion:

The serpentine and non serpentine areas on the Witwatersrand are part of the grassland biome (Reddy *et al.*, 2001). Hemicryptophytes (plants whose buds survive adverse conditions resting on or just below the soil surface) are the dominant life form among the grassland forbs (Reddy *et al.*, 2009); similarly hemicryptophytes were the most common life form in this sub-set of the serpentine flora. Hemicryptophytes as well as geophytes usually possess large underground storage organs and annual above ground parts. These types of life forms ensure persistence during unfavourable conditions such as regular fires and harsh winters.

The only therophytes (annuals) amongst the study taxa, *N. hottentotica* and *P. maderaspatensis*, were in the more common on serpentine soil category.

Therophytes maximise reproduction by allocating a relatively greater proportion of their resources to reproduction than to vegetative growth or storage (Aronson *et al.*, 1993). Hemicryptophytes then would be expected to do the converse, that is put more resources into vegetative growth and storage than into reproduction. From the reproductive indices, it would appear that the reproductive allocation for the study taxa were similarly small for all life forms, even the two therophytes.

Both the species in the family Leguminosae, *I. hedyantha* and *T. capensis*, showed greater reproductive productivity on serpentine soils, with *Indigofera* having more flowering shoots and *Tephrosia* more fruit. Leguminous plants often have symbiotic nodules on their roots which contribute significantly to the nitrogen, organic matter contents and physical properties of soils (Russell, 1894). Thus they actively contribute to soil fertility under salt, heat, and acid stresses and can sometimes do so under the effect of heavy metals (Zahran, 1999; Younis, 2002). This may ameliorate the situation on the Witwatersrand serpentine soils and thus enable these taxa to perform well on this soil type.

Most of the study taxa were fertile for long periods (5 – 11 months). Flowering occurred mainly during spring and summer whilst fruiting occurred mainly during summer and autumn, with the fruiting period of a few taxa, *S. sphacelata*, *S. venosus* and *E. racemosa*, extending into winter. Insects and wind appeared to be the main pollinating agents of the study taxa. With regard to the insect vectors, most of the study taxa appear to be generalists (visited by many different insect species). This implies that the study taxa, at least, may still need the genetic flexibility that is brought about by outcrossing. However, most of the study taxa in all three categories, are either facultatively or obligately autogamous (capable of self fertilization). Xenogamy was not very common in all three categories. This indicates that this environment is transitional and reproductive capacity is

maximised by being able to self fertilize when necessary (Stebbins, 1957; Lloyd & Schoen, 1992).

Seeds must have enough resources to accomplish germination and establishment (Willson & Traveset, 2000), therefore if the seeds appear thin and underdeveloped, this implies that there are inadequate resources available to accomplish these functions. The length to width ratio is an indication of fruit (or seed) shape. Fruit (or seed) width is the single best indicator for weight but using length and width gives a substantial improvement on results (Shifriss *et al.*, 1989). A higher ratio indicates narrower fruits (or seeds), while lower ratios indicate rounder fruits (or seeds) (Kremer *et al.*, 2009). So the well developed, apparently viable, unbagged seeds of *N. hottentotica*, are an indication that the plants growing on serpentine soils allocate more resources towards seeds than the plants on non serpentine soils, which would then account for it being more common on serpentine than non serpentine soils. The bagged fruit of *S. venosus*, a taxon equally common on both soil types, also showed this trend, i.e. the bagged fruit of plants growing on serpentine soils may possibly have viable seeds. However, when the bagged fruit of *S. venosus* was compared with the unbagged fruit of plants from the non serpentine soils, the unbagged fruit ratio was still significantly greater. *Senecio venosus* (Table 3B) was found to be facultatively autogamous, so it is possible that being autogamous on serpentine soils is a way of overcoming the serpentine condition by conserving rare genes that are adapted to these conditions. However, the results were not significant for all the taxa examined, so it cannot be validly concluded that the study taxa growing on serpentine soils allocate more resources towards seed production, neither can it be concluded that autogamy is a way of overcoming the serpentine condition on the Witwatersrand.

Seed dispersal mechanisms may either be autochorous (physical, often explosive, expulsion) or achorous (passive dispersal) or seeds may be dispersed by other agents. Achorous seeds by their nature, do not disperse very far from the parent

plants. Long distance dispersal by a few seeds is often the best strategy and appears to be the norm amongst most plants, while local dispersal by most seeds allows offspring to retain the benefits of the parental environment to which they are genetically favourably adapted, through the inheritance of genes that are adapted to those conditions (Bolker & Pacala, 1999; Uller, 2008). In autochorous dispersal, wind dispersal tends to disperse propagules further than explosive dispersal, especially in low growing herbaceous taxa (Vander Wall & Longland, 2004). Of the study taxa, only five of the 18 taxa studied were wind dispersed. This would indicate that the majority of the study taxa are locally dispersed over relatively short distances into areas for which they are adapted, thus assisting in dispersal over a short distance.

Life history and breeding systems have fecundity implications (Bazzaz *et al.*, 1989). If plants put more energy into the production of vegetative biomass then this would lead to healthier individuals capable of surviving adverse periods, but not expanding the population. If plants put more energy into reproduction, thus increasing reproductive biomass, then the parent plant may not survive adverse periods but it will ensure that there is enough genetic diversity in the population. This will also contribute towards an increase in the population size and genetic diversity. These new individuals, if strong enough, will be able to better survive changing conditions. During favourable periods, plants will therefore tend to put more energy into vegetative biomass production and during adverse conditions into reproduction and reproductive biomass. A third strategy proposed by Grime (1979) is that of stress tolerators. These plant taxa live in areas of high intensity stress and low intensity disturbance and respond to environmental stresses through physiological variability (Kazakou *et al.*, 2008). Species that have adopted this strategy generally have slow growth rates, long-lived leaves, high rates of nutrient retention, and low phenotypic plasticity. The Witwatersrand flora, including the serpentine flora consists of mainly long-lived hemicryptophytes (forbs and geophytes) and some phanerophytes (trees and shrubs). The hemicryptophytes

have mainly short-lived leaves, but the underground organs are usually large and are used for storage. This study has shown that phenotypic plasticity among the study taxa was low as there were no differences between the taxa growing on and off serpentine soils. In addition the Witwatersrand grasslands experience very harsh winters as well as frequent fires. All these factors indicate that plants of the Witwatersrand are probably stress tolerators.

Sinking more resources into reproduction is also a strategy for coping with a harsh, unstable environment. The type of breeding system exhibited by a taxon could be an indication of the stability of its environment. An inbreeding system could be an indication of a relatively stable environment whereas an outbreeding mode could indicate an unstable one. Most of the taxa in this study appear to adopt a “best of both worlds” strategy in that they appear to be facultative. Facultative reproductive strategies, a combination of sexual outcrossing and autogamy, may give plants optimal versatility (Yeboah Gyan & Woodell, 1987). While most plant taxa exhibit only a single type of breeding system, there are a few cases where they differ within a taxon (Cruden, 1976; Kaul *et al.*, 2002). However, the type of breeding system appears to be the same on and off serpentine soils, so this could indicate either that the system is in a transition phase or that it is not the soil toxicity that is driving this process.

The vegetative biomass of most of the taxa, with significant results, was greater on serpentine soils than the non serpentine soils. This could indicate that the serpentine areas are perhaps more productive than the non serpentine areas. The nutrients from the tree litter, as well as the droppings from birds that perch on the trees increases the nutrient content and hence the productivity of herbaceous species growing under savanna trees (Belsky, 1994). The serpentine areas of the study sites are rocky outcrops that provide fire protection for tree and shrub species. As a result, there is an increase in leaf litter under these trees which would increase the soil nutrient content and make these areas more productive. In

addition, leaf litter under shady trees conserves moisture (Sydes & Grime, 1981; Belsky, 1994) which is then available to the herbaceous species that occur in the area.

Grassland fires tend to be surface fires which are spread by fuels that are close to the ground in the grass layer (Bond & van Wilgen, 1996), but do not affect trees taller than 2 – 4 m (Bond & Keeley, 2005). These tree canopies provide shady habitats, so that forbs growing here may be moister and retain moisture levels for longer into the dry season (Higgins *et al.*, 2000), causing fires to burn with low intensities in these areas. Fire intensity will affect the loss of nutrients through volatilization (Vestergaard & Alstrup, 1996; Fynn *et al.*, 2003). The lower the fire intensity, the lower the nutrient loss (Trabaud, 1994). The low fire intensities may also increase the rate of survival of plants growing on rocky outcrops (Bond & van Wilgen, 1996). This would enable them to have more biomass than the plants growing on the more level non serpentine areas.

The reproductive biomass (inflorescence) of two taxa common on non serpentine also showed significantly more inflorescence biomass on serpentine than off; Putting more energy into inflorescence production than the serpentine plants, implies that these non serpentine taxa are perhaps better established and stable on non serpentine soils and therefore a greater reproductive output is necessary on serpentine soils (Bonser & Aarssen, 2003; Jacquemyn *et al.*, 2006). Again, this is not representative of all the data so it cannot be conclusively stated that plants growing on serpentine soils have a greater reproductive output.

Conclusions:

The results of this study indicate that reproductive and life history data do not separate the study taxa into ecotypes. The implications are that all *bodenvag* serpentine taxa investigated on the Witwatersrand could either be pre-adapted for

the serpentine condition and therefore they are similar both on and off serpentine soils or, because the Witwatersrand serpentine soils are not very toxic, the serpentine conditions are similar to that of the Highveld grasslands; hence there is no selection pressure for the formation of ecotypes. It is thus likely that other factors, such as the harsh climatic conditions in the form of severe Highveld winters, being part of a fire dependent grassland and occurring at a high elevation, exert a stronger influence on these plants than does a relatively mild serpentine condition. The results also imply that gene flow occurs amongst the study taxa growing on and off serpentine soils.

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Appendix 1. Summary of some of the productivity data used in this study. No. = number, flring = flowering , flrs = flowers, inflr = inflorescence, S = serpentine, NS = non serpentine.

Taxon	No. branches/plant mean $\pm$ SD (n)		No. flring shoots mean $\pm$ SD (n)		No. flrs/inflr mean $\pm$ SD (n)		No. heads or flrs/plant mean $\pm$ SD (n)		No. rachi/head mean $\pm$ SD (n)		No. spike/rachis mean $\pm$ SD (n)		No. flowers/branch mean $\pm$ SD (n)	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
Common on serpentine														
<i>Bewsia biflora</i>			1.3 $\pm$ 0.8 (6)	2.3 $\pm$ 1.2 (3)			1.3 $\pm$ 0.8 (6)	2.3 $\pm$ 1.2 (3)	9.3 $\pm$ 1.4 (6)	10 $\pm$ 4.4 (3)	9.3 $\pm$ 2.6 (6)	15.2 $\pm$ 4.27 (5)		
<i>Indigofera hedyantha</i>	7.8 $\pm$ 2.1 (7)	7.8 $\pm$ 2.6 (6)	13 $\pm$ 11.6 (21)	9.7 $\pm$ 6.8 (12)	5.7 $\pm$ 18.1 (9)	4 $\pm$ 2.6 (4)								
<i>Ipomoea crassipes</i>	5.8 $\pm$ 5.95 (6)	10.4 $\pm$ 5.9 (7)	5.7 $\pm$ 4.4 (6)	5 $\pm$ 3.1 (6)									3.9 $\pm$ 2.4 (14)	6.2 $\pm$ 2.5 (6)
<i>Nidorella hottentotica</i>	4.3 $\pm$ 4.8 (7)	1 $\pm$ 0 (6)	9 $\pm$ 4.9 (7)	12 $\pm$ 6.5 (6)			176.3 $\pm$ 163.4 (4)	56.5 $\pm$ 28.4 (4)						
<i>Phyllanthus maderaspatensis</i>	6 (1)	7 $\pm$ 9.9 (2)	2 $\pm$ 0.7 (5)	no data	2.5 $\pm$ 0.7 (2)	3.3 $\pm$ 0.5 (4)							20 $\pm$ 7.8 (3)	11.3 $\pm$ 8.4 (3)
<i>Tephrosia capensis</i> var. <i>capensis</i>	4.3 $\pm$ 1.9 (7)	8.2 $\pm$ 4.0 (5)	16 $\pm$ 5.4 (7)	23.5 $\pm$ 10.0 (4)										
Equally common														
<i>Asclepias adscendens</i>	5.1 $\pm$ 2.7 (11)	no data	3.4 $\pm$ 2.2 (14)	1 (1)	16.6 $\pm$ 5.7 (20)	16.2 $\pm$ 4.8 (4)	2.9 $\pm$ 2.3 (13)	1 (1)						
<i>Chaetacanthus costatus</i>	9.4 $\pm$ 5.7 (17)	4.5 $\pm$ 1.5 (6)	5.5 $\pm$ 4.0 (24)	4 $\pm$ 2.7 (3)			7.4 $\pm$ 5.3 (19)	3.8 $\pm$ 1.7 (4)						

Taxon	No. branches/plant mean $\pm$ SD (n)		No. flring shoots mean $\pm$ SD (n)		No. flrs/inflr mean $\pm$ SD (n)		No. heads or flrs/plant mean $\pm$ SD (n)		No. rachi/head mean $\pm$ SD (n)		No. spike/rachis mean $\pm$ SD (n)		No. flowers/branch mean $\pm$ SD (n)	
<i>Chlorophytum fasciculatum</i>			1.7 $\pm$ 0.5 (6)	1.6 $\pm$ 0.8 (7)	11.3 $\pm$ 8.6 (9)	19.3 $\pm$ 5.8 (9)								
<i>Hermannia depressa</i>	1.9 $\pm$ 0.9 (11)	3.5 $\pm$ 1.3 (4)	3.6 $\pm$ 3.0 (11)	1.5 $\pm$ 1 (4)			6.7 $\pm$ 5.3 (11)	3.5 $\pm$ 5 (4)						
<i>Senecio venosus</i>	6.1 $\pm$ 1.6 (12)	5.8 $\pm$ 1.6 (16)	1.8 $\pm$ 1.1 (12)	4.3 $\pm$ 5.1 (16)	23.6 $\pm$ 14.0 (12)	18.5 $\pm$ 9.5 (16)								
<i>Setaria sphacelata</i> var. <i>sphacelata</i>	1 $\pm$ 0 (4)	1 $\pm$ 0 (7)	1 $\pm$ 0 (6)	1 $\pm$ 0 (7)	121.7 $\pm$ 47.0 (3)	77.3 $\pm$ 22.8 (4)								
Common on non serpentine														
<i>Bulbostylis oritrephes</i>			12.2 $\pm$ 11.3 (9)	14.8 $\pm$ 7.1 (10)	3.3 $\pm$ 0.7 (9)	3.5 $\pm$ 0.5 (9)	17.7 $\pm$ 8.4 (3)	43.3 $\pm$ 60.3 (3)						
<i>Eragrostis racemosa</i>			8.4 $\pm$ 5.2 (20)	no data	14.1 $\pm$ 4.1 (15)	no data								
<i>Helichrysum nudifolium</i>				1 $\pm$ 0 (12)		40.7 $\pm$ 15.4 (7)		109 $\pm$ 76.5 (4)						
<i>Kohautia amatymbica</i>	5.1 $\pm$ 4.7 (12)	4.5 $\pm$ 3.8 (6)	2.5 $\pm$ 1.8 (24)	2 $\pm$ 1.3 (20)			7.6 $\pm$ 3.2 (27)	6.2 $\pm$ 1.8 (20)						
<i>Ledebouria marginata</i>			2.1 $\pm$ 1.3 (9)	no data	27.8 $\pm$ 11.3 (8)	no data								
<i>Scabiosa columbaria</i>	1.6 $\pm$ 0.8 (11)	1.8 $\pm$ 0.8 (6)	4.5 $\pm$ 3.7 (17)	3.4 $\pm$ 2.0 (14)			2.5 $\pm$ 1.1 (8)	4.36 $\pm$ 2.0 (14)						

**Chapter 5: An analysis of gene flow patterns in two
Indigofera taxa from serpentine soils in South Africa.**

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An analysis of gene flow patterns in two *Indigofera* taxa from serpentine soils in South Africa

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Abstract: *Amplified Fragment Length Polymorphism (AFLP) markers were used to investigate whether ecotype formation occurred among populations of *Indigofera hedyantha*, a taxon that appeared to be most common on serpentine soils on the Witwatersrand. Five natural populations were investigated; two on and two off serpentine soils from the Witwatersrand and one from the serpentine soils of the Barberton Greenstone Belt area. A similar study was undertaken on two natural populations of *I. dregeana* from the Barberton Greenstone Belt area in order to determine whether ecotypes are more likely to arise on the more toxic serpentine soils of this area. Twenty three leaf samples of *I. hedyantha* were collected on individuals growing on serpentine soils and 24 on non serpentine soils on the Witwatersrand while eight samples were located on serpentine soils in the Barberton area. Twenty samples of *I. dregeana* were collected on non serpentine soils and 19 from serpentine soils. For *I. hedyantha* AFLP primers gave rise to 39 discernible DNA bands while 41 bands were obtained for *I. dregeana*. At the species level, high genetic diversity was detected with N_m : 16.13; H_T : 0.245; H_S : 0.238 for *I. hedyantha* at the main study site, Ruimsig, and N_m : 2.95; H_T : 0.244; H_S : 0.208 for *I. dregeana*. Despite some gene flow, the F_{ST} value for the *I. dregeana* populations indicated that the two populations were genetically different. This was not the case for the populations of *I. hedyantha* where some gene flow (N_m :1.48) occurred among all *I. hedyantha* populations.*

It was concluded that genetic differentiation does not occur on serpentine soils when the concentration of heavy metals in the soils is low.

Keywords: serpentine soils, *Indigofera hedyantha*, *Indigofera dregeana*, AFLP, gene flow, metal tolerance, ecotype formation.

Introduction:

Many serpentine soils are known to support a unique flora with many endemic species (McNair, 1992; Westerbergh and Saura, 1992; Williamson *et al.*, 1997; McCallum and Balkwill, 2004). These endemic serpentine plants provide useful tools for studying how natural selection may result in speciation events (Gögler *et al.*, 2009). The endemic taxa that occur here may either be paleoendemics, growing on serpentine soil refugia, or neoendemics (Williamson *et al.*, 1997). As a result, serpentine soils have long been considered as living laboratories in which to study the mechanisms of plant speciation and consequently evolution (McNair, 1992; Westerbergh and Saura, 1992; Brady *et al.*, 2005). This is because serpentine soils are usually characterized by high concentrations of heavy metals such as nickel, chromium and cobalt; low Ca:Mg ratios in which calcium is present at significantly lower concentrations relative to non serpentine areas, and a high water deficit, all of which can adversely affect plant performance (Alados *et al.*, 1999).

Tolerance to extreme levels of heavy metals in the soil has provided a fertile field for botanical investigation and has been considered as a typical example of local adaptation (Pollard *et al.*, 2002). Individuals taken from soils contaminated by mine workings have much greater tolerance to heavy metals than individuals of the same species taken from closely adjacent areas of uncontaminated soil (Jain and Bradshaw, 1966; McNeilly, 1968; McNeilly and Bradshaw, 1968; Antonovics and Bradshaw, 1970; Antonovics *et al.*, 1971). Soils with elevated concentrations of heavy metals are phytotoxic, therefore these areas represent very harsh and

restrictive habitats for plants (Antonovics *et al.*, 1971). Exposure to these toxic soils can result in changes in genetic population structure, due to founder effects, hybridization and adaptation, and this often results in changes in genetic diversity (Yap *et al.*, 2007). Although gene flow between the local populations can be great, the selection to tolerance for toxic soils can be strong enough to create and maintain genetic population structure. Selection, coupled with restricted gene flow, can maintain ecotypes or sub populations that are adapted to local conditions (Kittelson and Maron, 2001).

Ecotype formation depends upon the interaction between genotypes and the environment. Since genotype performance varies in different environments, these interactions are potentially reversible. Adaptations to specific environments do not always result in obvious morphological differences (Bickford *et al.*, 2006). Whether a species evolves as a cohesive unit depends critically on the dynamic balance between homogenizing gene flow among populations and potentially disruptive local adaptation (Sambatti and Rice, 2006; Garant *et al.*, 2007). High dispersal between patches, or patches with similar environmental conditions may prevent local adaptation (Allendorf, 1983).

With advances in molecular techniques, the earlier, indirect, view of the effectiveness of gene flow and its relevance to the genetic structuring of natural populations (Levin *et al.*, 1970; Ehrlich and Raven, 1976; Levin and Kerstner, 1978; Levin, 1981) has been challenged (Linhart and Grant, 1996). The three most common techniques for multilocus genomic fingerprinting are random amplified polymorphic DNAs (RAPDs), inter simple sequence repeats (ISSRs) and AFLP.

The AFLP technique is a quick and powerful process, which provides results within a relatively short space of time (Castiglioni *et al.*, 1999; Najimi *et al.*, 2002; Lin *et al.*, 2005). No prior sequence knowledge of the DNA of interest is

required (Vos *et al.*, 1995; Muluvi *et al.*, 1999). It can simultaneously detect polymorphisms in many loci allowing for an assessment of genome-wide variation (Gobert *et al.*, 2002; Meudt and Clarke, 2007) and a large number of highly reproducible, informative bands can be produced from a single PCR reaction (Castiglioni *et al.*, 1999; Miyashita *et al.*, 1999; Vanhala *et al.*, 2004). The ability to differentiate individuals in a population makes the technique useful for paternity analyses, for gene flow studies (Robinson and Harris, 1999) and the construction of genetic maps for species with large and/or poorly investigated genomes (Schlötterer, 2004). AFLP band patterns are species-specific and can be used to identify closely related taxa that would be difficult to identify by other methods (Han and Ely, 2002). AFLPs have proved successful in genetic diversity studies of a number of leguminous crop plants (Guzmán *et al.*, 2005). The technique is based on the amplification of fragments created by digestion with restriction enzymes (Vos *et al.*, 1995) and can be performed with very small amounts of DNA (Castiglioni *et al.*, 1999; Najimi *et al.*, 2002).

On the Witwatersrand, gene flow might either act as a cohesive force in populations or divergence may take place despite gene flow. If gene flow still occurs between *bodenvag* taxa (taxa apparently indifferent to the serpentine condition), then the implications are that the *bodenvag* taxa are cohesive genetic units but the possibility should not be ruled out that divergence could still occur despite gene flow. If gene flow is limited, then gene flow between *bodenvag* taxa would be limited and one would expect there to be ecotypic differentiation. However, gene flow could still be maintained if seeds are dispersed over longer distances (Hanson *et al.*, 2007; García *et al.*, 2007) and are capable of surviving on serpentine soils.

The serpentine areas on the Witwatersrand, unlike those of the Barberton Greenstone Belt area, consists of relatively small patches (Reddy *et al.*, 2001) of serpentine areas surrounded by large patches of non serpentine areas (Figure 1).

Soil analyses showed that the serpentine soils on the Witwatersrand were less toxic than the Barberton or Swaziland serpentine areas (Reddy *et al.*, 2001). The southern African serpentine rocks are far older than many of the northern hemisphere serpentine rocks, but there is uncertainty as to the length of exposure of these rocks (Anhaeusser, 1973). However, the southern hemisphere did not experience as much glaciation as the northern hemisphere during the ice ages (McCarthy & Rubidge, 2005), therefore it is possible that these areas were exposed for a longer period, providing a longer length of time for plants to evolve. Although the grassland biome, where the Witwatersrand, Barberton and Swaziland serpentine floras occur, only began to reflect modern conditions about 7,000 years BP (Bredenkamp *et al.*, 2002), it may have originated between 200 – 300 million years ago (Brooks, 1987). This indicates that there has possibly been sufficient time for the formation of endemic taxa in these serpentine areas. The Barberton area has 29 endemics and Swaziland serpentine outcrops have six, but no endemic taxa occur on the Witwatersrand serpentine soils. Diversity studies showed that adjacent non serpentine areas appeared to be more similar to the serpentine sites than to each other (Reddy *et al.*, 2009) and that there was a high, but similar, diversity on serpentine and non serpentine soils (Reddy *et al.*, 2009). An investigation of the reproductive and life history data (Chapter 4) indicated that the study taxa did not appear to separate into ecotypes, but was it possible that ecotypes could have differentiated without any morphological changes?

The aim of this study was to determine whether gene flow occurs between populations of *Indigofera hedyantha* Eckl. and Zeyh., a common serpentine tolerant taxon, and whether the sub populations are genetically different on and off serpentine soils. *Indigofera hedyantha* does not appear to be as common on the Barberton Greenstone Belt (BGB) serpentine soils as it is on the Witwatersrand serpentine soils. Thus, in order to compare gene flow patterns in a plant from a more toxic serpentine soil, *I. dregeana* E. Mey., a species related to *I. hedyantha*, that appeared to be *bodenvag* on the Barberton serpentine soils, was investigated.

It was predicted that gene flow still occurs amongst *I. hedyantha* populations on the Witwatersrand and that subpopulations on and off serpentine soils were genetically similar. It was also predicted that reduced gene flow was more likely to occur in the BGB serpentine area because of the more toxic soils that occur there thus enabling ecotype formation to occur.

MATERIALS AND METHODS:

Study sites and sample collection —

Table 1 summarises the location of the populations and some of the soil properties of these locations. A geological map of the area identified the serpentine and non serpentine areas for the Barberton samples and rock samples collected from the potential non serpentine area was confirmed as being non serpentiniferous by Prof C.R.Anhaeusser of the Economic Geology Research Institute of the University of the Witwatersrand. Soil data for the Barberton serpentine soils were taken from the literature for this area (Morrey *et al.*, 1992, S. Williamson unpublished data).

Witwatersrand:

Indigofera hedyantha appeared to occur more commonly on serpentine than non serpentine soils (Reddy *et al.*, 2009). It was thus selected for further studies in order to determine whether or not it had differentiated or was in the process of differentiating into serpentine and non serpentine ecotypes.

Forty samples of *I. hedyantha* were collected in March 2007 from the Ruimsig Entomological Reserve (26° 04' S 27° 51' E, 1550 m) (Figure 1, site 3). Twenty samples each were collected from the serpentine and adjacent non serpentine soils. Ten from each soil type were collected close to the interface area of the two soil types and the remaining ten each from about 500 m deeper into the contrasting soil types. Results were obtained for three samples from the Diamond Four serpentine site (Figure 1, site 5) and for four samples from Honingklip, its

relatively close non serpentine site. The distance between these two study sites is about 4.7 km.

Table 1. A summary of the location of the study populations and some of the soil properties at these locations. ND = no data. (Soil data for the Barberton serpentine area from Morrey *et al.*, 1992, S. Williamson unpublished data).

Population	species	Location (n)	latitude (South)	longitude (East)	altitude (m)	Mg:Ca	Fe	Total Ni	Cr
1	<i>I. hedyantha</i>	Ruimsig ns (20)	26°04 71	27°51 26	1540	1.1	51300	203.89	614.4
2		Ruimsig s (20)	26°05 36	27°52 09	1540	1.5	45520	559	976.89
3		Diamond Four s (3)	26° 01'	27° 48'	1520	2.3	60700	1259.4	1191.6
4		Honingklip ns (4)	26° 01'	27° 44'	1540	1.5	32070	147.56	544.21
5		Barberton s (8)	26° 00 24	30° 52 50	850	4.3	73684	1452.2	2569
6	<i>I. dregeana</i>	Barberton ns (20)	26° 00 39	30° 55 19	870	ND	ND	ND	ND
7		Barberton s (19)	26° 00 37	30° 55 22	876	4.3	73684	1452.2	2569

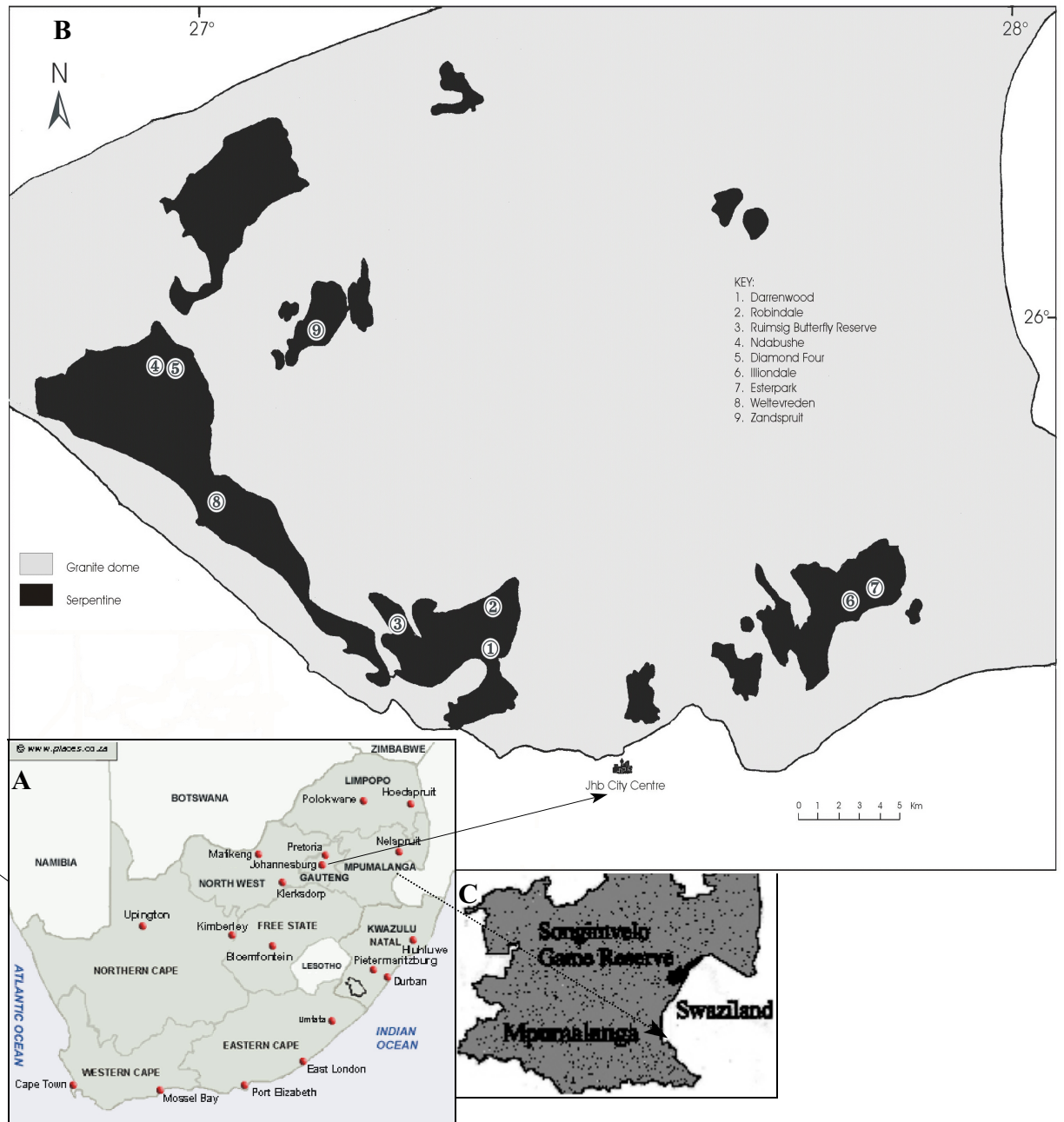


Figure 1. Maps indicating the localities of the study sites. A: map showing the provinces, B: the Witwatersrand sites, C: the Barberton site.

Barberton:

In order to compare the results from the Witwatersrand serpentine soils with the results obtained from a more toxic serpentine soil, an attempt was made in May 2008 to collect specimens of *I. hedyantha* from the Songimvelo Nature Reserve (31°01'59"E, 25°55'0"S) in the Barberton Greenstone Belt area, approximately 400 km east of Johannesburg (Figure 1). Unfortunately, *I. hedyantha* is not as common in this area as on the Witwatersrand and only eight samples, from serpentine soils, were located at the Songimvelo Game Reserve. It was then decided to collect another species of *Indigofera* from the Barberton Greenstone Belt area that grows both on and off serpentine soils in order to determine whether the results were any different from those obtained for the *I. hedyantha* samples from the Witwatersrand. *Indigofera dregeana* was found to grow both on and off serpentine soils in the Barberton Greenstone Belt area. Nineteen samples were collected on the serpentine soil and 20 on the adjacent non serpentine soil.

All samples were collected into silica gel, weighed and stored at -75°C until the DNA was extracted.

Study species — *Indigofera hedyantha* is a fairly widespread species. It is a perennial herbaceous shrublet, up to about 300 mm tall. Stems are many, erect and densely leafy. The leaf rachis is about 15 mm long. Leaves are once compound with 2 – 5 pairs of pinnae, each often folded upwards along the mid-rib, glabrous above and silky hairy below. Inflorescences consist of short, compact spikes of up to five per stem. The spikes are longer than the leaves and elongate further when fruiting. The calyx is densely covered in fine black hairs. Buds are crowded with black or brownish appressed hairs on the outer surface. The flowers are dark red and 8 – 12 mm long. Pods are cylindrical. Life history and breeding system studies (Reddy *et al.*, in prep.) indicated that this taxon was a facultatively autogamous, spring-flowering, summer-fruiting hemicryptophyte. *Indigofera hedyantha* did not set seed when pollinators were excluded indicating that

outcrossing was preferred. The fairly heavy, rounded seeds were dispersed explosively, but not very far from the parent plant. However, the shape of the seeds ensured that they could roll a small distance away from the parent plant if the ground is not level or could be rolled further along during rainstorms. No insects were observed visiting the plants, but flower colour implies that it is probably bee pollinated.

Indigofera dregeana is a dwarf shrub up to 500 mm tall. Leaflets are pale green above and darker green and hairy below, the terminal leaflet is narrowly obovate and up to 17 mm long. Flowers are red or purple within, the standard has brown hairs on the outer surface. Pods are long, cylindric and are spreading to erect. Life history data were not collected for this taxon.

Both taxa belong in the section Pinnatae of the tribe Indigoferae. In both taxa flowering and fruiting occur from late spring to mid summer (van Wyk and Malan, 1997; Pooley, 1998; Retief and Herman, 1997) and both occupy grassland habitats.

DNA extraction, AFLP technique, staining and scoring procedures —

Approximately 0.1 g of leaf tissue was taken from each sample and ground in liquid nitrogen. DNA was extracted using the Qiagen DNeasy[®] plant mini kit according to the manufacturer's protocols. The extracted DNA was stored at -20°C. AFLP analysis was performed on nuclear DNA using a modified method of Vos *et al.* (1995) using MseI and ApoI adaptors and primers and MilliQ[®] water (Millipore) throughout. The double stranded adaptor sequences used were as follows: ApoI adaptor forward, 5' CTC GTA GAC TGC GTA CC and reverse CAT CTG ACG CAT GGT TAA-5'. MseI adaptor forward 5' GAC GAT GAG TCC TGA G and reverse TA CTC AGG ACT CAT-5' (Durrant *et al.*, 2000; Angus, 2005).

The pre-selective ApoI primer contained no selective nucleotides, while the MseI primer contained one selective nucleotide. The sequences used were as follows: ApoI primer (WD16): 5' CTC GTA GAC TGC GTA CCA ATT 3' and MseI primer (WD19): 5' GAC GAT GAG TCC TGA GTA A 3' (Sanchez *et al.*, 2000; de Torres Zabela, *et al.*, 2002; Angus, 2005). Pre-selective PCRs of 40 µl were performed containing 100 ng of each pre-selective primer, 20 µl of Fermentas 2X PCR Master Mix and 10 µl of the digested/ligated DNA mixture. Only one set of primers was used for each species as this was found to produce sufficient numbers of polymorphic bands to allow meaningful analysis. The pair of primers used for *I. hedyantha* did not give clear bands for *I. dregeana*. Several combinations of primers were then tested for *I. dregeana* and the best pair selected. Selective PCR primers chosen contained one selective nucleotide for both the ApoI and MseI primers for *I. hedyantha* and the ApoI primer of *I. dregeana* and no selective nucleotide for the MseI primer of *I. dregeana*. The sequences used were as follows: *I. hedyantha*: ApoI primer (WD18): 5' GAC TGC GTA CCA ATT CCC A 3' and MseI primer (WD38 + C): 5' GAT GAG TCC TGA GTA ACT C 3'. *I. dregeana*: ApoI primer (EcoC): 5' GAC TGC GTA CCA ATT CC 3' and MseI primer (WD30): 5' GAT GAG TCC TGA GTA A 3'. For both study taxa, 30 µl selective PCRs were performed containing 3 µl diluted pre-selective PCR products and 15 ng ApoI selective primer, 30 ng MseI selective primer, 15 µl of 2X PCR Master Mix.

The 10% polyacrylamide gels, made up of 10% acrylamide, 0.25% methylene bisacrylamide, and 7.5 M urea in 1X TBE (0.089 M tris, 0.089 M boric acid, and 0.002 M EDTA, pH 7.8), were cast in a 18 cm x 16 cm x 1 mm gel apparatus. A 10 well comb with well sizes of 0.8 cm x 1.5 cm was used. Gels were run at 50 V for 30 minutes and then run at 500 V for approximately 120 minutes. Following electrophoresis, gels were removed from the plates and stained immediately. The method of Angus (2005), which had been adapted from Blum *et al.* (1987) as well as that of Creste *et al.* (2001) (Table 2), was followed to stain the gels. The

duration of rinses was decreased (Table 2) to retain more pre-treatment and impregnate solutions in the gels, thus increasing band staining. The gels were developed for longer (Table 2) and the development reaction was not stopped. This ensured that bands developed to their maximum (Angus, 2005). The gels were stored overnight at 4° C; which shrank the gels and concentrated the bands, making them easier to resolve and score. The gels were placed between two glass sheets on a light table and the bands were scored manually by determining the presence (1) or absence (0) of bands between approximately 75 and 1500 base pairs in size. Gels were also scanned using an Epson Expression 1640XL scanner in order to have a permanent, digital record, of each (Figure 2).

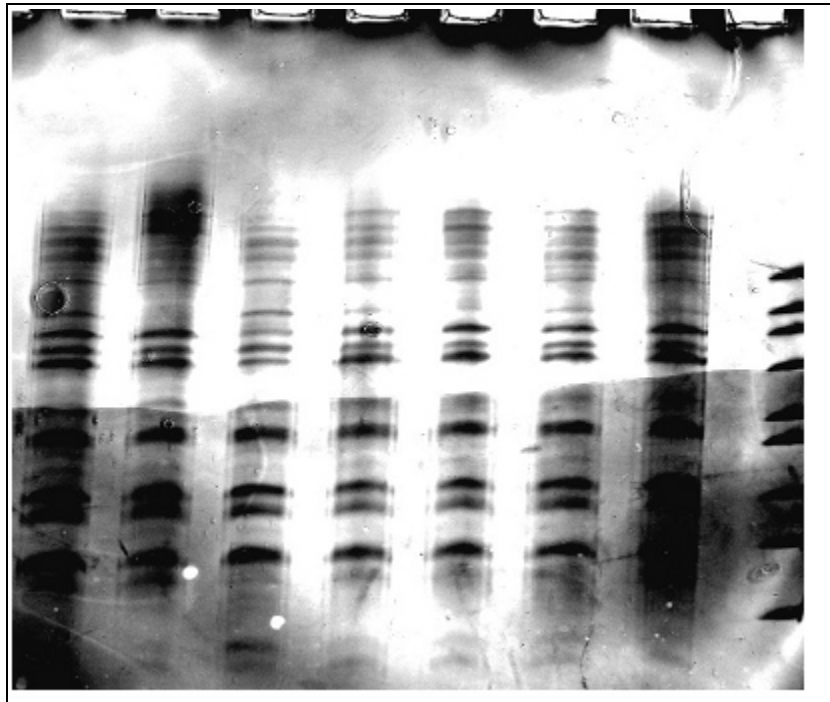


Figure 2. A sample silver stained polyacrylamide gel showing amplified fragment length polymorphisms from *I. dregeana*.

Table 2: Silver staining protocol, adapted from Angus (2005) and Creste *et al.* (2001).

Steps	Solutions	Time of treatment
1. Fix	1% ethanol, 5% acetic acid	10 minutes
2. Wash	MilliQ® water	1 minute
3. Pretreat	1.5% Nitric Acid	3 minutes
4. Wash	MilliQ® water	1 minute
5. Impregnate	0.2% silver nitrate	20 minutes in the dark
6. Rinse	MilliQ® water	2 x 5 seconds
7. Develop	6% Sodium carbonate, 0.0008% sodium thiosulphate, 37% formaldehyde	1 x 30 minutes, 1 x 20 minutes
8. Store	50% methanol	Overnight

Statistical Analysis — All calculations assume Hardy Weinberg equilibrium. POPGENE v. 1.3 (Yeh *et al.*, 1997) was used to estimate polymorphism parameters at both the population and species levels, the number and percentage of polymorphic bands (regardless of allele frequencies), the Shannon information index (I), an estimate of the relative degree of genetic variation within population and Nei's (1973) gene diversity statistic (h), a statistic similar to 'I'. In addition, POPGENE v. 1.3 was used to determine Nei's gene diversity statistics for overall population ($H_T = H_S + D_{ST}$), the diversity within sub- populations (H_S) and the estimated amount of gene flow (Nm). The output from Nei's genetic distance matrices were then used in MEGA version 4 (Tamura *et al.*, 2007) to generate a dendrogram of populations using the unweighted pair grouping (UPGMA) method.

The program AFLP-SURV (Vekemans *et al.*, 2002) was used to calculate Wright's fixation index (F_{ST}). The gene diversity within populations and the population genetic structure were calculated according to the Lynch and Milligan (1994) method. This value indicates the reduction in heterozygosity that has occurred in a group because of genetic drift and is calculated using the formula: $F_{ST} = (H_T - H_S / H_T)$. Permutation tests for genetic differentiation among groups were performed;

for this test the null hypothesis was that there is no genetic differentiation among the groups.

Principal Coordinate Analysis (PCoA) was performed on the standardised distance matrix using the NTSYSpc 2.02 program (Rolf, 1997) to estimate genetic distances among the major groups. The SIMQUAL option with a simple matching (SM) coefficient was used. The matrix was then double centred and used in the ordination to obtain two dimensional scattergrams for the populations from the Ruimsig and Barberton sites.

RESULTS

I. hedyantha

Band Patterns

Thirty-nine bands were observed for *I. hedyantha*. The Ruimsig populations had the highest number of polymorphic bands, on the Witwatersrand, *I. hedyantha* had 28 (71.8 %, $\bar{x} = 11$) and 16 (41.0 %, $\bar{x} = 10$) polymorphic bands respectively at the Ruimsig and Diamond Four/Honingklip sites (Table 3). The Ruimsig site showed a high degree of polymorphism both on and off serpentine soils, but this could be because of the smaller sample sizes from the other study areas.

Table 3. Genetic structure of *I. hedyantha* samples from all localities, h = Nei's (1973) gene diversity, I = Shannon's information index.

1= Ruimsig ns, 2 =Ruimsig s, 3 = Diamond Four s, 4 = Honingklip s, 5 = Barberton s; ns = non serpentine, s = serpentine.

Population	No. Individuals	No. polymorphic bands (%)	$h (\bar{x} \pm \text{S.D.})$	$I (\bar{x} \pm \text{S.D.})$
1	20	27 (69.2)	0.208 ± 0.173	0.324 ± 0.253
2	20	28 (71.8)	0.267 ± 0.204	0.393 ± 0.286
3	3	16 (41.0)	0.147 ± 0.187	0.222 ± 0.276
4	4	16 (41.0)	0.156 ± 0.198	0.231 ± 0.288
5	8	14 (35.9)	0.157 ± 0.219	0.224 ± 0.310

Genetic Analysis:

The Ruimsig serpentine population (2) had a higher gene diversity than the adjacent non serpentine area (1) (Table 3), while the converse was true of the Diamond Four/Honingklip pair (3 & 4) where the non serpentine site displayed a higher gene diversity. The Barberton sample (5) had a similar gene diversity to that of the Honingklip non serpentine site on the Witwatersrand.

The genetic distances indicate that each serpentine and its nearby non serpentine site are more closely related to each other than to the other populations (Table 4). The Barberton population is on a distinct branch from the Witwatersrand ones (Figure 3). At both Witwatersrand sites, within group diversity was the major contributor towards total variation, indicating a low genetic differentiation between populations from geographically separated areas. The Ruimsig site, perhaps because of the geographic proximity of the two sub-populations, has a higher within group diversity than the Diamond Four/Honingklip site. The genetic distance of the samples between adjacent sites or sites closer to each other is closer than between sites situated further apart.

As expected, the genetic identities and distances (Nei, 1978) of *I. hedyantha* between the paired serpentine and non serpentine populations were very high (Table 4). The Ruimsig serpentine population (2) had the greatest affinity to the Barberton serpentine population (5), in fact, both the serpentine and non serpentine populations from Ruimsig showed a slightly closer genetic identity to the Barberton serpentine than the Diamond Four/Honingklip sites.

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for all populations of *I. hedyantha*.

1= Ruimsig ns, 2 =Ruimsig s, 3 = Diamond Four s, 4 = Honingklip s, 5 = Barberton s; ns = non serpentine, s = serpentine.

Population	1	2	3	4	5
1	****	0.98	0.87	0.91	0.69
2	0	****	0.85	0.9	0.74
3	0.13	0.16	****	0.93	0.63
4	0.1	0.11	0.1	****	0.67
5	0.38	0.3	0.47	0.4	****

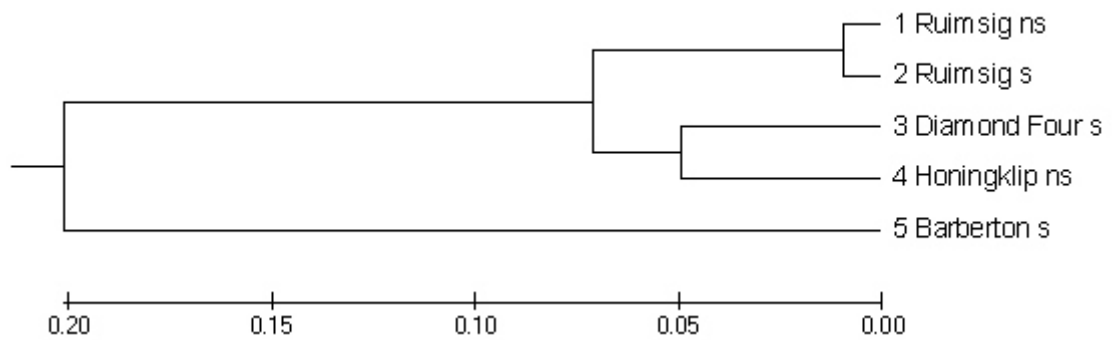


Figure 3. Dendrogram of all *I. hedyantha* study sites based on Nei's (1972) genetic distance derived from the allele frequency of 39 AFLP markers within each population. The scale indicates genetic distance between populations as clustered with UPGMA.

Intra-population Nm at the Ruimsig site was the highest (Table 5). This is possibly because the two areas are very close to each other. The Diamond Four/Honingklip areas are about 4.7 km apart, so it is to be expected that gene flow will be less but even this value indicates that gene flow does occur. The greater distance between the Diamond Four serpentine site and the Honingklip non serpentine site meant that the within group diversity was lower for this pair of sites than for the Ruimsig site.

All the Witwatersrand sites of *I. hedyantha* are closer to each other than to the Barberton site, thus the within group diversity of the Witwatersrand populations is less than the within group diversity between the Witwatersrand and Barberton sites, indicating a higher level of genetic differentiation between these areas.

Table 5. An estimate of Nm and Nei's gene diversity statistics for subdivided populations, showing the genetic structure of the overall population of samples of *I. hedyantha* from the Witwatersrand and Barberton. H_T = total genetic diversity, H_S = diversity within groups.

Site	Nm	$H_T (\bar{x} \pm \text{S.D.})$	$H_S (\bar{x} \pm \text{S.D.})$
Witwatersrand	1.48	0.260 ± 0.027	0.195 ± 0.019
Ruimsig	16.13	0.245 ± 0.037	0.238 ± 0.034
Diamond Four/ Honingklip	1.89	0.191 ± 0.038	0.151 ± 0.027
Witwatersrand vs Barberton serpentine	0.64	0.340 ± 0.025	0.190 ± 0.017

The observed F_{ST} value for the Ruimsig populations was 0.024 (S.E.: ± 0.122) and upper 99% limit, 0.050 ($P = 0.054$), and the observed F_{ST} value for Diamond Four serpentine/Honingklip non serpentine data was 0.024 (S.E.: ± 0.064) and the upper 99% limit was 0.160 ($P = 0.362$). At both the Ruimsig and Diamond Four/Honingklip sites, these values are lower than the values of F_{ST} lying at the 1% uppermost part of the distributions. Thus in both cases the null hypothesis is accepted, implying that the groups at each site are not genetically differentiated. However, this is a very small sample which is also indicated by the fact that the standard error is larger than the mean, so more data is required to confirm this observation. When all four of the Witwatersrand populations were compared, then the observed F_{ST} value was 0.106 (S.E.: ± 0) and the upper 99% limit was 0.213 ($P = 0.0350$). This value is also lower than the expected value of F_{ST} lying at the 1% uppermost part of the distribution. Again the null hypothesis is accepted, indicating that all the groups being tested are similar to each other. When the Barberton

population of *I. hedyantha* is included, the observed F_{ST} value for all five populations was 0.301 (S.E.: ± 0.103) and the upper 99% limit was 0.220 ($P=0.0030$). Thus the observed value is higher than the value of F_{ST} lying at the 1% uppermost part of the distribution. Implying that the null hypothesis is rejected and it can therefore be concluded that there is genetic differentiation among the groups being tested. Which is to be expected, given the distance between the Witwatersrand and Barberton sites, perhaps a study of geographically intermediate populations may show otherwise.

I. dregeana

Band Patterns:

Forty-one bands were observed for *I. dregeana*; the mean number of bands per individual was 13.3. The total number of polymorphic bands was 23 for the non serpentine soil population and 27 for the serpentine soil population (Table 6). The non serpentine population (6) had fewer polymorphic bands. There was a negative correlation between fragment sizes and frequencies.

Table 6. Genetic structure of samples of two Barberton populations of *I. dregeana*. h = Nei's (1973) gene diversity, I = Shannon's information index. 6 = Barberton ns, 7 = Barberton s; ns = non serpentine, s = serpentine.

Population	n	No. polymorphic bands (%)	$h (\bar{x} \pm \text{S.D.})$	$I (\bar{x} \pm \text{S.D.})$
6	20	23 (56.1)	0.197 ± 0.208	0.292 ± 0.296
7	19	27 (65.9)	0.220 ± 0.211	0.327 ± 0.296

Genetic Analysis:

The serpentine population (7) showed a greater, but not significant, gene diversity than the non serpentine population (6) (Table 6). The Nm value between the serpentine and non serpentine groups was 2.95 which indicates that gene flow does occur between these two populations. The total genetic diversity was 0.244 (± 0.045 SD) and diversity within the group was 0.208 (± 0.035 SD). The observed F_{ST} value for the two *I. dregeana* populations was 0.183 (S.E.: ± 0.047) and the upper 99%

limit was 0.083 ($P = < 0.00001$). This is higher than the expected value of F_{ST} lying at the 1% uppermost part of the distribution. Thus the null hypothesis is rejected and it can be concluded that there is genetic differentiation between the groups being tested.

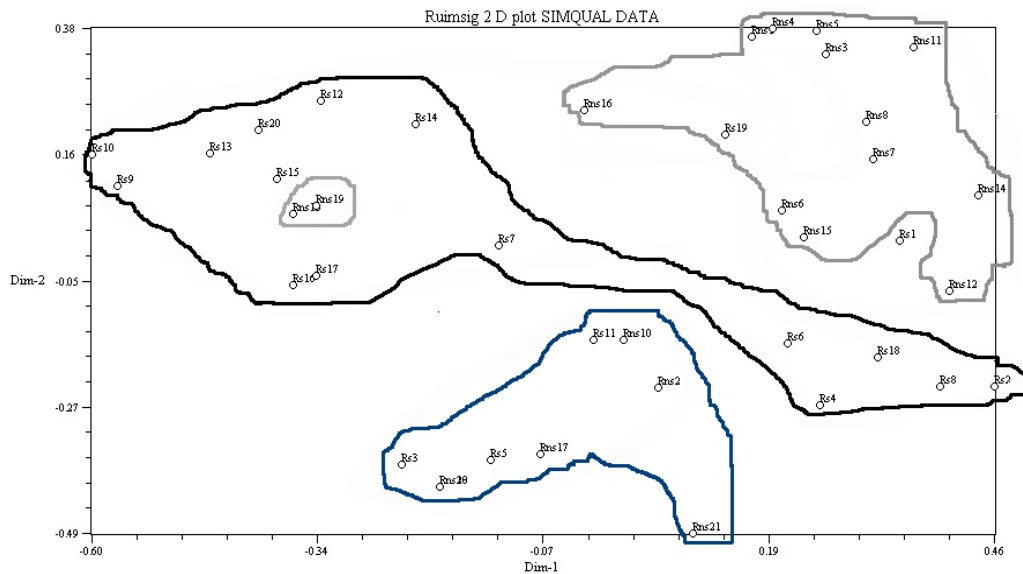


Figure 4. PCoA plot of AFLP bands from the distribution of the serpentine and non serpentine populations of *I. hedyantha* at the Ruimsig site. The grey areas consist of mainly the non serpentine samples, the black area consists of mainly the serpentine samples and the blue area consists of a mixture of serpentine and non serpentine samples.

Figure 4 graphically illustrates that gene flow does occur at the Ruimsig study site because there is an intermingling of the serpentine and non serpentine populations. The percentages of variance revealed by the first and second axes were 19.7 and 13.2 %, respectively. The populations of *I. dregeana* from the Barberton Greenstone Belt area (Figure 5) appear to form two distinct groups, with the exception of sample Bns 7. The percentages of variance revealed by the first and second principal components axes were 22.9 and 18.6 %, respectively. It is possible that sample Bns 7 was either collected on a non serpentine microsite or it may be serpentine ecotype seed that germinated on non serpentine soil.

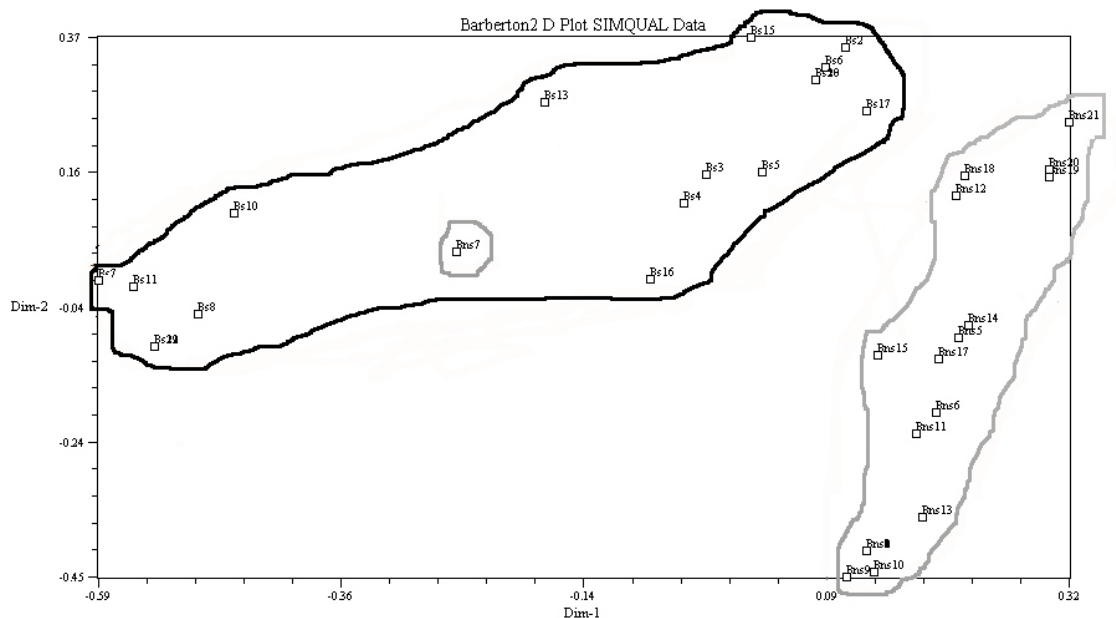


Figure 5. PCoA plot of AFLP bands from serpentine and non serpentine populations of *I. dregeana* at the Songimvelo, Barberton site. The grey area includes the non serpentine samples, the black area includes all of the serpentine samples. Bns 7 is an aberrant non serpentine sample.

DISCUSSION

A comparison of the gene flow amongst all the Witwatersrand populations indicates that gene flow occurs, to some extent, among all the populations on the Witwatersrand (Nm : 1.48). Estimates of $Nm < 1.0$ indicate relatively little gene flow (Soltis and Soltis, 1987). Geographic distance among populations may affect the magnitude of Nm and the analysis of adjacent populations may result in a higher value of estimated Nm than in populations sampled over greater distances, however for species with high interpopulational gene flow values ($Nm > 2$), this source of bias is probably small (Slatkin, 1985). The Diamond Four/Honingklip sites are separated by a distance of 4.7 km, but the Nm value (1.89) indicates that gene flow still occurs and the value is fairly high so it is unlikely that the distance between the sites causes a bias in the results. However, the distance between the Diamond Four serpentine

and Honingklip non serpentine sites as well as the reduced gene flow between these two sites, as compared to the adjacent populations at the Ruimsig and Barberton sites, results in fewer polymorphic bands in common occurring at these sites since the number of polymorphic bands in common decreases with geographic distance (Miyashita *et al.*, 1999). This is because genetic diversity increases with geographical distance (Oiki *et al.*, 2001).

The Ruimsig and the Barberton sites have comparable spatial arrangements but there is slightly more within group diversity at the Barberton site than at the Ruimsig site. The *N_m* value at the Barberton site is very low when compared to the Ruimsig site, but higher than the Diamond Four/Honingklip site. This pattern is not common only in natural populations growing on toxic soils because Angus (2005) also found a similar within group genetic differentiation pattern for two *Indigofera* taxa on artificially created, highly toxic mine dumps on the Highveld. The Witwatersrand serpentine soils have been found to be less toxic than the Barberton Greenstone Belt (BGB) serpentine soils (Reddy *et al.*, 2001) and are also less toxic than the soils of mine dumps. It is to be expected then, that plants growing on the serpentine soils on the Witwatersrand are not growing under as stressed conditions as are the plants from the BGB serpentine soils or the mine dumps. Thus there may be less selective pressure to form ecotypes on the Witwatersrand serpentine soils since adaptive diversification is driven by selection in ecologically different environments (Räsänen and Hendry, 2008).

The genetic diversity for both the Ruimsig and the Barberton sites was higher on serpentine soils. A study comparing the genetic variation in populations of *Arrhenatherum elatius*, a perennial grass, growing in natural pasture with populations growing on mine spoils reported a similar finding and suggests that the tolerant populations may have been built up from a large number of tolerant genotypes emanating from the normal pasture populations in which tolerance genes are common (Ducousso *et al.*, 1990). If there is a large difference in selective effects

between two environments, then even large amounts of gene flow are unable to prevent the formation of population differentiation along steep clines (Endler, 1973; Joshi *et al.*, 2001; Bischoff *et al.*, 2006; Becker *et al.*, 2008). There appears to be a large difference in soil chemical concentrations between the serpentine and non serpentine soils of the BGB area, so it is likely that the *I. dregeana* populations consist of serpentine tolerant ecotypes. Indeed, the PCoA results seem to suggest that ecotype formation has taken place within these populations.

F-statistics analyse genetic structure within and among population subdivisions (Gibson *et al.*, 2008) and indicate the reduction in heterozygosity due to genetic drift. Thus the F-statistic is also known as the inbreeding coefficient. F_{ST} is a mean measure of relatedness of individuals within a sub population. High F_{ST} values indicate high levels of differentiation within rather than between groups. If the observed F_{ST} value is higher than the value of F_{ST} lying at the 1% uppermost part of the distribution, the null hypothesis is rejected, and it can be concluded that the groups being tested are more genetically differentiated than random assemblages of the individuals making up those groups (Vekemans *et al.*, 2002). All the Witwatersrand taxa appeared to be genetically similar, both at each site (or for a pair of sites) and collectively. In contrast, the data for *I. dregeana* showed that there was more of a difference between the serpentine and non serpentine soil populations, despite there being very little physical distance between the two populations. Again, this could imply that ecotype formation is more likely to have occurred at the Barberton site.

The within group diversity values are high and the F_{ST} values for all the Witwatersrand sites ($F_{ST} = 0.106$, $P = 0.0350$) are similar. This indicates either that gene flow still occurs on the Witwatersrand or has done so until recently. In contrast, there is a lower gene flow rate for *I. dregeana* at the Barberton site, where the populations are adjacent to each other, and the F_{ST} value ($F_{ST} = 0.183$, $P = 0$) shows that there is genetic differentiation occurring between the groups. It is

possible that these two populations of *I. dregeana* could in time differentiate into ecotypes or have already done so.

Gene flow could be due to pollen flow since pollination is carried out by insect vectors, most probably bees. The seeds of these species are rather heavy and fall close to the maternal plants, it is likely, however, that secondary dispersal of these rounded seeds occurs during rainstorms and this may influence gene flow.

However, Reddy *et al.* (in prep.) found no evidence from the life history and breeding system of *I. hedyantha* that any differentiation had occurred. This evidence, however, cannot show conclusively that no ecotype formation had occurred because no reciprocal transplant experiments to test for chemical tolerance were conducted.

The broader implications of this study are that ecological factors play an important role in local adaptation and possibly, in the long term, in speciation. A better understanding of the geographical structure of genetic variation and the local adaptation of plant species across their distribution range would contribute to our understanding of the ecological and genetic processes at different scales (Picó and van Groenendael, 2007; Becker *et al.*, 2008). Local adaptation is an important aspect of nature conservation because the transfer of individuals is often proposed as a technique to replenish genetic variation in order to reduce the negative effects of low genetic variation (Raabová *et al.*, 2007). So, transferring plants may disrupt local adaptation and lead to reduction in offspring fitness or the transferred plants may not be able to survive under unfavourable conditions. A knowledge of local adaptation is important when considering the effects of transfers between populations, especially along clines (Raabová *et al.*, 2007; Becker *et al.*, 2008).

There were several limitations to this study; a lack of difference between the Witwatersrand populations could be due to the failure to detect small differences due to small sample size. It is possible that if more primers were used, then a

greater number of polymorphic bands obtained could have given greater resolution, especially for the Witwatersrand populations. The number of populations on the Witwatersrand was limited because very few of the study sites had adjacent non serpentine areas. Zandspruit was the other area that had adjacent non serpentine soils, but the initial samples collected from this site gave no results because the DNA was stored for too long and lack of access to the site prevented more samples being collected.

Future studies should investigate reciprocal transplant pot experiments for *I. dregeana* in order to determine whether or not the non serpentine populations are capable of surviving on serpentine soils, more populations from different areas should also be investigated, especially in the Barberton area and studies should involve the use of more combinations of primers for better resolution. A study of phylogenetics can perhaps help to further elucidate the degree of separation between the two populations. It would also be interesting to investigate populations of other taxa, especially in the Barberton area, in order to discover how many taxa have differentiated or are differentiating and to conduct long term studies to follow the outcome of the separations.

CONCLUSIONS

The high genetic diversity, low fixation index and high Nm values implies a high degree of gene flow between populations at the Ruimsig site. In contrast, the *I. dregeana* populations showed slightly lower genetic diversity than that of the Ruimsig populations, a higher fixation index and the Nm value was very low, this despite the Barberton populations occurring adjacent to each other, as do the Ruimsig populations. Although no soil testing was undertaken during this study for the Barberton soils, other studies (Morrey *et al.*, 1992, S. Williamson unpublished data) show that the Mg:Ca ratio and concentration of total Ni is much higher in the Barberton serpentine soils (Table 1). These factors could be contributing to the ecological differentiation of the *I. dregeana* populations.

The prediction that gene flow still occurs in the selected *bodenvag* taxon on and off serpentine soils on the Witwatersrand and that sub populations of the study taxon growing on and off serpentine soils on the Witwatersrand are genetically similar is correct. The initial work done for the Barberton Greenstone Belt serpentine taxon indicates a reduced gene flow pattern between two closely situated sites indicating that ecotype formation has possibly occurred here.

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Chapter 6: General Discussion.

General discussion

1. Characteristics of the flora on the serpentine outcrops on the Witwatersrand

Serpentine soils and their floras are extremely variable in soil composition (and include mature oxisols, serpentinised peridotite, laterites, dunites, harzburgites, olivines, pyroxenites and serpentinites), exposure (1– 75 million years), elevation (0 – 2900 m) and climate (tropical, sub-tropical, Mediterranean and temperate) (Table 1); this makes generalizations difficult (Borhidi, 1992; Ferrari *et al.*, 1992; Hunter & Horenstein, 1992; Jaffré, 1992; Jeffrey, 1992; Slingsby, 1992; McCallum, 2007). As a result, each area needs to be studied separately in order to draw a complete picture of what constitutes a serpentine flora and how this flora differs from the surrounding floras. In southern Africa botanical studies have been conducted on the vast areas of serpentine soils of the Great Dyke in Zimbabwe (Proctor & Craig, 1978; Proctor, Burrow & Craig, 1980) and the Barberton Greenstone Belt area in South Africa and Swaziland (Morrey *et al.*, 1989; Morrey *et al.*, 1992; Williamson, 1995; Balkwill *et al.*, 1997; Williamson *et al.*, 1997; Moffett, 1999; Changwe & Balkwill, 2003; McCallum & Balkwill, 2004; McCallum, 2007). The Witwatersrand serpentine area consists of relatively small, isolated patches that are confined to the edges of a granite dome. Both the serpentine and granite rock types are of an Archean origin (Anhaeusser, 1973). The small extent of this area has not, until now, warranted an in depth investigation of this area. The aim of this study, therefore, was to gain an understanding of the relationship between serpentine soils and their associated flora.

An analysis of the serpentine soils on the Witwatersrand showed that these soils were potentially not as toxic as the soils of the Great Dyke or Barberton areas (Chapter 2). No endemic, nickel-hyperaccumulating or indicator taxa have been recorded on the serpentine sites on the Witwatersrand, indicating that the concentrations of these metals in serpentine soils might be too low to affect plant growth.

Table 1. Summary and comparison of the southern African serpentine areas with some of the larger serpentine areas in the world;
a = age, hyperacc. = hyperaccumulating.

Locality	Latitude	Elevation	T°(°C) / Climate	Rainfall (mm/yr)	Area (km ²)	Age of rock (a)/ or exposure	Ni	Total Fe	Mg:Ca	soil texture	soil type	soil pH	no. hyperacc. taxa	endemics
Cuba (Brooks, 1987; Borhidi, 1992; Reeves <i>et al.</i> , 1996)	21° 30 N	0 – 1100	20 – 29 sub-tropical climate	1380 May – October	5300	1 million (young) 10 – 30 million (old)	0.1 – 5.0 % Sol.				young – magnesian sialitic & ferralitic soils Old - mature oxisols	6.0 – 6.5	80	920 (30% of total flora) 14% young, 81% old, 5% common
California (Reeves <i>et al.</i> , 1983; Kruckeberg, 1984; Martens & Boyd, 1994)	34°– 43°N	0 – 2900	12 – 29 Mediterranean	480 October – April	2860 (1% of California)	10 million	1530 – 3100 µg/g total		2.5 – 17.4		serpentinised peridotite	6.0 – 7.5	3	215 (10%)
New Caledonia (Brooks, 1987; Jaffré, 1992)	20 – 23° S		20 – 27 tropical	1000 – 2000	5500	35 – 40 million	1300 – 10400 µg/g total				laterites	5	50	253 (28%)
Great Dyke (Wild, 1974; Proctor & Cole, 1992)	16°27'51"S (north end) 20°50'22"S (south end)	1000 – 1500	12 – 26 wet-dry tropical	450 – 880	44000	? 75 million	31.1 ± 22.4mg/kg 4838 ± 2583 total	0.8 ± 0.97	0.9 – 3.7	Loam/ fine sandy loam	dunites harzburgites olivine bronzitites & pyroxenites & narrow layers of chromitite	6.1 – 6.6	5	20

Locality	Latitude	Elevation	T°(°C) / Climate	Rainfall (mm/yr)	Area (km ²)	Age of rock (a)/ or exposure	Ni	Total Fe	Mg:Ca	soil texture	soil type	soil pH	no. hyperacc. taxa	endemics
Barberton Greenstone Belt (Morrey <i>et al.</i> , 1992; van Wyk & Smith, 2001; Mucina & Rutherford, 2006)	25° 55" – 26° 10" S	350 – 1650	16 – 32 sub-tropical	950 – 1470 Nov – Mar	137	3.5 – 3.2 billion (a)	143.6 ± 79.0 mg/kg 1452.2 ± 762.4 (total)		1.04– 15.9	Clay/ loam/ coarse sandy loam	serpentinite	5.4 – 7.9	5	29
Swaziland (McCallum, 2007)	26° 20' S & 25° 40' S	1340 – 1420 (high) 880 – 960 (low)	11 – 22 Highveld 15 – 29 Lowveld sub-tropical	130 – 150 Nov – Feb	52	3.5 – 3.2 billion (a)	41.43 ± 9.44 mg/kg 17 – 100 µg/g 750 ± 120 (total)	400 mg /kg – 12500 mg/kg	0.32 – 0.61	high clay and a low sand content	serpentinite sequence & a sequence of serpentinous & tremolitic schists & tremolite amphibolite	4.34 – 6.01	2	6
Witwatersrand (Chapter 2; van Wyk & Smith, 2001; Mucina & Rutherford, 2006)	26° 08' S	1450 – 1750	10 – 23 mean = 16 temperate	570 – 730 Nov – Feb	c. 10	c. 3.3 – 3.2 billion (a)	94.9 ± 58.0 mg/kg sol. 969 ± 515.1 (total)	226.1 ± 60.8	0.5 – 2.3	Loam/ coarse sandy clay/ loam/ clay loam	serpentinite	4.9 – 6.2	0	0

Island Biogeography Theory (MacArthur & Wilson, 1967) contributes towards an understanding of the effects of habitat fragmentation. This theory proposes that the species diversity of an area is the result of an equilibrium between colonization and extinction (MacArthur & Wilson, 1967). There is also a strong relationship between area and richness, in which species richness increases with area (MacArthur & Wilson, 1967; Rosenzweig, 1995). In large areas, the larger local population sizes ensure that extinctions are less frequent and as a result they will support a greater diversity of species (MacArthur & Wilson, 1967). Colonization of islands and fragmented areas is influenced by the distance from the colonizing source as well as by fragment size because the probability of immigration declines with distance (MacArthur & Wilson, 1967; Yao *et al.*, 1999). On islands near a species rich source, most species will be present through direct colonization (Heaney, 2000), while evolution in relatively isolated habitats may result in endemic species that can differ dramatically from their ancestors (MacArthur & Wilson, 1967). On serpentine soils it was found that the greater the area, the greater the number of endemics present (Kruckeberg, 1984). Conversely, small islands, or remnant habitat fragments, will support fewer endemic species but an increased abundance of widespread generalist species (Harrison & Bruna, 1999) because plants that are good colonisers and good persisters tend to show limited island effects and endemism (Bond, 1991). McCallum (2007) found this to be the case in the relatively small Swaziland serpentine patches.

The length of exposure of the serpentine rocks to weathering and as a substrate for plant growth is considered as a potential contributing factor to patterns in serpentine endemic diversity. Most serpentine studies, in the northern hemisphere at least (Brooks, 1987; Jaffré, 1992; Reeves *et al.*, 1996; Harrison *et al.*, 2004; Alexander *et al.*, 2006), show that the longer the exposure of serpentine soils (10 – 30 million years BP), the greater the number of endemics. The age of exposure of most serpentine soils in California is Late Pliocene (3.7 – 2.6 million years BP) to Early Quaternary (2.6 – 1.8 million years BP) (Alexander *et al.*, 2006). The southern African serpentine rocks are far older (3.5 –

3.2 billion years) and the grassland biome may have originated between 200 – 300 million years ago (Brooks, 1987). However, the length of exposure of these rocks, as a substrate for growth, in Swaziland and on the Witwatersrand is uncertain (Anhaeusser, 1973). If these rocks have only been recently exposed, then this could imply that the Swaziland and Witwatersrand serpentine floras are relatively young and could possibly explain why there are few or no endemic taxa compared to other major serpentine areas.

The diversity between serpentine and adjacent non serpentine areas (Chapter 2, Table 6) showed that the serpentine flora is more similar to the adjacent non serpentine flora than to other serpentine sites. It was found that the vegetation on Witwatersrand serpentine sites, although physiognomically appearing to belong in the savanna biome, is part of the grassland biome (Chapter 2). The serpentine patches on the Witwatersrand represent isolated areas consisting of small patches surrounded by large patches of non serpentine flora. The total area covered by serpentine soils on the Witwatersrand is approximately 10 km², with patches ranging in size from 0.02 – 6 km². The small sizes of the patches therefore means a close proximity to source species and a high rate of immigration resulting in a similar flora on and off serpentine soils with no endemic taxa (Chapter 2).

Three taxa (*Cineraria austrotransvaalensis* Cron, *C. longipes* S. Moore and *Habenaria mossii* (G. Will.) J.C. Manning) that occur on serpentine soils are listed as near endemics on the Gauteng list of rare and endangered species, but these taxa also occur on non serpentine soils on the Witwatersrand, so there are no rare or endangered taxa unique to the Witwatersrand serpentine area. It was thus concluded that the serpentine areas on the Witwatersrand did not support a unique flora (Chapter 2).

The high, but similar, diversity on serpentine and non serpentine soils on the Witwatersrand indicates that soil factors do not play a significant role in determining diversity on potentially toxic soils in the area. However, beta-diversity was higher

between serpentine plots than between non serpentine plots (Chapter 3). The higher turnover rate on serpentine soils could be because the serpentine areas on the Witwatersrand encompass grassland and rocky outcrops thus providing a more heterogenous habitat than the non serpentine areas which consisted of fairly flat grasslands. This could possibly be because, like most rocky ridges on the Witwatersrand, the rocky serpentine koppies provide protection against fires (Mogg, 1961) enabling the growth of trees and other woody vegetation; so that the serpentine outcrops appeared to support a distinct serpentine community (Chapter 3).

The optimal growth strategy of a plant is to maximize fitness. Perennial plants on nutrient-poor and dry soils have been found to invest a proportionally high amount of resources into the root system, this limits the resources available for the above ground plant parts (Gleeson & Tilman, 1990). The reproductive index of the study taxa (Chapter 4) showed that all taxa invested less in reproductive biomass than in vegetative biomass.

The Witwatersrand Highveld grassland experiences severe, frosty winters, is fire dependent and occurs at a relatively high elevation (Rutherford & Westfall, 1994; Ellery *et al.*, 2001; Chapter 3). Most of the taxa occurring in this area, including the 18 taxa in the three categories investigated in Chapter 4 (common on serpentine soils, common on non serpentine soils and equally common on both soil types), were long-lived hemicryptophytes or geophytes that are capable of surviving these conditions by maximising vegetative biomass. Thus these harsh conditions could exert a stronger influence on these plants than the relatively mild serpentine condition making ecotype formation unlikely. Reproductive and life history data (Chapter 4) did not separate the study taxa into ecotypes.

Indigofera hedyantha, a taxon that appeared to be more common on serpentine than non serpentine soils (Chapter 3), was selected to explore the gene flow patterns occurring

between serpentine and adjacent/close non serpentine populations. Life history and breeding system studies, on and off serpentine soils (Chapter 4) indicated that this taxon was facultatively autogamous although no seeds were set when pollinators were excluded, indicating that outcrossing was preferred. No insects were observed visiting the plant, but flower colour and structure suggests that it is probably bee pollinated. The fairly heavy, rounded seeds were dispersed explosively, but not very far from the parent plant. From the genetic study (Chapter 5) it was concluded that gene flow between populations of *I. hedyantha* occurs on and off serpentine soils on the Witwatersrand and that sub-populations are genetically similar. Given the nature of seed dispersal, it is likely that gene flow is the result of pollen flow rather than seed dispersal, emphasizing the importance of biotic pollinators for this taxon.

2. Speciation in serpentine habitats

Plant speciation is characterized by the evolution of barriers to genetic exchange between previously interbreeding populations (Rieseberg & Willis, 2007). Speciation may either be allopatric (isolation of populations by some geographic barrier), sympatric (without any physical or geographical separation of the two populations but with reproductive isolation) or parapatric (between neighboring populations that share zones of contact but where a difference in some other physical condition limits gene flow). Adaptive divergence is therefore of central importance to the process of speciation. One way in which such divergence may proceed is via the evolution of ecotypes that are adapted to different habitats (Abbot & Comes, 2007).

Selection in ecologically marginal populations may result in specialized adaptations, as populations at the margin of distribution of a species are commonly in a state of flux (Lewis, 1962; Broyles *et al.*, 1994; Lindén & Knape, 2009). When gene flow in these populations becomes restricted, there is intense selection and ecotypes develop (Lindén & Knape, 2009), for serpentine soils, this would separate a species into serpentine tolerant and serpentine intolerant gene pools (Kruckeberg, 1991). This may isolate the

sub-populations into demes which then have smaller effective sizes enabling genetic drift to then fix certain alleles (Kittelson & Maron, 2001) and prevent gene exchange between the serpentine and non serpentine soil populations. Further divergence in structural and functional traits, e.g., flower shape and colour, flowering times and an increase in self-fertility could occur within the serpentine tolerant population (Brady *et al.*, 2005; Antonovics, 2006) and may result in reproductive isolation and the formation of two genetically distinct, though not always morphologically distinct, populations (Kruckeberg, 1984; Kruckeberg, 1991; Sandoval & Nosil, 2005). The evolution of metal tolerance in plants growing on metal contaminated soils is an example of the operation of divergent selection between adjacent populations (Jain & Bradshaw, 1966; Antonovics *et al.*, 1971). Measurement of metal tolerance and other key traits across mine tailing boundaries revealed that high levels of genetic differentiation are possible over short distances even when there was substantial gene flow (McNeilly, 1968; Antonovics *et al.*, 1970; Endler, 1977; Angus, 2005).

Like fragmented habitats, serpentine areas with high levels of toxicity can be regarded as edaphic ‘islands’. They are of great importance because they have permitted differentiation of new species and have ensured the survival of relict species under conditions of unfavourable mineral nutrition because these species benefitted from less severe interspecific competition (Whittaker, 1954). They have also acted as refuges for rare and endemic species of conservation value (Morrey *et al.*, 1989; Jaffré, 1992).

Populations of *Indigofera dregeana*, a taxon that grows on both serpentine and adjacent non serpentine soils in the Barberton Greenstone Belt area, were investigated (Chapter 5) in order to determine whether a similar pattern of gene flow, to that of the Witwatersrand, occurs in populations growing on more toxic serpentine soils. The initial work done for this taxon indicated that, while moderate levels of gene flow occurs between the two closely situated populations, divergence and possibly ecotype formation has occurred here and that, with time, the serpentine and non serpentine

populations could form separate species. The results (Chapter 5) confirm that there is a difference between the sub-populations of *I. dregeana* growing on different soil types.

The Barberton serpentine areas are relatively large so that there is a proportionately smaller area of contact between the serpentine and non serpentine populations of *I. dregeana*. In contrast, the Witwatersrand serpentine areas are small, so there is a proportionately larger zone of contact between plants growing on and off serpentine soils. This results in a high rate of gene flow between the serpentine and non serpentine sub-populations (Chapter 5). Life history and breeding system studies (Chapter 4) also did not show differences between adjacent serpentine and non serpentine sub-populations on the Witwatersrand. As a result of the high rates of gene flow, the close proximity and large zone of contact between the serpentine areas and the non serpentine areas as well as the low toxicity of the serpentine soils, the conditions do not satisfy the requirements for the formation of serpentine tolerant and intolerant sub-populations on the Witwatersrand.

There has been renewed interest in the idea that the macroevolutionary phenomenon of speciation is the result of the microevolutionary process of ecologically-based divergent selection; this is because speciation models have been reclassified from a geographic scheme (i.e. sympatric vs. allopatric) to one that focuses on mechanisms for the evolution of reproductive isolation (Rundle & Nosil, 2005). Ecological speciation is defined as the process by which barriers to gene flow evolve between populations or subsets of a single population by adaptation to different environments or ecological niches as a result of ecologically-based divergent natural selection (Rundle & Nosil, 2005; Schluter, 2009). Divergent selection is a consequence of the interaction of individuals with their environment during resource acquisition (Rundle & Nosil, 2005) and acts in contrasting directions between environments, which in turn drives the fixation of different alleles advantageous in one environment but not in the other (Schluter, 2009).

Most scientists share a common conception of species as evolutionary groups and that organisms within a species share a closer evolutionary history, as well as the processes of genetic drift and adaptation, with each other than with organisms in other species (Hey, 2006). The biological species concept (BSC) (Mayr, 1963) requires that there be reproductive isolation before a taxon can be considered a species while the phylogenetic species concept (PSC) requires that diagnosably distinct taxa with independent evolutionary histories are considered to be species irrespective of whether or not there is reproductive isolation (Zink, 2006). Thus under PSC, gene flow can still occur between diagnosably distinct taxa. A good criterion for whether speciation has occurred is the existence of stable multilocus genetic differentiation in the face of potential gene flow, here separate species exist when selection dominates gene flow, so that distinguishable genotypic or genomic clusters are stable in parapatry (Mallet, 2006).

In the current study, there appeared to be a separation of *I. dregeana* into two genetically “diagnosably different” serpentine and non serpentine populations on the more toxic Barberton serpentine soils despite the moderate rate of gene flow (Chapter 5). This study therefore shows evidence of the processes that may eventually result in parapatric speciation because the two sub-populations appear to be genetically different. It also indicates that the degree of soil toxicity, as well as the area of the contrasting soil types plays an important role in determining whether or not divergence between adjacent sub-populations occurs. The results also lend support to the PSC in that apparent separation has occurred between the serpentine and non serpentine sub-populations, despite appearing to be morphologically similar and despite gene flow occurring between the sub-populations. An in-depth study of *I. dregeana* should be undertaken, over its entire distribution range, in order to confirm whether or not any barriers to gene flow are developing or are in place. Aspects of life history and the breeding system, as well as reciprocal pot transplant experiments and further genetic studies at a finer level may confirm that there is differentiation, and possibly ecotype formation, between serpentine and non serpentine plant sub-populations of *I. dregeana* in the Barberton area.

3. Conservation of serpentine habitats on the Witwatersrand

Presently there are approximately 8,500 protected areas in the world which amount to only 5.2 % of the Earth's land surface ((IUCN, http://www.unep-wcmc.org/protected_areas/categories/). The grassland biome, of which the serpentine habitats are a part (Chapter 2), plays an important role in recreation and is of economic importance in the form of game farming, hunting, ecotourism, agriculture and mining with related job provision and contribution to the economy in South Africa. This biome is globally significant for its biodiversity; at the 1000 m² scale, with the average species richness (82 species) of the grassland biome being even higher than that of fynbos (68 species) communities (Cowling *et al.*, 1989).

Currently in South Africa 30% of the total grassland area has been irreversibly transformed mainly by anthropological factors such as commercial afforestation, agriculture, urban and industrial development, mining, alien plant invasion and road construction (Reyers *et al.*, 2007). This increase in land use and resource requirements associated with a growing human population leads to transformation and extreme fragmentation of the landscape placing valuable biodiversity at risk (Reyers *et al.*, 2007). In South Africa, only 1.9 % of this highly fragmented grassland is formally conserved (Reyers *et al.*, 2005). Most of the taxa that occur in the study areas are long-lived hemicryptophytes (Chapter 3) that invest more biomass into vegetative growth than reproductive output (Chapter 3). This implies that once a patch of grassland habitat is lost, the population sizes of many species are reduced which, over time, could lead to a loss of biodiversity.

A major challenge for conservation assessments is to identify priority areas that incorporate biological patterns and processes. For the maintenance of healthy populations, large, continuous areas are needed. Large-scale processes are mostly oriented along environmental gradients, so in order to accommodate them Rouget *et al.* (2006) proposed that regional-scale corridors, based on systematic conservation

planning principles, such as representation and persistence, be designed to capture these gradients. This is necessary because the connectedness of a protected area is important in maintaining species diversity and abundance. In order to achieve this, corridors of natural habitat need to be opened up in order to allow immigration and dispersal (Harrison & Bruna, 1999). These corridors should be large enough to support their own population in order for areas to be regarded as connected (Harrison & Bruna, 1999).

On the Witwatersrand, ridges are areas of high biodiversity and are particularly suitable as future refuges for biodiversity in an urbanized landscape as they function as islands even within a natural landscape (Pfab, 2001). The high species turnover for the serpentine sites (Chapter 3) supports this observation and high levels of beta-diversity among fragments (a measure of species turnover among sites) may increase the total species richness in the landscape (Ewers & Didham, 2005). Ridges and outcrops form natural wildlife corridors, which promote ecological processes and benefit regional and local biodiversity ([http://www.deat.gov.za/soer/reports/gauteng/Chapter 8 Biodiversity.pdf](http://www.deat.gov.za/soer/reports/gauteng/Chapter%208%20Biodiversity.pdf) accessed 02/11/09). While the development policy guideline for ridges in the Gauteng province does not cover rocky outcrops, because of their small area of coverage, it does regard them as sensitive areas characterized by high biodiversity to which the no-go development policy should be applied (Pfab, 2001). The serpentine outcrops, with their apparently distinct plant communities (Chapter 3) and as part of the corridor system, should therefore warrant conservation.

The survival and behaviour of invertebrates, many of which are important pollinators, are often dependent on the ridge environment (Pfab, 2001) and plant-arthropod associations can be maintained in patchy environments irrespective of the width of the corridor provided that human disturbance is kept to a minimum (Bullock & Samways, 2005). Fragmentation may lead to a reduction or loss of biotic pollinators because studies have shown that plants in fragmented habitats are visited less often by pollinators (Bullock & Samways, 2005). This is because the pollinators are unable to

find the small isolated pockets of plants, so plants at these sites may not be effectively cross-pollinated resulting in fewer seeds being set and thus lower reproduction (Boshier *et al.*, 1995; Didham *et al.*; 1996; Aguilar *et al.*, 2006).

Fourteen of the 18 study taxa (Chapter 4) potentially depend on insect pollinators, which indicates that biotic pollinators play an important role in gene flow. A reduction in plant population sizes together with a loss of effective cross pollination by biotic pollinators could therefore lead to a reduction in the diversity of gene pools and subsequently a loss of plant fitness. Inbreeding depression and the loss of genetic diversity in the small population could then lead to a decrease in plant diversity and possibly slow extinction (Piessens *et al.*, 2005). However the data for tropical tree species (Dick *et al.*, 2008) and for *I. hedyantha* (Chapter 5) suggests that it is possible for gene flow to occur over long distances emphasising the importance of maintaining the ridges and koppie corridors on the Witwatersrand in order to facilitate and maintain effective pollination.

Conservation management is important for scientific research, wilderness protection, preservation of species and genetic diversity, maintenance of environmental services, protection of specific natural and cultural features, tourism and recreation, education, sustainable use of resources from natural ecosystems and maintenance of cultural and traditional attributes (IUCN, (http://www.unep-wcmc.org/protected_areas/categories/)). In the past, population growth and poverty were seen as the main causes of environmental degradation so people were excluded from protected environments. This approach led to serious conflicts between local communities and resulted in the transformation in thinking and recognition that biodiversity and environmental protection has to be effective in linking conservation with human needs (IUCN, http://www.unep-wcmc.org/protected_areas/categories/). As a result, the IUCN recognised six protected area categories:

1. Strict protection (Nature Reserve / Wilderness Area),
2. Ecosystem conservation and recreation (National Park),

3. Conservation of natural features (Natural Monument),
4. Conservation through active management
(Habitat/Species Management Area),
5. Landscape/seascape conservation and recreation
(Protected Landscape/Seascape) and
6. Sustainable use of natural ecosystems
(Managed Resource Protected Area).

All categories are considered important but the degree of human intervention varies. The latter three categories require greater intervention and modification than the first three. Of the six categories, only categories 5 and 6 recognize that maintenance of biodiversity is not always the primary reason for protection and that management choices may be determined by cultural values, environmental management, sustainable land use and recreational needs. These categories are thus suited for sustainable use by indigenous and urban communities, tourism and small-scale agriculture (IUCN, http://www.unep-wcmc.org/protected_areas/categories/).

The Witwatersrand region is a highly developed area with a dense population and urbanization is the major contributor to habitat loss. Currently most serpentine areas on the Witwatersrand are tiny islands surrounded by development or are ear-marked for development. Development leads to fragmentation of already small serpentine patches. Possible solutions to curbing environmental threats should include educating human populations surrounding protected natural areas on sustainable utilisation, the removal of alien plants and accurate environmental impact assessments for proposed urban development (Grant *et al.*, 1998). In South Africa, the National Environmental Management Acts (No. 107 of 1998 and No. 57 of 2003)(NEMA) established principles for decision-making on matters affecting the environment and sought to address the above issues. Should the ridge development policy guidelines be strictly implemented, then the rocky serpentine outcrops should remain safe from development and thus contribute towards the richness of the flora of the area and act as corridors and

refuges of local biodiversity. Thus on the Witwatersrand any protected area would most likely fall into categories 5 and 6.

Nine serpentine sites were investigated in this study. In the west, two of the sites, Robindale and Emma Park (Figures 1 & 2), are urban parks currently protected by the City of Johannesburg metropolitan municipality public open spaces by-laws of 21 May 2004. The Ruimsig Entomological Reserve (Figure 3), is a protected area with a conservation management strategy in place (Deutschlaender & Bredenkamp, 1999). Dombeya/Ndabushe site, a former private nature reserve, is now a private game farm with a limited number of luxury cluster houses on it (Figure 4); it does however have a conservation management plan (http://www.letamo.co.za/emp_rar.asp, accessed 25/03/10). Thus, four of the nine sites investigated are currently receiving some legal protection. However, none of these areas are proclaimed as protected areas under the NEMA: Protected Areas Act (57 of 2003) or under the provincial nature conservation ordinance, so the future of these areas is still uncertain.

The sites that are not legally protected are fenced off and so access is currently restricted. Without legal protection, however, the prolonged existence of these unprotected sites is tenuous. All that remains of the formerly 0.2 km² of serpentine area at the Darrenwood site, is the rocky koppie surrounded by development (Figure 5) and small patches of highly disturbed former grassland. Diamond Four (Figure 6), a private smallholding, which was formerly unoccupied, now has a building on the property. The Weltevreden site (Figure 7), once a small lot between two developed properties, now has been developed and only a narrow, grassy strip remains while the major part of this site is now paved. The Zandspruit site (Figure 8), which offers the most obvious interface between serpentine and non serpentine, is surrounded by a housing development with some commercial activity occurring within the fenced off area. Neither of the two eastern sites visited is under any protection; Estherpark (Figure 9), the largest of the sites and once an open veld, has now been developed on one side and a

railway line, built over what was once a seepage area, now passes close to the rocky koppie. Parts of the Edenvale/ Illiondale site (Figure 10), always a highly disturbed area with power pylons running through it, is now commercially developed, increasing the level of disturbance at this site.

Conclusions

The Witwatersrand serpentine rocks are as old as those of the Barberton area and unlike the serpentine areas in other parts of the world, the floral diversity on the South African serpentine soils is high and very similar to the adjacent non serpentine areas. The temperature ranges, winter temperatures and the annual rainfall on the Witwatersrand is comparable to other more temperate, more toxic serpentine areas, for example the Great Dyke and California. The Witwatersrand serpentine areas also consist of very small, isolated patches that occur at a higher latitude and altitude, in a fire maintained grassland that is characterised by dry, severe winters, and soils that are not as toxic. Thus the main differences between the Witwatersrand serpentine areas and other serpentine areas are that the serpentine soils on the Witwatersrand are less toxic than the soils of other areas and the small, isolated patches are in close proximity to the non serpentine areas. All these factors could contribute towards the absence of serpentine indicator plants or endemics on the Witwatersrand and to the similarity between the serpentine and non serpentine areas. The results obtained for the gene flow patterns of the Barberton *Indigofera* taxon and the Witwatersrand taxon implies that soil toxicity does contribute towards ecotype formation. The distinct serpentine community observed on the serpentine outcrops on the Witwatersrand is more likely to be due to physical characteristics of these rocky outcrops rather than to soil chemical characteristics since these outcrops provide protection against fire which creates a microclimate that is suitable for the growth of woody vegetation.



Figure 1. Robindale Park has been properly fenced. The inset shows that access to the park is now controlled.



Figure 2. Emma Park was considered as part of the Darrenwood area. An attempt has been made to fence off this urban park, but, as can be seen, the fence was damaged but has subsequently been repaired.



Figure 3. Ruimsig Entomological Reserve. The fence is currently maintained, but has, on occasion, been cut to provide illegal access to the reserve.



Figure 4. Ndabushe/Dombeya is now a low density, high cost game estate. The inset shows that there are 17 plots for sale at this site.



Figure 5. Darrenwood site, now just a koppie surrounded by an office park.



Figure 6. Diamond Four is still fairly untouched, although a structure has now been built on the property.



Figure 7. The Weltevreden site is almost completely built over, only a narrow strip of grassland remains. The inset shows the front of the property which has been completely paved over.



Figure 8. Most of the Zandspruit site is untouched. It now has been fenced off, but there are gaps in the fencing. This site is surrounded by housing development and within the fenced off area Johannesburg Water is undertaking some development (inset).



Figure 9. The koppie at Esther Park still exists, but a railway line now passes close to it. On the right one can see the high density housing close to this site. The inset shows what remains of the wetland area.



Figure 10. The Illiondale site always had the powerlines, but the insets show some of the development that has and is taking place in the upper areas of this site.

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