Ecotypes of wild rooibos \textit{(Aspalathus linearis} (Burm. F) Dahlg., Fabaceae) are ecologically distinct

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Abstract

\textit{Aspalathus linearis} (Burm. F) Dahlg., Fabaceae is cultivated by small- and large-scale commercial farmers of the Cederberg and Bokkeveld Plateau in the Cape Floristic Region of South Africa, for the production of an herbal beverage called ‘rooibos’ or ‘rooibos tea’. Small-scale farmers also harvest \textit{A. linearis} from the wild and market the tea as an organic and fair-trade certified product. However, little is known about the apparent ecotypes of wild \textit{A. linearis}. We hypothesized that 1) rooibos ecotypes are ecologically distinct (occurring in different plant communities defined by environmental variables); 2) rooibos ecotypes are functionally distinct due to variance in water use efficiency; and 3) rooibos ecotypes are associated with threatened vegetation types/species, making populations of wild rooibos ecotypes worthy of conservation. Ecotypes of wild rooibos were identified based on plant habit and local knowledge. Plant communities were classified using Twinspan, environmental factors were tested as predictors of ecotype distribution and water use efficiency of ecotypes growing across a rainfall gradient was determined from foliar natural abundance of $^{13}$C. Wild rooibos was not generally associated with endangered vegetation types but was associated with plant species having endangered status. Wild rooibos occurred in four plant communities and comprised five wild rooibos ecotypes: shrub, tree, upright, salignus and prostrate types. Although some ecotypes clearly co-occurred, evidence is provided for habitat preference between the ecotypes: Prostrate and upright ecotypes occurred at higher elevations (>400-600 m). Shrub ecotypes occurred at lower rainfall sites (<200 mm p.a.) and the salignus ecotype occurred at higher rainfall sites (>500 mm p.a.). Foliar $^{13}$C indicated greater water use efficiencies by ecotypes in relatively drier areas. The extent to which this is a plastic or inherent response requires further investigation. Considering that wild rooibos ecotypes differ both ecologically and possibly also functionally and genetically, it is concluded that populations of wild rooibos ecotypes should be considered as distinct and worthy of conservation. This distinctness should be considered when farmers apply for both harvesting and ploughing rights on land with wild rooibos ecotypes.

Keywords: Elevation; Isotopes; Local knowledge; Nutrient acquisition strategies; Participatory action research; Precipitation

1. Introduction

\textit{Aspalathus linearis} (Burm. F) Dahlg. (Fabaceae) is a legume endemic to South Africa, and the Swedish botanist Carl Thunberg first reported its use by the Khoisan to produce a beverage in 1772 (Morton, 1983). The name ‘rooibos’ refers to the herbal tea produced from \textit{A. linearis} but is also often used locally to refer to the plant. In this paper ‘wild rooibos’, ‘cultivated rooibos’ or ‘rooibos ecotype’ all refer to the plant and ‘rooibos’ or ‘rooibos tea’ refers to the beverage. \textit{A. linearis} is both farmed and harvested from the wild to make this tea,
which is marketed both locally and internationally. Small-scale farmers also harvest wild rooibos and market the tea as an organic and fair-trade certified product to niche markets abroad, a process culminating from participatory action research and community exchange (Oettlé et al., 2004). *A. linearis* has a limited geographical range extending from the north-western to western region of the Fynbos biome in the Cape Floristic Region (CFR), South Africa (Fig. 1), one of the 25 global biodiversity hotspots (Myers et al., 2000). As a result, rooibos tea is a candidate for the geographical indications type certification, where a product name, e.g. Rooibos or Champagne, is protected and linked to produce from a specific geographical area and associated climate and soils (Biénabe et al., 2009; Bramley et al., 2009).

The species is adapted to deep, well-drained sands which are oligotrophic and acidic (Muofhe and Dakora, 2000). Rooibos regenerates by re-seeding or re-sprouting after fire (Van der Bank et al., 1999) where Fynbos vegetation is characterized by a fire return of 4 to 50 years. *A. linearis* has an ecological role as a N$_2$-fixer in the post-fire environment, persisting in abundance for <5 years (Cocks and Stock, 2001), although plants as old as 20 years exist (Morton, 1983). *A. linearis* exists as a series of partially allopatric populations (Van der Bank et al., 1999) which differ in a number of respects, specifically growth form, fire-survival strategy, vegetative and reproductive morphology (Malgas et al., 2010), isozyme patterns, flavonoids (Dahlgren, 1968; Van der Bank et al., 1999), fermentation characteristics/taste/appearance (Morton, 1983) and genetics (Malgas et al., 2010). All of these studies and especially that of Malgas et al. (2010) strongly indicate that some rooibos populations are genetically distinct as ecotypes or even subspecies. Thus wild rooibos and associated plant communities can be considered as a conservation priority for several reasons, namely 1) rapid habitat loss, 2) uncharacterized and unprotected ecotypes of wild rooibos and 3) potential loss of genetic resource for both biodiversity and the rooibos industry.

Regarding 1), the biodiversity of habitats where wild rooibos grows naturally is threatened mostly by rooibos and potato farming (Botha, 2009; Van Wyk, 2002) but also by excessive burning, over-grazing, over-harvesting, periodic drought and fragmentation (Low and Rebelo, 1998). Protection levels of vegetation types where wild rooibos grows, or potentially could be cultivated, are often poor, despite some areas having endangered ecosystem statuses (e.g. Léipoldt Sand Fynbos). At the species level, land-clearing over the last five years has resulted in habitat loss of many vulnerable and critically endangered plants (CREW database, Custodians of Rare and Endangered Wildflowers, South Africa). Recently the Rooibos Biodiversity Initiative, a partnership between the South African Rooibos Council (SARC) and the statutory conservation organization CapeNature, has been mandated to provide sustainable farming guidelines to protect biodiversity in this area via the Right Rooibos initiative (Botha, 2009).

Regarding 2), four apparent ecotypes of *A. linearis* were identified on the basis of growth form (Malgas and Oettlé, 2007) which were later classified morphologically and.

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Fig. 1. Google Earth map showing the distribution and ecotypes of *Aspalathus linearis* (Burm. F) Dahlg. at each collection site. The inserted map of South Africa shows the general study area in the Cederberg and Bokkeveld Mountains (grey shaded area) as well as all reported collection sites of *A. linearis* (black dots, from Dahlgren (1968) and Malgas et al. (2010); white dots, from this study).
genetically as being only three distinct forms (shrub (or sometimes called ‘bush’) and prostrate forms, which re-sprout; and tree-like or upright forms, which are re-seeders only; Malgas et al. (2010). Seven ecotypes have been identified on the basis of flavonoids and tea colour (Van Heerden et al., 2003). The commercially grown ‘Nortier’ form is a shrub-type re-seeder and is thought to originate from the Pakhuis area of the northern Cederberg, but clear records of plant selection are not publicly available. The genetic distinction of these forms has been convincingly suggested from previous work (Malgas et al., 2010; Van der Bank et al., 1995), but it is unknown whether these apparent ecotypes cross-pollinate or how seed dispersal occurs (Malgas et al., 2010). This knowledge is essential if proper conservation management strategies of wild rooibos are to be formed. For example, if conservation management treated genetically distinct and reproductively isolated ecotypes as one type, significant loss of diversity could result. Therefore, wild rooibos populations (and associated habitats) are under threat due to both habitat destruction and loss of genetic resources including possible genetic dilution by the ‘Nortier’ type that is cultivated.

Regarding 3), the potential of ecotype variety as a resource for the rooibos industry is completely unknown. Recognition of ecotypes, including what was to become the cultivated ‘Nortier’ type, depended originally on local knowledge. The cultivated Nortier type was selected from plantings by Dr. P le Frais Nortier and associates in the 1930s using seeds from the Pakhuis Mountains of the Cederberg collected by himself and local Khoisan people (Cheney and Scholtz, 1963; Morton, 1983). The selection of the Nortier type was based on priorities at the time, such as growth rate, seed production and especially taste. Certain wild rooibos types such as the prostrate type, were not always regarded as valuable for producing rooibos and were mostly regarded as unpalatable while others were used to improve flavour (Dawie de Villiers, pers comm., 2007, SARC). More recently, rooibos tea from wild prostrate rooibos has been utilized as a stand-alone product (Heiveld Co-op, pers comm., 2007). However, not only taste is important for the industry, but also pest resistance and drought resistance. Pest resistance has become an increasing problem (Donaldson et al., 2003) and an industry based on one A. linearis selection made ca. 80 years ago incurs a risk of large-scale crop decimation. Local knowledge suggests that wild re-sprouting rooibos shrubs of the Suid Botkeveld have higher drought resistance than the cultivated type (Heiveld Co-op, pers comm., 2007). Use of wild rooibos plants may therefore buffer the rooibos industry from changing temperatures and rainfall conditions within the context of climate change. As such, wild rooibos represents an untapped resource for the industry.

Considering the potential of wild rooibos and associated habitats as conservation priorities, we hypothesized that 1) rooibos ecotypes are ecologically distinct (occurring in different plant communities defined by environmental variables); 2) rooibos ecotypes are functionally distinct due to variance in water use efficiency; and 3) rooibos ecotypes are associated with threatened vegetation types/species, making populations of wild rooibos ecotypes worthy of conservation.

2. Materials and methods

2.1. Location of wild rooibos populations

Populations of wild rooibos were chosen at 13 sites between Nieuwoudtville in the north, Wupperthal in the east and Citrusdal in the south of the rooibos production area. The selection of populations was based on three criteria: 1) importance to local people for wild harvesting; 2) location near potentially endangered vegetation types and 3) accessibility. Location of wild rooibos was based on local knowledge of small-scale rooibos harvesters and farmers from the Heiveld and Wupperthal Cooperatives, as well as the PRECIS (Pretoria Computerized Information System) data on A. linearis from the National Herbarium (South African National Biodiversity Institute, SANBI, Pretoria) and the South African Rooibos Council. Small and large-scale farmers participated in the studies by providing essential knowledge not only on population location but also land-use and fire history, which ensured that only populations of similar age were compared.

2.2. Site assessment

Ten plots (5 m × 5 m) were spaced at regular intervals along one 100-m transect for each of the 13 sites and subject to vegetation analyses. Environmental variables of soil, rainfall, elevation, slope and aspect were also assessed. Transects through diverse sites were located to exclude as much as possible differences of age after fire, elevation, slope, aspect, geology, soil and rock cover.

2.3. Plant identification

Plants were identified using field guides (Manning and Goldblatt, 2007; Trinder-Smith, 2003; Van Rooyen and Steyn, 1999) or by using keys for the Cape Floristic Region (Goldblatt and Manning, 2000) and/or interactive identification keys for restios (Linder, 2006), ericas (Volk and Forshaw, 2004) and succulents (Interactive mesembs, SANBI database). Species that could not be identified due to lack of floral parts at the time of collection were labelled Species 1, 2, 3 etc. and included in cluster analysis.

2.4. Vegetation survey

Percentage cover by a species, species richness and α-diversity were determined for each site. Plant species were grouped into habits and functional types (the most important in Fynbos are growth form (shrub, tree, graminoid-grass, graminoid-restio, graminoid-sedge, herbaceous annual, herbaceous perennial, bulb) and nutrient acquisition guilds (Holmes and Richardson, 1999; mycorrhizal, non-mycorrhizal, cluster rooted including proteoid and dauciform roots, N₂-fixing) based on published results (Allsopp and Stock, 1993) and inspection of roots in the Proteaceae, Restionaceae and rooibos (i.e. for proteoid roots, dauciform roots and N₂-fixing nodules, respectively).
Communities of plants were classified according to Campbell (1985) and Cowling and Holmes (1991) and vegetation types according to Mucina et al. (2005). Plant communities were classified using Twinspan (Community Analysis Package v. 2.13, Pisces Conservation, UK). For Twinspan analysis, raw percentage cover was used with the following user-defined categories (%): 0, 2.5, 5, 10 and 50. Environmental factors were tested as predictors of variance in species distribution using canonical correspondence analysis (Environmental Community Analysis v. 1.37, Pisces Conservation, UK).

2.5. Environmental variables

Elevation, slope, aspect, geology, soil type, soil depth and rock cover were determined for each site to account for any variance in vegetation data due to environmental variables. Average monthly rainfall data over 2 to 100 years (depending on availability) was obtained from the South African Weather Bureau.

Soil cores (0–200 mm, \( \varnothing = 50 \) mm, \( n = 4 \)) and root samples were collected under rooibos canopies at each site. Soil C, N and P were determined on dried, 2-mm sieved soil. Soil nitrogen was determined by combustion using the FP-528 Nitrogen Analyser (Leco Corporation, St. Joseph, USA). Soil C was determined using the Walkley Black method (Nelson and Sommers, 1982). Soil was prepared for P analysis by extracting 6.6 g soil in Bray II solution (Bray and Kurtz, 1945) before filtering and analysing using inductively coupled plasma atomic emission spectrometry (Varian Vista MPX ICP-AES, Australia).

2.6. Ecosystem status, protection level and conservation value of sites

On a scale of 1:50 000, the ecosystem status, protection level and conservation values of each site were assessed using the National Spatial Biodiversity Assessment (NSBA) (Driver et al., 2005) on the SANBI Biodiversity GIS website http://bgis.sanbi.org. The vulnerability status of plants was determined using the IUCN Red Data List available on the SANBI website http://www.sanbi.org/biodiversity/reddata.htm as well as by consulting the CREW database on rare plants in rooibos and potato farming areas.

2.7. Foliar \(^{13}\text{C} \) analysis

Leaf samples (ca. 20 g fresh mass) from different plants (\( n = 4 \)) of wild rooibos (shrub, prostrate, tree, upright and salignus) from five sites (L1, A1, K, Kaf and G, respectively) were sampled and compared to one another as well as with cultivated rooibos from the site Kafkraai. The latter was the only site where cultivated and a wild rooibos ecotype co-occurred closely in this study. Oven-dried leaf samples of wild and cultivated rooibos were ground using a mortar and pestle before sub-samples were weighed (ca. 2.8 mg) into tin capsules prior to Dumas combustion. Isotope ratios were measured with a Delta XP mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) at the University of Cape Town and were expressed as \( \delta ^{13}\text{C} \) (in ‰) relative to a lentil standard following the equation:

\[
\delta ^{13}\text{C} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where \( R \) is the ratio of \(^{13}\text{C} \) to \(^{12}\text{C} \). Analytical precision on the internal lentil standard was 2‰.

2.8. Statistics

Species data was square root transformed to allow better separation of groups prior to Twinspan analysis. Growth forms and nutrient acquisition strategies between sites were compared using post-hoc Tukey tests after one-way ANOVAs (Statistica v. 8, Statsoft Inc., USA). Alpha-diversity within sites was calculated (Species Diversity and Richness, v. 3.02, Pisces Conservation, UK) and compared using \( t \)-tests, as was the difference in water use efficiency between cultivated and upright rooibos growing in the same rainfall area. Environmental predictors of ecotype occurrence were determined using multiple regression analysis (Statistica v. 8). The relationship between mean annual precipitation (MAP) and water use efficiency (WUE) of ecotypes was determined using linear regression (Statistica v. 8).

3. Results

3.1. Characterization of wild rooibos sites

Wild rooibos sites were characterized according to environmental variables (Table 1). Wild rooibos was found at an average altitude of 682 m across all sites, with a maximum of 1040 and minimum of 350 m. Slopes ranged between 1 and 27° with an average across sites of 6°. Most sites had a southern aspect, with some northern or eastern aspects. Rainfall in the area ranged between about 120 mm in the north to about 700 mm in the south. Rock cover was 19% across sites and ranged between very sandy with small, rounded ferricrete stones typical of areas where rooibos grows, and >70% rock cover. Average soil depth was shallow at 27 cm although this was likely to have been underestimated due to the difficulty of estimating soil depth in rocky soils. Soils were significantly deeper with more soil C at most southern sites (except Hotbergfontein in the north) compared to other sites, being lowest at Landskloof in the north (\( P < 0.0001 \)). While soil C differed between sites, all sites had low C between 0.17 and 1.47%. There was no significant difference in soil N or P between sites. There was no correlation between soil C, rockiness or soil depth (\( P > 0.05 \)).

3.2. Ecosystem status, conservation value and protection level of vegetation types

Rooibos populations assessed were distributed in mountain Fynbos from Nieuwoudtville in the north to the Citrusdal area in...
the south, encompassing a major part of the natural range of rooibos (Table 2; Fig. 1). All the wild rooibos surveyed grew in dry to mesic restioid or proteoid Fynbos. Most sites with wild rooibos tea grew within Sandstone or other types of Fynbos that are classified as least threatened (Table 2). For example, Bokkeveld Sandstone Fynbos (in the north) and Cederberg/Olifants Sandstone Fynbos (in the south) are classified as least threatened by the NSBA and have a conservation value of 0. As a result most of these areas are poorly or moderately protected at present. Only one site was classified as vulnerable (Graafwater Sandstone Fynbos; Table 2). Despite a conservation value of 50 this vegetation type is not protected at present. Other vegetation types encountered (Swartruggens Quartzite Fynbos and Northern Inland Shale Band Vegetation) are also classified as least threatened by the NSBA and have a conservation value of 0.

### 3.3. Occurrence of rare and endangered plant species with wild rooibos

Only between three and one plant species with some threatened status (near threatened [NT], vulnerable [VU], endangered [EN] or critically endangered [CR]) were found per wild rooibos site (Table 2). Most of the threatened species identified were geophytes. Species included *Romulea sabulosa* (VU), *Geissorhiza subridgida* (CR), *Babiana vanzyliae* (NT), *Babiana klaverensis* (EN), *Babiana ecklonii* (CR) (Iridaceae); *Bulbinella latifolia* subsp. *latifolia* (VU) (Asphodelaceae);

### Table 1
Environmental components for each site as well as across sites, where site names may be referenced from site codes in Table 2. n=10 for each site and 110 across sites, except for soil depth and soil N, P and C where n=4 for each site and 44 across sites, and for elevation, slope and rainfall where n=1 for each site and n=11 across sites. Different letters indicate significant difference at the P < 0.05 level (Tukey post-hoc test) after a one-way ANOVA.

<table>
<thead>
<tr>
<th>Site code</th>
<th>Annual rainfall (mm)</th>
<th>Elevation (m)</th>
<th>Slope (°)</th>
<th>Aspect</th>
<th>Soil depth (cm)</th>
<th>Rock (%)</th>
<th>Soil P (mg kg⁻¹)</th>
<th>Soil C (%)</th>
<th>Soil N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>400</td>
<td>739</td>
<td>1</td>
<td>S</td>
<td>59.9±8.0</td>
<td>1±0</td>
<td>13.50±3.18</td>
<td>1.06±0.07</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>L1</td>
<td>117</td>
<td>478</td>
<td>4</td>
<td>S</td>
<td>3.3±0.04</td>
<td>4±1</td>
<td>9.75±0.63</td>
<td>0.17±0.04</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>L2</td>
<td>117</td>
<td>533</td>
<td>1</td>
<td>E</td>
<td>22.6±2.9</td>
<td>43±3</td>
<td>7.00±1.96</td>
<td>0.57±0.10</td>
<td>0.11±0.06</td>
</tr>
<tr>
<td>A1</td>
<td>224</td>
<td>826</td>
<td>1</td>
<td>E</td>
<td>19.1±0.6</td>
<td>46±2</td>
<td>8.00±2.04</td>
<td>0.77±0.18</td>
<td>0.09±0.006</td>
</tr>
<tr>
<td>A2</td>
<td>224</td>
<td>893</td>
<td>1</td>
<td>E</td>
<td>29.7±4.5</td>
<td>49±9</td>
<td>12.00±2.16</td>
<td>0.47±0.09</td>
<td>0.08±0.004</td>
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<tr>
<td>K</td>
<td>217</td>
<td>664</td>
<td>1</td>
<td>N</td>
<td>23.0±7.9</td>
<td>15±3</td>
<td>9.50±2.72</td>
<td>1.47±0.10</td>
<td>0.12±0.01</td>
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<tr>
<td>LK1</td>
<td>217</td>
<td>1040</td>
<td>9</td>
<td>S</td>
<td>20.2±5.1</td>
<td>39±5</td>
<td>9.75±0.85</td>
<td>0.91±0.02</td>
<td>0.09±0.003</td>
</tr>
<tr>
<td>LK2</td>
<td>217</td>
<td>995</td>
<td>27</td>
<td>N</td>
<td>8.7±2.4</td>
<td>1.4±0.2</td>
<td>10.00±1.78</td>
<td>0.83±0.01</td>
<td>0.08±0.01</td>
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<tr>
<td>Kaf</td>
<td>499</td>
<td>429</td>
<td>4</td>
<td>S</td>
<td>25.4±8.9</td>
<td>1±0</td>
<td>11.00±1.78</td>
<td>0.90±0.11</td>
<td>0.08±0.005</td>
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<tr>
<td>W</td>
<td>499</td>
<td>537</td>
<td>12</td>
<td>S</td>
<td>30.8±5.2</td>
<td>4.5±0.8</td>
<td>11.00±1.96</td>
<td>1.13±0.04</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>G</td>
<td>699</td>
<td>352</td>
<td>1</td>
<td>S</td>
<td>29.1±1.8</td>
<td>7.2±1.6</td>
<td>12.00±1.47</td>
<td>0.46±0.04</td>
<td>0.16±0.06</td>
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<tr>
<td>P (ANOVA)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a</td>
<td>n.a</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>0.5689</td>
<td>&lt;0.0001</td>
<td>0.5956</td>
</tr>
<tr>
<td>All sites</td>
<td>314±54</td>
<td>682±21</td>
<td>6±0.7</td>
<td>Mostly S</td>
<td>27.4±1.6</td>
<td>19±2</td>
<td>10.32±0.58</td>
<td>0.79±0.09</td>
<td>0.10±0.01</td>
</tr>
</tbody>
</table>

### Table 2
Location, ecosystem status, conservation value and protection level of vegetation types with wild rooibos populations. Vegetation types are based on Driver et al., 2005. Protection levels, ecosystem status and conservation values based on the National Spatial Biodiversity Assessment (Driver et al., 2005) and associated Biodiversity GIS software.

<table>
<thead>
<tr>
<th>Site code</th>
<th>Site name</th>
<th>Area</th>
<th>Easting</th>
<th>Southing</th>
<th>Vegetation type</th>
<th>Ecosystem Status</th>
<th>Conservation value</th>
<th>Protection level</th>
<th>Species with threatened status</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Hothbergfontein</td>
<td>Nieuwoudtville</td>
<td>19.1079</td>
<td>31.4046</td>
<td>Bokkeveld Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Poorly</td>
<td>3</td>
</tr>
<tr>
<td>L1</td>
<td>Landskloof</td>
<td>Suid Bokkeveld</td>
<td>19.09221</td>
<td>31.8182</td>
<td>Bokkeveld Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Poorly</td>
<td>1</td>
</tr>
<tr>
<td>L2</td>
<td>Landskloof</td>
<td>Suid Bokkeveld</td>
<td>19.08451</td>
<td>31.8285</td>
<td>Bokkeveld Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Poorly</td>
<td>1</td>
</tr>
<tr>
<td>A1</td>
<td>Achterstefontein</td>
<td>Biedouw</td>
<td>19.36008</td>
<td>32.21843</td>
<td>Swartruggens Quartzite Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Poorly</td>
<td>1</td>
</tr>
<tr>
<td>A2</td>
<td>Achterstefontein</td>
<td>Biedouw</td>
<td>19.34076</td>
<td>32.17203</td>
<td>Swartruggens Quartzite Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Poorly</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>Kleinvlei</td>
<td>Wupperthal</td>
<td>19.18797</td>
<td>32.29416</td>
<td>Cederberg Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Moderately</td>
<td>2</td>
</tr>
<tr>
<td>LK1</td>
<td>Langkloof</td>
<td>Wupperthal</td>
<td>19.24042</td>
<td>32.40158</td>
<td>Northern Inland Shale Band Vegetation</td>
<td>LT</td>
<td>0</td>
<td>Moderately</td>
<td>2</td>
</tr>
<tr>
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<td>Wupperthal</td>
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<td>0</td>
<td>Moderately</td>
<td>2</td>
</tr>
<tr>
<td>Kaf</td>
<td>Kalkraal</td>
<td>Citrusdal</td>
<td>18.88436</td>
<td>32.34108</td>
<td>Graafwater Sandstone Fynbos</td>
<td>VU</td>
<td>50</td>
<td>Not</td>
<td>0</td>
</tr>
<tr>
<td>W</td>
<td>Witels</td>
<td>Citrusdal</td>
<td>18.80205</td>
<td>32.33857</td>
<td>Cederberg Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Moderately</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>Gecko Creek</td>
<td>Citrusdal</td>
<td>18.98821</td>
<td>32.39745</td>
<td>Olifants Sandstone Fynbos</td>
<td>LT</td>
<td>0</td>
<td>Moderately</td>
<td>0</td>
</tr>
</tbody>
</table>

*a EN—endangered, VU—vulnerable, LT—Least threatened where Least Threatened ecosystems are still largely intact; Vulnerable ecosystems are reasonably intact, but are nearing the threshold beyond which they will start to lose ecosystem functioning; endangered ecosystems have lost significant amounts of their natural habitat, impairing their functioning; critically endangered ecosystems have so little natural habitat left that not only has their functioning been severely impaired, but species associated with the ecosystem are also being lost. The different levels thus also reflect importance in terms of national biodiversity conservation targets.

*b Includes near threatened, vulnerable, endangered and critically endangered species.
Fig. 2. Wild rooibos tea ecotypes (a) shrub, (b) prostrate, (c) tree, (d) salignus, e.g. upright) and cultivated rooibos tea (h).
Heliophila arenaria var. agtertuinensis (Vu., (Brassicaceae) and Gnedia deserticola (rare) (Thymelaeaceae). It is likely that the number of threatened species was underestimated as >50% of geophytes could not be identified due to lack of flowering parts. The endangered plant species Babiana klaverensis (Iridaceae) co-occurred with wild rooibos in seven of the eleven sites, i.e. in the northern drier sites.

3.4. Wild rooibos ecotypes

Four wild rooibos ecotypes identified in a preliminary field and social survey (Malgas and Oettlé, 2007) were also observed in the present study. These were the shrub, prostrate, tree and upright types (Figs. 1 and 2a, b, c and e). In addition, another apparent ecotype was observed at Witelskloof in the Citrusdal area (Figs. 1 and 2d), which has been tentatively referred to here as a ‘salignus’ ecotype. Although this apparently new ecotype was not exhaustively characterised as described by Malgas and Oettlé (2007), it was characterised in the field as being A. linearis (from flowers and seeds), as having a habit very obviously different from other ecotypes in that it was ca. 2 to 3 m tall, salignus (meaning Salix or willow like), with internodes ca. 20 to 50 cm apart and having a sparse canopy (Fig. 2d). Also, at least three upright types were observed (Fig. 2e–g) that may be different ecotypes but were all referred to as upright types pending genetic characterization. Therefore a total of five wild rooibos ecotypes based on plant habit are referred to here: shrub, prostate, tree, salignus and upright types. The cultivated Nortier type (Fig. 2h) has a habit similar to that of the shrub and upright types.

3.5. Vegetation survey

Twinspan analysis split the eleven wild rooibos tea sites into a drier northern restiod complex and wetter southern proteoid complex, each complex comprising two main plant communities (Table 3). The four different plant community types that wild rooibos grew in were: Dry to Mesic Restiod Fynbos characterized by geophytes and a prostrate wild rooibos with an unusual red-keeled flower growing on relatively deep, relatively carbon-rich, sandy-loam soils (Northern complex A1); Dry Restiod Fynbos dominated by asteraceous shrubs and restios, and characterized by the shrub and tree wild rooibos (Northern complex B1 and B2) where B1 was characterized by the restio Willdenowia incurvata (Restionaceae) and B2 by the restio Thamnochortus bachmanii and fabaceous shrubs such as Nylandia spp.; Dry to Mesic Proteoid and Restiod Fynbos characterized by Leucadendron spp. and ephemerals and the prostrate wild rooibos (Southern Complex A1 and 2); Mesic Proteoid Fynbos characterized by Protea spp., ephemerals and the salignus wild rooibos (Southern complex B1 and 2). The greater diversity and cover of ephemerals in the Southern complex simply reflected the longer rainy season in the south compared to the north, where ephemerals had already partially senesced at the time of sampling. All the above divisions had Eigenvalues above the average (0.4924). The northern sites had shrub, prostrate and tree type wild rooibos, while the southern sites had the prostrate, upright and salignus types. It was confirmed that the most commonly found wild rooibos type in the Suid Bokkeveld was the shrub type (Malgas and Oettlé, 2007). Twinspan suggested that variation in plant communities, and rooibos ecotypes in particular, was associated with a N–S gradient. From our observations, most ecotypes co-occurred at the regional scale, e.g. upright and prostrate or shrub and prostrate, while only a few co-occurrences at the local scale were observed, e.g. prostrate and upright. One ecotype (salignus) appeared to be restricted to the southern wetter and lower elevation site (Fig. 3). The following analysis provides further evidence of variation in habitat preference between the ecotypes and suggests that the ecotypes are ecologically distinct.

3.6. Predictors of vegetation and wild rooibos distribution

Canonical correspondence analysis of species data using environmental variables was undertaken with annual rainfall, elevation, slope, aspect, soil depth, rock cover and soil C as explanatory variables for both vegetation and wild rooibos distribution. A stepwise forward addition of each environmental variable revealed that the best predictors of all species distributions were elevation, slope and rainfall where these

Table 3

Recruitment, distribution, associated vegetation complex and predictors of occurrence of wild rooibos ecotypes and cultivated rooibos. Vegetation complexes were determined using Twinspan and predictors of occurrence of these complexes were determined using canonical correspondence analysis.

<table>
<thead>
<tr>
<th>Description a,b</th>
<th>Recruitment a</th>
<th>Distribution a,b</th>
<th>Vegetation complex</th>
<th>Predictor b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrub type</td>
<td>Re-sprouter and re-seeder</td>
<td>Suid-Bokkeveld, Biedouw</td>
<td>Northern complex, Dry Restiod Fynbos</td>
<td>Low rainfall &lt;200 mm</td>
</tr>
<tr>
<td>Tree type</td>
<td>Re-seeder</td>
<td>Wupperthal, Suid-Bokkeveld, Biedouw</td>
<td>Northern complex, Dry Restiod Fynbos</td>
<td>Unknown</td>
</tr>
<tr>
<td>Prostrate type with red-keeled flower</td>
<td>Re-sprouter</td>
<td>Wupperthal, Biedouw, Citrusdal,</td>
<td>Southern complex, Dry to Mesic Restiod Fynbos</td>
<td>High elevation &gt;600 m</td>
</tr>
<tr>
<td>Prostrate type</td>
<td>Re-sprouter</td>
<td>Wupperthal, Agterpakhuis</td>
<td>Southern complex, Dry to Mesic Proteoid and Restiod Fynbos</td>
<td>High elevation &gt;400 m</td>
</tr>
<tr>
<td>Upright type</td>
<td>Re-seeder</td>
<td>Wupperthal, Biedouw, Citrusdal,</td>
<td>Southern complex, Dry to Mesic Proteoid and Restiod Fynbos</td>
<td>High elevation &gt;400 m</td>
</tr>
<tr>
<td>Salignus type</td>
<td>Re-seeder</td>
<td>Citrusdal Extensive, originated in Pakhuis area</td>
<td>Southern complex, Mesic Proteoid Fynbos</td>
<td>High rainfall &gt;500 mm</td>
</tr>
<tr>
<td>Cultivated type</td>
<td>Re-seeder</td>
<td>Citrusdal Extensive, originated in Pakhuis area</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

a Malgas and Oettlé (2007), Malgas et al. (2010).
b This study.
explained 4.87, 4.30 and 4.06% of the variation, respectively. If soil C was included, this variable explained 3.57% of the data.

Regarding the rooibos ecotypes, multiple regression analysis revealed that, in particular, elevation (but not rainfall or slope) was a good predictor of where prostrate rooibos would be found, i.e. high elevation, \( P < 0.0001 \), (Fig. 3) while rainfall (but not elevation or slope) was a good predictor of where salignus \( (P < 0.0002) \) and shrub rooibos \( (P < 0.01) \) would be found (Fig. 3). Shrub ecotypes tended to be found at low rainfall, northern sites while the salignus type was found at the southern high rainfall areas. Elevation was also a good predictor of where the upright ecotypes would be found (higher elevations, \( P < 0.0001 \)) but as there were apparently several types of upright ecotypes and some uncertainty about whether these were upright or prostrate, further predictions about the distribution of the upright ecotype requires genetic typing. Evidently the prostrate rooibos type at Hotbergfontein was different to other types, occurring on a locally richer soil, high rainfall and altitude site (Table 1) and also displays red-keeled flowers similar to those in the Wupperthal area, but from which it is geographically isolated. The growth form, height, recruitment strategy, distribution and predictors of distribution for the five ecotypes therefore varied (Table 3) and some displayed distinct habitat preferences (Fig. 3) making them ecologically distinct.

3.7. Alpha diversity

There was a significant difference in alpha diversity (H) between sites \( (P < 0.0001) \) with an incremental increase in diversity from northern to southern sites where Landskloof and Kleinvlei in the north had lower H than Witelskloof and Kafkraal in the south \( (P < 0.05) \). An exception was Hotbergfontein, the northern-most site, which had an H comparable to that of the southern sites Witelskloof and Kafkraal.

3.8. Functional types

The wild rooibos sites were characterized by shrubs and restios, with a small but consistent presence of geophytes and herbaceous perennials (Fig. 4a). There was no N–S trend in habit types across sites \( (P > 0.05) \). All sites were dominated by arbuscular mycorrhizal plants (most woody shrubs and herbaceous perennials) and cluster rooted plants (Proteaceae and some Restionaceae, Fig. 4b). Roots comprising cluster roots and arbuscular mycorrhizal roots while also engaging in a rhizobial symbiosis to fix nitrogen (AM, N2, C, Fig. 4b) represented the wild rooibos plants found. Cluster roots and nitrogen fixing nodules were evident on all wild and cultivated rooibos plants sampled, regardless of fertilizer regime (e.g. organic Landskloof and non-organic Achterstefontein fertilized with 500 kg P ha\(^{-1}\)). There was no N–S trend in root functional types across sites \( (P > 0.05) \).

3.9. Water use efficiency of wild rooibos ecotypes

There was a negative correlation between water use efficiency (less negative \( \delta^{13}C \) signatures) of various rooibos ecotypes and mean annual rainfall \( (R^2 = 0.768) \). Rooibos
ecotypes found growing in relatively drier areas (shrub, prostrate and tree types) had less negative foliar $\delta^{13}C$ (greater WUE) compared to those growing in wetter areas (upright and salignus, Fig. 5). It is not known to what extent the WUE was the result of plastic or inherent drought tolerance of the ecotypes. In contrast to the common perception among local farmers that wild rooibos is more drought resistant than cultivated rooibos, there was no difference between the $\delta^{13}C$ signatures of cultivated and upright rooibos from the same rainfall area ($P = 0.7926$).

4. Discussion

A total of five wild rooibos ecotypes are referred to in the present survey: shrub, tree, upright, salignus and prostrate types. The salignus or willowy growth form, also known amongst local farmers as wild rooibos, is poorly documented. A similarly described form of *A. linearis* is known to have been misidentified in the past as *A. pendula*, a sister species that closely resembles *A. linearis* (Dahlgren, 1968) and the identity of this species clearly requires genetic typing. In addition, several ecotypes of wild rooibos are referred to locally in Afrikaans as ‘upright’ but the variation in phenotype within this description suggests more than one genotype. For example, both prostrate and upright individuals were found adjacent to one another at Langkloof and may have been genetically different, and upright types at Kriedouw and Kafkraal displayed noticeable green and blue-green patinas on the leaves respectively, and may be different ecotypes.
This is the first report of all wild rooibos ecotypes having three root adaptations to acquire nutrients: cluster roots (which mobilize very poorly available phosphorus), arbuscular mycorrhiza (which transport poorly available phosphorus to plants) and a rhizobial symbiosis to fix nitrogen (Marschner, 1995).

These root adaptations, together with the known role of *A. linearis* as a post-fire colonizer (Cocks and Stock, 2001), make wild rooibos a valuable pioneer plant and soil conditioner. In addition, rooibos has tap roots extending to 2 m or more (Morton, 1983) and probably aids in soil water replenishment and supply of water to understorey plants by way of hydraulic redistribution, similar to proteas (Hawkins et al., 2009).

On the biome scale, about 45% of Fynbos is classified as vulnerable, endangered or critically endangered and has been identified as one of the nine priority biomes to conserve in South Africa, and as a global biodiversity hotspot (http://bgis.sanbi.org; Myers et al., 2000). Exceptions were the endangered Graafwater Sandstone Fynbos at Kafkraal and endangered Sand Fynbos (from previous records). The latter could not be verified and local farmer and landowner knowledge supported the idea that wild rooibos had not grown at these sites, or other Sand Fynbos sites further north. Alternatively, it is possible that wild rooibos had occurred at these sites in the past, and has since been replaced through agricultural land-use practices or alien invasive plants that were visible at the Sand Fynbos sites investigated. Other very small, far-south populations of rooibos exist that we did not sample, e.g. Klaasjagersberg near Simons Town, Kogelberg Nature Reserve, and De Hoop Nature Reserve, but these also occur on the least threatened Sandstone Fynbos (PRECIS database) and are not large enough to be a local resource. Therefore, the study sites in the present survey were representative of the natural range of wild rooibos and largely indicated that, within the parameters of these selected sites, wild rooibos is not associated with endangered vegetation types. However, the low protection status of this vegetation type poses a threat in itself, in that the large-scale destruction and often illegal clearing of Fynbos for rooibos and potato cultivation is met with no resistance from authorities. The numbers of threatened species per site were low (1 to 3). However, it is very likely that this was an underestimation as >50% of the geophytes present were not flowering and could not be identified as already mentioned. Despite a limited number of threatened species being identified to co-occur with wild rooibos, it is well-known that areas in the northern range (southern Bokkeveld area) of wild rooibos are of high botanical value due to the bulb and annual plant biodiversity.

This study presents evidence for habitat preference by the rooibos ecotypes in terms of a N–S rainfall gradient and elevation, which was supported by the occurrence of ecotypes in different plant communities. While some ecotypes clearly co-occurred in the different plant communities, some did not, suggesting that the ecotypes are ecologically distinct.

Three wild rooibos types (shrub, prostrate and salignus) were morphologically very distinct and had distinctive rainfall and elevation cut-offs. Prostrate and upright ecotypes occurred at higher elevations (>400–600 m). Since the rainfall gradient was roughly north–south, the distribution of the ecotypes that could be predicted by rainfall (shrub, salignus) also had a roughly north–south distribution. Shrub ecotypes occurred mostly in the north where the annual rainfall was <200 mm, while the salignus ecotypes occurred in the south where annual rainfall was >500 mm. Soil characteristics (depth, rockiness, N, P and C contents) were less useful than rainfall and elevation in explaining differences in community structure between sites. This was since most characteristics (except for rockiness), were in the same range, i.e. N, P and C were all low even when significantly different.

Salignus ecotypes were found in plant communities with relatively high species diversity, while shrub ecotypes were found in communities with relatively lower species diversity. An exception in many respects was the northern-most site, Hotbergfontein in Nieuwoudtville, which had the highest species diversity and the wild rooibos ecotype present was of the prostrate, not shrub type. Nieuwoudtville and the entire Bokkeveld Plateau on which it is situated is world-renown as a bulb capital and centre of biodiversity. The relatively higher rainfall is not surprising since Nieuwoudtville lies on the Bokkeveld Plateau and receives frontal rain from the Atlantic while all the other northern sites were in the south of the Bokkeveld where rainfall drops off to <300 mm. Species diversity of both animals and primary productivity (Reed and Fleagle, 1995) are known to be positively correlated with rainfall. In addition, Hotbergfontein had a soil which was not purely sandstone, but was a mixture of sandstone and richer clays from tillite and dolerite (Manning and Goldblatt, 2007) with relatively higher carbon contents and this, along with rainfall, may be linked to high biodiversity at this site.

Isotopic data indicated that rooibos ecotypes found growing in relatively drier areas were more water use efficient, and thus, potentially more drought tolerant and functionally distinct. Local knowledge has indicated that certain rooibos ecotypes are more drought tolerant than others and that wild rooibos was more drought tolerant than the cultivated type (Heiveld Co-op farmers, pers comm. 2007). However, to determine whether the response of rooibos ecotypes was plastic depending on rainfall or inherent depending on the genotype, the same ecotypes would have to be grown across the entire rainfall gradient. The similar water use efficiencies of the upright and cultivated type at one site favours the idea that water use efficiency in rooibos may be plastic dependent on the prevailing rainfall conditions. This remains an exciting question requiring further investigation.

5. Conclusions

It is clear from this study and that of Malgas et al. (2010) that wild rooibos comprises a diverse group of ecotypes and possibly even sub-species. We have provided for habitat preference between the ecotypes where these ecotypes occurred in different Fynbos plant communities and separated along an environmental gradient. Considering that wild rooibos ecotypes differ both ecologically and possibly also functionally and genetically, it is concluded that populations of wild rooibos ecotypes should be considered as distinct and that each ecotype is worthy of conservation. This distinctness should be considered when farmers apply for both harvesting and ploughing rights on land.
with wild rooibos ecotypes. In addition, ecological, functional and
genetic distinction between the rooibos ecotypes implies an
expanded genetic resource for the rooibos industry, a resource that
may prove invaluable in the event of global change influencing
the production range of rooibos, or the disease resistance thereof.
Some rooibos ecotypes may even be able to grow outside
threatened vegetation types, e.g. Sand Fynbos, and thus relieve
cultivation pressure on threatened habitats.

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