Comparative water relations of indigenous and invasive Australian Proteaceae in fynbos.

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ABSTRACT

Water-use efficiency (WUE) as reflected in the leaf carbon stable isotope ratio was compared between co-occurring indigenous fynbos proteoids and invasive Australian hakeas *H. sericea*, *H. gibbosa* and *H. suaveolens*. At the driest site, *H. suaveolens* was slightly more WUE than several co-occurring proteoids; there was no significant difference between hakeas and proteas at the other sites. Transpiration rates of shoots and of whole trees were compared between *Hakea sericea* and *Protea repens* growing on Stellenboschberg northeast of Cape Town. Both measurements showed no real difference between the species and it is concluded that differences in water relations are not responsible for the highly competitive growth rates of hakeas in fynbos. It is hypothesised that hakeas may be able to vegetatively outcompete proteoids as a consequence of monopodial architecture and some ability to prevent shade-limitation of photosynthesis. A rough estimate of water loss due to transpiration and interception by *H. sericea* stands indicates that this species may have a significant effect on catchment water loss relative to open-canopy proteoid fynbos. This effect would be due not to transpiration rates of individual trees but to consistently high densities of mature hakea stands.

Abbreviations used:

F - transpirational flow rate
IRGA - infra-red gas analyser
LSA - leaf surface area
PAR - photosynthetically active radiation
WUE - water-use efficiency (carbon assimilation rate A / transpiration rate E)
XPP - xylem pressure potential

INTRODUCTION

Three members of the Australian genus *Hakea* Schrader (subfamily Grevilloideae) are significant invaders of Cape fynbos. *Hakea sericea* is highly invasive and presently covers 14% of the area of mountain fynbos (Richardson *et al* 1987). *Hakea gibbosa* and *H. suaveolens* are moderately invasive (Richardson *et al* 1987) and confined to the Cape Peninsula (Richardson *et al* 1996). Invasion of fynbos by hakeas has serious implications in terms of threats to biodiversity (Richardson *et al* 1996) and reduction in water delivery from catchments (van Wilgen *et al* 1997). The ability of hakeas, especially *H. sericea*, to disperse into fynbos in large numbers is hypothesised to be due mainly to enhanced reproductive characteristics as they produce large numbers of highly dispersable seeds which are protected from local seed predators in hard woody follicles (Richardson 1998; Richardson *et al* 1996). However, reproductive factors alone do not account for the success of hakeas in fynbos. The shrubs need to be able to grow competitively in the fynbos environment via preadaptation not only to fire but also to low nutrients and the prevailing moisture regime. Hakea*s* must have high rates of vegetative growth in order to outcompete their fynbos 'equivalents', the serotinous non-sprouting members of the genera *Protea, Leucadendron* and *Leucospermum* in the subfamily Proteoideae.

This study examined water relations of invading *Hakea* spp. in order to determine whether the apparently superior vegetative physiology of *Hakea* is related to different patterns of water use relative to Proteoideae and in order to compare catchment transpirational losses due to hakeas and indigenous equivalents.

Water relations were compared at three levels over different timescales using the following measurements:

- water-use efficiency over the lifetimes of leaves via analysis of leaf carbon isotope ratios;
- instantaneous transpiration rates of excised twigs; and
- whole-plant transpiration rates over a period of two weeks using deuterium (heavy water) as a chemical tracer.

STUDY SITES

Each site (Figure 1) consisted of stands of proteoid mountain fynbos in the process of being invaded by a different species of *Hakea*.

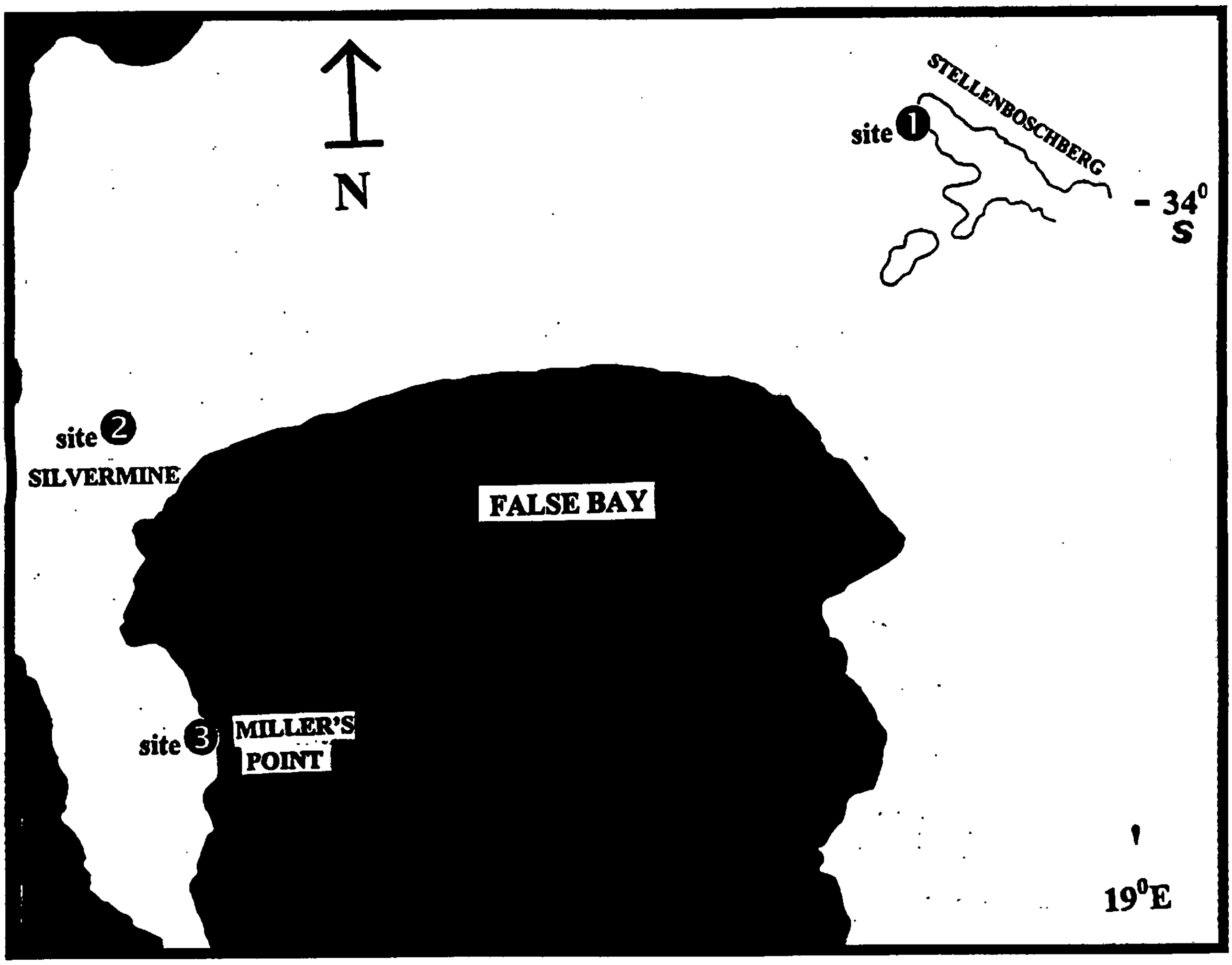

Figure 1: Study sites on the Cape Peninsula and Stellenbosch, southwestern Cape.
Site 1: west-facing slope at the northern end of Stellenboschberg, Stellenbosch (33° 58' S; 18° 53' E) containing *Hakea sericea.*
Site 2: north-facing slope at the northern end of the Silvermine Nature Reserve, Cape Peninsula (34° 24''S; 18° 26' E) containing *Hakea gibbosa*
Site 3: east-facing slope above Miller's Point, Simonstown, Cape Peninsula (34° 14'S; 18° 27' E) containing *Hakea suaveolens.*

All three sites were used for carbon isotope analysis of water use efficiency. Transpiration rates were compared at Site 1 between coexisting *H. sericea* (the most successful of the *Hakea* invaders) and *Protea repens*, a common and widespread fynbos protea.

METHODS AND MATERIALS

1. Water-use efficiency

One *Hakea* species and at least three indigenous proteoid species were sampled at each site. Five individuals of each species growing in open-canopy stands were sampled. Leaves were taken from topmost, outer branches at the northern aspect of the canopy and only unshaded shoots were sampled. Leaves were dried for 48 hours at 50°C and ground through a size 40 mesh. Care was taken to clean the grinder thoroughly between samples in order to prevent the possibility of contamination, which is a particular problem due to the very small amounts of leaf powder used in mass spectrometric analyses (0.05 - 0.08 mg). Carbon 13/12 isotopic composition of the leaves was measured with a Finnegan MAT 252 dual inlet gas isotope ratio mass spectrometer. Readings were corrected by linear regression to the known value of an internal standard and are reported as $\delta^{13}C$-values (parts per thousand or per mille $^0/_{00}$) relative to the PDB carbonate standard.

At each site, the $\delta^{13}C$ values of the *Hakea* sp were compared to those of each indigenous species in turn; and to the mean value for all the indigenous proteas. The Mann-Whitney U test for non-parametric data was used to test the significance of differences between the means.

2. Transpiration rates:

Transpiration rates of *H. sericea* and *Protea repens* were compared at site 1. Two alternative methods were used in this comparison.

(a) Cut-shoot method

Weight-loss was measured simultaneously for pairs of twigs so that each pair experienced exactly the same conditions during the weighing period. Similarly-sized shoots were excised simultaneously from one *H. sericea* and one *P. repens* individual. The mass of each twig was measured every two minutes over a ten-minute period (Dye *et al* 1990) using a two-place decimal balance placed inside a box to minimise effects of wind. Between weighing, shoots were held upright in full sun to simulate orientation on the tree. This was repeated thirteen times with twigs from different trees. The diameter of each twig stem was measured in order to calculate stem basal area.

Leaf surface area:

Protea repens:

The leaves were removed from six shoots, run through a Li-Cor LI-3000 portable leaf-area meter and oven-dried at 70°C for 24 hours. The ratio of leaf surface area (LSA) to dry mass from these six shoots was used to calculate specific leaf area. Dry mass was regressed against measured LSA to obtain an equation which was used to calculate estimated LSA for all twigs.

H. sericea:

This species has terete leaves which are cylindrical in cross-section and so a true estimate of LSA cannot be obtained from a leaf-area meter. The length and mid-point diameter of individual needles were thus measured in order to calculate LSA assuming each needle is a cylinder. Two samples of approximately 70 needles each were measured in this way and oven-dried as above to obtain leaf dry mass. The ratio of dry mass: surface area was calculated and used to estimate LSA for all twigs.

(b) Deuterium tracing method

(i) 'TOTAL COUNTS' METHOD

This method involves the use of deuterated water (D_20) as a tracer (Calder *et al* 1986; Calder 1992). The deuterium (minimum isotopic purity 99.8%) was injected into holes drilled around the base of the trunk. Clear plastic bags tied around shoots were used to collect condensate each day. The concentration of deuterium in the condensate (D/H % above background) provides an estimate of transpiration rate since the greater the amount of water moving through the tree during the study, the more diluted the tracer will be. Samples of water transpired from bagged shoots prior to injection of the tracer are used to obtain the background D/H ratio for each tree. The conditions for use of this method are that complete mixing of the tracer with the transpirational stream must occur before the stream splits to separate branches, and that all the tracer must move through the tree during the study period(Calder et al 1986). Estimates of transpirational flow rate F are calculated according to the sum of the area under the curve of plotted daily D/H concentration.

$$F = M / \sum_{i=1}^{i=T} (C_i \, \Delta t_i) \qquad (1)$$

where C_i is the concentration of tracer in the sample from the i^{th} time increment (H/D % above background)
and
Δt_i is the duration of the i^{th} time increment

(Calder 1992)

(ii) AVERAGED METHOD

Studies using deuterium as a tracer have measured the concentration of D in each bag each day. Over a 14 - day period, with three bags per tree, this would require spectrometric analysis of 45 samples per tree. Due to the expense of such analyses, the number of replicates of each species which can be measured is greatly reduced. Combining the samples from the bags into a daily sample reduces the number of analyses required to some extent but replication is still limited. A recent paper by Calder (1998) showed that the analysis per tree can be reduced to one sample by averaging the concentrations of the daily samples. The resulting estimate of F represents a mean flow rate for the period of the experiment:

$$F = M / (T \Delta t\, Cm) \qquad (2)$$

where

F is the mean flow rate
M is the volume of tracer injected into the trunk
T is the last time increment in which tracer is present in the system
Δt is the duration of the average time period between sample collection
and
Cm is the concentration of deuterium in the sample.

(Calder 1998)

PROCEDURE:

Prior to deuterium insertion, two shoots per tree were bagged and condensate collected over one to several days to obtain a measure of baseline deuterium concentration.

The drilling and injecting took place on hot, sunny days between noon and 4: 00 pm. Deuterium was injected into holes (2.5 - 5.0 mm diameter) drilled around the base of the trunk using a new sterile needle each time on a graduated syringe. Between 0.5 and 1.25 ml of deuterium was injected per tree, depending on the size of the tree. The deuterium was distributed equally between the holes. After injection, the holes were stoppered with flexible wood putty which was then painted with lacquer to ensure waterproofing.

One ml of condensate was collected each day from each of three bags positioned at different heights in the canopy. A fresh pipette tip was used each time to ensure no cross-contamination between bags. Collection times were kept as close as possible to the original injecting time in order to minimise differences in the time Δt over which each bag was integrating. A new bag was placed around the same shoot after each collection. In some cases, a bagged shoot became damaged during the course of the experiment. If this occurred I averaged samples from only two bags to calculate F (trees 2, 4 & 7 in Table 4).

On the three days in which there was some rain during collection, care was taken not to allow rainwater into the collected condensate or onto the leaves while the bags were being changed.

Daily samples were collected from four *H. sericea* and four *P. repens* individuals. For one member of each species, sample was collected for seventeen days following injection and the concentration of the pooled daily condensate (0.3ml from each bag in the canopy) determined in order to obtain a 'total counts' estimate of F. This served as a control to compare the accuracy of the time-averaged estimate of F and also to check whether the assumption that all the tracer had moved through the trees during the study period was met. Equal amounts (0.3ml) of all samples were then combined to obtain a single averaged sample for each tree over the two-week study period.

Deuterium concentrations were measured using a Finnegan MAT 252 dual inlet mass spectrometer. Torsten's H2 was used as a reference gas and the two internal standards were Cape Town municipal water and a premixed standard of 3µl D_20 made up to 10 ml with tap water.

Within the constraints of the project, leaf surface area for each tree could only be estimated, not measured directly. Due to potential scaling errors arising from such estimation, it was decided to compare transpiration rate on the basis of basal trunk area as this quantity could be directly measured as trunk diameter. The significance of differences in the F values for *P. repens* and *H. sericea* were tested using Lord's Range Test for small samples.

Analysis:

Statistical tests were performed using Statistica for Windows version 5.1, Statsoft Inc 1996

RESULTS:

1. Water-use efficiency

Table 1 shows the $\delta^{13}C$ values in parts per thousand $(^{0}/_{00})$ relative to the PDB standard. Indigenous species had very similar values to *H. sericea* and *H. gibbosa* at Sites 1and 2. *Protea lepidocarpodendron* and *P. nitida* were depleted relative to *H. suaveolens* at site 3. At site 1 where transpiration rates of *H. sericea* and *P. repens* were compared, the hakea was slightly enriched relative to the protea but this difference was not statistically significant. Hakeas had similar or slightly higher values than the average for co-existing proteoids.

Table 1: $\delta^{13}C$ values $(^{0}/_{00}$ mean ± sd) for leaves of *Hakeas* and co-existing indigenous proteoids. At each site, the mean for the *Hakea* species is compared to that of each indigenous species using a Mann-Whitney U Test. NS = not significantly different from the *Hakea* value at the 5% level ie $p>0.05$. Where this test found significant differences between the means, the p-level is reported. ***L. salig*** = *Leucadendron salignum;* ***L. xanth*** = *Leucadendron xanthoconus;* ***Lsp. cono*** = *Leucospermum conocarpodendron;* ***P. lepi*** = *Protea lepidocarpodendron;* ***P. neri*** = *Protea neriifolia.*

Site 1			Site 2			Site 3		
Hakea sericea	**-26.82 ± 0.72**	p-level	***Hakea gibbosa***	**-27.94 ± 1.21**	p-level	***Hakea suaveolens***	**-24.76 ± 0.68**	p-level
P. repens	-26.21 ± 1.23	NS	*P. lepi*	-28.37 ± 0.57	NS	*P. lepi*	-26.70 ± 0.42	0.009
P. neri	-27.32 ± 0.83	NS	*Lsp. cono*	-29.28 ± 0.68	NS	*Lsp. cono*	-26.04 ± 0.57	0.028
P. nitida	-26.22 ± 0.97	NS	*L. laure*	-28.70 ± 0.92	NS	*P. nitida*	-24.18 ± 0.35	NS
L. salig	-27.81 ± 1.95	NS				*L. xanth*	-24.78 ± 0.76	NS
mean for indigenous spp:	-26.89 ± 1.40	NS		-28.34 ± 0.80	NS		-25.50 ± 1.14	NS

2. Transpiration rates:

The transpiration measurements took place in spring when hot sunny days were interspersed with rare overcast rainy days. Prior to the start of the experiment, the site received a week of rainy weather which would have recharged the soil water so presumably the trees were transpiring maximally during subsequent sunny days. Weather data are not presented here as the study trees co-occurred and so were subject to identical weather during the course of the experiment.

(a) Cut-shoot method

Table 2 shows the data for the pairs of excised twigs. Weight loss of twigs over the first four minutes of measurement was used as this was found to be sufficient time to maximise transpirational loss while remaining within the time period of linear mass loss. Transpiration rate compared on the basis of both twig basal area and twig leaf surface area gave greater values for the hakea but these differences were not statistically significant in either case.

Table 2: Measurement of transpiration rates from excised twigs of *Protea repens* and *Hakea sericea*. Values are mean ± standard deviation for 13 samples. Differences in transpiration rates per unit twig basal area for pairs of twigs are not significant (Wilcoxon's Matched Pairs Test; Z = 0.384; p = 0. 7). Differences in transpiration rates per unit estimated leaf surface area are also not significant (Wilcoxon's Matched Pairs Test; Z = 0.235; p = 0. 81). Mean twig basal areas were: *P. repens* 0.427 ± 0.076 cm^2; *H. sericea* 0.259 ± 0.075 cm^2. Mean leaf surface areas per twig were: *P. repens* 850 ± 216 cm^2; *H sericea* 493 ± 246 cm^2.

Transpiration rate (ml / 4 min)		Transpiration per unit twig basal area (ml / 4 min / cm^2)		Transpiration per unit estimated leaf area (ml / 4 min / mm^2) X 100	
P. repens	*H. sericea*	*P. repens*	*H. sericea*	*P. repens*	*H. sericea*
0.400 ± 0.193	0.318 ± 0.325	0.937 ± 0.395	1.097 ± 0.844	4.82 ± 1.94	6.45 ±4.92

(b) Deuterium tracing method

(i) 'TOTAL COUNTS' METHOD

Results of analyses of the daily samples from the two trees studied for seventeen days are shown in Table 3.

Table 3: Data for calculation of transpiration rates using the 'total counts' technique for one individual of *P. repens* and one *H. sericea* over seventeen days.

	P. repens			***H. sericea***		
T	Δt_i (days)	C_i (D/H above background)	$C_i \,.\, \Delta t_i$	Δt_i (days)	C_i (D/H above background)	$C_i \,.\, \Delta t_i$
1	0.989583	2.88E-06	2.85E-06	1.04875	0.000158	0.000166
2	1.020833	6.03E-05	6.15E-05	1.020833	0.000186	0.000189
3	1.015	0.000122	0.000124	0.993056	5.62E-05	5.58E-05
4	0.9825	7.15E-05	7.03E-05	0.975833	2.78E-05	2.71E-05
5	0.867917	4.07E-05	3.54E-05	0.867917	1.75E-05	1.52E-05
6	1.135417	2.61E-05	2.97E-05	1.135417	1.77E-05	2.01E-05
7	0.98625	1.75E-05	1.72E-05	0.98625	9.31E-06	9.18E-06
8	0.989583	9.32E-06	9.23E-06	0.98625	6.02E-06	5.94E-06
9	1.072917	6.9E-06	7.4E-06	1.1075	4.54E-06	5.02E-06
10	0.992917	5.04E-06	5E-06	0.996667	4.38E-06	4.37E-06
11	0.944583	4.44E-06	4.19E-06	0.944583	4.22E-06	3.98E-06
12	1.065833	4.35E-06	4.63E-06	1.069583	2.71E-06	2.9E-06
13	0.992917	2.38E-06	2.36E-06	0.989583	2.88E-06	2.85E-06
14	1.020833	1.12E-06	1.14E-06	1.020833	2.15E-06	2.2E-06
15	0.972083	1.38E-07	1.34E-07	0.972083	1.88E-06	1.83E-06
16	0.95125	5.63E-07	5.35E-07	0.947917	2.21E-06	2.1E-06
17	0.885417	-4.9E-07	-4.3E-07	0.885417	1.86E-06	1.64E-06
		$\Sigma\, C_i \,.\, \Delta t_i$	**0.000375**		$\Sigma\, C_i \,.\, \Delta t_i$	**0.000515**

Transpiration rates calculated from Table 3 using equation (1) are:

$$\text{Protea: } F = M \,/ \sum_{i=1}^{i=T} (C_i\, \Delta t_i) = 0.001 \text{ litres} \,/\, 0.000375\ C_i.\text{days} = 2.67 \text{ litres / day}$$

$$\text{Hakea: } F = M \,/ \sum_{i=1}^{i=T} (C_i\, \Delta t_i) = 0.0008 \text{ litres} \,/\, 0.000515\ C_i\ .\text{days} = 1.55 \text{ litres / day}$$

Figure 2 shows the time course of tracer movement in the transpirational stream of the same two trees. The shape of the concentration/time curve for movement of tracer through the trees is similar to other studies using eucalypts (Calder *et al* 1986; Calder 1998; Dye *et al* 1992) and *Prunus serrulata* (Dugas *et al* 1993). In these studies the D/H ratios in the condensate also peaked after 1 to 5 days and concentrations took between 6 and 27 days to return to baseline levels, depending on the amount of tracer injected.

(ii) AVERAGED METHOD

Figure 2 shows that deuterium concentration in the sap stream essentially returned to baseline levels within 14 days of injecting. Thus the condition that all the tracer must move through the tree within the course of the experiment was satisfied and transpiration rates could be calculated using the averaging method of Calder (1998). These values as well as descriptive features of the trees are given below (Table 4). Transpiration rates were well correlated to trunk basal area (BA)for the eight study trees (F= 0.123BA- 0.887; correlation coefficient r = 0.97; p=0.0001). This indicates a linear increase in conducting area with increasing stem area for both species.

Table 4: Characteristics and estimated transpiration rate F for the eight trees used in the deuterium study. There were no significant differences between the species for transpiration rate per unit basal area (Lord's Range Test for small samples; L = 0.218; p> 0.05)

	amount of D_20 injected (litres)	D/H above b/g	T (days)	F (total counts method) (litres / day)	F (time-avg'd method) (litres/day)	**F/ basal area** (litres/cm²/day)	height (m)	trunk basal area (cm²)
P repens								
1	0.001	1.00E-05	17	2.67	2.59	**0.11**	2.20	24.01
2	0.00125	1.25E-05	14		21.01	**0.12**	3.30	174.98
*3	0.001	1.00E-05	14		1.00	**0.03**	2.60	31.40
4	0.001	1.00E-05	14		1.94	**0.07**	2.80	25.95
				mean	**6.64**	**0.08**	**2.73**	**64.09**
				stdev	9.60	**0.04**	0.46	74.00
H sericea								
5	0.0008	8.00E-06	17	1.55	1.56	**0.13**	1.90	11.94
6	0.0005	5.00E-06	14		1.46	**0.11**	2.70	13.52
7	0.00125	1.25E-05	14		12.46	**0.14**	2.70	87.37
8	0.001	1.00E-05	14		7.39	**0.08**	2.00	89.87
				mean	**5.72**	**0.12**	**2.33**	**50.68**
				stdev	5.28	**0.03**	0.43	43.83

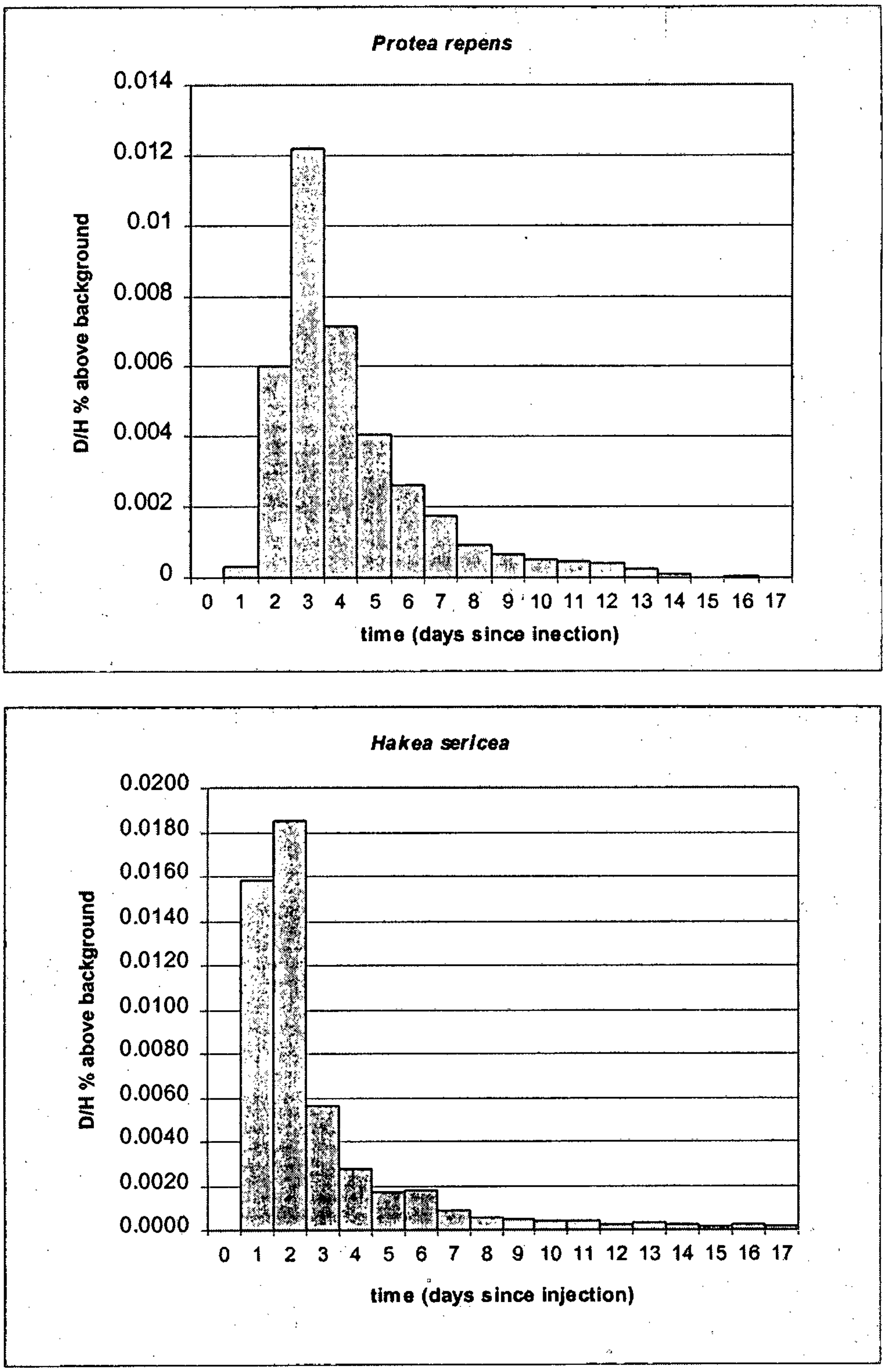

Figure 2: Concentration of deuterated water in condensate collected over seventeen days. The concentrations represent condenstate collected from three bags per canopy and pooled to make one daily sample. *P. repens* = tree 1 in Table 4; *H. sericea* = tree 5 in Table 4.

Transpiration rates calculated using both the 'total counts' and the averaged methods were in close agreement (Table 4) showing that the latter was a good approximation of the more rigorous daily estimation. One of the *P. repens* individuals (tree 3, marked * in Table 4) had a transpiration rate per unit trunk area far lower than its sister trees. This may be due to the fact that this tree had several dead branches giving it a leaf surface area disproportionately small in comparison to its basal trunk area.

As was found in the cut-shoot measurements, *H. sericea* had slightly greater mean transpiration rates but the difference was not statistically significant and would be even smaller if tree 3 were omitted.

Leaf surface area:

Specific leaf area (leaf surface area divided by dry mass) was calculated to be **83.82** $cm^2.g^{-1}$ dry mass for *H. sericea* and **74.64** $cm^2.g^{-1}$ dry mass for *P. repens* (using a double-sided measure of leaf surface area for the protea since both sides of the leaf are transpirationally active).

DISCUSSION:

THE DEUTERIUM TRACING TECHNIQUE:

This study represents the first use of the deuterium tracing technique to measure transpiration in fynbos and the first use of the technique on non-plantation species. It is also the first experiment to measure whole-plant transpiration of Cape Proteaceae.

The main finding was that water relations of invasive needle-leaved hakea species do not appear to be different from those of broad-leaved indigenous proteoids, both at the leaf level and that of the whole plant.

The deuterium tracing method gave plausible estimates of transpiration relative to other studies which report flow rates on a stem-diameter basis. Both *Hakea* and *Protea*, being small shrubs, would be expected to have lower values than eucalypts and studies on *Eucalyptus* spp. using both deuterium and heat pulse velocity techniques gave transpiration values ranging from 0.20 - 0.97 litres/cm^2/day (Calder *et al* 1986; Dye *et al* 1992). This compares well with the findings shown in Table 4. The results obtained in the present study are consistent between trees as higher rates of transpiration were recorded in the larger individuals. The fact that values obtained via the 'total counts' method and the averaged method were in close agreement is another indication that the estimates are reasonable. The disappearance of tracer from the trees during the time of the experiment indicates that appropriate amounts of D_2O were injected and that there was no storage of tracer in the stem (Kalma *et al* unpubl.).

Further support for the result obtained by each of the three methods (deuterium, cut-shoot and carbon isotope analysis) lies in the fact that all three techniques had similar findings: that although *H. sericea* on average seem to use slightly more water than *P. repens*, there is no real difference between them in this regard.

Calibrations of the deuterium method with heat pulse, gravimetric and porometer methods of measuring water loss have supported the favourability of using D_2O as a tracer (Dye *et al* 1990; Dye *et al* 1992; Calder *et al* 1992; Dugas *et al* 1993; Kalma *et al* unpub). The present study also supports the applicability of the deuterium technique to measure sap flow rates in the field. It has a number of advantages over other methods of transpiration measurement. For example the artificial environment of the leaf chamber makes measurements IRGA of transpiration questionable (Groom & Lamont 1996) and any instantaneous measures of WUE may miss critical seasons or times of day (Lajtha & Marshall 1994). Measures on parts of trees for example using cut shoots or individual leaves require large replications in order to account for inherent between-shoot variability (Dye *et al* 1990; Ehleringer & Field 1993). The heat pulse velocity technique, although it also measures whole-plant transpiration, is more complex than the deuterium method, requiring detailed data about wood characteristics of each measured tree (Dye *et al* 1992).

The deuterium method is thus an appropriate technique for measuring whole-plant transpiration rates: it is technically fairly simple and convenient, not unreasonably time-consuming and the use of single-samples in the time-averaged method makes it inexpensive. It will be very applicable to estimations of average transpiration from stands of many different types of trees and shrubs.

TRANSPIRATION RATES:

Measurement of transpiration from excised shoots is valuable mainly on a comparative basis as scaling up to the whole plant is liable to result in an overestimate (Lajtha & Marshall 1994). On a purely comparative basis the results obtained with this method support the finding that flow rates are very similar between the two species, with *Hakea* consistently slightly higher on both a twig basal area and leaf surface area basis. *Protea repens* stems are thicker than those of *H. sericea* but both species had a very similar ratio of leaf surface area to twig basal area (cm^2 leaf area / cm^2 twig area = 1991 for *P. repens* and 1903 for *H.*

sericea). Leaf specific area values were also very similar [results section 2(a)]. This is surprising as needle-shaped leaves have a lower surface-area:volume ratio due to their circular cross-section and indicates a greater proportion of dry matter in *P. repens* leaves. Groom *et al* (1994) examined differences between broad and terete leaves produced by a single species (the two-leaved hakea *H. trifurcata*) thus eliminating interspecific differences. They found that terete leaves are more xeromorphic and function more efficiently under hot, dry high-light conditions, while the advantage of broad leaves is in increased light interception and greater carbon gain when water is not limiting. This and another study comparing flat and needle-like leaves (Groom *et al* 1994; Groom & Lamont 1996) indicate that the latter have lower photosynthetic capacity but reduced transpiration rates and greater drought tolerance. Broad leaves have greater heat load problems and so require more transpirational cooling. Under hot dry conditions broad leaves thus do not have as much recourse to stomatal control of water loss and so function at a more negative xylem pressure potential (XPP) than the terete leaves. It would thus be expected that *H. sericea* would be capable of reduced transpiration relative to *P. repens*.

However, both of the methods I employed to compare transpiration rates indicate no real difference in sapflow between the indigenous and the invasive species. It is well established that plants show strong seasonal fluctuations in water use (Dye *et al* 1998; van der Heyden & Lewis 1989). The present study was conducted in spring when water was not limiting and it is unlikely that either species was exerting stomatal control on transpiration. It is possible that hakeas and proteas will respond differently to the summer dry period and that this is when differences in transpirational rates, if any, will become apparent (but see interpretation of carbon isotope values below).

A higher flow rate in the hakea would have been indicative of possibly greater carbon assimilation rate since it would indicate more open stomata and thus greater internal CO_2 concentrations. The fact that transpiration rates are similar means that this is not likely to be the means by which *H. sericea* maintains high vegetative growth rates in the southwestern Cape mountains.

WATER-USE EFFICIENCY:

In C3 plants, carbon isotope composition is determined primarily by the ratio of intercellular to ambient CO_2 concentration (c_i/c_a). This reflects CO_2 assimilation rate relative to transpirational water loss, since when stomata are open and c_i is high, water loss will also be high. When CO_2 assimilation rate is reduced c_i should decrease and $\delta^{13}C$ increase and $\delta^{13}C$ will thus be the least negative in the most water-use efficient species (Farquhar *et al* 1982). The studies on *Hakea* spp in Australia, although they did no carbon isotope analyses, indicate that terete leaves should be more WUE than flat leaves, assuming no other species-specific differences (Groom *et al* 1994; Groom & Lamont 1996). Site 3 was the only site where the *Hakea* species was found to show greater WUE. This site appears to be the driest since $\delta^{13}C$ values for proteoids here are higher than for the same species from Site 1 and Site 2. What appears to be happening is that at the site where water is limiting, the *Hakea* species is able to show a greater increase in WUE than the proteoids. In sites where water is more available (Sites 1 & 2) the hakeas maintain a water-spending strategy. Thus the transpiration measurements for site 1 indicate a situation where no stomatal control is exerted and show that hakeas are capable of transpiration rates as great, if not greater than, *P. repens*. This is surprising in light of the studies of Groom & Lamont (1996). Similar WUE in broad and terete leaves may be due to differences in stomatal control. At any rate, several IRGA studies found the same result (equivalent instantaneous WUE of broad and terete leaves) but discounted them on the assumption that the leaf chamber alters the leaf microenvironment and so affects transpiration (Groom & Lamont 1996).

DIFFERENCES IN PHYSIOLOGY BETWEEN *H. sericea* AND *P. repens*:

Water is not often limiting for deep-rooted species such as Proteaceae in fynbos (Stock *et al* 1992), so differences in water use are not likely to become apparent except at certain sites or during drought. Thus it appears that leaf-level WUE does not explain the ability of *H. sericea* to thrive in fynbos. The $\delta^{13}C$ results indicate that leaves of *H. sericea* and *P. repens* function at very similar c_i levels. The similarity in their transpiration rates indicates that there is unlikely to be any difference in the rate of carbon assimilation between the two species, unless there are differences in the carboxylation efficiency such that one species can maintain higher rates of carbon gain at similar c_i levels.

What characteristics allow such competitive growth and reproductive abilities in hake*a*? Escape from specialised Australian predators in the fynbos allows for elevated seed numbers but since the major predators of hakea are seed-eaters reduced predation will not aid vegetative growth. Invading hakeas do not appear to be diverting resources away from seed production to vegetative structures. What then are the mechanisms by which hakeas grow so successfully? One of the most obvious differences between *H. sericea* and *P. repens* is in the leaf morphology.

In the native habitat of the genus *Hakea*, terete-leaved species dominate in hotter, more arid parts and are more drought-tolerant than co-occurring broad-leaved species (Groom & Lamont 1996). Richards *et al* (1997) found that adult mortality due to drought was directly related to leaf shape, with broad-leaved species being more susceptible to water-stress damage. Terete-leaved species were able to maintain lower transpiration rates during summer drought. These results suggest the possibility that a reason for its vegetative competitiveness in fynbos is that *Hakea* survival may be greater than that of Proteoideae during summer drought. My study did not compare drought-tolerance in the two species but previous studies on fynbos water relations indicate that summer drought is in fact not often experienced by proteoids (van der Heyden & Lewis1989; Stock *et al* 1992) which due to their deep root systems are able to maintain relatively constant transpiration rates without much reduction in XPP throughout the dry summer months. Thus enhanced drought tolerance is unlikely to be a consistently advantageous feature of hakeas over proteas. Circumstantial support is lent to this fact by distribution maps of hakea invasion which show that they have spread into areas with a wide range of mean annual precipitations (200 - +3000 mm; Richardson 1998).

Adult proteas escape drought via their deep roots but the same is not true of seedlings. As a consequence of serotiny, with almost all germination taking place in the first winter after fire (Cowling & Lamont 1987 in Richards *et al* 1997) seedlings face their first drought within a few months of emergence, when roots have not penetrated deeply enough to avoid low soil water potentials. Proof that seedlings experience greater water stress than adults was provided by Richardson & Kruger (1990) who measured summer predawn XPP's of -2.0 MPa in *P. nitida* and -3.0 MPa in *P. repens* seedlings. Adults had XPP of only -0.5 MPa. Studies on *Hakea* seedlings in southwestern Australia indicate that survival at this growth stage is greatly enhanced by possession of terete as opposed to broad leaves (Richards & Lamont 1996; Lamont *et al* 1993 cited by Richards *et al* 1997). Since seedling mortality is likely to greatly limit recruitment, greater drought tolerance in seedlings will confer a definite competitive advantage on *Hakeas*. However, Midgley (1985) showed no increase in summer mortality of protea seedlings, indicating that drought tolerance of seedlings does not greatly limit recruitment. Southwestern Australia is more arid than the southwestern Cape, especially the fold mountains where orographic rain may interrupt dry periods, so drought tolerance is not as important in fynbos. Thus it seems that the answer to how hakeas vegetatively outcompete proteas does not lie in the field of water relations.

Mitchell & Allsopp (1984) found that *H. sericea* seeds contain more than four times the phosphorous per g dry mass of *P. repens* and *P. nerifolia* seeds. This might indicate a mechanism of enhanced phosphorous nutrition in the hakea relative to proteoids. However, the most striking differences between the species relate to the effects of combined architecture and leaf characteristics.

ARCHITECTURE AND LIGHT:

Hakeas are taller with smaller lateral branches than *P. repens* (pers obs). Thus part of the reason for their growth ability may lie in architectural differences. Unlike many proteas with their multi-branched growth requirement to maximise terminal cone production (Bond 1992), hakeas have no reproductive requirement for repeated branching (hakea flowers are produced along the length of branches in the leaf axils). Thus initially hakeas may be able to overtop proteas for purely architectural reasons while still maintaining greater cone production (Bond *et al* 1995). Bond (1992) describes the vegetative trade-offs of unbranched and highly branched growth: plants which form broad, low canopies maximise exposure of foliage to light but are vulnerable to overtopping by neighbours. Plants which grow tall with less secondary branching benefit by avoiding shading from competitors but the cost is increased shading of their own lower canopies.

Hakeas appear to produce more leaves inside the canopy compared to proteas which concentrate their leaves around the canopy edge (pers obs). This should result in a greater leaf-area index for the hakea. Increased LAI may be the reason that individual *Hakea* trees can maintain relatively high transpiration rates despite their terete leaves. Needle-like leaves have been shown to cause less self -shading (Schulze 1982) so photosynthesis may be spread more evenly throughout the canopy. It also appears probable that hakea leaves possess the ability to photosynthesise under lower light conditions than those of proteas. Circumstantial evidence for this is the fact that in Australia *H. sericea* commonly grows as an understory species in eucalypt woodlands (Richardson 1998) where the eucalypt canopies will intercept a large proportion of the PAR; and also that *Hakea* grows vigorously at extremely high densities which must result in a large amount of shading from neighbours. I have observed *H. sericea* individuals flourishing and producing many follicles in dense shade under large wattles and eucalypt stands at Site 1. If hakeas can decrease self-shading by reason of possessing terete leaves they can escape from the self-shading limitations of relatively less-branched growth. Maintenance of growth in shade indicates that their leaves may also be capable of maintaining photosynthetic rates under low-light conditions. The benefit of such enhanced light utilisation per unit ground area occupied would outweigh any decreases in light capture at the leaf level imposed by the reduced surface area of needle leaves (Groom & Lamont 1996).

I suspect that differences in canopy light penetration and/or photosynthetic light requirements might explain the ability of hakeas to vegetatively outcompete proteas in fynbos. Aspects of this hypothesis may be easily testable by measuring light intensities at different levels within protea and hakea canopies and by constructing light response curves for leaves.

CATCHMENT WATER LOSS DUE TO TRANSPIRATION AND CANOPY INTERCEPTION:

Recent studies have highlighted the impacts of alien plants on water resources (van Wilgen *et al* 1997). However, direct measures of rates of water use by plants are often technically difficult and so estimates of water loss due to alien invasion are frequently based on indirect measurements, such as observed rates of soil water depletion and changes in streamflow. These can easily lead to erroneous conclusions (Dye *et al* 1998) for example soil water depletion measurements will not show deeper groundwater depletion by deep-rooted eucalypts. The deuterium technique offers a relatively simple method for measuring whole-plant transpiration rate; such measures are important baseline knowledge even though scaling up from the individual plant can lead to large errors (Lajtha & Marshall 1994). Prior to this study, no direct measures of *Hakea* water use had been made so it was not known if these plants have inherently greater transpiration rates than fynbos. I have shown that a single *H. sericea* individual does not transpire at a much greater rate than a typical *P.repens* individual. However, hakea infestations consistently reach greater densities and cover larger areas than stands of proteas, with dense infestations having more than 9000 hakea plants per ha (0.9 trees per m^2) (Richardson 1998).

A rough estimate of transpirational water loss from dense stands of *Hakea sericea* can be made on the basis of my results. Shrubs growing at high densities will have lower individual leaf surface areas than shrubs in open canopies (Dye *et al* 1998) so under such conditions a slightly reduced transpiration rate per tree is expected. If we assume that an average individual growing in an open canopy situation transpires an average 5.72 litres / day (Table 4) then an (arbitrarily chosen) 20% correction for reduced leaf surface area in closed canopy gives an average of 4.58 litres per tree per day. If stand density is 0.9 trees per m^2 and we ignore the effects of understory vegetation (which is negligible in dense stands of *Hakea*) then water loss due to transpiration will be of the order of 4.1 mm of soil water per day. This value is likely to be an overestimate since my results reflect transpiration measured (presumably) during conditions of unlimited water availability, and is also not representative of the diversity of transpiration rates between individuals. As such it is not intended to be a true estimate of transpirational losses due to hakeas. However, this rough estimate does indicate that transpiration from dense stands of *H. sericea* may be comparable to that from stands of adult *Eucalyptus grandis* (4-7 mm per day, Dye *et al* 1998). This is despite the fact that individual *E. grandis* trees can each transpire several hundred litres of water per day and is due to the fact that the eucalypts grow at far lower densities than *Hakea* (Dye *et al* 1998).

Since individual tree transpiration rate is seemingly less important than stem density, my results indicate that transpiration rates from proteoid fynbos may be considerable in older stands with closed canopies, while stands in earlier stages of post-fire regeneration with lower stem densities and sparser canopies will have far lower rates of transpirational water loss. In general, it is unlikely that *P. repens* stands will reach the density and extent of dense *H. sericea* infestations (Richardson & Kruger 1990). Total transpiration from mountain fynbos is of course more than that due to proteoids alone because of the vigorous herbaceous layer below protea stands. This is still unlikely to rival water loss from dense stands of hakeas, especially if canopy interception by the hakeas is taken into account. Studies have shown that canopy interception and re-evaporation of rainfall by stands of pine (terete-leaved) is about three times higher than that from plantations of broad-leaved eucalypts (Dye *et al* 1998). Terete-leaved *Hakea* canopies are thus likely to have maximal interception abilities which may approach 20 - 40% of gross rainfall (values from studies in temperate forests; Dye *et al* 1998). Thus it is probable that *Hakea sericea* invasion of fynbos mountain catchments has significant effects on catchment hydrology relative to the original vegetation.

CONCLUSIONS:

Hakea sericea does not use more water than *P. repens*; nor does it appear to have more efficient use of water. Vegetative attributes of *H. sericea* responsible for invasive success are thus probably unrelated to water use. Instead, monopodial architecture and the ability to avoid light-limitation due to shading may give hakeas a growth advantage over proteas. Transpiration rates calculated for *H. sericea* indicate that this species will have a significant effect on water loss due not to transpiration rates of individuals but to the high density and large extent of stands.

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