

Sexual dimorphism in reproductive allocation and water use efficiency in *Maireana pyramidata* (Chenopodiaceae), a dioecious, semi-arid shrub

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Abstract. Sexual dimorphism in dioecious plant species is widely attributed to the differential impacts of reproduction on male *v.* female plants. We investigated sexual dimorphism in reproductive, morphological and physiological traits of *Maireana pyramidata* (Benth.) Paul G. Wilson (Chenopodiaceae), a dioecious, semi-arid shrub endemic to Australia. We estimated reproductive allocation for each sex by calculating the relative biomass allocated to flowers and fruits per gram of leaf tissue, based on one branch per sample plant. Morphological measurements included leaf mass, stem mass, specific leaf area, plant height and plant leaf area index. We also measured leaf nitrogen and chlorophyll, gas exchange and $\Delta^{13}\text{C}$. Reproductive allocation was nine times greater in females than in males. No significant difference between the sexes in photosynthetic rate or transpiration could be detected but instantaneous water use efficiency (photosynthesis/transpiration) was significantly lower in females than in males during the fruiting period. $\Delta^{13}\text{C}$ did not differ between the sexes. The results indicate that greater reproductive allocation in females has an immediate impact on their capacity for conservative water use but does not lead to long-term differences in water use efficiency.

Introduction

Sexual dimorphism in dioecious plants is often attributed to sexual specialisation, a theory based on the assumption that male and female reproductive functions have contrasting requirements. In general, female fitness is predicted to be more limited by resources than by male fitness (Bateman 1948; Lloyd and Webb 1977; Delph 1999). Whilst hermaphrodites might be viewed as representing a compromise between optimal function for each sex, when the sexes are separate, each sex may be independently subjected to selective forces that enhance fitness. This disruptive selection is thought to lead to individually specialised sexes with greater efficiency and therefore greater fitness (Darwin 1877; Lloyd and Webb 1977; Bawa 1980; Givnish 1980; Kohorn 1994; Geber 1995).

For dioecious plant species, differing reproductive demands can cause the sexes to respond to environmental conditions in different ways. For example, the higher reproductive demand of females may cause greater drought stress relative to males, leading to differential survival and fitness over soil moisture gradients (Freeman *et al.* 1976; Fox and Harrison 1981; Freeman and McArthur 1982; Zimmerman and Lechowicz 1982; Dawson and Ehleringer

1993). In these studies, females have greater reproductive output in wetter habitats relative to more xeric sites. Alternatively, female plants can survive in dry conditions by using water more efficiently than males (Dawson and Bliss 1989; Gehring and Monson 1994; Retuerto *et al.* 2000).

The few studies that have compared the physiology of the sexes of dioecious species typically involve Northern Hemisphere deciduous species (but see Dawson and Ehleringer 1993). Arid environments provide a unique set of environmental pressures on plant morphology and physiology. Approximately one third of the land area of Australia is arid (Barlow 2001). Plant species native to Australia's arid regions, in particular those of the Chenopodiaceae, have evolved strategies to withstand a climate typified by low, unpredictable rainfall and hot, dry summers (Graetz and Wilson 1984; Leigh 2001). In regions where water availability is low and often variable, one might expect selection to favour female plants with a greater capacity for water conservation. To test this hypothesis, we studied *Maireana pyramidata*, a dioecious chenopod shrub. We investigated reproductive, morphological and physiological characteristics of *M. pyramidata* in north-western Victoria, Australia. We predicted that female

plants would have a greater reproductive allocation than males and that this would impact on their capacity for efficient water use.

Materials and methods

Study species

Maireana pyramidata (Benth.) Paul G. Wilson (Chenopodiaceae), black bluebush, is a hemispherical, perennial shrub, growing up to 1.5 m tall. Its leaves are small, obovoid and fleshy; flowers are very small and unisexual. Fruits are pale green when fresh and dark brown and papery when mature. *M. pyramidata* is a common and widespread plant in many of the semi-arid regions of Australia. It is drought-resistant, growing on calcareous sands and loams, often alongside other *Maireana* and *Atriplex* species and in association with mallee and belah woodland (Harden 1990; Cunningham *et al.* 1992).

Field site

During October 2000, a population of *M. pyramidata* plants was studied at Lake Tyrrell, a salt lake surrounded by farmland in north-western Victoria, Australia. The species grows abundantly (0.5–2 m apart) along the side of a dirt road circling the lake, approximately 200 m from the lake edge. The road is elevated relative to the lake and shoreline by approximately 1–1.5 m, with *M. pyramidata* individuals existing only on the upper side of the road. The roadside verge has experienced heavy soil disturbance, probably within the previous 10 years and many of the plants directly adjacent to the road have their lower stems covered by earth.

The approximate age of the plants in the population was estimated by making cross-sections through a small number of dead basal stems and counting growth rings. This ageing method is thought to be reliable for *Maireana* in south-eastern Australia as these plants produce new layers of vascular tissue every spring when soil moisture is relatively high and maximum temperatures are high but not extreme (J. Ash, pers. comm. 2000). The estimated age range of *M. pyramidata* at Lake Tyrrell was 0.5–20 years with older plants and very young seedlings predominating. We estimate that individuals reach reproductive maturity at 2–3 years of age.

Study plants were selected from within a 100-m × 3-m plot. Although selection of individuals was random, plants were rejected if multiple buried stems prevented distinguishing them from other individuals or if they did not appear to be reproductively mature. The sex ratio of the population was determined by walking two 300-m transects, parallel and 3-m apart along the roadside, and recording the sex of the plant at every 2-m interval along each transect. The 2-m interval was used in order to ensure that different individuals were sampled at each interval. Soil moisture (down to approximately 7-cm depth) was measured beneath each plant with a Thetaprobe Model ML1 soil moisture meter.

Reproductive and vegetative allocation

Reproductive allocation (RA) was estimated when the majority of fruits on female plants were mature. A representative branch from 28 randomly chosen plants (14 of each sex) was used to obtain an estimate of RA per plant as it was not logistically feasible to calculate RA on a whole plant basis. Because the branches collected from each plant differed in size (10–20 cm in length, bearing many small branchlets, 2–4 cm in length) a ratio of reproductive biomass to leaf biomass was used to standardise measurements across plants. RA was measured as reproductive biomass/leaf biomass (g dry mass).

Each branch was collected and placed in a paper bag to prevent loss of foliage, flowers and fruits. For each sample, stems, leaves, flowers and fruits were separated, oven-dried at 60°C for a minimum of 5 days and weighed. The total reproductive biomass of a branch was calculated as

the dry mass of flowers that did not develop into fruits plus the dry mass of fruits (if present). The numbers of flowers and fruits were also recorded. It is possible that the number and mass of flowers were underestimated because our data collection was based on a 'snapshot' estimate in time of all reproductive output for a season. Similarly, a number of fruits may also have shed since maturation. Therefore, if RA was underestimated, this underestimate is likely to be greater in females due to fruits being appreciably larger than flowers (see Results).

For comparisons of vegetative characteristics, height, specific leaf area (SLA), the number of leaves per mm of stem and leaf mass (g) per gram of stem mass were determined on 20 plants per sex. For SLA measurements, samples were brought back to the laboratory in dampened paper bags on ice to prevent leaf tissue from drying and shrinking. Twenty healthy leaves from the tips of branchlets of each plant were digitally scanned and the area was analysed using the public domain NIH Image program (<http://rsb.info.nih.gov/nih-image/>). Leaves were then oven-dried and weighed. For each plant, the total area of the 20 leaves was divided by their total mass to obtain SLA. The number of leaves per millimetre of stem was estimated from three branchlets per plant. A ratio of leaf to stem mass was obtained by using the 28 sample branches used for estimating RA (see above).

Leaf area index (LAI, leaf area per area of ground, Welles 1990) was measured with a LAI-2000 plant-canopy analyser (LI-COR Inc.) on 40 plants. In this study, a 90° optical sensor was used to take one reading above each plant and four readings beneath each plant canopy in four different directions. Because *M. pyramidata* individuals varied in the degree of shading from neighbouring foliage, the two outermost ring sensors were masked when calculating LAI to ensure consistent readings. Thus, the canopy of each plant was analysed at angles ranging between 0° and 38° from the zenith.

Leaf nitrogen and chlorophyll

Nitrogen content was determined by a semi-micro Kjeldahl technique (AOAC 1984) on leaves of 10 randomly selected study plants of each sex, with leaves from female plants taken from fruiting branches. Duplicate samples for each plant were digested at 250°C for 15 min and then at 460°C for 1 h in a Tectator 2012 heating block with 6 mL sulfuric acid and a selenium catalyst. Cooled samples were then distilled and titrated with a Gerhardt Vapodest-5 apparatus. The nitrogen readings for the replicates were averaged to obtain an estimate of percentage nitrogen for each plant.

To determine leaf chlorophyll content, leaves of the same 10 plants selected for nitrogen analysis were brought back to the laboratory on ice and scanned by using NIH Image to obtain leaf area. Samples were then frozen in liquid nitrogen and stored at –80°C until used for chlorophyll extraction and spectrophotometric determination (with a DU 640 Spectrophotometer, Beckman Instruments Inc.) following the method of Porra *et al.* (1989). Chlorophyll concentration was divided by leaf area to obtain an estimate of leaf chlorophyll content (chl *a* + chl *b*, μmol m⁻²) for each plant.

Carbon isotope discrimination $\Delta^{13}\text{C}$

Leaf material from 10 plants of each sex was oven-dried and ground in a cyclone mill and passed through a 0.4-mm screen. Samples were subjected to a controlled combustion, catalytic oxidation and reduction, using a Carlo Erba 1110 Elemental Analyser. The resulting gas was analysed for $\delta^{13}\text{C}$, the ratio of $^{13}\text{C}:^{12}\text{C}$ relative to a Pee Dee belemnite (PDB) standard (Farquhar *et al.* 1989), using a VG Isochrom-EA Mass Spectrometer. The negative values of δ were converted to Δ , which denotes the degree of ^{13}C discrimination by a plant (Farquhar *et al.* 1989). A higher value for $\Delta^{13}\text{C}$ indicates higher discrimination and therefore less carbon fixed per unit of water lost (low water use efficiency). Thus, $\Delta^{13}\text{C}$ is an inverse measure of water use efficiency, integrated over the lifetime of the foliage measured.

Table 1. Reproductive allocation in *Maireana pyramidata*

Results of one-way ANOVA tests for differences between male and female *M. pyramidata* plants in reproductive biomass, measured from one branch per individual. Mass measurements are grams dry mass

Trait	Male mean	Female mean	<i>F</i> -value _(df)	<i>P</i> -value
Number flowers/leaf mass	62.73	147.81	19.85 _(1,26)	<0.001
Flower mass/leaf mass	20.53	38.03	8.40 _(1,26)	<0.008
Mean flower mass	3.2×10^{-4}	2.4×10^{-4}	7.83 _(1,27)	<0.009
Number of fruits/leaf mass	2.00	103.00	16.41 _(1,18)	<0.001
Fruit mass/leaf mass	0.54	141.33	5.04 _(1,2)	<0.034
Mean fruit mass	2.47×10^{-3}	8.85×10^{-3}	61.35 _(1,18)	<0.000
Reproductive mass/leaf mass	18.83	168.55	5.61 _(1,24)	<0.025
Reproductive mass/stem mass	33.56	213.63	6.02 _(1,26)	<0.021

Table 2. Vegetative allocation in *Maireana pyramidata*

Results of one-way ANOVA tests for differences between male and female *M. pyramidata* plants in morphological traits. Mass measurements are grams dry mass

Trait	Male mean	Female mean	<i>F</i> -value _(df)	<i>P</i> -value
Leaf mass/stem mass	1.70	1.42	2.73 _(1,26)	>0.111
Specific leaf area (SLA) (m ² g ⁻¹)	7.78	8.03	0.22 _(1,18)	>0.641
Plant height (cm)	92.25	90.50	0.05 _(1,38)	>0.824
Leaf area index (LAI)	17.86	17.02	0.21 _(1,38)	>0.653

Gas exchange

All gas exchange measurements were carried out on 22 study plants (11 of each sex). The measurements were conducted in October 2000, immediately after an unusually heavy rainfall event, with maximum temperatures averaging 30°C. Plants had passed peak flowering and an estimated 90% of female branches bore large quantities of mature fruits. For each plant, gas exchange measurements were made on the tip of a north-facing branchlet. On female plants, both a non-fruiting and fruit-bearing branchlet was used for measurements. Fruiting branchlets bore fruit along their length but gas exchange was measured on leaf tissue only. Gas exchange was measured with a LI-COR 6400 portable photosynthesis system (LI-COR Inc.). Light was provided with a LI-COR chamber-mounted red/blue light source. Prior to measurements a series of light-response curves were made to determine saturating light levels for *M. pyramidata* at Lake Tyrrell. On the basis of preliminary light-response curves, all measurements were taken with photon flux density set to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Relative humidity was set at 30% to represent the average conditions at the site. Measurements were made between 0800 hours and 1230 hours or were terminated when leaf temperatures could not be maintained at 35°C. Because the leaves of *M. pyramidata* are small and cylindrical, it was not practicable to estimate the area of foliage within the sample chamber. Therefore, the foliage used for each measurement was removed from the plant, oven-dried and weighed in the laboratory and all gas exchange measurements were re-calculated on a mass basis ($\mu\text{mol g}^{-1} \text{s}^{-1}$). From the photosynthesis and transpiration values obtained via this method, instantaneous water use efficiency was calculated for each plant by dividing photosynthesis by transpiration.

Data analysis

Data were analysed by one-way ANOVA and contingency table analysis. The Tukey–Kramer HSD test was used for *a posteriori* means comparisons. Analyses were carried out with the software package JMP (SAS Institute Inc.). The level of statistical significance was set at $\alpha = 0.05$.

Results

Patterns of reproductive and vegetative allocation

The sex ratio of males to females, 1:1.18 (males, $n = 94$; females, $n = 111$), was not significantly different from 1:1 ($\chi^2_{(1)} = 1.41$, $P > 0.24$). Male flowers were larger than female flowers but females had significantly more flowers per gram of leaf (Table 1). A small number of male plants bore fruits. However, when male fruits were present, these were few and less than one third of the mass of female fruits (Table 1). Thus, females had significantly greater RA (calculated as the ratio of total reproductive biomass per gram of leaf tissue) than did males (Table 1).

Despite a greater female biomass allocation to reproduction, no between-sex patterns in vegetative allocation could be found. The sexes did not differ in SLA, LAI, plant height or leaf biomass per gram of stem (Table 2). Neither was there a significant difference in the moisture of the soil beneath plants of each sex (ANOVA: $F_{(1,20)} = 1.50$, $P > 0.234$).

Physiology

No significant difference in photosynthetic rate, transpiration or conductance could be detected among female fruiting, female non-fruiting or male branchlets (photosynthesis, ANOVA: $F_{(2,29)} = 0.90$, $P > 0.417$; transpiration and conductance, ANOVA: $F_{(2,29)} = 1.71$, $P > 0.199$; Fig. 1). Both types of female foliage had significantly lower instantaneous water use efficiency (photosynthesis/transpiration) than male foliage (ANOVA: $F_{(2,29)} = 5.17$, $P < 0.012$; Fig. 1). In female fruiting

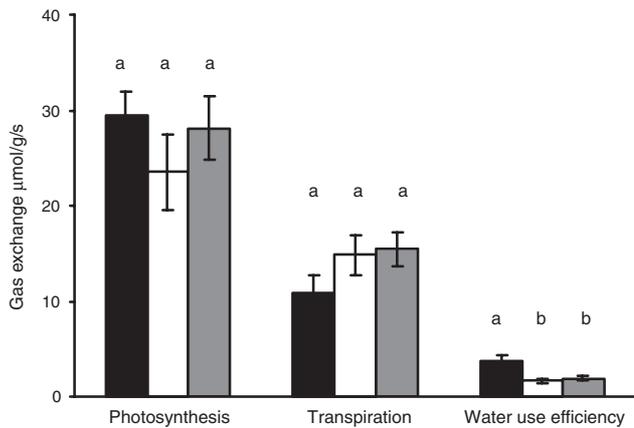


Fig. 1. Comparison of photosynthesis, transpiration and instantaneous water use efficiency (photosynthesis/transpiration) among male branches (black bars), female fruiting branches (white bars) and female non-fruiting branches (shaded bars) in *Maireana pyramidata* plants. Means with different letters were significantly different at the 0.05 level (Tukey–Kramer HSD-test).

branchlets, low water use efficiency was due to a slightly lower photosynthetic rate and higher transpiration than in males. In female non-fruiting branchlets, this result was due to the combined effects of a similar photosynthetic rate and a higher transpiration rate than in males (Fig. 1).

In contrast to the patterns of instantaneous water use efficiency, long-term water use efficiency, indicated by the $\Delta^{13}\text{C}$ content of the leaves, did not differ between male and female plants (Table 3). Male plants had slightly higher leaf nitrogen and chlorophyll than did female plants but this difference was not significant (Table 3).

Discussion

Reproductive allocation in female *M. pyramidata* plants was substantially greater than that in males. We did notice low numbers of fruits on a small proportion of male plants, suggesting that the population we examined is, at some level, gynodioecious. Among other dioecious species, low-level hermaphroditism is not uncommon, particularly in the form of males producing small numbers of bisexual flowers and fruits (A. Leigh, pers. obs.). Male ‘lability’ might represent vestigial gynodioecy or, alternatively, mark the emergence of increasing numbers of hermaphrodites amongst a once strictly dioecious population. In the case of *M. pyramidata*,

both the low numbers of fruits as well as their apparently poor quality, indicated by size, suggests that the seed contribution of male plants to the next generation is negligible. Therefore, the observed fruit-producing male plants are probably functionally ‘male’. Taking into account the fruit produced by male plants, female *M. pyramidata* plants allocated nine times more biomass to reproduction than did males. This represents a between-sex difference in RA at the higher end of the range found in dioecious plants, where RA generally is 1–10 times greater for females than males (Putwain and Harper 1972; Wallace and Rundel 1979; Allen and Antos 1988; Cipollini and Stiles 1991; Freeman *et al.* 1993; Gehring and Linhart 1993; Nicotra 1999).

High resource investment in reproduction can constitute an indirect cost in the form of reduced vegetative activity. In dioecious species, female plants can flower less frequently, have lower growth rates and shorter life spans than males (Delph 1999). However, for *M. pyramidata*, we found no evidence of lower female leaf biomass, foliage density or plant height. The sex ratio of the population was close to 1:1, indicating that if differential growth or mortality occurs between the sexes, it is not marked. With no apparent vegetative effects, one might expect female carbon gain to differ from males, either manifested as an adaptation to offset reproductive costs or as a cost itself.

During fruit maturation in spring, patterns of carbon gain in *M. pyramidata* females differed from those of male plants. Water use efficiency was significantly higher in males than females in both the predominant fruiting female foliage and the less abundant non-fruiting foliage. It is not uncommon for female plants of dioecious species to suffer greater water stress than males do (Freeman *et al.* 1976; Wallace and Rundel 1979; Fox and Harrison 1981; Freeman and McArthur 1982; Zimmerman and Lechowicz 1982; Dawson and Bliss 1989; Dawson and Ehleringer 1993). Female plants of different species vary in their physiological response to drought conditions. Relative to males, females might decrease stomatal conductance (and so transpiration), thereby lowering carbon gain but increasing water use efficiency (Dawson and Bliss 1989). Alternatively, females may be less able to reduce conductance in response to dry conditions, leading to lower water use efficiency, despite a consequently higher carbon gain (Dawson and Ehleringer 1993). In the present study, non-fruiting foliage of *M. pyramidata* females displayed a pattern similar to the

Table 3. Physiological characteristics of *Maireana pyramidata*

Results of one-way ANOVA tests for differences in physiological characteristics between male and female *M. pyramidata* plants

Trait	Male mean	Female mean	<i>F</i> -value _(df)	<i>P</i> -value
Leaf chlorophyll content (chl <i>a</i> + chl <i>b</i> , $\mu\text{mol m}^{-2}$)	181.46	158.47	1.99 _(1,18)	>0.175
Leaf nitrogen content (%)	2.88	2.70	1.32 _(1,18)	>0.266
C-isotope discrimination ($\Delta^{13}\text{C}$)	17.39	17.10	0.49 _(1,18)	>0.492

latter case, with relatively high levels of photosynthesis and conductance. On the other hand, female fruit-bearing foliage exhibited a third possible response: slightly lower photosynthetic and higher transpiration rates than in males. Although neither photosynthesis nor transpiration on its own was significantly different between the sexes, the observed sex-related patterns in these two variables combined to produce a significant between-sex difference in water use efficiency (photosynthesis/transpiration).

Transpiration has been found to be higher in females of other dioecious species (Dzhaparidze 1969; Houssard *et al.* 1992). However, most researchers, while attributing the higher transpiration in females to higher reproductive costs, offer no explanation as to *how* reproductive processes should lead to an increase in stomatal conductance. Sex-based differences in root allocation are thought to be the cause of higher female transpiration (Dawson and Ehleringer 1993) and water stress (Stark 1970). Given the importance of deep root systems among semi-arid chenopods, reduced root growth would potentially cause stress in *M. pyramidata* females. Alternatively, it may be that female plants were not suffering water stress at the time of sampling. Soil moisture was relatively high at Lake Tyrrell in spring. As such, water loss via increased conductance would not present a problem to a plant if there were no immediate risk of desiccation. Thus, by maintaining high conductance, female plants would maximise carbon gain during fruit maturation, albeit at a reduced rate in fruiting, relative to non-fruiting foliage.

Despite both fruiting and non-fruiting female foliage having relatively high levels of transpiration, fruit-bearing foliage displayed a slightly, although not significantly, decreased photosynthetic rate relative to non-fruiting foliage, suggesting that carbon gain might be affected by fruit production. Decreased photosynthetic rates can reflect a translocation of resources, such as nitrogen, from photosynthetic to reproductive processes (e.g. Poot *et al.* 1996). Leaves of female *M. pyramidata* fruiting branches had slightly lower leaf nitrogen and chlorophyll than did male leaves, suggesting that translocation of nitrogen to fruit adjacent to leaves may occur in female plants.

In spite of the immediate impacts of reproduction on carbon gain and water use efficiency, $\Delta^{13}\text{C}$ did not differ between the sexes. Why is it that high female RA and low instantaneous water use efficiency are not reflected in the patterns of long-term water use, growth and mortality? In other dioecious species, a decrease in female gas exchange does not always lead to a negative impact on growth over the lifetime of a plant (Gehring and Monson 1994; Nicotra *et al.* 2003). One reason for this is that females are capable of up-regulating photosynthetic rate when reproductive demands are high (DeJong 1986; Gucci *et al.* 1995; Li *et al.* 2001). Although we did not measure gas exchange prior to or during flowering, *M. pyramidata* females may have been photosynthesising at a higher rate than males at this time. A

higher female carbon fixation earlier in the year might compensate for the low rate we observed to the point that overall $\Delta^{13}\text{C}$ did not differ from males.

Flowers and fruits are capable of 'paying' a degree of their carbon cost if they contain chlorophyll and are able to photosynthesise (Bazzaz *et al.* 1979; Reekie and Bazzaz 1987). *M. pyramidata* fruits are pale green during maturation, suggesting that they have the capacity to photosynthesise. Photosynthetically active fruits vary in the degree to which they can contribute to their own production, depending, for example, on the degree of fleshiness (Hogan *et al.* 1998) and light availability (Cipollini and Levey 1991). *M. pyramidata* fruits have a high surface to mass ratio and light is unlikely to be a limiting factor at Lake Tyrrell where tree growth is sparse. Therefore, it is likely that *M. pyramidata* fruits offset at least some of their own production and maintenance costs.

Concluding remarks

Our data indicate that fruit production has an instantaneous physiological impact on *M. pyramidata* females in the form of a short-term reduction in water use efficiency. Decreased water use efficiency might be viewed as a cost of reproduction for female plants. However, the lack of long-term impacts on growth and physiology suggest that females have become 'specialised' to overcome the demands of both reproduction and water stress over the lifetime of the plant.

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