Sex-specific differences in key functional traits in the dioecious shrub *Dodonaea viscosa*: an exploratory study.

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**Abstract**

Disproportionate investment in reproduction by female plants of dioecious species may produce sex-specific disadvantages under a changing climate, leading to sex ratio biases which can ultimately threaten the long-term persistence of species. This is particularly valid for regions of projected increasing aridity, such as South Australia. We studied several functional traits of the dioecious *Dodonaea viscosa*, a wide-ranging shrub and key species in many southern Australian ecosystems. Here we compare sexes and reveal significantly greater values for specific leaf area, stomatal density, and potential maximum stomatal conductance for female plants. These differences may reflect female-specific morpho-physiological responses to overcome or compensate for their disproportionate reproductive investment. This is the first study to investigate sex-specific differences in key functional traits in *Dodonaea viscosa* and reveals clear differences between the sexes that merit future research.

**Key Words:** *Dodonaea viscosa*, dioecious, functional traits, reproductive allocation, South Australia

**Introduction**

Plants have three main life history activities which compete for a limited energy and resource budget. These activities are a) vegetative growth and general maintenance, b) reproduction, and c) defence or predation avoidance. The Principle of Allocation (Lloyd 1988) describes these competing demands and postulates that an increase in the allocation of resources to any one of these activities should result in a decrease in allocation to one or both of the others. Dioecious plants offer a unique opportunity to study sex-specific differences in resource allocation, as female and male sexual reproductive functions are separated into distinct individual plants. This division of sexual functions means that each sex has different reproductive tasks, and consequently, may differ in their resource demands and resource allocation (Obeso 2002).

When the number of reproductive structures is equivalent, females of dioecious species allocate more resources to reproduction than males due to the overall greater cost by females in producing flowers, fruit and seeds to that of males producing only flowers and pollen (Putwain and Harper 1972; Delph *et al.* 1993; Dawson and Geber 1999; Delph 1999; Obeso 2002; Leigh and Nicotra 2003). This disproportionate allocation of resources by females can result in reduced allocation in the other life history activities (ie. vegetative growth and/or defence). For example, male *Rumex acetosella* flowers accounted for 10% of their total plant biomass, whereas females allocated 33% of their biomass to the production of seeds. This greater reproductive allocation by female *R.* *acetosella* came at the expense of allocation in, and therefore development, of vegetative offshoots and roots (Putwain and Harper 1972).

Some female plants have compensatory eco-physiological responses to overcome the resource deficit caused by a greater reproductive investment (Obeso 2002). For example, in a study of the Australian dioecious shrub, *Maireana pyramidata*, females were found to allocate up to nine times more biomass to reproduction than their male counterparts despite no apparent differences in above ground vegetative allocation (Leigh and Nicotra 2003). It was revealed that female plants exhibited a lower instantaneous water-use efficiency during fruiting than males over the same time period, for which the authors hypothesised could be attributed to an upregulated photosynthetic rate in order to balance their greater reproductive investment (Leigh and Nicotra 2003). This compensatory response could theoretically increase the vulnerability of female *M. pyramidata* to water stress, especially in arid environments or under drought conditions.

Owing to the disproportionate investment in reproduction by females of some dioecious species, and the resulting reduced allocation to other life history activities and/or compensatory physiologies that account for this deficit, female individuals may have a greater risk of mortality compared to their male counterparts (Hultine *et al.* 2016). This elevated sex-specific rate of mortality may become further pronounced in regions where aridity is predicted to increase under forecast climate change. Ultimately, the resulting sex ratio biases may threaten the long-term persistence of dioecious species in these regions (Hultine *et al.* 2016).

Plant functional traits can inform us on how a plant interacts with its environment and the strategy for resource allocation within a particular environmental matrix (Walker *et al.* 2017). Therefore, by measuring functional traits of dioecious plants and comparing between sexes, we can gain insight on whether a certain sex may be disproportionately threatened by climate change and the potential outcomes of distortion on population and community structure. In this study we measured the key functional traits, leaf area (LA), specific leaf area (SLA), stem density, stomata size and density, and maximum potential water loss through open stomata (*g*wmax), of a native Australian dioecious shrub, *Dodonaea viscosa* (Sm.) J.G.West (Sapindaceae). This species is ubiquitous, and an important component of the vegetation in southern Australia (Foulkes and Gillen 2000).

We ask, are there sex-specific differences in these functional traits that could indicate differences in resource allocation between sexes and suggest potential implications of a changing climate on the persistence of this species. To our knowledge, this exploratory study is the first to compare key functional traits between sexes of *D. viscosa*.

**Methods**

*Study species*

*Dodonaea viscosa* is a species complex with a cosmopolitan distribution across six continents (Harrington and Gadek 2009). Australia, as the evolutionary origin of *D. viscosa* (Harrington and Gadek 2009), has the greatest diversity with seven intergrading subspecies (West 1980). The focus of this study is *Dodonaea viscosa* (presumablysubsp*. spatulata)*, a perennial dioecious woody shrub that may grow up to 4m tall (Figure 1a). It is distributed across temperate and semi-arid Southern Australia, growing in a variety of habitats and soil types, and shows a robust tendency for regeneration following disturbances (i.e. grazing, drought, and fire) (West 1980). Because of this regenerating quality and its broad distribution, *D. viscosa* is widely used in revegetation projects in Australia (Baruch *et al.* 2017).

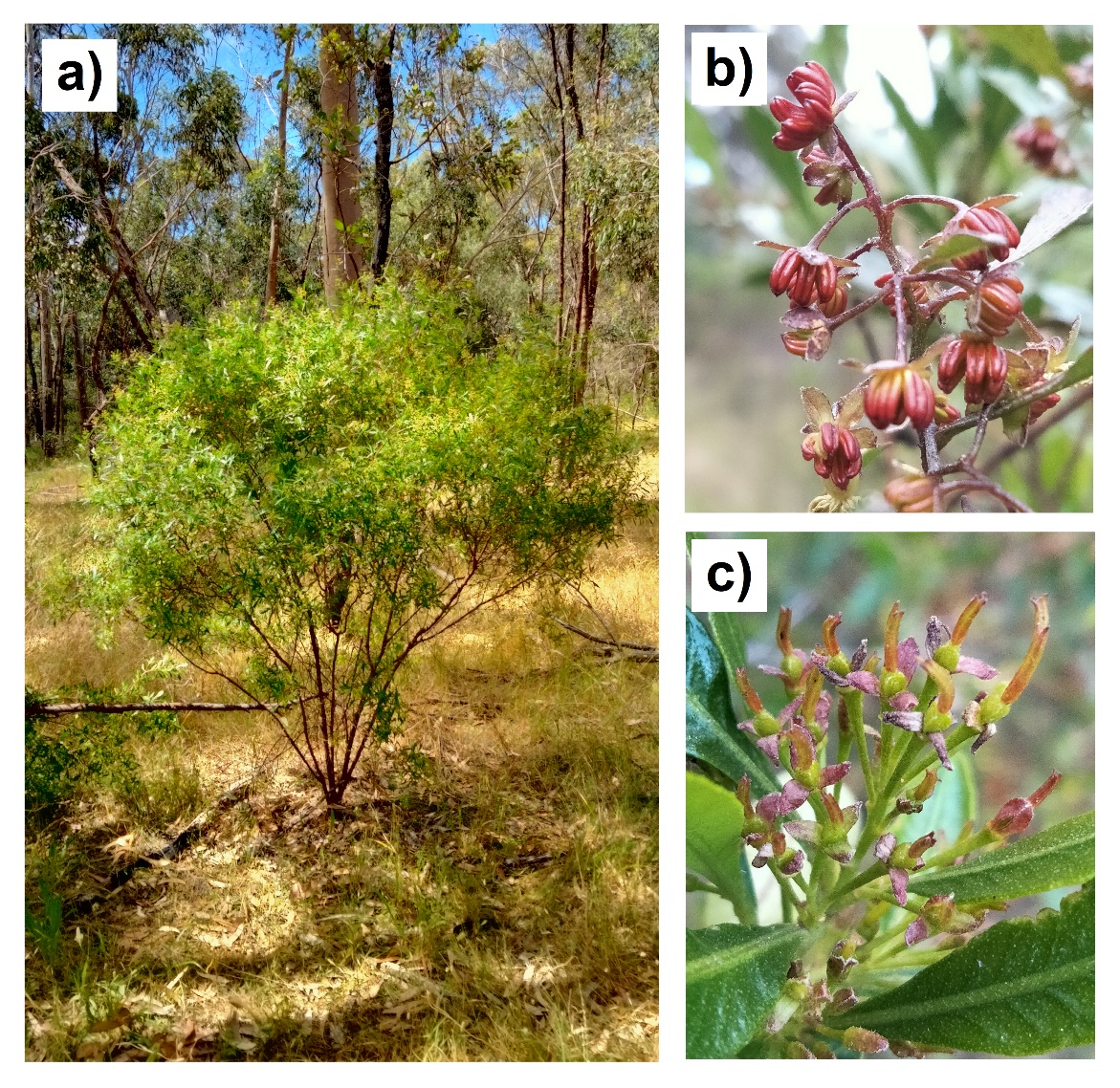
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Figure 1. Photographs showing a) a mature D. viscosa plant, b) male flowers, and c) female flowers (Photos by JT).

*D. viscosa* is likely wind pollinated as it has small, inconspicuous flowers that lack petals (Figure 1b and 1c) and nectar production, and therefore provide little attractant for pollinators besides pollen which is produced in ‘copious amounts’ (West 1980). However, the reproductive system of this species is complex as individuals from the sampled population appear to be aposematic, meaning that female plants can produce seeds in absence of fertilisation (Baruch, Z, personal observation).

*Study site and sampling*

The plant material was collected in October 2019 along a ~5km stretch of revegetation in the River Torrens Linear Park Trail, between the suburbs of Windsor Gardens and Dernancourt, Adelaide, South Australia (-34.86762, 138.67977) (Figure 2). Adelaide has a typical Mediterranean climate with mild, wet winters and dry, hot summers and a mean annual rainfall of approximately 550mm. Twenty-two pairs of male and female plants were sampled (at a distance no greater than 5m from one another) across the study area. From each plant, new but fully expanded leaves were sampled, as was a small length of mature woody branch. Samples from each plant were individually sealed in zip-locked bags and stored at 4°C until processing.

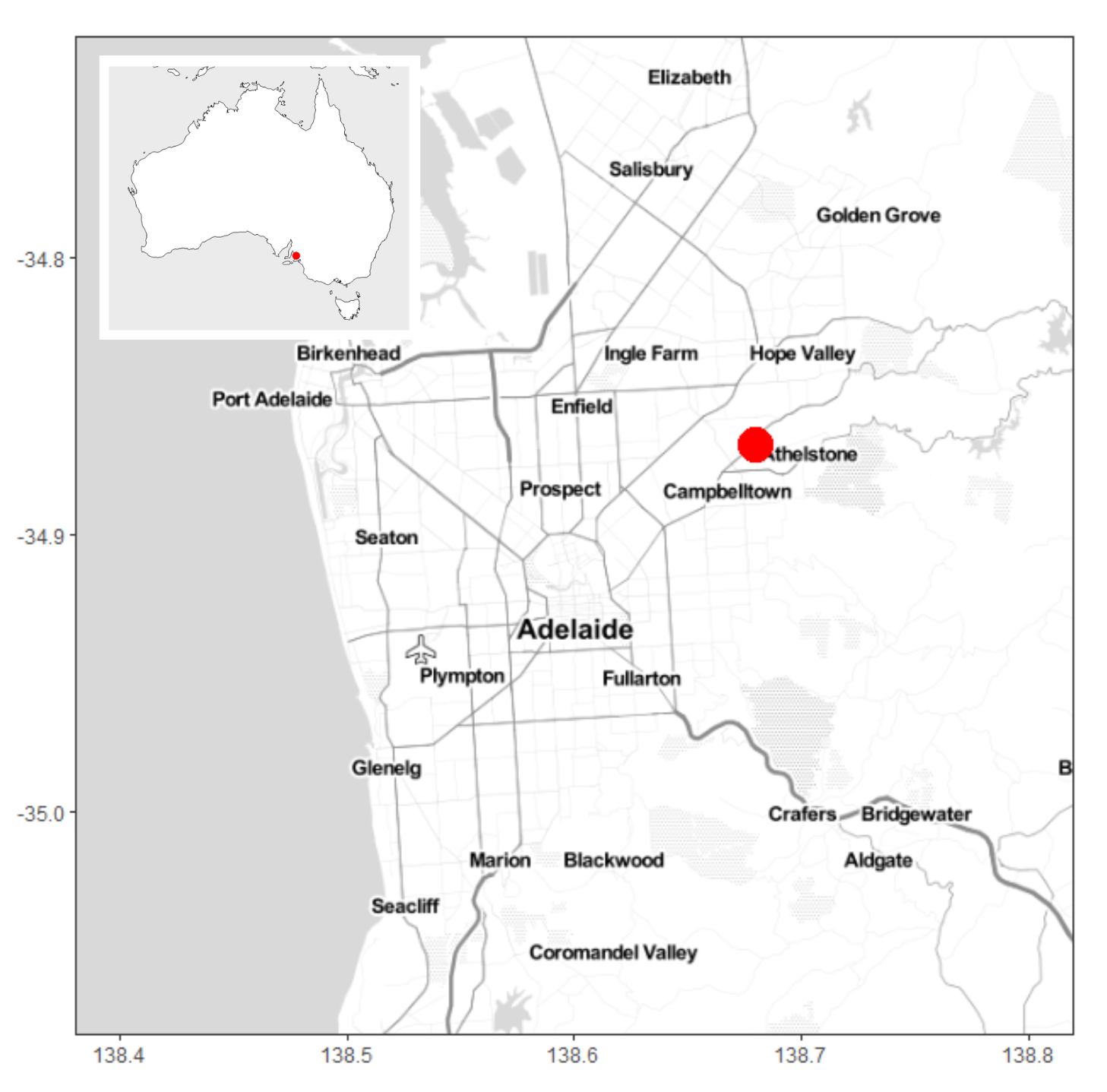


Figure 2. Map showing the location of study site (red circle) in the context of the Adelaide suburbs and insert map showing the location of Adelaide (red circle) in the context of Australia.

*Functional traits*

*Leaf area and specific leaf area*

Ten leaves from each sampled individual (n = 220 from each sex) were selected on basis of approximate uniformity. Each leaf was scanned with a HP Scanjet 200 and the leaf area (mm²) measured in FIJI: ImageJ (Schindelin *et al.* 2012). Each leaf was subsequently oven-dried at 60°C for 72 hours before being individually weighed. The value of the fresh leaf area over dry leaf weight determines the specific leaf area (cm2.g−1).

*Stem density*

A 1-2 cm uniform segment of woody stem (including bark) was cut from each sample (n = 43 (one sample missing)). Care was taken to ensure cuts were perpendicular to the length of the stem, as to produce an approximate cylindrical shape. The diameter (*d*) and height (*h*) of each cylinder was measured with a digital caliper to determine volume (*V*) (Equation 1).

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Each stem segment was then placed in a 60°c oven for 96 hours before weighing. The stem density value (*D*) was determined from the dry weight (*g*) divided by the volume (*V*) of the stem (g/cm3) (Equation 2).

(2)

*Cuticle preparation and stomatal measurements*

One leaf was sampled from each of the 22 male and 22 female individuals and an approximately 1 cm2 segment was cut from the centre of each leaf. Cuticle removal, preparation and imaging followed Hill *et al.* (2015).

Three micrographs of the abaxial surface were captured for each of the cuticle segment (n = 132). The length and width of the guard cells of five stomata per micrograph were measured in ImageJ (Schindelin *et al.* 2012). These measurements were then multiplied together to obtain the stomata size (µm²). Stomata density was estimated by a count of the number of stomata occurring within a 200 x 200µm square in each micrograph. These measurements of stomatal size and density were then used to calculate *g*wmax (mol m-2 s-1) (Equation 3) where *d* is the diffusivity of water vapour in air (m2 s-1), *v* is the molar volume of air (m3 mol-1), *D* is stomatal density (stomata per mm2), *amax* is maximum pore area of an open stoma (µm2) and *l* is inferred stomatal pore depth (µm) (half the width of the guard-cell pair width) (Farquhar and Sharkey 1982; Franks and Beerling 2009).

(2)

*Statistical analysis*

Results are shown as boxplots and ANOVAs tested differences between sexes for each functional trait (RStudio; Team 2015).

**Results**

The results of the ANOVAs revealed significant differences between males and females for three of the six variables in this study (Table 1). Compared with males, females had significantly greater values for SLA, stomatal density, and *g*wmax (Table 1).

The raw data used in these analyses is presented as supplementary data in Tables S1 and S2.

Table 1. ANOVA results for comparisons of functional traits of female and male *D. viscosa*. Significant differences are presented in bold text.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Functional trait** | **Female mean ± std** | **Male**  **mean ± std** | ***F*-value (df)** | ***P*-value** |
| Leaf area (cm2) | 4.29±1 | 4.45±0.9 | 2.71 (1, 428) | 0.101 |
| Specific leaf area (cm2.g-1) | 133±22.4 | 128±17.3 | 8.78 (1, 438) | **0.003** |
| Stem density (g cm-3) | 0.59±0.1 | 0.6±0.1 | 0.02 (1, 41) | 0.891 |
| Stomata size (µm2) | 579.48±113 | 567.49±117 | 1.79 (1, 658) | 0.181 |
| Stomatal density (stomata mm-2) | 263.64±53.8 | 243.94±52.5 | 4.62 (1, 130) | **0.033** |
| gwmax (mol m-2 s-1) | 1.71±0.3 | 1.53±0.3 | 10.09 (1, 130) | **0.002** |

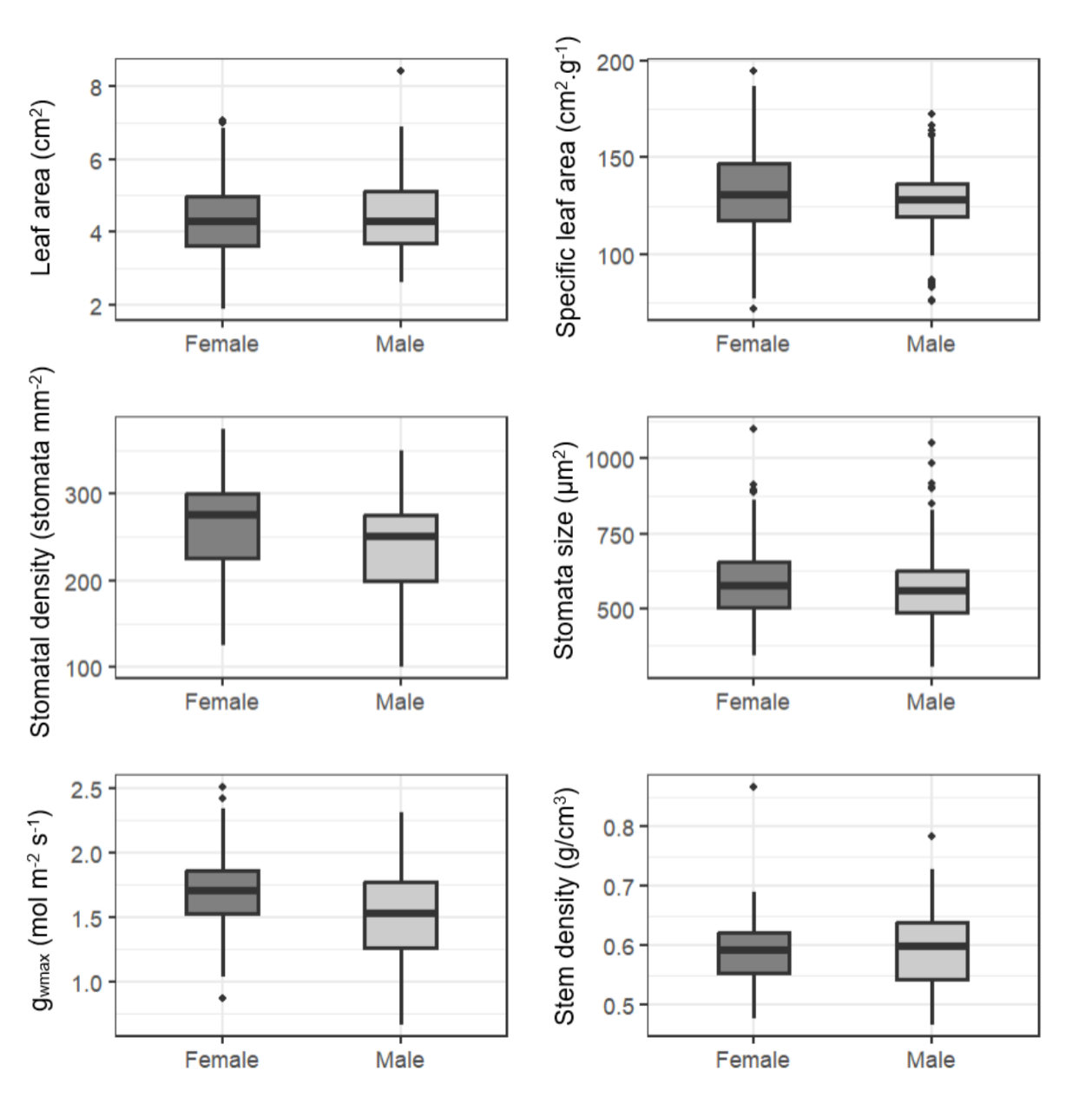


Figure 3. Boxplots displaying the values of functional traits for female and male D. viscosa.

**Discussion**

The aim of this work is to determine whether there are sex-specific differences in functional traits of *Dodonaea viscosa* that could indicate differences in resource allocation between sexes and suggest potential implications of a changing climate on the persistence of this Australian ubiquitous species.

Female individuals displayed significantly greater values for specific leaf area, stomatal density, and potential maximum stomatal conductance (Table 1). These differences in key functional traits may reflect female-specific morpho-physiological adaptive responses to overcome or compensate for their disproportionate reproductive investment.

Specific leaf area is a calculated value describing the leaf area produced per unit biomass invested and is almost universally employed in plant ecophysiological studies as it is associated with several other important leaf functions. For example, SLA points at a trade-off between carbon investment and leaf longevity with higher SLA values indicating less investment and lower longevity (Wilson *et al.* 1999; Westoby *et al.* 2002; Wright *et al.* 2004; Perez-Harguindeguy *et al.* 2016). Furthermore, species with higher SLA have been linked to greater photosynthetic nitrogen-use efficiency and possibly a higher rate of photosynthesis (Poorter and Evans 1998). The higher SLA of female *D. viscosa* (Table 1 and Figure 3) could indicate a strategy in which a greater reproductive allocation is compensated by having a higher photosynthetic rate while investing less carbon per unit of leaf mass.

Although our mean LA values are similar to those reported by Baruch *et al.* (2017; 2018) for *D. viscosa*, interestingly, our SLA values are somewhat greater. This may be a consequence of the relatively enriched urbanised habitat (ie. increased water and nutrient availability compared to a natural environment) in which the study population of *D. viscosa* was growing or that this population is a revegetation planting of unknown provenance. These unnatural factors may be somewhat confounding our results and may differ from the values of a natural, wild population of *D. viscosa* grown in a similar region.

Stem density is negatively associated to growth rate and positively associated to longevity (Williamson and Wiemann 2010). In this study no significant differences in stem density were observed between sexes of *D. viscosa* (Table 1). However, it must be mentioned that our method differed somewhat from the recommended standard in terms of stem length and uniformity (Perez-Harguindeguy *et al.* 2016). For future studies, longer stem lengths and a more accurate measuring method such as the water-displacement method as specified in Perez-Harguindeguy *et al.* (2016) should be used.

The *g*wmax value sets the theoretical upper limit for a plant’s stomatal conductance (Franks and Beerling 2009), and therefore can serve as a proxy for maximum potential photosynthetic rate (Mott *et al.* 1982). Our results revealed no significant differences in stomatal size, but significantly greater stomatal densities for females, which consequently produced a significantly greater *g*wmax value in females (Table 1). This could indicate the potential for female *D. viscosa* to achieve a greater maximum photosynthetic rate than males and couldtherefore be an adaptation to compensate for their assumed higher reproductive allocation. The gwmax values reported here fall within the range of those reported by Hill *et al.* (2015)in a study of *D. viscosa* subsp. *angustissima* which measured stomatal traits over a mean maximum temperature gradient but did not consider sex. The male and female gwmax values in the present study similarly align with those from the cooler and warmer ends (respectively) of the temperature gradient published by Hill *et al.* (2015). It was postulated that the greater gwmax values of the populations from the warmer end of the gradient allow *D. viscosa* subsp. *angustissima* the ability to efficiently take advantage of the short winter growing period with rapid resource acquisition (Hill *et al.* 2015). Similarly, in the present study, a higher gwmax exhibited by females may allow them a greater ability to acquire resources and assimilate carbon during the reproductive season as a compensatory physiological mechanism for disproportionate reproductive allocation.

Another potential compensatory strategy observed in females of dioecious species is the ability of the reproductive structures to photosynthesise (see references in Obeso 2002). The majority of the floral components (Figure 1c) and the immature fruits of female *D. viscosa* are green which suggests some photosynthetic capacity, and therefore may have the ability to offset some of their own carbon cost of production and maintenance. This may be particularly so for the fruit of *D. viscosa* which has a long immature stage (almost one-year) over which time it remains green, only changing colour shortly before dispersion (West 1980).

Higher photosynthetic rates exhibited by females has been observed numerous times in dioecious species (Dawson and Bliss 1989; Dawson and Ehleringer 1993; Laporte and Delph 1996; Dawson *et al.* 2004). A higher photosynthetic rate should be coupled with a higher uptake of resources (Bazzaz and Grace 1997) and therefore where resources are limited (for example under drought conditions) may result in reduced reproductive capacity or increased stress (Hultine *et al.* 2016). Climate change forecasts for southern Australia indicate that precipitation is likely to become increasingly variable resulting in more periods of drought (CSIRO and Bureau of Meteorology 2022). Under such conditions, female *D. viscosa* may be specifically disadvantaged, leading to reduction of reproductive capacity and increased mortality in the short-term, and sex-biases and risk of extinction in the long-term (Hultine *et al.* 2016). This is a real risk for *D. viscosa* as the only study on sex ratios found that all of the four populations exhibited male:female sex ratio biases, one significantly so (West 1980).

Eco-physiological dimorphisms of dioecious plants have been less studied than other dimorphic expressions (Dawson and Geber 1999) and as yet, no patterns of generality have emerged (Juvany and Munné-Bosch 2015). Further research on *D. viscosa* shouldbe conducted in a holistic physiological approach. For example, investigating natural populations across environmental gradients and incorporating the responses of functional traits such as assimilation rate, and water and nitrogen use efficiency, would offer more complete patterns of generality.

**Conclusions**

To our knowledge this is the first study to compare functional traits between sexes of *Dodonaea viscosa*. Whilst a preliminary study, we revealed important results with significant differences for SLA, stomata density and *g*wmax between females and males that agree with predicted resource allocation theory. This study therefore forms a basis for further research to examine these differences in greater depth and to investigate their potential implications for the future of a key species of ecosystems in southern Australia, especially regarding ongoing climate change.

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