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Morphological variation of *Solanum elaeagnifolium* in south-eastern Australia

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Summary

Solanum elaeagnifolium (silverleaf nightshade) is an invasive perennial weed in Australia, with aerial growth commencing in spring from either the perennial root system or the soil seedbank, with senescence occurring in autumn. A total of 642 *S. elaeagnifolium* individuals were collected at flowering from 92 locations in south-eastern Australia to study morphological variation and its implications for management. Large morphological variation was found between individuals from different locations. Leaf length, width and area ranged from 1.44 to 10.6 cm, 0.39 to 4.09 cm and 0.41 to 25.8 cm^2 respectively. Plants from higher rainfall regions were significantly taller and had larger leaves, suggesting a possible correlation between rainfall and morphology. Scanning electron microscopy comparison of leaf surfaces showed lower trichome and stomatal densities on the adaxial surface (67.0 ± 3.3 trichomes mm^{-2} and 603.4 ± 29.2 stomata mm^{-2} respectively) than on the abaxial surface (131.9 ± 7.2 trichomes mm^{-2} and 813.7 ± 30.5 stomata mm^{-2} respectively). The morphological plasticity of *S. elaeagnifolium* highlighted in this study could probably contribute to its adaptability and partly explain its establishment and continuing expansion in Australia.

Keywords: invasive weed, growth, silverleaf nightshade, size variability, stomata, trichomes.

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Introduction

Solanum elaeagnifolium Cav. (silverleaf nightshade) is a weed of worldwide significance that originated from south-western USA and northern México (Stanton *et al*., 2009). It infests at least 0.35 million hectares in Australia, covering various climatic zones and has the potential to infest 398 million hectares (Feuerherdt, 2009). The species occurs over the southern cereal cropping zone of Australia (Stanton *et al*., 2009), which has an annual rainfall of 250–600 mm (Parsons & Cuthbertson, 2001). *Solanum elaeagnifolium* reproduces sexually and vegetatively. In Australia, the above-ground shoots emerge from perennial root systems or soil seedbank from September (spring) and the shoots senesce in May (autumn) (Stanton *et al*., 2009).

Morphological variation of *S. elaeagnifolium* such as prickle density, leaf size and shape has been reported previously in the USA (Bryson *et al*., 2012), Greece (Economidou & Yannitsaros, 1975) and Australia, based on limited herbarium samples (Symon, 1981; Bean, 2004). *Solanum elaeagnifolium* plants are usually between 10

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and 100 cm high (Encomidou & Yannitsaros, 1975), with leaf length and width ranging from 3 to 6.5 cm and 0.8 to 1.4 cm respectively (Bean, 2004). Prickles are absent or present on the stem, leaf and calyx. Prickle density on *S. elaeagnifolium* stems may increase under dry conditions (Encomidou & Yannitsaros, 1975). Under glasshouse condition with reliable water supply, plants can grow 50–120 cm high with prickles present on calyx, leaf and stem (Bryson *et al.*, 2012).

Morphological variation has been highlighted in many *Solanum* species, including eggplant (*Solanum melongena* L.) (Prohens *et al.*, 2005), *Solanum bahamense* L. (canker berry), *Solanum capsicoides* All. (cockroach berry) and *Solanum carolinense* L. (carolina horsenettle) (Bryson *et al.*, 2012). Prohens *et al.* (2005) compared the morphological characters of 28 Spanish eggplant cultivars and found considerable variation, especially regarding fruit length, ranging from 9.3 to 25.8 cm. Bryson *et al.* (2012) observed morphological characters and variations of 18 weedy and non-weedy prickly nightshades (*Solanum* spp.) in the south-eastern USA and provided diagnostic features to identify these species.

Morphological variation has been studied in many weed species under controlled environment conditions, including *Oryza sativa* L. (weedy rice) (Fogliatto *et al.*, 2010) and *Brassica juncea* (L.) Czern. (Indian mustard) (Huangfu *et al.*, 2009), or in field surveys, such as of *Solidago canadensis* L. (Canada goldenrod) (Weber, 1997). *Oryza sativa* from north-west Italy were divided into three groups: awnless, mucronate and awned (Fogliatto *et al.*, 2010). The flag leaf length of awnless *O. sativa* was significantly shorter than the awned one, while the awned and awnless populations were significantly taller than the mucronate population (Fogliatto *et al.*, 2010). Weber (1997) sampled 379 *S. canadensis* field individuals from Europe and found that leaf length and width ranged from 6.5 to 20.7 cm and 0.8 to 35 cm respectively.

These morphological variations may be attributed to genetic (Clements & Ditommaso, 2011) and/or phenotypic plasticity in response to abiotic factors such as light (Xu *et al.*, 2012), habitats (Sharma & Esler, 2008) and nutrition (Hejcman *et al.*, 2012). Phenotypic plasticity increases the adaptability of invasive species and contributes to their success (Clements & Ditommaso, 2011). For example, habitats significantly impacted on morphology of *Echium plantagineum* L. (Paterson's Curse), including plant height, seed size and seed weight (Sharma & Esler, 2008). Such morphological plasticity was also reported in *S. elaeagnifolium*, with a significant increase in plant height (around 30–90 cm), total leaf area (around 80–200 cm^2), biomass (around 200–500 g per plant) and seed production (95–3420 per plant) under higher water availability (Travlos, 2013).

Variations of leaf morphology, such as leaf size (Richburg *et al.*, 1994), trichome density (Huangfu *et al.*, 2009) and stomatal density (Ricotta & Masiunas, 1992), can affect foliar herbicide uptake. Richburg *et al.* (1994) reported that small leaf size of *Cyperus* spp. (nutsedge) affected herbicide droplet coverage and retention and reduced foliar herbicide efficacy. In addition, dense trichomes can form a water repellent surface that blocks herbicide uptake (Brewer *et al.*, 1991). Huangfu *et al.* (2009) observed that glyphosate uptake in *Brassica juncea* was negatively correlated ($r = -0.65$) with trichome density on the adaxial leaf surface. However, herbicides can penetrate into leaves through the guard cells of stomata (Wang & Liu, 2007). For example, Ricotta and Masiunas (1992) detected a high negative correlation between acifluorfen tolerance and stomatal density ($r = -0.895$) in different tomato genotypes.

Effective management strategies for *S. elaeagnifolium* are very limited, especially for large and dense infestations, and are often herbicide based. A large-scale study of the morphological variation of *S. elaeagnifolium* across Australia is required for improving the identification, understanding and management of this weed. The objective of this study was to assess the morphological variation of *S. elaeagnifolium* in south-eastern Australia and to identify possible relationships between morphology and abiotic factors such as rainfall.

Materials and methods

Plant material

A total of 642 individuals were collected from 92 locations across New South Wales (NSW), South Australia (SA), Victoria (VIC) and Queensland (QLD) in February 2010 (Appendix 1). Sampling locations and rainfall areas are shown in Fig. 1. All individuals were collected within 3 weeks, to minimize the environmental impacts on morphology. One to 12 individuals at flowering stage were randomly collected at each location, depending on the level of infestation. One individual was sampled if there was only one patch of *S. elaeagnifolium* present, while at locations with large populations (more than 1 ha), up to 12 individuals were sampled and spaced at least 50 m apart from each other to reduce the probability of sampling clonal individuals. Individuals were chosen from open fields with few trees or buildings to avoid any shading effects. An above-ground shoot from each individual was cut, placed in a zip-lock plastic bag and kept in an

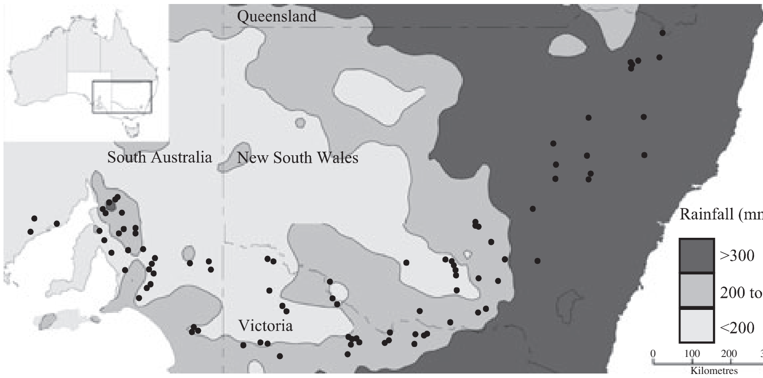

Fig. 1 *Solanum elaeagnifolium* sampling points and total rainfall between September 2009 and February 2010; rainfall data obtained from Bureau of Meteorology, Australia.

insulated container for transport. Digital photographs of the sixth, seventh and eighth leaves from the shoot apex were taken within 24 h after sampling for subsequent measurement of leaf size and shape. These leaves were chosen because they are at similar maturity stage and fully expanded. Additionally, the chosen leaves would have to be available from the shortest plants and have to be clean (not too close to the ground). The shoots were then pressed and dried for scanning electron microscopy (SEM) examination.

Morphological evaluation

All individuals were measured for 10 morphological traits, as follows: plant height, leaf length, width, area and roundness, trichome density on the adaxial and abaxial leaf surfaces, and prickle density on stem, leaf and calyx.

Plant height was measured in the field from the ground to the highest shoot apex. Digital photographs of the sixth, seventh and eighth leaves were processed using Image J software (Schneider *et al.*, 2012) to measure leaf length, width and area. Leaf roundness was also calculated according to formula: $4 \times$ leaf area/π(major axis)^2 using the same software, with a value of 1.0 indicating a perfect circle, where major axis indicates the long axis of the best fitting ellipse.

Trichome density was visually assessed on fresh leaves on the adaxial and abaxial surfaces and classified into three groups: low, medium and high densities according to the degree of silvery-white colour on the leaf surface. Preliminary investigations suggested that a visual rating of the 'medium' category equated to a trichome density from 40 to 80 trichomes mm^{-2}. This visual rating was verified by trichome counts on SEM images from 77 randomly selected individuals (described in the next section).

Stem prickles were visually assessed on a 5 cm length of the mid-stem and classified into three density levels: low (<40 prickles), medium (40–60 prickles) and high (more than 60 prickles). Calyx prickles were visually assessed as low, medium and high density (<30, 30–50 and >50 prickles/calyx respectively). Leaf prickles were classified as absent, present on abaxial leaf surface only, or present on both surfaces.

Trichome and stomatal densities assessed by SEM

Scanning electron microscopy (JCM 5000 NeoScope, JEOL, Japan) images of 77 individuals from 33 locations were used to study trichome density (Appendix 1). Three mature leaves were randomly chosen from each individual. For each leaf, small areas were cut from the adaxial and abaxial surfaces, adhered to 12-mm carbon tabs (ProSciTech, Australia) and observed by SEM. The adaxial trichome density was counted from three SEM images (0.2–3.7 mm^2, according to trichome density) per leaf (Fig. 2A). Due to the presence of multiple trichome layers on the abaxial surface (Christodoulakis *et al.*, 2009), trichomes were shaved using a scalpel under a dissecting microscope, and the trichome basal cell density was counted on a single SEM image (0.2–3.7 mm^2, according to trichome density) per leaf (Fig. 2B). Stomatal density was determined from a subset of 41 individuals from 23 locations. Adaxial and abaxial trichomes were shaved, and adaxial stomata were counted from three SEM images (0.02–0.15 mm^2, according to stomatal density) per leaf (Fig. 2C), while the abaxial stomata were counted from a single image (0.02–0.15 mm^2, according to stomatal density) per leaf (Fig. 2D).

Statistical analysis

Coefficients of variation and a histogram plot were calculated for each morphological character. Mean, standard error of mean, and correlation matrix were calculated for quantitative traits. Plant height was square root transformed, while the other quantitative traits were log-transformed before analysis to normalize variances. Individuals from the same rainfall areas (Fig. 1) were considered as a population and subjected

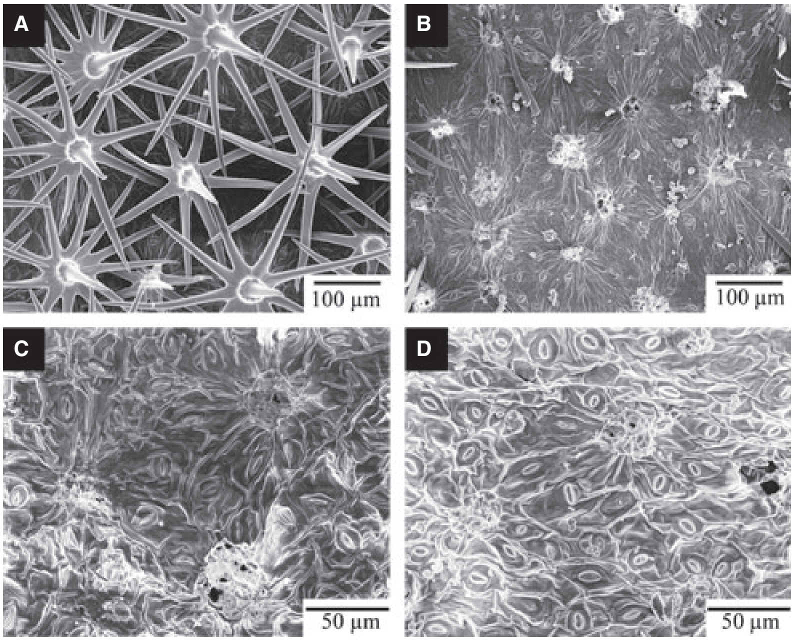

Fig. 2 Scanning electron microscopy images of trichome and stomatal densities in *Solanum elaeagnifolium*; (A): trichomes on the adaxial leaf surface; (B): trichome basal cells on shaved abaxial leaf surface; (C): stomata on shaved adaxial leaf surface; and (D): stomata on shaved abaxial leaf surface.

Fig. 3 Variation of *Solanum elaeagnifolium* leaf size and shape. All leaves were the seventh leaf from the shoot apex of flowering plants, showing the adaxial surface only.

to unbalanced analysis of variance (ANOVA). Means were separated by Fisher's LSD at the 5% level. Principle component analysis (PCA) was performed for quantitative traits using the multivariate analysis model. The relation between trichome and stomatal densities was tested by linear regression analysis. All analyses were performed using Genstat 14th edition (Payne *et al*., 2011).

Results

Morphological variation

Considerable morphological variation was present in quantitative traits of the 642 individuals of *S. elaeagnifolium*. Leaf length ranged from 1.44 to 10.6 cm and leaf width from 0.39 to 4.09 cm (Figs 3 and 4). Leaf area had the largest coefficient of variation at 69.5%. The largest leaf area was 25.8 cm^2, which was 63 times greater than the smallest area (0.41 cm^2). Most individuals (88.2%) had a leaf area of 0.41–12 cm^2. Leaf roundness ranged from suborbicular (0.61) to narrowly strap shaped (0.15). Plant height varied from 7 to 73 cm, with an average of 33.6 cm. There were 510 individuals (79.4%) between 20 and 50 cm in height.

High correlations were found among leaf length, width and area (Table 1). Leaf roundness showed weak correlation with leaf width and area (r = 0.43 and 0.22 respectively). In addition, a weak, negative correlation was found between plant height and leaf roundness (r = −0.24).

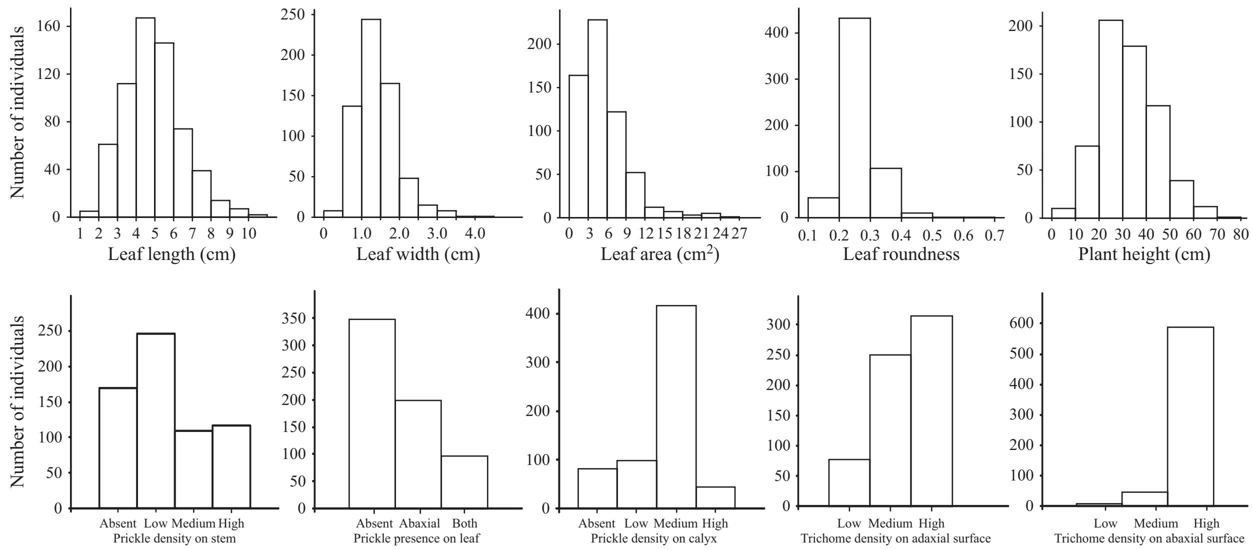

Fig. 4 Frequency distributions of the morphological traits of *Solanum elaeagnifolium*.

Table 1 Correlation matrix for quantitative traits of *Solanum elaeagnifolium*

Traits	Leaf length	Leaf width	Leaf area	Leaf roundness
Leaf length	–			
Leaf width	0.855***	–		
Leaf area	0.923***	0.943***	–	
Leaf roundness	−0.030	0.434***	0.223***	–
Plant height	0.057	−0.109**	−0.004	−0.240***

Correlation is significant at **: $P < 0.01$ level and ***: $P \leq 0.001$ level.

High variation was found in qualitative traits (Fig. 4), with the coefficient of variation of prickle density on the leaf, stem and calyx at 45.6%, 46.1% and 29.5% respectively. Individuals with no or low stem prickle density represented 64.5% of the 642 individuals, while similar numbers of individuals had medium and high stem prickle densities (109 and 117 individuals respectively). More than half of the individuals (54.1%) did not have prickles on the leaves, while 96 (15%) individuals had prickles on both leaf surfaces. Prickles were always present on the abaxial leaf surface if they were present on the adaxial surface. The majority of individuals (65%) had a medium level of prickles on the calyx (30–50 prickles).

There were 164 individuals (25.6%) without prickles on either the stems or leaves. Six individuals (0.9%) had prickles on the leaves but not on the stem, and 183 individuals (28.5%) had prickles on the stem but not the leaves. The remaining 289 individuals (45.0%) had prickles on both the stem and the leaves. By contrast, no obvious correlation was found between calyx prickle density and other morphological traits.

The majority of individuals (91.6%) had a high trichome density on the abaxial leaf surface (more than 80 trichomes mm^{-2}). However, 77, 250 and 315 individuals were classified with low, medium and high adaxial leaf trichome densities respectively.

Plant height and leaf shape characteristics were significantly different between rainfall areas (Table 2). In areas of greater than 300 mm of rainfall, individuals had significantly ($P < 0.001$) larger leaf length, width and area (5.81 cm, 1.58 cm and 6.88 cm^2 respectively) as compared to plants from low (<200 mm) rainfall areas (4.55 cm, 1.34 cm and 4.71 cm^2 respectively). In the higher rainfall areas, plants were taller and had more pointed leaves. In addition, plants from high rainfall areas tended to have fewer stem prickles (Fig. 5). Plants from low (42.9%) and medium (36.6%) rainfall areas had a larger proportion of medium and high prickle density individuals than those from high rainfall areas (19.5%).

Individuals with a larger leaf area had lower ($P < 0.001$) adaxial trichome density. The average leaf area of the individuals with low adaxial trichome density (T_{AL}) was 7.99 ± 0.64 cm^2, almost twice the size of individuals with high trichome density (T_{AH}, 4.10 ± 0.16 cm^2). Most of the T_{AH} individuals (73%) had a relatively small leaf area between 0.41 and 6 cm^2, while the majority (78%) of T_{AL} individuals had a leaf area between 2 and 12 cm^2, with 8% of individuals with a leaf area of more than 18 cm^2 (Fig. 6).

Table 2 Relationship between growing season rainfall areas and morphological traits of *Solanum elaeagnifolium*

	Rainfall September 2009 – February 2010 (mm)					
	<200		200–300		>300	
Traits	Mean	SE	Mean	SE	Mean	SE
Leaf length (cm)	4.55[a]	0.098	4.68[a]	0.092	5.81[b]	0.120
Leaf width (cm)	1.34[a]	0.032	1.38[a]	0.035	1.58[b]	0.043
Leaf area (cm^2)	4.71[a]	0.247	5.05[a]	0.229	6.88[b]	0.321
Leaf roundness	0.28[a]	0.004	0.27[b]	0.004	0.25[c]	0.004
Plant height (cm)	29.3[a]	0.734	34.2[b]	0.708	39.0[c]	0.871

SE, standard error of mean.
Values sharing the same letters within each row are not significantly different according to Fisher's LSD (P = 0.05).

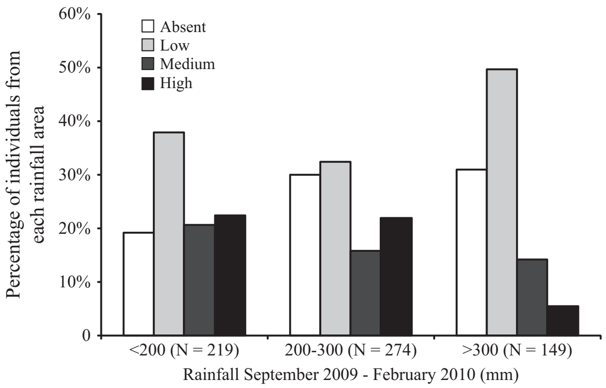

Fig. 5 Frequency distributions of stem prickle density for *Solanum elaeagnifolium* individuals from different rainfall areas. Numbers in the parentheses indicated the sample size in the particular rainfall area.

Principal component analysis

The first two components of PCA explained 83.3% of the total variation (Fig. 7). The first principal component accounted for 57.8% of the variation and was mainly contributed by length, width and area of the leaves (Table 3). The second component explained 25.5% of the variation with dominance of plant height and leaf length. A negative correlation was found with leaf roundness. Individuals were randomly dispersed, and no well-separated groups were identified (Fig. 7).

Trichome and stomatal densities observed by SEM

Trichome density varied between individuals, with a coefficient of variation of 42.3% and 47.9% for the adaxial and abaxial leaf surfaces respectively (Fig. 8). Trichome density on the adaxial leaf surface ranged from 21.9 to 196.0 trichomes mm^{-2}, with a mean of 67.0 ± 3.3 trichomes mm^{-2}, which is lower than the density on the abaxial surface (57.4–395.3 trichomes mm^{-2}, with a mean of 131.9 ± 7.2 trichomes mm^{-2}).

The coefficient of variation for stomatal density was 29.5% and 24.0% for the adaxial and abaxial leaf surfaces respectively. Stomatal density ranged from 284 to 942 (mean of 603.4 ± 29.2) and 455 to 1519 (mean of 813.7 ± 30.5 stomata mm^{-2}) on the adaxial and abaxial leaf surfaces respectively (Fig. 8).

There was a weak positive correlation in trichome density between the adaxial and abaxial leaf surfaces (r = 0.43). A weak positive correlation was also found on stomatal density between the adaxial and abaxial leaf surfaces (r = 0.59). For all individuals, trichome and stomatal densities were always higher on the abaxial surface. No correlation was found between trichome and stomatal densities.

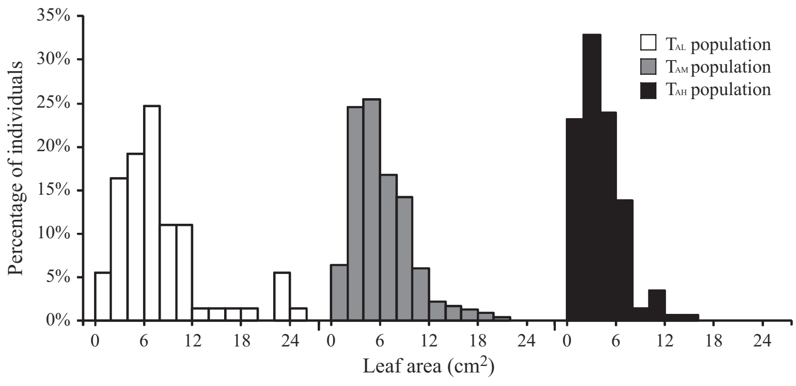

Fig. 6 Frequency distributions of leaf area (cm^2) for *Solanum elaeagnifolium* individuals with low (T_{AL}, n = 77), medium (T_{AM}, n = 250) and high (T_{AH}, n = 315) adaxial trichome densities.

The reliability of the visual assessment method of trichome density based on the degree of the silvery-white colour on leaf surface was confirmed with the direct measurement of trichome densities from SEM observation. The SEM observation indicated that those individuals visually assessed as having low, medium and high trichome densities were successful validated to have a trichome density of <40, 40–80 and more than 80 trichomes mm^{-2}, respectively, with an error rate of about 6.5% (five out of 77).

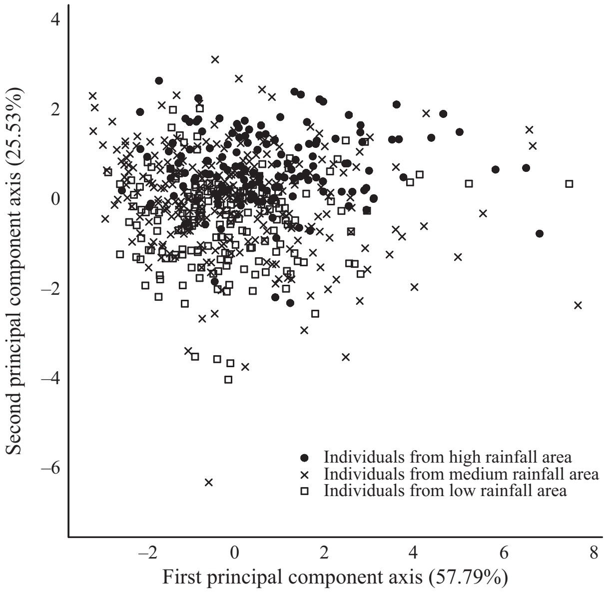

Fig. 7 Principle component analysis for 642 *Solanum elaeagnifolium* individuals from Australia.

Table 3 Principal component analysis of 642 *Solanum elaeagnifolium* individuals from Australia; showing the percentage of variation accounted by the first two principle components

Variables	PC1	PC2
Leaf length	0.53922	0.28436
Leaf width	0.57962	−0.08693
Leaf area	0.57622	0.10267
Leaf roundness	0.19835	−0.66912
Plant height	−0.04365	0.67329

Discussion

This is the first large-scale morphological study of *S. elaeagnifolium* in south-eastern Australia. High morphological variation was found for all traits, except trichome density on the abaxial leaf surface. The ranges in *S. elaeagnifolium* leaf length and width reported here were greater than reported previously in Australia (3.0–6.5 cm in length and 0.8–1.4 cm in width) (Bean, 2004), but similar to those reported from Greece (2–19 cm in length and 0.5–5.2 cm in width) (Encomidou & Yannitsaros, 1975). The morphological difference may be associated with genetic diversity of this weed (Zhu *et al.*, 2012) or edaphic and climatic difference between locations. Individuals collected from higher rainfall areas are often taller and have larger leaves, indicating a possible correlation between phenotypic plasticity and water availability. A recently study has shown that plant height and total leaf area of *S. elaeagnifolium* reduced from around 90 to 30 cm and 200 to 80 cm^2 in response to dry conditions respectively (Travlos, 2013). Phenotypic plasticity has been reported in response to habitats for *Echium plantagineum* (Sharma & Esler, 2008), light for *Alternanthera philoxeroides* (Mart.) Griseb. (alligator weed) (Xu *et al.*, 2012) and nutrition for *Rumex crispus* L. (curled dock) (Hejcman *et al.*, 2012). Such phenotypic plasticity plays an important role in weed establishment and increases the adaptability of invasive species to novel environments (Dawson *et al.*, 2012).

In addition, this study indicated that individuals from low rainfall areas tended to have more prickles on the main stem. Encomidou and Yannitsaros (1975) also reported that *S. elaeagnifolium* growing under dry conditions in Greece were more likely to have more prickles. Prickles are structural defence adaptations that help protect plants from herbivores and are more likely to be developed under suboptimal conditions,

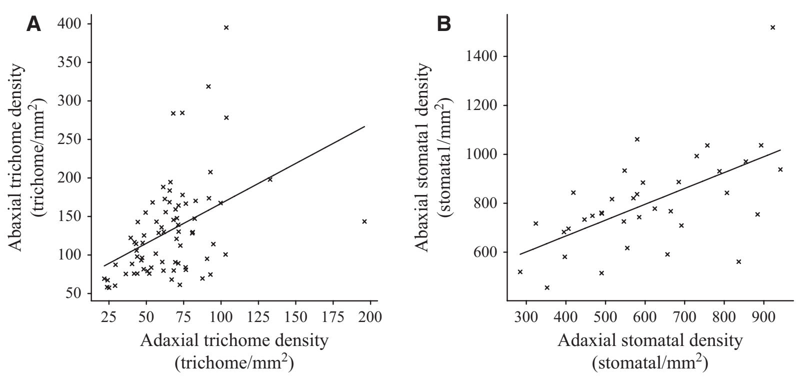

Fig. 8 Relationship between trichome (A) and stomata (B) densities on *Solanum elaeagnifolium* adaxial and abaxial leaf surfaces observed by scanning electron microscopy.

such as low rainfall, because the drier conditions may reduce overall plant growth, leading to increased resources being available from photosynthesis for development of trichomes and prickles (Hanley *et al.*, 2007).

Scanning electron microscopy observation indicated that trichome densities ranged from 22 to 196 trichomes mm^{-2} on the adaxial surface and from 57 to 395 trichomes mm^{-2} on the abaxial surface, which is much higher than other investigated *Solanum* species (0.84–7.13 trichomes mm^{-2}), including six species of *S. arboreum* group and two species of *S. deflexiflorum* group (de Rojas & Ferrarotto, 2009) and eggplant (Leite *et al.*, 2003). Recently, Blonder *et al.* (2012) showed that most leaves shrink 10–30% when dried; thus, the densities reported here may be higher than that in fresh leaves. High trichome density might help protect leaves from high summer temperatures and reduce transpiration rates (Perez-Estrada *et al.*, 2000), solar radiation (Jordan *et al.*, 2005) and herbivores (Levin, 1973). Jordan *et al.* (2005) highlighted that open vegetation usually associates with dense trichomes or papillae in Proteaceae species, which indicated the photoprotection function of trichomes. Trichomes on *S. elaeagnifolium* create a silver-white surface, which could reflect and reduce the light that reaches leaf surfaces and protects leaves from damage due to high summer temperatures and solar radiation. Trichomes also play an important role against herbivores including molluscs and leaf chewing and sap-sucking insects (Hanley *et al.*, 2007), which probably explains the very limited insect damage found on *S. elaeagnifolium* leaves during our sample collection.

The stomatal density on both leaf surfaces detected in this study is extremely high compared with other studies (e.g. Beaulieu *et al.*, 2008). A high density of stomata on both leaf surfaces is probably associated with increasing CO_2 conductance and photosynthetic capacity under optimal conditions (Beaulieu *et al.*, 2008).

The morphological variation of leaf area and trichome and stomatal densities highlighted in this study could potentially impact herbicide control of *S. elaeagnifolium*. Plants with larger leaves may intercept more herbicide droplets than those of smaller leaves (Richburg *et al.*, 1994), thus increasing the amount of herbicide available for uptake. Dense trichomes can form a hydrophobic barrier on the leaf surface (Brewer *et al.*, 1991), reduce droplet retention, create air pockets and block herbicide uptake (Kraemer *et al.*, 2009), hence may restrict herbicide efficacy on *S. elaeagnifolium*. However, this study also showed high stomatal densities on both leaf surfaces, which may aid in chemical management of *S. elaeagnifolium*, as Ricotta and Masiunas (1992) reported that guard cells are more permeable than other leaf cells, due to a thinner cuticle and better connection with subjacent cells. Further research may focus on suitable adjuvants and concentrations to improve foliar herbicide efficacy on *S. elaeagnifolium*, through increasing leaf penetrability. The potential difficulties in uptake of foliar herbicide also suggest that root absorbed residual herbicides should be used in conjunction with the foliar applied herbicides to improve control of *S. elaeagnifolium*.

In conclusion, high morphological variation was found in *S. elaeagnifolium* from south-eastern Australia. The ranges of the leaf size parameters were larger than previous records in Australia. In addition, individuals from high rainfall areas had a larger leaf area than those from low rainfall areas, suggesting a possible relationship between rainfall and phenotypic plasticity. However, the relationship between leaf size and trichome/stomatal density is not well understood. Further studies are needed using clonal material under controlled environments to determine the impact of abiotic factors on any such correlation. Variation in leaf size and trichome and stomatal densities highlighted in this study could potentially impact the retention and uptake of foliar applied herbicides, thus influencing the control of this weed. High trichome and stomata densities on both leaf surfaces suggested that effective management of *S. elaeagnifolium* may require the use of soil-applied herbicide through root uptake and the use of appropriate adjuvants to improve the efficacy of foliar applied herbicide.

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Appendix 1 The total rainfall of growing season, sampling size and locations of *Solanum elaeagnifolium* collected from different sate of Australia: New South Wales (NSW), South Australia (SA), Victoria (VIC), Queensland (QLD) and Western Australia (WA). A number of samples used for trichome density observation (TO) and for stomata observation (SO) were also included. Rainfall in the growing season from September 2009 to February 2010: H = total rainfall > 300mm, M = total rainfall between 200 and 300, and L = total rainfall < 200 (See Fig. 1).

Location	State	Rainfall	Longitude/Latitude	Sample size	TO	SO
Adelaide	SA	L	−34°40/138°41	11	0	0
Angas Valley	SA	L	−34°44/139°19	10	0	0
Annadale	SA	L	−34°24/139°21	2	3	1
Appila 1	SA	M	−33°01/138°26	6	1	1
Appila 2	SA	M	−33°00/138°28	2	1	1
Avon	SA	L	−34°15/138°20	10	0	0
Balranald	NSW	M	−34°56/143°28	2	0	0
Bingara 1	NSW	H	−29°52/150°33	9	0	0
Bingara 2	NSW	H	−29°48/150°32	4	0	0
Bingara 3	NSW	H	−29°49/150°32	10	0	0
Blyth	SA	M	−33°50/138°30	1	1	1
Boree Creek	NSW	L	−35°08/146°27	4	0	0
Bridgewater	VIC	M	−36°38/143°54	10	3	3
Burra	SA	M	−33°41/138°55	4	2	2
Calivil 1	VIC	M	−36°21/144°07	5	1	1
Calivil 2	VIC	M	−36°17/144°05	12	1	1
Cambrai	SA	L	−34°39/139°15	2	0	0
Cartwrights Hill	NSW	M	−34°56/147°25	4	0	0
Carwarp	VIC	L	−34°28/142°10	5	0	0
Clare	SA	M	−33°43/138°37	9	2	2
Coonabarabran	NSW	H	−31°05/149°33	8	0	0
Corowa	NSW	M	−35°53/146°18	10	3	3
Crystal Brook	SA	M	−33°19/138°12	6	0	0
Culcairn	NSW	M	−35°41/146°58	10	3	1
Delungra	NSW	H	−29°45/150°42	6	0	0
Dimboola	VIC	L	−36°25/142°00	3	0	0
Dookie 1	VIC	M	−36°13/145°40	4	2	0
Dookie 2	VIC	M	−36°12/145°42	6	2	1
Dubbo	NSW	H	−32°11/148°48	10	0	0
Dunedoo	NSW	H	−31°58/149°30	12	0	0
Echuca	VIC	M	−36°07/144°52	10	4	3
Eudunda	SA	L	−34°11/139°05	9	0	0
Finley	NSW	M	−35°37/145°35	10	0	0
Ganmain	NSW	M	−34°53/146°59	6	0	0
Gilgandra	NSW	H	−31°40/148°42	4	0	0
Griffith	NSW	L	−34°26/146°11	6	0	0
Gulgong	NSW	H	−32°23/149°36	10	0	0
Hay	NSW	L	−34°29/145°17	3	0	0
Hopetoun 1	VIC	L	−35°36/142°26	10	0	0
Hopetoun 2	VIC	L	−35°31/142°22	5	0	0
Inglewood	QLD	H	−29°05/151°17	2	3	3
Inverell	NSW	H	−29°39/151°12	10	0	0
Jarklin 1	VIC	M	−36°16/143°58	3	0	0
Jarklin 2	VIC	M	−36°14/143°56	8	4	0
Keith 1	SA	L	−36°06/140°16	10	0	0
Keith 2	SA	L	−36°04/140°17	10	0	0
Keith 3	SA	L	−36°06/140°21	3	0	0
Koonoona	SA	M	−33°49/138°56	10	0	0
Lake Boga	VIC	M	−35°28/143°39	10	4	0
Langhorne Creek	SA	L	−35°19/139°00	8	0	0
Leeton	NSW	L	−34°27/146°22	5	0	0
Lochiel	SA	L	−33°57/138°10	2	0	0
Longerenong	VIC	M	−36°40/142°18	10	0	0
Loxton 1	SA	L	−34°28/140°37	10	0	0

Appendix 1 (Continued)

Location	State	Rainfall	Longitude/Latitude	Sample size	TO	SO
Loxton 2	SA	L	−34°38/140°41	10	0	0
Mangalo	SA	L	−33°29/136°31	5	0	0
Mannum	SA	L	−35°00/139°14	10	1	1
Mitchelville	SA	L	−33°35/137°04	5	0	0
Morven	NSW	M	−35°35/147°09	10	3	3
Mount Priscilla	SA	L	−33°46/136°24	5	0	0
Mudgee	NSW	H	−32°31/149°33	10	0	0
Murray Bridge	SA	L	−35°04/139°13	10	0	0
Nanneella	VIC	M	−36°20/144°49	3	2	0
Narrandera	NSW	L	−34°46/146°25	7	0	0
Nhill 1	VIC	M	−36°24/141°27	1	0	0
Nhill 2	VIC	L	−36°24/141°49	9	2	0
Parkes	NSW	H	−33°13/148°13	11	2	2
Port Pirie	SA	M	−33°16/138°09	9	3	0
Red Cliffs	VIC	L	−34°24/142°00	7	0	0
Rochester	VIC	M	−36°23/144°46	9	2	2
Scone	NSW	H	−31°58/150°51	7	0	0
Sedan	SA	L	−34°33/139°18	3	1	1
Serpentine	VIC	M	−36°24/143°58	10	3	0
Shepparton	VIC	M	−36°25/145°27	10	5	3
Snowtown	SA	M	−33°44/138°05	10	0	0
Spalding	SA	M	−33°19/138°35	2	1	0
Swan Hill	VIC	M	−35°19/143°31	2	1	0
Tamworth	NSW	H	−31°03/150°51	6	0	0
Tarlee	SA	M	−34°12/138°43	1	0	0
Temora	NSW	M	−34°24/147°36	10	0	0
Ungarie 1	NSW	M	−33°39/146°59	12	0	0
Ungarie 2	NSW	M	−33°38/146°58	5	0	0
Ungarie 3	NSW	M	−33°36/146°55	10	0	0
Walpeup	VIC	L	−35°09/142°03	5	0	0
Wellington	NSW	H	−32°31/148°48	10	0	0
West Wyalong	NSW	M	−34°00/147°15	2	0	0
Wirrabara	SA	H	−33°02/138°16	9	4	3
Wunghnu	VIC	M	−36°10/145°28	10	3	1
Wunkar	SA	L	−34°29/140°12	10	1	1
Yanco 1	NSW	L	−34°38/146°25	2	0	0
Yanco 2	NSW	L	−34°34/146°23	3	0	0
Young	NSW	H	−34°27/148°19	11	3	0
Total				642	77	41