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Intrusive trichome bases in the leaves of silverleaf nightshade (*Solanum elaeagnifolium*; Solanaceae) do not facilitate fluorescent tracer uptake[1]

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- *Premise of the study: Solanum elaeagnifolium* (silverleaf nightshade), having originated in the Americas, is now a serious summer-growing, perennial weed in many countries, including Australia. Most surfaces of the plants have a dense covering of trichomes, giving them a silvery-white appearance, hence the common name. We aimed to identify structural and functional properties of its leaves, especially the trichomes, that may affect the uptake of foliar-applied tracer dyes.
- *Methods:* The structure of leaves of *Solanum elaeagnifolium* was examined by light and scanning electron microscopy. The potential for transport of materials between trichomes and veins was studied with symplastic (carboxyfluorescein diacetate) and apoplastic (lucifer yellow) tracer dyes.
- *Key results:* Mature leaves had a dense covering of complex, stellate trichomes on both surfaces, particularly the abaxial. The basal cells of *Solanum elaeagnifolium* trichomes penetrated into the underlying palisade mesophyll layers. The innermost lobes of these basal cells sometimes contacted the bundle sheath of the veins, but were not observed to directly contact the xylem or phloem. We found that neither symplastic nor apoplastic dyes were transferred between the basal cells of the trichomes and the vascular tissues. The trichome layer repelled water-based tracer dyes, while one of four adjuvants tested facilitated entry of both symplastic and apoplastic dyes.
- *Conclusions:* Our results did not support a transport function for the trichomes. The trichomes may protect the mesophytic leaves from invertebrate herbivory, while also probably decreasing radiation absorbed resulting in cooler leaves in this summer-growing species.

Key words: adjuvant; amphistomatous; herbicide; isobilateral; silverleaf nightshade; *Solanum elaeagnifolium*; stomatal density; tracer dyes; trichome; weed.

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.; Solanaceae) is considered native to the Americas, with a possible initial range of North America and subsequent introduction to South America (Levin et al., 2006). *Solanum elaeagnifolium* is a herbaceous perennial that resprouts in late spring by forming adventitious buds on the extensive horizontal root system, grows through summer (depending on the amount of rainfall), then the aerial parts die back in late fall with the onset of the first frosts (Stanton et al., 2009). It is now a widespread and serious weed in many countries, including Australia, India, Greece, Spain, and South Africa (Stanton et al., 2009). *Solanum elaeagnifolium* has several characteristics that make it difficult to control and virtually impossible to eradicate using current strategies (Boyd et al., 1984; Green et al., 1987; Mekki, 2007; Stanton et al., 2009).

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Solanum elaeagnifolium has two features that are potentially interrelated—its leaves have a dense covering of trichomes that give the plant a silvery appearance (hence the common name) (Fig. 1A), and it is difficult to control using foliar-applied herbicides (Boyd et al., 1984; Green et al., 1987; Mekki, 2007; Stanton et al., 2009), possibly related to interception of herbicide by the trichomes. Previous research has shown that these trichomes have a very unusual structural feature, an intrusive base that can come close to the veins (Bruno et al., 1999; Dottori et al., 2000; Bothma, 2002; Christodoulakis et al., 2009). Some authors (Bothma, 2002; Christodoulakis et al., 2009) indicated that the veins and the trichomes seem to be connected or in contact, which suggests a possible transport function. We investigated this possible connection using apoplastic and symplastic tracer dyes, which may provide important insights into basic biology, as well as herbicide control, of *S. elaeagnifolium*. An understanding of leaf morphology and anatomy, as well as herbicide uptake, underpins techniques to improve control strategies for this species.

Trichomes have many functions, including mechanical defense against herbivores, leaf cooling by reducing the absorption of short-wave radiation, and reducing transpiration by increasing the effective boundary layer (Gutschick, 1999; Haworth and McElwain, 2008; Skelton et al., 2012). Trichomes

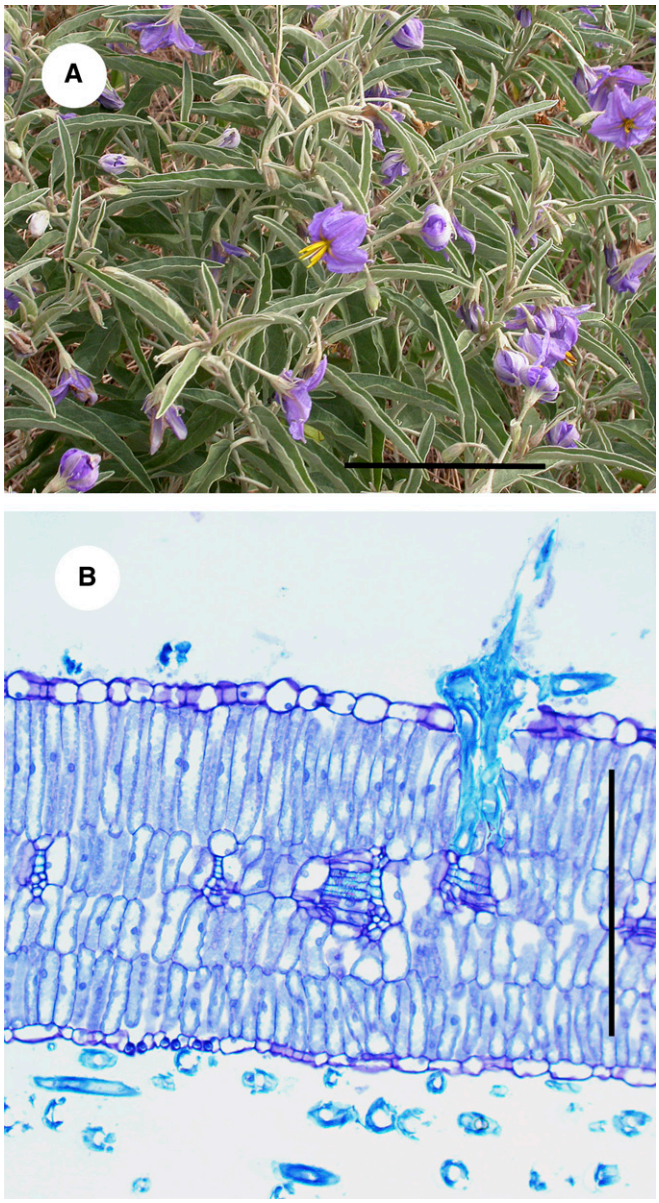


Fig. 1. *Solanum elaeagnifolium.* (A) Shoots showing that almost all plant surfaces (except for the petals and stamens) are densely covered in trichomes. Note that the adaxial leaf surface is a darker green than the abaxial. Scale bar = 5 cm. (B) Transverse section of mature leaf. Note the four layers of palisade mesophyll, with the cells of the adaxial layer longer than those beneath it. Scale bar = 200 μm.

can also decrease leaf wettability, i.e., keeping the epidermis dry, in both natural ecosystems (e.g., Brewer and Smith, 1997; Brewer and Nuñez, 2007) and in spray applications of herbicides. Keeping water from the leaf surface can allow for better gas exchange, while also providing a barrier to herbicide entry (Hess and Falk, 1990; Huangfu et al., 2009). Consequently, herbicides are often most efficacious when applied with wetting oils or other adjuvants (Zabkiewicz, 2000; Xu et al., 2010). Adjuvants come in a wide range of formulations, but in general they assist in the spread and penetration of the herbicide. The trichome functions mentioned can be highly species specific because trichomes vary markedly in density and structure (Werker, 2000).

While plants can absorb a wide range of chemicals through their leaves (e.g., water, nutrients, hormones, herbicides, atmospheric pollutants) the influence of trichomes on these processes is not consistent. For example, in a recent review Fernández and Brown (2013) note that the role of trichomes in foliar-applied nutrient uptake is "currently unclear". Trichomes have been associated with increased absorption by providing additional leaf surface area for uptake (Kannan, 2010), and also decreased absorption by suspending spray droplets above the epidermal surface (Hess and Falk, 1990). The lack of consensus on this topic may arise from the great diversity of trichome morphology, densities and physiology, all of which can change during the life of a leaf or plant (Hull, 1970).

The "hairs" of plants develop from either trichomes or emergences. Trichomes originate from epidermal cells only, while emergences develop from epidermal and subepidermal cells (Werker, 2000). The vast majority of "hairs" are trichomes, and in almost all species that possess trichomes, their development is superficial, i.e., outward growth from cells of the epidermis. However, *S. elaeagnifolium* possesses an unusual trichome structure; the basal stalk can feature intrusive cells that penetrate between the mesophyll cells and terminate near the veins (Bruno et al., 1999; Dottori et al., 2000; Bothma, 2002; Christodoulakis et al., 2009). Apparently similar structures in *Microlepis oleaefolia* (Melastomataceae) have been associated with apoplastic transport between the emergences and the vascular system (Milanez and Machado, 2008). In some epiphytes, specialzed leaf trichomes can facilitate water transfer between the external environment and internal leaf tissue (e.g., *Tillandsia* spp.; Benzing et al., 1976; Martin, 1994). Thus, we hypothesized that the unusual structure of the *S. elaeagnifolium* trichomes might influence uptake of tracer dyes, which can be used as proxies for some herbicides.

There have been several general studies of *S. elaeagnifolium* anatomy (Pilar, 1937; Cosa et al., 1998; Dottori et al., 2000; Bothma, 2002; Christodoulakis et al., 2009), a study of leaf trichome ontogeny (Bruno et al., 1999) and images of trichome morphology (McCleery, 1907; Roe, 1971; Sanders et al., 1980; Boyd et al., 1984; Maiti et al., 2002; El Naggar and Abdel Hafez, 2003). Several authors noted that the intrusive trichome base seems to contact the vascular tissue (Bruno et al., 1999; Dottori et al., 2000; Bothma, 2002; Christodoulakis et al., 2009) and thus may have a transport function. Here, we aimed to provide a detailed analysis of the leaf trichomes, with an investigation into their potential functional connection with vascular tissue using fluorescent tracer dyes. Other leaf anatomical features that might be associated with herbicide uptake and leaf function, such as stomatal density and size (Wanamarta and Penner, 1989; Ricotta and Masiunas, 1992), were also examined.

MATERIALS AND METHODS

***Plant material*—**Material for the anatomical and scanning electron microscope (SEM) studies was collected from mature *S. elaeagnifolium* plants growing in the field from various locations in New South Wales (NSW), Australia, while the tracer studies were conducted on plants propagated from root segments grown in soil, in pots in a shadehouse (40% of full sunlight) at Wagga Wagga, NSW. The study concentrated on samples from the lamina but also included petiole and stem samples. Lamina samples were taken from fully expanded leaves, midway along the lamina and midway between the midvein and margin.

***Anatomical and micromorphological analysis*—**Material was fixed in 50% formalin–acetic acid–alcohol, dehydrated through a graded ethanol series and

infiltrated with Leica HistoResin (Leica Microsystems, Heidelberg, Germany) under a slight vacuum for a minimum of 7 d. The samples were placed in pharmaceutical gelatin capsules containing HistoResin and polymerized overnight at 60°C. They were then sectioned at 5 μm using tungsten–carbide-tipped steel blades fitted to a motorized retraction microtome (Leica RM 2265). Sections were stained with 0.5% aqueous toluidine blue and observed using bright-field microscopy. Ten leaves from seven plants were cut in transverse section (TS), and serial paradermal sections were cut of 13 leaves from seven plants. Petioles and stems were also cut in transverse and paradermal section.

Adaxial trichome density was measured accurately in SEM images, but not abaxial density because the multiple layers of trichomes meant that many trichomes were obscured from surface view (Fig. 3C), thus abaxial densities were measured from paradermal sections.

The high density of trichomes meant that replica techniques (e.g., nail polish) could not be used to measure stomatal density. Instead a thin, flexible razorblade was used to shave the trichomes from selected areas of the lamina and then a thin paradermal slice of epidermis (and some underlying mesophyll tissue) was taken. The slice was mounted in water, coverslipped and photographed. Stomata in a 285 × 360 μm area were counted (three to five areas from at least two leaves from each of nine plants). Stomatal density was also assessed in the paradermal resin sections.

Small areas of the adaxial and abaxial leaf surfaces were excised for SEM examination. Samples were adhered to an SEM mounting plate, transferred into an Oxford Instruments (Oxfordshire, UK) CT1500 Cryo Preparation System and cooled under vacuum to −180°C. After rapid transfer into the cryostage of a Cambridge Instruments (Cambridge, UK) S360 SEM, specimens were etched at −90°C for a few minutes to remove ice by sublimation. Specimens were then cooled to −180°C, moved back into the cryo preparation unit and coated with 10 nm of gold. Specimens were then returned to the cryostage within the SEM chamber (held at −180°C) for examination. Individual trichomes removed from leaves were sputter-coated with gold in an Emitech K550X (Quorum Technologies Ltd, Kent, UK) and examined in a JEOL (Peabody, Massachusetts, USA) NeoScope SEM.

***Lignin and suberin staining*—**Trichomes were removed from fresh leaves with fine forceps, then rapidly processed through staining solutions, while retained in a 10 mm × 10 mm diameter container with a Millipore 30 μm nylon mesh filter on its base. For total lignin staining, trichomes were immersed in 1% phloroglucinol (Sigma, St. Louis, Missouri, USA) in 100% ethanol for 1–2 min and then transferred into concentrated HCl until a red color appeared, which indicated the presence of lignin (Jensen, 1962). The Mäule reaction was used to detect syringyl lignin (Chapple et al., 1992). Trichomes were immersed in 1% (w/v) aqueous $KMnO_4$ for 1–2 min, rinsed in water, transferred to 10% HCl and left to decolorize for 10-20 min. After another rinse in water, trichomes were mounted in 29% (v/v) NH_4OH and examined by bright field microscopy. Red staining indicated normal syringyl lignin, altered lignin (slightly reduced) appeared pink, while very reduced lignin appeared yellow.

Suberin was assessed by staining with berberine sulfate, which fluoresces bright green in suberin-impregnated cell walls (Brundrett et al., 1988; Lux et al., 2005). Hand sections of fresh leaves were immersed in 0.5% (w/v) aqueous berberine sulfate for 30 min, counterstained briefly with 0.1% (w/v) aqueous toluidine blue, rinsed in water and observed with a confocal laser scanning microscope (CLSM, Leica TCS SP2, excitation 405 nm, emission 520–580 nm for berberine sulfate, emission 420–470 nm for cell walls and excitation 633 nm, emission 650–700 nm for chloroplasts).

***Tracer analysis*—**Two tracer dyes were used to assess uptake via the leaf trichomes. The first was the apoplastic tracer, lucifer yellow carboxy hydrazide (LYCH, molecular weight [MW] 443, Sigma), used at 0.5 μg/mL in distilled water, which travels in the cell wall space and long-distance via the xylem (Botha et al., 2008). The second was the symplastic tracer, 5(6)-carboxyfluorescein diacetate (CFDA, Sigma), used at a final concentration of 100 μg/mL in distilled water, made fresh each day from 10 mg/mL stock solution in dimethyl sulphoxide. CFDA is nonfluorescent until it enters living cells where the acetate groups are cleaved to form fluorescent carboxyfluorescein (CF, MW 376), which is then confined to the symplasm and can travel from cell-to-cell in the symplasm and long-distance in the phloem (Botha et al., 2008). These are the smallest readily available tracer dyes, which although somewhat larger than common herbicides applied to *S. elaeagnifolium* (e.g., glyphosate MW 169, fluroxypyr MW 255, picloram MW 242, 2,4-D amine MW 266; Ensbey, 2011), have been widely used as tracers of both symplastic and apoplastic short-distance and vascular transport (e.g., Grignon, et al., 1989; Oparka, 1991; Wang and Fisher, 1994).

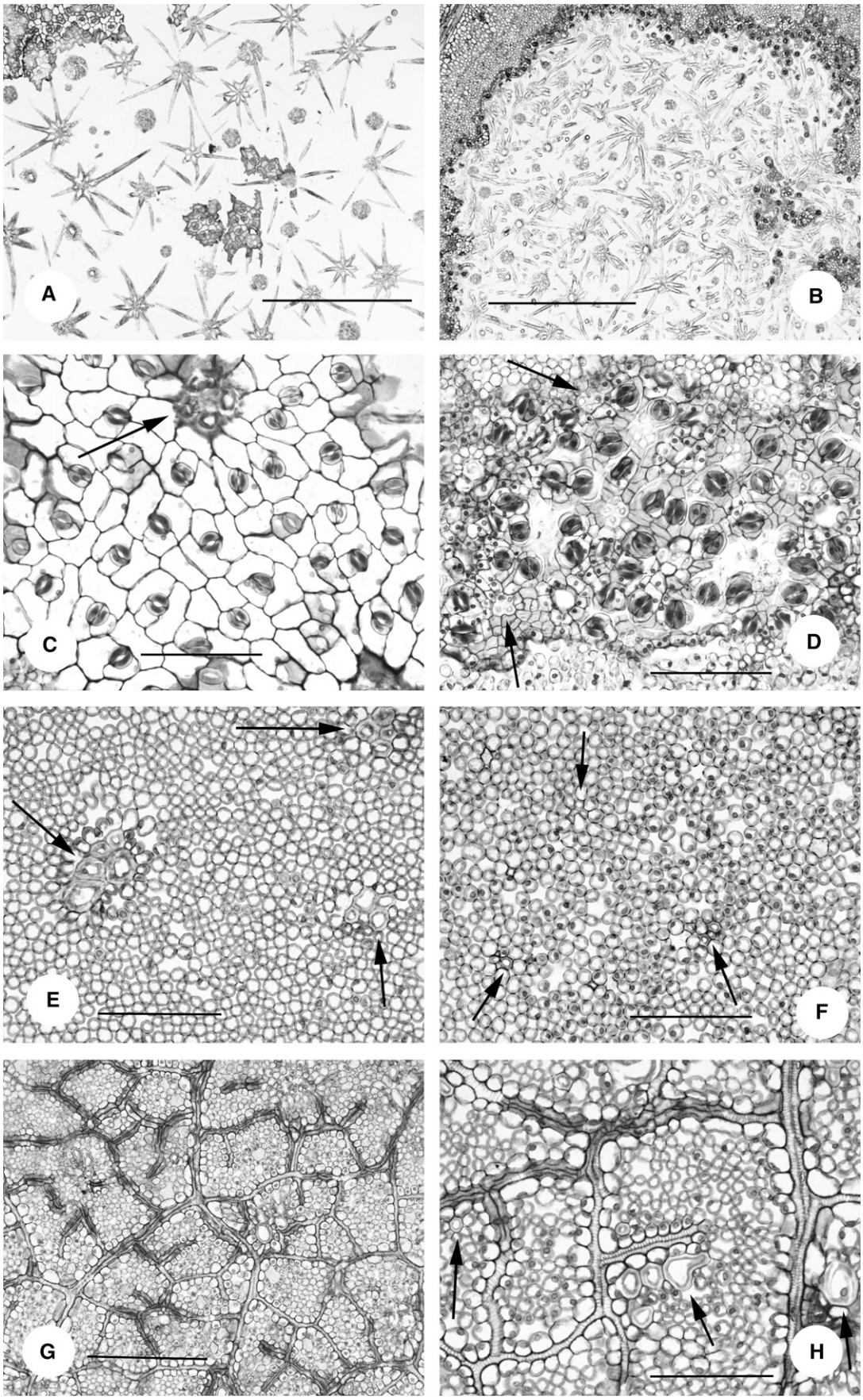
Dyes were applied as 50–200 μL droplets to the adaxial surface of mature leaves of plants growing in pots, and entry into the multicellular trichomes and internal leaf tissues was monitored. Mature leaves were held horizontally for 30 min to allow droplets to dry onto the surface. Additional leaves were cut from these plants, and the cut end of the petiole placed immediately into dye solution. Entry of tracer dye into the vascular system was monitored, together with any diffusion into adjacent tissues and, from there, into the trichomes. Plants were placed under a combination of moderate fluorescent and incandescent illumination (~200 $\mu E \cdot m^{-2} \cdot s^{-1}$) to maintain transpiration during dye uptake.

At 1, 6, and 24 h after tracer application, leaf surfaces were examined with a fluorescence stereomicroscope (Zeiss Stereo Lumar, GFP long-pass filter) or on a CLSM (Leica TCS SP2, excitation 488 nm, emission 510–550 nm and 660–700 nm for CF; excitation 488 nm, emission 520–580 nm and 660–700 nm for LYCH). Tracer movement in internal tissues was analyzed after 1, 6 and 24 h in leaves hand-sectioned under silicone oil to limit diffusion of the aqueous dyes during sectioning (Botha et al., 2008), and immediately examined with either the fluorescence stereomicroscope, as above, a fluorescence upright microscope (Zeiss Axioimager) or the confocal laser scanning microscope. The experiments were repeated twice, with at least three leaves used for each time point.

***Adjuvant trials*—**Four commonly used adjuvants (Somervaille et al., 2012) were tested for their effects on the uptake of both apoplastic and symplastic tracers. The adjuvants were 1020 g/L polyether modified polysiloxane (Sprinta; Victorian Chemical, Melbourne, Australia), 582 g/L paraffinic oil (Uptake; Dow AgroSciences, Sydney, Australia), 704 g/L esterified vegetable oil (Hasten; Victorian Chemical) or 1000 g/L alcohol alkoxylate (BS1000; Crop Care Australasia, Brisbane, Australia). Each was diluted to a final concentration of 1% in distilled water, a common concentration used with herbicides that are applied to *S. elaeagnifolium* (e.g., Meat & Livestock Australia, 2009). For tracer analysis, stock solutions of either LYCH or CFDA were added to give final concentrations of 0.5 μg/mL and 100 μg/mL, respectively. Tracer dyes with adjuvants were applied as 50–200 μL droplets to the surfaces of mature leaves and allowed to dry while the leaves were held horizontally for 30 min. Leaves were removed from the plant 6 and 24 h after treatment and examined with a fluorescence microscope as described above. Untreated leaves were also removed from mature plants and their petioles immersed in LYCH or CFDA for 6 or 24 h to determine whether dye would move into trichomes from the vascular tissue. Leaves were examined using an upright fluorescence microscope as described previously.

RESULTS

***General leaf anatomy*—**Leaf thickness (cuticle to cuticle) averaged 281 μm (range 234–317 μm), while the trichomes (to the approximate "surface level" of the rays—see below) added, on average, another 50 μm depth on the adaxial surface and 190 μm on the abaxial surface (Fig. 1B) (for a total leaf "thickness" of ~500 μm). Palisade mesophyll cell distribution was isobilateral (Fig. 1B); however, of the four palisade layers, the adaxial layer was much thicker (average depth 106 μm) than the three lower layers (average depth 48 μm) (Fig. 1B). The veins were embedded in the uppermost of the three lower layers of palisade mesophyll (Fig. 1B). Except for the layer with the veins, the other palisade layers were almost completely continuous because no bundle sheath extensions, as often found in xerophytic leaves, were present. There was little difference in cell density or diameter between the upper and lower palisade layers (Fig. 2E, F). Numerous idioblasts (presumably of calcium oxalate "sand") were present, mainly in the two middle palisade layers and were almost completely absent from the adaxial palisade layer. A cuticle was present but was very thin (<1 μm) on both leaf surfaces (Fig. 1B). While transverse sections showed that the palisade mesophyll was apparently very tightly packed (Fig. 1B), the


A
B
C
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H

paradermal sections revealed an extensive system of intercellular air spaces (Fig. 2E, F).

***Trichome structure*—**Leaves possessed trichomes that were large (usually 400–500 μm diameter, Fig. 3A-C) and abundant, that gave the leaf surface a silvery appearance (Fig. 1A). The multicellular trichomes consisted of a basal stalk that was attached to a central "hub", from which radiated numerous rays (parallel to the leaf surface) and a single spine (perpendicular to the leaf surface) (termed porrect stellate trichomes) (Fig. 3). A TS of the basal stalk (paradermal section of the leaf) showed that it usually comprised four cells connected together along their long axis (Fig. 2C-F although some adaxial trichomes had five or six basal cells (Fig. 2E). A variable number (usually 12–16) of single-celled rays, mostly between 150–200 μm in length, extended from the hub (Fig. 3), together with a single-celled spine that projected from the top of the hub. The walls of all these cells were relatively thick (3–5 μm). Trichome density was relatively low on the adaxial surface (see below, Figs. 2A, 3B), such that the epidermal cells were visible between the trichome rays (Fig. 3B), which were all at about the same height (~50 μm) above the epidermis.

Trichome density was approximately three times greater on the abaxial surface, which completely obscured the epidermis (Fig. 3C). To accommodate this high trichome density, the stalk cells were of different lengths; thus, the rays formed at different heights above the leaf surface. For trichomes with a short basal stalk, only the projecting spine (if anything at all) appeared through the overlapping rays of the taller layers (Fig. 3C). Trichomes on the adaxial surface had thicker cell walls than those on the abaxial surface (e.g., Fig. 2A-D).

The basal cells of these trichomes were either nonintrusive or intrusive (Figs. 1B, 2C-F), with the degree of intrusion being quite variable (Fig. 2E-H). On the abaxial leaf surface, the stalk cells of many trichomes did not develop extensively into the mesophyll, and the basal cells of those that did, often did not extend farther in than the lowermost palisade layer (Fig. 2F). Almost all basal cells of adaxial trichomes extended through the upper palisade layer (Figs. 1B, 2E), and in older leaves, some basal cells extended into the abaxial palisade layers (Fig. 4A, B). Investigation of the paradermal sections indicated that the cells of the basal stalk were of different lengths and shapes, and the tips of the basal cells often did not extend to the depth of the veins (Fig. 2G, H), especially the abaxial trichomes. When they did, the tips often abutted the bundle sheath cells and were not observed to contact the xylem and phloem directly (Fig. 2H). In short, given the high density of trichomes on both surfaces, at the level of the veins, relatively few tips of the trichome basal cells were observed.

Stellate trichomes were also present on petioles and stems, and these were exclusively nonintrusive. In the petiole and stem trichomes, the basal stalk appeared to consist of a stack of 4–5 cells high and four cells in TS (wide), meaning that the stalk was composed of 16–20 cells.

Flakes of cuticular wax were seen on the trichomes in SEM or detected as small autofluorescent spots in confocal microscopy. In younger fully expanded leaves, the trichome rays contained nuclei and chloroplasts (Fig. 4E-G). In older leaves collected in late summer or early autumn, nuclei were still observed in ray and stalk cells of trichomes on both leaf surfaces. All cells of mature trichomes had elaborate pit connections between the four basal cells and the central hub cell, and also between the hub cell and the lateral ray cells (Fig. 4C, D).

Cell wall composition was investigated with phloroglucinol and Mäule staining of isolated trichomes to detect total lignins and syringyl lignin, respectively. Phloroglucinol stained the walls of trichome stalk cells red with little or no staining in trichome rays in younger leaves, but strong staining of the trichome walls in older leaves (Appendix S1, see Supplemental Data with the online version of this article). Trichome stalk cells stained red with Mäule stain, the stalk cells of trichomes became red showing the presence of syringyl lignin, whereas the yellow stain in trichome ray cells indicated the presence of reduced syringyl lignin (Appendix S1). Staining with berberine sulfate indicated the presence of suberin in the unlignified trichome ray cells of leaves from glasshouse-grown plants (Fig. 4A-D).

***Trichome and stomatal densities*—**On average, there were 35.0 (SE 2.2) and 100.5 (SE 4.7) trichomes/mm^2 on the adaxial and abaxial surfaces, respectively. The ratio of abaxial to adaxial trichomes/mm^2 averaged 2.9 : 1.

For the leaves sectioned paradermally, on average, 340.3 (SE 16.6) and 589.2 (SE 19.4) stomata/mm^2 were present on the adaxial and abaxial surfaces, respectively. The ratio of abaxial to adaxial stomata/mm^2 averaged 1.7 : 1. For the leaves that were examined fresh ("shaved"), there were, on average, 298.4 (SE 11.8) and 505.1 (SE 13.9) stomata/mm^2 on the adaxial and abaxial surfaces, respectively. The average ratio of abaxial to adaxial stomata/mm^2 was the same as for the prepared slides (1.7 : 1). On average, guard cells were 5% longer on the adaxial surface compared with the abaxial (22.0 [SE 0.24] and 20.9 μm [SE 0.24], respectively).

***Tracer studies*—**The symplastic tracer dye CFDA rarely entered the leaf trichomes when applied to the adaxial or abaxial leaf surface in water or with addition of adjuvants BS1000, Hasten, or Uptake (Fig. 5A, B, D, F; Appendix S2b–e, see online

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Fig. 2. Bright-field micrographs of paradermal sections of mature leaves of *Solanum elaeagnifolium*. (A) Section through or just above the adaxial surface. Trichome density is about 25 trichomes/mm^2. Scale bar = 500 μm. (B) Section through or just below the abaxial surface. Note that some trichomes have been sectioned through the "basal stalk", some through the rays, and some through the terminal spine. Note that the trichomes are less robust than those shown in (A). Trichome density is about 120 trichomes/mm^2. Scale bar = 500 μm. (C) Adaxial epidermis with a stomatal density of ~360 stomata/mm^2. Note the large, thick-walled cells of the basal stalk cells of a trichome (arrowed). Scale bar = 100 μm. (D) Abaxial epidermis with a stomatal density >600 stomata/mm^2. Note the small, relatively thin-walled cells of the basal stalk cells of several trichomes (arrowed). Scale bar = 100 μm. (E) Adaxial palisade mesophyll. Note the thick-walled basal cells of three trichomes (arrowed). Although cells are densely packed, an extensive network of intercellular airspaces is present. Scale bar = 100 μm. (F) Abaxial palisade mesophyll. Note that the basal cells of only three trichomes are present (arrows) and that these cells are relatively small and thin walled. Scale bar = 100 μm. (G) Section through the veins and the second layer of palisade mesophyll from the adaxial surface. Note the complex and densely reticulated pattern of vascular tissue. Note also that few of the inner tips of the trichome basal cells have reached this level. Scale bar = 200 μm. (H) Detail of a section similar to that shown in (G) showing that the tips of some of the trichome basal cells (arrowed) are adjacent to the bundle sheath cells. Scale bar = 100 μm.

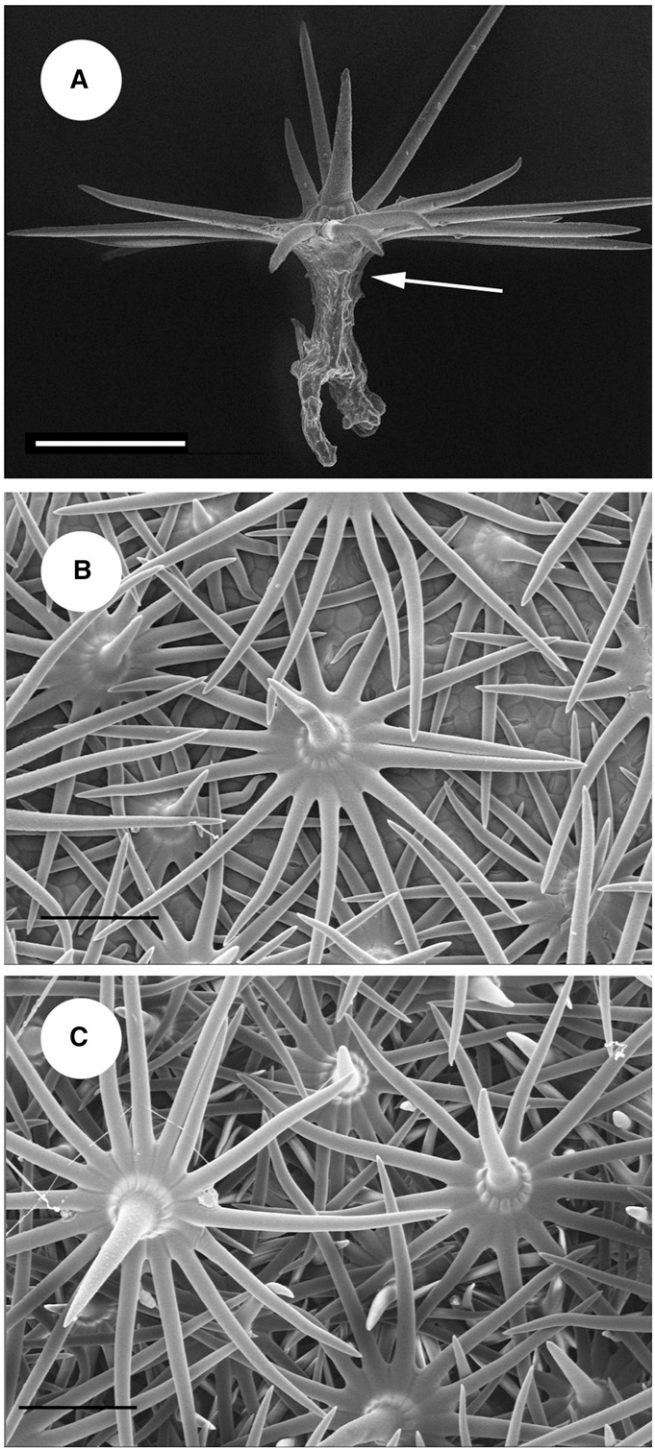

Fig. 3. Scanning electron micrographs of mature leaves of *Solanum elaeagnifolium*. Scale bars = 100 μm. (A) Trichome removed from adaxial surface. Note downward projections of the intrusive basal cells. Arrow indicates the approximate leaf surface level. (B) Adaxial leaf surface. Note the stomatal complexes visible between the rays of the trichomes. (C) Abaxial surface. Note the upper level trichome on the left that would have a long basal stalk and the lower level trichomes where only the terminal spine is visible.

Supplemental Data). Even when entry occurred, the dye did not spread into other cells. Small amounts of dye that dried onto the surface of trichome ray and central cells were readily detected (Appendix S2f), but transverse sections revealed that entry into the basal trichome cells was rare (one example is shown in Appendix S2i), and the dye never appeared to penetrate past the bundle sheath. Background green autofluorescence was minimal (Appendix S2f). If CFDA reached the epidermis, it entered epidermal cells directly through the thin cuticle and, rarely, also appeared to enter via the abundant stomata (Appendix S2d, arrow). More trichomes contained dye 24 h after application, but no dye was detected spreading through the vascular system. Addition of the adjuvant Sprinta allowed rapid entry of CFDA into the trichomes (Appendix S2h), and the dye also penetrated deeply into the leaf mesophyll and vascular tissues (Appendix S2h). In all analyses of applications of tracers to leaf surfaces, results from 1, 6, and 24 h after application were indistinguishable.

The membrane-impermeant dye LYCH, when applied to the upper or lower leaf surface, could be seen as fluorescent flecks dried onto the surface of ray and central trichome cells (Appendix S2j), and it also occasionally stained trichome cell walls (Fig. 5F). The dye was only seen within the trichome cells if they had been damaged in some way before the application of the tracer. With the addition of the adjuvant Uptake, more dye was retained on the leaf surface (Appendix S2e), especially in ray cells (Appendix S2k), and the addition of Sprinta allowed dye entry into the leaf epidermis and mesophyll, indicated by cell wall staining (Fig. 5G).

When the cut end of a petiole was placed into CFDA solution, the dye moved into the vascular tissues and throughout the dense network of leaf veins. Observation of leaf surfaces showed labeling of the underlying veins (Fig. 6A) but no labeling of trichomes, which appeared in negative relief (Fig. 6B). Transverse sections of midveins taken midway along the lamina (Fig. 6C) and longitudinal profiles of minor veins (Fig. 6D) showed that CFDA had spread into the vascular parenchyma where it was cleaved, forming the fluorescent CF. CF also spread farther into bundle sheath cells but not into the trichomes (Fig. 6C, D). There was virtually no background green or yellow autofluorescence (Appendix S2a). When the cut petioles were placed into LYCH solution, the dye could be detected in the cell walls of xylem vessels and adjacent tissues (Fig. 6E, F). In lamina transverse sections, the dye was also seen in walls of cells around the midvein (Fig. 6E) and around minor veins (Fig. 6F) and occasionally in mesophyll cells closest to the veins, but did not enter or accumulate in trichomes. There was limited transport into vascular tissues after 1 h, and very strong fluorescence after 24 h, by which time leaves were beginning to wilt. All results presented are from 6 h dye uptake.

DISCUSSION

Leaves of *S. elaeagnifolium* plants had a high density of structurally complex, stellate trichomes. As in previous studies, we found that the trichomes possessed an unusual intrusive base, the tips of which could come in close proximity to the veins. Contrary to earlier suggestions, we found no direct physical contact between the trichome basal cells and the xylem or phloem. Evidence from applied tracer dyes indicates the trichomes were more of a barrier than a gateway to the interior

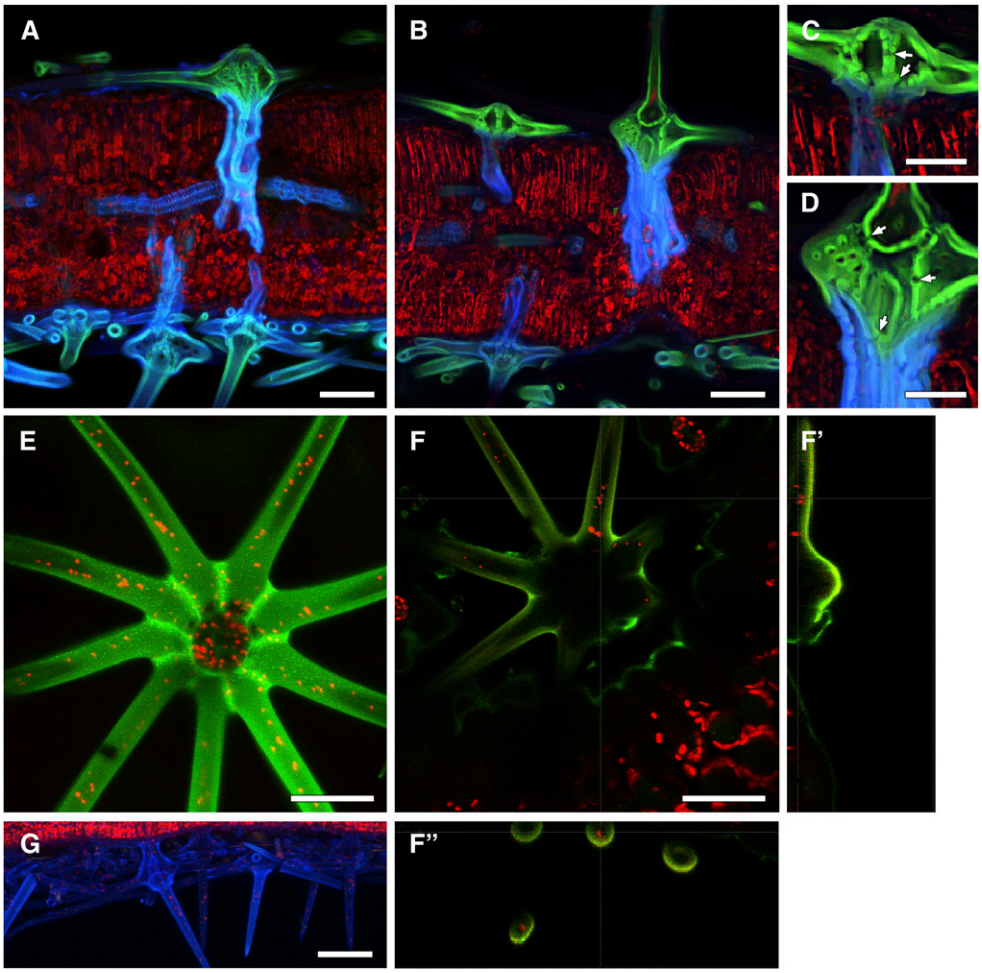

Fig. 4. Confocal fluorescence images of *Solanum elaeagnifolium* leaves and trichomes showing details of insertion and living chloroplasts. (A-D) Hand-cut transverse sections of leaves stained with berberine sulphate showing suberin green and autofluorescence blue. (A, B) The larger adaxial trichome basal cells penetrated close to (A) and considerably beyond (B) the central vascular tissue, whereas the basal cells of abundant abaxial trichomes are seen only in the abaxial mesophyll. Trichome ray cells show green fluorescence indicating suberin. Basal cells show blue autofluorescence. (C, D) Enlargement of trichomes in (B) showing pit connections (arrows) between trichome cells. (E) Composite image of surface view of unstained trichomes showing small chloroplasts (red) inside the trichome ray cells (green). (F) Single confocal section of trichome showing chloroplasts inside the cells. (F′) View of (F) from the right, (F″) view of (F) from below; chloroplasts are enclosed within the ray cells. (G) Multiple trichomes in side view with chloroplasts (red) within the ray cells (blue cell wall autoflorescence). Scale bars = 50 μm (A, B, E, F), 25 μm (C, D), 100 μm (G).

of the leaf, while use of adjuvants could, in some cases, substantially alter dye uptake. The high trichome and stomatal densities recorded in this study have important implications for the growth of *S. elaeagnifolium* during hot, dry summers (the main growth period) and chemical control of this species.

***General anatomy*—**The main aspects of *S. elaeagnifolium* leaf anatomy appear quite consistent, with plants collected from widely differing locations in Australia (present study), South Africa (Bothma, 2002), South America (Cosa et al., 1998), and Greece (Christodoulakis et al., 2009) all having isobilateral, amphistomatous leaves, with four layers of palisade mesophyll. Even variability in leaf thickness is relatively consistent in these studies (Bothma, 2002, ~210 μm; Christodoulakis et al., 2009, 230–250 μm; present study average, 280 μm). As noted, the distinctive stellate trichomes have been described by numerous authors, while their greater abaxial density and/or the intrusive base have been recorded by Bruno et al. (1999), Dottori et al. (2000), Bothma (2002), and Christodoulakis et al. (2009).

***Stomatal density*—**While previous authors reported that the leaves of *S. elaeagnifolium* are amphistomatous (Bothma, 2002; Christodoulakis et al., 2009), there appears to be no records of stomatal density or of the greater stomatal density on the abaxial surface. The average for the adaxial surface was in the range of 300–340 stomata/mm^2, and the abaxial surface was in the range of 500–589 stomata/mm^2. Various values from studies of over 50 species (Peat and Fitter, 1994; Hetherington and Woodward, 2003; Zarinkamar, 2007; Beaulieu et al., 2008; Tay and Furukawa, 2008; Loranger and Shipley, 2010) indicate that greater than 400 stomata/mm^2 could be considered a very high density. It could be assumed that many of the species in these studies had glabrous leaves or leaves with a relatively low density of simple trichomes that would allow the epidermal replica method to work. It would be interesting to compare stomatal densities of leaves that are glabrous to those with high trichome densities. Skelton et al. (2012) speculated that smaller, denser stomata may be associated with or compensate for the presence of a dense pubescence layer.

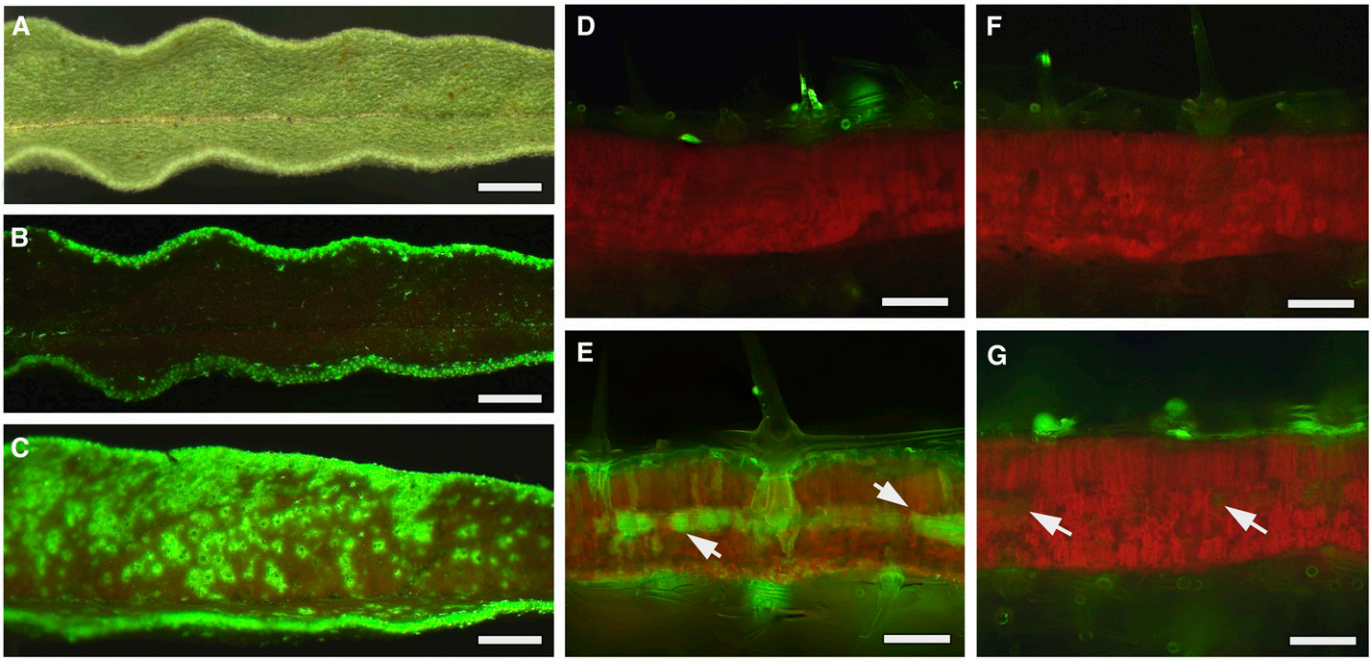

Fig. 5. Mature leaves of *Solanum elaeagnifolium* 1 h after application of fluorescent tracer dyes to the leaf surface. In all cases, the dye fluoresces green; red background fluorescence is from chlorophyll. (A) Overview, bright-field image and (B, C) fluorescence images of leaves after application of CFDA with (B) adjuvant BS1000, showing little dye entry or (C) adjuvant Sprinta showing considerable dye entry into leaves. (D–G) Leaf transverse sections, cut in silicone oil to prevent dye spread, showed little entry of CFDA applied in (D) water. With addition of Sprinta (E), dye rapidly spread over the leaf surface, then accumulated in cells around the vascular bundles (arrow). The apoplastic dye LYCH applied in water (F) did not enter the leaves, but could enter and stain cell walls if applied with (G) Sprinta, and is seen as faint staining of xylem elements in the veins (arrows). Scale bars = 2 mm (A–C), 100 μm (D–G).

The high stomatal densities of *S. elaeagnifolium* are of interest from two perspectives—gas exchange for photosynthesis and transpiration and as a possible pathway for herbicide uptake. Beaulieu et al. (2008) note that high stomatal densities are associated with greater stomatal conductances, greater transpiration rates, and maximization of CO_2 diffusion during periods optimal for photosynthesis. Herbicide absorption is facilitated by cuticular or stomatal penetration (Wanamarta and Penner, 1989). Higher numbers of stomata can result in greater penetration of herbicides into leaf tissue (Currier et al., 1964; Wanamarta and Penner, 1989; Ricotta and Masiunas, 1992; Eichert and Burkhardt, 2001; Burkhardt et al., 2012), with both the stomatal pore and the guard cells of importance. For example, in 11 *Lycopersicon* (Solanaceae) genotypes, a strong correlation between higher stomatal density and decreased tolerance to the herbicide acifluorfen was found (Ricotta and Masiunas, 1992). Thus, the discovery of high stomatal densities on both leaf surfaces of *S. elaeagnifolium* has important implications for both herbicide uptake and basic leaf physiology.

Stomatal size (guard cell length) shows a range of about 10–80 μm worldwide, with smaller stomata tending to show a greater water-use efficiency (Hodgson et al., 2010). The studies of Hetherington and Woodward (2003) and Beaulieu et al. (2008) indicate that high stomatal density is associated with short guard cells, and *S. elaeagnifolium* (average stomatal length 21–22 μm) fits this trend.

Trichomes and tracers—Trichomes develop only from the cells of the protoderm/epidermis, while emergences have cells from both the epidermis and subepidermal layers (Werker, 2000). The developmental study of Bruno et al. (1999) indicates that *S. elaeagnifolium* stellate hairs are trichomes, not emergences, because intrusive and nonintrusive variants develop from a single epidermal cell. Both emergences and trichomes with intrusive-type growth have been recorded in at least three very different species, *S. elaeagnifolium* (Solanaceae), *Microlepis oleaefolia* (Melastomataceae) (Milanez and Machado, 2008), and *Jovetia humilis* (Rubiaceae) (Guédès, 1975). As the detection of intrusive trichomes requires leaf dissection or sectioning, it is possible that the intrusive trichome type might be more common than the literature indicates. Stellate trichomes with an external morphology like those found on *S. elaeagnifolium* have been described in many species of *Solanum*, in the Solanaceae in general and in other families. For example, most members of *Solanum* subgenus *Leptostemon* (~350–450 species) have stellate trichomes (Levin et al., 2006). It would be remarkable if the intrusive stellate trichome type was restricted to *S. elaeagnifolium*.

The leaf trichomes of *S. elaeagnifolium* are elaborate, multicellular structures with the basal stalk consisting of four (or more) cells that may penetrate through the epidermis and mesophyll. Christodoulakis et al. (2009) indicated there was only a single basal cell, while Bothma (2002) described a biseriate stalk and also that the nonintrusive trichomes had a multicellular stalk, while the intrusive type had fewer but much larger stalk cells. This latter observation is similar to that of Bruno et al. (1999) and the present study. Some of the multilobed internal ends of the basal stalk contacted the bundle sheath cells, and some penetrated into the mesophyll on the opposite side of the leaf. This proximity of trichome base and vein might suggest a functional role, but our data do not support a transport function, either from the trichome to internal tissues or vice

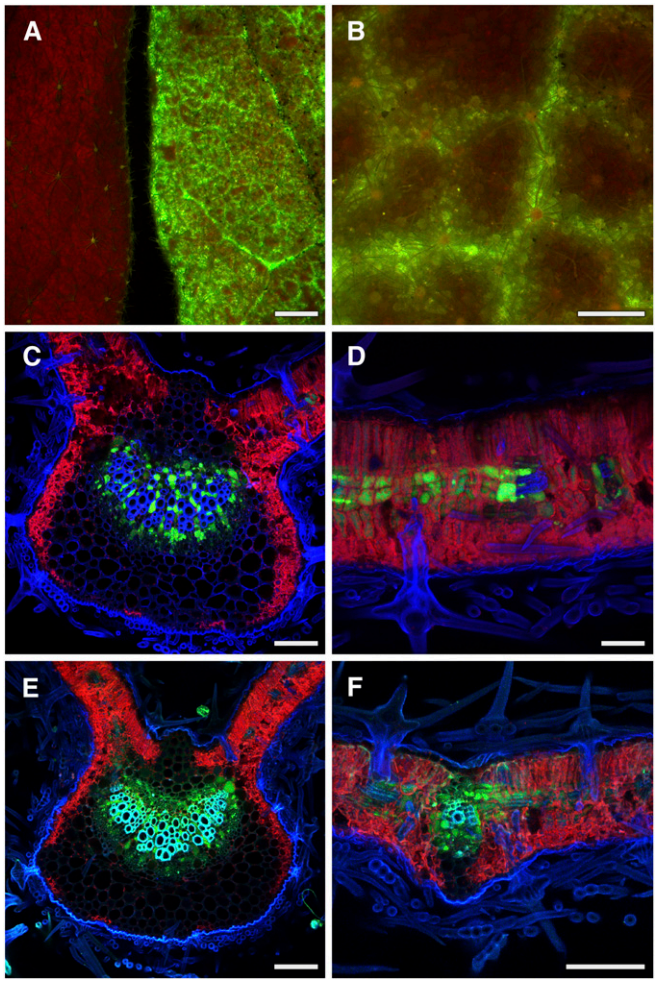

Fig. 6. Surface views and sections of *Solanum elaeagnifolium* leaves after a 6-h uptake of fluorescent tracer dyes through the cut petiole. (A) Fluorescence image of surface of control leaf (left) with cut petiole in water; leaf after uptake of CFDA through the petiole (right) in which dye (green) has penetrated throughout the dense network of large and small veins. (B) Higher magnification shows that although dye has entered the veins, it has not entered the trichomes. (C–F) Leaf transverse sections cut in silicone oil and observed with confocal microscope show CFDA (pseudocolored green) in cells next to the xylem (thick-walled cells pseudocolored blue) of main veins (C) and in cells around smaller veins (D). After taking up the apoplastic dye LYCH, cell walls of xylem elements (thick-walled cells pseudocolored cyan) and much of the surrounding tissues are stained, especially around main veins (E); the dye (pseudocolored green) also spread into walls of tissues around smaller veins (F). Background blue fluorescence is from cell walls stained with calcofluor white; chloroplasts autofluoresce red. Scale bars = 1 mm (A), 0.5 mm (B), 100 μm (C, E, F), 50 μm (D).

versa, as was suggested for *Microlepis oleaefolia* by Milanez and Machado (2008).

The relationship of the intrusive stalk base and the veins is important. Bothma (2002, pp. 44, 46) noted that "the stalks seem to reach the vascular bundles" and "seem to penetrate to the point of making contact with the xylem", Christodoulakis et al. (2009, p. 435) recorded that the stalk "seems to be connected to the conductive tissue", while Bruno et al. (1999) indicated that the intrusive part of the trichome reaches the vascular tissues and partially surrounds them. These studies were based on leaf transverse sections. Our transverse sections also seemed to show a close association between the tips and the bundle sheath (Figs. 1B, 4A, 4B), but the much clearer serial paradermal sections showed that the tips rarely, if ever, directly contacted the xylem or phloem (Fig. 2H). When thicker hand sections were cleared and stained, the deep penetration of the adaxial basal cells was more easily seen as their highly three-dimensional structure was more apparent. Other areas of variation between these anatomical studies of *S. elaeagnifolium* exist. Christodoulakis et al. (2009) indicated that the stalk is lignified, while the rays are nonlignified, while Bruno et al. (1999) and Bothma (2002) indicated that the "emerged" part is lignified, while the intrusive part has little or no lignification. Our study shows that trichomes have lignified walls in the stalk, but lignified ray cells appeared only in older leaves. These differences are likely to arise from different growing conditions for the plants examined. Bothma (2002, p. 46) recorded that "all cells of the intrusive stellate trichomes… seem to be dead", while our observations of nuclei and small chloroplasts would indicate they remain alive for most of the life of a leaf.

As noted, the leaf emergences of *Microlepsis oleaefolia* have some remarkable similarities in basic structure to the intrusive trichomes of *S. elaeagnifolium*. In *Microlepsis oleaefolia* the cell walls of the "arms" were nonlignified and nonsuberized, and an apoplastic transport path was indicated to be present between the vascular system and the emergences (Milanez and Machado, 2008). Fahn (1986) floated the leaves of three xerophytic and three mesophytic leaves with trichomes on a solution containing an apoplastic tracer. The tracer was not found in the trichome walls of the xerophytes, but was in the mesophytes. Sclereids are usually associated with a support function, but Heide-Jørgensen (1990) found that the extensive osteosclereids in the leaves of *Hakea suaveolens* also had a transport function, acting as vein extensions. The highly modified (but nonintrusive) leaf trichomes of *Tillandsia* species (air plant) have an absorption role (Benzing et al., 1976; Martin, 1994). Taken together, these studies show that the relationship between trichomes and transport into and out of leaves is highly complex and needs to be assessed on an individual species basis.

Solanum elaeagnifolium grows during the hot, dry Mediterranean-type summers of southern inland Australia, a period when many other species, both native and introduced, are dead (annuals) or dormant (herbaceous perennials) (e.g., Burrows, 1999) and most native species that maintain functioning leaves during this period are highly sclerophyllous (Burrows, 2001). Although *S. elaeagnifolium* grows during a particularly stressful part of the year, the leaves show little external evidence of damage, either biological or physical. The anatomical sections show that the leaves of *S. elaeagnifolium* possess little sclerenchyma or other specialized mechanical tissues. Assuming the trichomes of *S. elaeagnifolium* are not primarily involved in transport into or out of the leaf, what other functions might they serve? Trichomes have been associated with numerous functions, but comparing between studies is fraught with difficulties given the often substantial differences in trichome structure and density.

A dense coat of hairs may be a leaf cooling or insulating mechanism, as for many other leaves in which a dense covering of trichomes has been shown to reflect incident solar radiation and/or dissipate absorbed heat (Pérez-Estrada et al., 2000; Jordan et al., 2005) or protect against UV damage. Interestingly, the leaves or phyllodes of some species that show a high reflectivity from numerous trichomes become dark green when wet and consequently absorb a much greater amount of photosynthetically

active radiation (Harrington and Clark, 1989; Yates, 1992). We have observed (data not shown) a similar change in color upon the wetting of leaves of *S. elaeagnifolium*. Because the cells of the trichomes are thick walled, heavily lignified, and form a complete "coat", at least on the abaxial surface, they may also protect the leaf from herbivores (Levin, 1973), in combination with the smaller glandular hairs on the leaves (Cosa et al., 1998; Christodoulakis et al., 2009). We have observed minimal leaf damage in *S. elaeagnifolium* populations across Australia at the end of the growing season (our unpublished observations). The cuticle is very thin, and stomata are very dense, making the epidermis delicate and susceptible to herbivore attack. The role of trichomes as an antitranspirant, by increasing boundary layer resistance and consequently reducing transpiration is complex (e.g., Haworth and McElwain, 2008; Jordan et al., 2008; Skelton et al., 2012) but has been reported for some species (Kenzo et al., 2008).

Trichome and stomatal densities are known to influence foliar herbicide uptake (e.g., Hess and Falk, 1990; Wang and Liu, 2007). Trichomes are associated with herbicide droplet interception and the creation of air pockets, consequently reducing the spread of droplets and the wetting of epidermal cells. Huangfu et al. (2009) reported that greater trichome density on the upper leaf surface in populations of *Brassica juncea* was correlated with reduced herbicide efficacy, while Xu et al. (2011) illustrated how a water droplet could be suspended above the leaf surface by trichomes and how some adjuvants improved spread while others did not. Brewer et al. (1991) indicated that leaves with trichomes were more water repellant, especially where trichome density was greater than 25 trichomes/mm^2. Presumably this value could be lower in species like *S. elaeagnifolium* that have large, spreading, and structurally complex trichomes. Once a herbicide droplet reaches the leaf surface, penetration into the leaf can occur via the cuticle, stomata, or guard cells. The relative importance of these pathways is not well understood for most weed species, although greater uptake with higher stomatal densities has been reported (Wanamarta and Penner, 1989).

Conclusion—It appears that *S. elaeagnifolium* stellate trichomes, alive at maturity, with heavily lignified intrusive bases that extend close to but do not directly contact the vascular tissue, do not take up symplastic or apoplastic dyes. Thus, the trichomes are likely to be a barrier to foliar-applied chemicals, even with certain adjuvants. It is possible that because of the high stomatal densities, that if the trichome barrier of *S. elaeagnifolium* is crossed, chemicals might be readily absorbed. While untested, the high densities of structurally complex and thick-walled trichomes could be providing protection from invertebrate herbivores, while also decreasing radiation absorption to keep leaves cooler during the summer growth period. The function of the unusual intrusive trichome bases remains unclear.

LITERATURE CITED

BEAULIEU, J. M., I. J. LEITCH, S. PATEL, A. PENDHARKAR, AND C. A. KNIGHT. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179: 975–986.

BENZING, D. H., K. HENDERSON, B. KESSEL, AND J. SULAK. 1976. The absorptive capacities of bromeliad trichomes. *American Journal of Botany* 63: 1009–1014.

BOTHA, C. E. J., N. AOKI, G. N. SCOFIELD, L. LIU, R. T. FURBANK, AND R. G. WHITE. 2008. A xylem sap retrieval pathway in rice leaf blades: evidence of a role for endocytosis? *Journal of Experimental Botany* 59: 2945–2954.

BOTHMA, A. 2002. Allelopathic potential of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). M.Sc. thesis, University of Pretoria, Pretoria, South Africa.

BOYD, J. W., D. S. MURRAY, AND R. J. TYRL. 1984. Silverleaf nightshade, *Solanum elaeagnifolium*, origin, distribution, and relation to man. *Economic Botany* 38: 210–217.

BREWER, C. A., AND W. K. SMITH. 1997. Patterns of leaf surface wetness for montane and subalpine plants. *Plant, Cell & Environment* 20: 1–11.

BREWER, C. A., W. K. SMITH, AND T. C. VOGELMANN. 1991. Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. *Plant, Cell & Environment* 14: 955–962.

BREWER, C. A., AND C. I. NUÑEZ. 2007. Patterns of leaf wettability along an extreme moisture gradient in western Patagonia, Argentina. *International Journal of Plant Sciences* 168: 555–562.

BRUNDRETT, M. C., D. E. ENSTONE, AND C. A. PETERSON. 1988. A berberine-aniline blue fluorescent staining procedure for suberin, lignin, and callose in plant tissue. *Protoplasma* 146: 133–142.

BRUNO, G., M. T. COSA, AND N. DOTTORI. 1999. Ontogenia de tricomas estrellados en *Solanum elaeagnifolium* (Solanaceae). *Kurtziana* 27: 169–172.

BURKHARDT, J., S. BASI, S. PARIYAR, AND M. HUNSCHE. 2012. Stomatal penetration by aqueous solutions—an update involving leaf surface particles. *New Phytologist* 196: 774–787.

BURROWS, G. E. 1999. A survey of 25 remnant vegetation sites in the South Western Slopes, New South Wales. *Cunninghamia* 6: 283–314.

BURROWS, G. E. 2001. Comparative anatomy of the photosynthetic organs of 39 xeromorphic species from subhumid New South Wales, Australia. *International Journal of Plant Sciences* 162: 411–430.

CHAPPLE, C. C. S., T. VOGT, B. E. ELLIS, AND C. R. SOMERVILLE. 1992. An *Arabidopsis* mutant defective in the general phenylpropanoid pathway. *Plant Cell* 4: 1413–1424.

CHRISTODOULAKIS, N. S., P.-N. LAMPRI, AND C. FASSEAS. 2009. Structural and cytochemical investigation of the leaf of silverleaf nightshade (*Solanum elaeagnifolium*), a drought-resistant alien weed of the Greek flora. *Australian Journal of Botany* 57: 432–438.

COSA, M. T., G. BRUNO, AND N. DOTTORI. 1998. Anatomía de los órganos vegetativos en *Solanum juvenale* y su comparación con *S. elaeagnifolium* (Solanaceae). *Anales del Instituto de Biología, Serie Botánica* 69: 9–22.

CURRIER, H. B., E. R. PICKERING, AND C. L. FOY. 1964. Relation of stomatal penetration to herbicidal effects using fluorescent dye as a tracer. *Weeds* 12: 301–303.

DOTTORI, N., G. BRUNO, AND M. T. COSA. 2000. Anatomía comparada de las plántulas de *Solanum elaeagnifolium* y *Solanum juvenale* (Solanaceae). *Boletín de la Sociedad Argentina de Botánica* 35: 115–123.

EICHERT, T., AND J. BURKHARDT. 2001. Quantification of stomatal uptake of ionic solutes using a new model system. *Journal of Experimental Botany* 52: 771–781.

EL NAGGAR, S. M., AND S. I. I. ABDEL HAFEZ. 2003. Leaf surface, pollen morphology, fungal biodiversity and leaf spots of some wild plants in Sinai, Egypt. *Feddes Repertorium* 114: 74–90.

ENSBEY, R. 2011. Noxious and environmental weed control handbook, 5th ed. New South Wales Department of Primary Industries, Orange, NSW, Australia.

FAHN, A. 1986. Structural and functional properties of trichomes of xeromorphic leaves. *Annals of Botany* 57: 631–637.

FERNÁNDEZ, V., AND P. H. BROWN. 2013. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science* 4: art. 289, 1–5.

GREEN, J. D., D. S. MURRAY, AND L. M. VERHALEN. 1987. Full-season interference of silverleaf nightshade (*Solanum elaeagnifolium*) with cotton (*Gossypium hirsutum*). *Weed Science* 35: 813–818.

GRIGNON, N., B. TOURAINE, AND M. DURAND. 1989. 6(5)Carboxyfluorescein as a tracer of phloem sap translocation. *American Journal of Botany* 76: 871–877.

GUÉDÈS, M. 1975. Intrusive hair sclereids in *Jovetia* (Rubiaceae). *Botanical Journal of the Linnean Society* 71: 141–144.

GUTSCHICK, V. P. 1999. Biotic and abiotic consequences of differences in leaf structure. *New Phytologist* 143: 3–18.

HARRINGTON, D. F., AND C. CLARK. 1989. Reduction of light reflectance of leaves of *Encelia densifolia* (Asteraceae) by trichome wetting. *Madroño* 36: 180–186.

HAWORTH, M., AND J. MCELWAIN. 2008. Hot, dry, wet, cold or toxic? Revisiting the ecological significance of leaf and cuticular micromorphology. *Palaeogeography, Palaeoclimatology, Palaeoecology* 262: 79–90.

HEIDE-JØRGENSEN, H. S. 1990. Xeromorphic leaves of *Hakea suaveolens* R. Br. IV. Ontogeny, structure and function of the sclereids. *Australian Journal of Botany* 38: 25–43.

HESS, F. D., AND R. H. FALK. 1990. Herbicide deposition on leaf surfaces. *Weed Science* 38: 280–288.

HETHERINGTON, A. M., AND F. I. WOODWARD. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424: 901–908.

HODGSON, J. G., M. SHARAFI, A. JALILI, S. DÍAZ, G. MONTSERRAT-MARTÍ, C. PALMER, B. CERABOLINI, ET AL. 2010. Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? *Annals of Botany* 105: 573–584.

HUANGFU, C. H., X. L. SONG, AND S. QIANG. 2009. Morphological disparities in the epidermal and anatomical features of the leaf among wild *Brassica juncea* populations. *Weed Biology and Management* 9: 234–242.

HULL, H. H. 1970. Leaf structure as related to absorption of pesticides and other compounds. *Residue Reviews* 31: 1–150.

JENSEN, W. A. 1962. Botanical histochemistry: Principles and practices. W. H. Freeman, San Francisco, California, USA.

JORDAN, G. J., R. A. DILLON, AND P. H. WESTON. 2005. Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. *American Journal of Botany* 92: 789–796.

JORDAN, G. J., P. H. WESTON, R. J. CARPENTER, R. A. DILLON, AND T. J. BRODRIBB. 2008. The evolutionary relations of sunken, covered, and encrypted stomata to dry habitats in Proteaceae. *American Journal of Botany* 95: 521–530.

KANNAN, S. 2010. Foliar fertilization for sustainable crop production. *In* E. Lichtfouse [ed.], Genetic engineering, biofertilisation, soil quality and organic farming, 371–402. Springer, Dordrecht, Netherlands.

KENZO, T., R. YONEDA, M. A. AZANI, AND N. M. MAJID. 2008. Changes in leaf water use after removal of leaf lower surface hairs on *Mallotus macrostachyus* (Euphorbiaceae) in a tropical secondary forest in Malaysia. *Journal of Forest Research* 13: 137–142.

LEVIN, D. A. 1973. The role of trichomes in plant defense. *Quarterly Review of Biology* 48: 3–15.

LEVIN, R. A., N. R. MYERS, AND L. BOHS. 2006. Phylogenetic relationships among the "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae). *American Journal of Botany* 93: 157–169.

LORANGER, J., AND B. SHIPLEY. 2010. Interspecific covariation between stomatal density and other functional leaf traits in a local flora. *Botany* 88: 30–38.

LUX, A., S. MORITA, J. ABE, AND K. ITO. 2005. An improved method for clearing and staining free-hand sections and whole-mount samples. *Annals of Botany* 96: 989–996.

MAITI, R. K., L. R. VILLARREAL, A. V. TREVIÑO, AND M. C. VALADES-CERDA. 2002. Some aspects on pharmacognosy of ten species of the family Solanaceae utilized in traditional medicine. *Caldasia* 24: 317–321.

MARTIN, C. E. 1994. Physiological ecology of the Bromeliaceae. *Botanical Review* 60: 1–82.

MCCLEERY, E. M. 1907. Stellate hairs and peltate scales of Ohio plants. *Ohio Naturalist* 7: 51–56.

MEAT & LIVESTOCK AUSTRALIA. 2009. 3D Weed management: Silverleaf nightshade [online]. Meat & Livestock Australia, North Sydney, Australia. Available at http://www.wool.com/Content/en-GB/3D_Weed_Guidelines_SilverleafNightshadeJune2009_lowresFINAL1.pdf.

MEKKI, M. 2007. Biology, distribution and impacts of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). *EPPO Bulletin* 37: 114–118.

MILANEZ, C. R. D., AND S. R. MACHADO. 2008. Leaf emergences in *Microlepis oleaefolia* (DC.) Triana (Melastomataceae) and their probable function: An anatomical and ultrastructural study. *Micron* 39: 884–890.

OPARKA, K. J. 1991. Uptake and compartmentation of fluorescent probes by plant cells. *Journal of Experimental Botany* 42: 565–579.

PEAT, H. J., AND A. H. FITTER. 1994. A comparative study of the distribution and density of stomata in the British flora. *Biological Journal of the Linnean Society* 52: 377–393.

PÉREZ-ESTRADA, L. B., Z. CANO-SANTANA, AND K. OYAMA. 2000. Variation in leaf trichomes of *Wigandia urens*: environmental factors and physiological consequences. *Tree Physiology* 20: 629–632.

PILAR, F. M. R. C. 1937. Contribución al estudio del *Solanum elaeagnifolium* Cav. (meloncillo del campo). *Anales de Farmacia y Bioquímica* 8: 23–44.

RICOTTA, J. A., AND J. B. MASIUNAS. 1992. Relationship of leaf surface characteristics to acifluorfen tolerance in tomato (*Lycopersicon esculentum*) and related species. *Weed Science* 40: 402–407.

ROE, K. E. 1971. Terminology of hairs in the genus *Solanum*. *Taxon* 20: 501–508.

SANDERS, K. D., B. E. DAHL, AND G. SCOTT. 1980. Bite-count vs fecal analysis for range animal diets. *Journal of Range Management* 33: 146–149.

SKELTON, R. P., J. J. MIDGLEY, J. M. NYAGA, S. D. JOHNSON, AND M. D. CRAMER. 2012. Is leaf pubescence of Cape Proteaceae a xeromorphic or radiation-protective trait? *Australian Journal of Botany* 60: 104–113.

SOMERVAILLE, A., G. BETTS, B. GORDON, V. GREEN, M. BURGIS, AND R. HENDERSON. 2012. Adjuvants—Oils, surfactants and other additives for farm chemicals, revised 2012 edition. Grains Research & Development Corp., Canberra, ACT, Australia.

STANTON, R. A., J. W. HEAP, R. J. CARTER, AND H. WU. 2009. *Solanum elaeagnifolium* Cav. *In* F. D. Panetta [ed.], The biology of Australian weeds, vol. 3, 274–293. RG & FJ Richardson, Melbourne, Australia.

TAY, A. C., AND A. FURUKAWA. 2008. Variations in leaf stomatal density and distribution of 53 vine species in Japan. *Plant Species Biology* 23: 2–8.

WANAMARTA, G., AND D. PENNER. 1989. Foliar absorption of herbicides. *Reviews of Weed Science* 4: 215–231.

WANG, C. J., AND Z. Q. LIU. 2007. Foliar uptake of pesticides-Present status and future challenge. *Pesticide Biochemistry and Physiology* 87: 1–8.

WANG, N., AND D. B. FISHER. 1994. The use of fluorescent tracers to characterize the post-phloem transport pathway in maternal tissues of developing wheat grains. *Plant Physiology* 104: 17–27.

WERKER, E. 2000. Trichome diversity and development. *Advances in Botanical Research* 31: 1–35.

XU, L., H. ZHU, H. E. OZKAN, W. E. BAGLEY, R. C. DERKSEN, AND C. R. KRAUSE. 2010. Adjuvant effects on evaporation time and wetted area of droplets on waxy leaves. *Transactions of the ASABE* 53: 13–20.

XU, L., H. ZHU, H. E. OZKAN, W. E. BAGLEY, AND C. R. KRAUSE. 2011. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. *Pest Management Science* 67: 842–851.

YATES, D. J. 1992. Short-term changes in spectral properties of phyllodes of brigalow (*Acacia harpophylla* F. Muell. ex Benth.) in response to wetting. *Australian Journal of Botany* 40: 27–35.

ZABKIEWICZ, J. A. 2000. Adjuvants and herbicidal efficacy—present status and future prospects. *Weed Research* 40: 139–149.

ZARINKAMAR, F. 2007. Stomatal observations in dicotyledons. *Pakistan Journal of Biological Sciences* 10: 199–219.