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Title:
The effects of rooting volume, greywater irrigation and reduced sunlight on climbing plants for indirect green façades in urban environments

Date:
2020

The effects of rooting volume, greywater irrigation and reduced sunlight on climbing plants for indirect green façades in urban environments

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Doctor of Philosophy

January 2021

Faculty of Science

School of Ecosystem and Forest Sciences

Submitted in total fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Melbourne

Abstract

Dense urban areas with limited ground space are often deprived of vegetation. By growing climbing plants on buildings as indirect green façades, vegetation can be implemented in a way that uses limited space and seamlessly integrates man-made structures with nature. Benefits of indirect green façades include air purification, noise reduction, micro-climate regulation, provision of natural habitats and improvement of the physical and psychological well-being of urban residents. However, in order to provide effective ecosystem services, indirect green façades need to develop great vegetation coverage to cover buildings, which can be challenging due to small rooting volumes, lack of potable water availability for irrigation and variable light conditions, imposed by site constraints and weight loading of containers on elevated structures. Therefore, it is important to research how indirect green façades can be successfully developed for cities in the presence of unfavorable growing conditions to ensure their sustainability. In this thesis, I have investigated how woody climbing plants respond to different growing conditions by evaluating their morphological and physiological traits. The three objectives of this thesis are:

1. To evaluate the effect of rooting volume on climbing plant growth, coverage and thermal tolerance in the first growing season (Ch 2);
2. To investigate whether greywater is a viable irrigation resource for indirect green façades in cities by assessing changes in substrate chemical properties and the growth response of six climbing plant species (Ch 3);
3. To explore how shade affects leaf traits and thermal tolerance of climbing plant species assisting green façade design and plant selection (Ch 4).

In chapter two, to examine the impacts of rooting volume on climbing plant species during the first growing season for their establishments, three rooting volumes (21, 42 and 63 L) were utilised to grow two climbing plant species; the slower-growing *Akebia quinata* and the faster-growing *Pandorea pandorana*, on east- and west-facing aspects. It was hypothesised that the reduced rooting volume will adversely affect growth, wall coverage and thermal tolerance of climbing plant species. The smallest rooting volume (21 L) significantly reduced plant biomass growth, specific leaf area and percentage wall coverage for both species over the measured six-month period. Surprisingly, neither climbing plant species significantly increased in growth and vegetation coverage when provided with the largest rooting volume (63 L) as compared with medium rooting volume (42 L). The shoot:root ratio and thermal tolerance of climbing plant species however remained unchanged across three different rooting volumes. A significant decline in the ratio of variable to maximum fluorescence (Fv/Fm) was observed for all climbing plants on the west-facing aspect due to heat stress, irrespective of rooting volume treatment.

The outcomes of chapter two suggests that, for a wall area of 2.8 m^2 with stainless-steel mesh (1.45 m (W) by 1.95 m (H)), 42 L rooting volume is adequate to support plant growth and coverage of indirect green façades for the first growing season. Practically, for every cubic metre of rooting volume, *A. quinata* and *P. pandorana* could approximately achieve 10.2 m^2 and 21.3 m^2 of wall coverage at the end of the first growing season.

In chapter three, an 18-week glasshouse experiment was conducted to evaluate the effects of greywater irrigation on substrate chemical properties (pH and electrical conductivity) and the growth of six climbing plant species. I hypothesised that domestic greywater would change substrate properties by increasing pH and electrical conductivity, and thereby adversely affect the growth and health of climbing plant species. Synthetic greywater formulated to conform with domestic greywater was used in this study. Three irrigation treatments were applied to container-grown climbing plants: (1) potable water irrigation, (2) greywater irrigation, and (3) greywater irrigation with a potable water ‘flushing’ once every three weeks. After 18 weeks, the results showed that substrate pH did not differ among treatments; however, increased substrate electrical conductivity was evident in containers receiving the greywater with potable water flushing treatment.

Both greywater treatments had no significantly detrimental effects on plant biomass, leaf area, gas exchange rates, or water use when compared with potable water irrigation. The outcomes indicated that domestic greywater can be safely used to irrigate climbing plants for indirect green façades, reducing the need for the use of potable water and supporting more sustainable design of indirect green façades in urban environments.

In chapter four, a 15-week field experiment was designed to assess how seven climbing plant species respond to changing light conditions from full-sun to shade, with respect to the adjustment in leaf morphology, physiology, and photochemistry for mature plants. The hypotheses were (1) a change from sun to shade will increase specific leaf area (SLA), leaf expansion rate, and chlorophyll content of climbing plants; whilst decreasing leaf photosynthetic rate (A_{1500}), stomatal conductance (g_s), light compensation point (LCP) and thermal tolerance (T_{50}), and (2) deciduous climbing plant species will be more plastic (greater changes in morphology and physiology) in response to shade than evergreen species.

Significant increases in SLA, and unchanged g_s, LCPs and T_{50} were observed in all seven climbing species after being provided shady conditions. In contrast, the variation in species response to reduced light intensity levels was evident in leaf expansion rates, A_{1500} and Chl *a*+*b*. Leaf expansion rate and A_{1500} remained unchanged for most of the climbing plant species by the shade treatment. However, significant increases in leaf expansion rate were observed in evergreen *Gelsemium sempervirens* and *Jasminum azoricum*. In addition, significant decreases in A_{1500} were recorded in deciduous *Ampelopsis brevipedunculata* and *Vitis* 'Ganzin Glory' as well as evergreen *Pandorea pandorana* under the shade treatment. On the other hand, leaf Chl *a*+*b* was significantly increased in most of the climbing plant species by the shade treatment, except for deciduous *V.* 'Ganzin Glory' and evergreen *G. sempervirens*.

Irrespective of light intensity levels, deciduous climbing plant species displayed significantly greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl *a*+*b*, whilst lower LCP than evergreen species in this study. On the contrary, deciduous climbing plant species were not more plastic than evergreen species in the overall response to the reduced light intensity levels. However, a greater reduction in A_{1500} in response to shade was observed in deciduous climbing species as compared with evergreen species.

The findings of chapter four demonstrated that these seven climbing plant species can maintain growth and health by adjusting leaf morphology and physiology when experienced changing light conditions from full-sun to shade. The findings suggested that the seven climbing plant species can maintain or increase vegetation wall coverage and provide ecosystem service benefits in cities with variable light conditions.

Overall, this thesis has indicated that climbing plants can grow successfully with relatively small substrate volume (42 L). It was also shown that a lower limit exists for the rooting volume required to support the early growth and vegetation wall coverage of indirect green façade climbing plants. This thesis also raises the possibility that domestic greywater can replace potable water irrigation of building indirect green façades in urban environments, as there was no deleterious effect on either substrate properties or climbing plant growth. Furthermore, the seven climbing species evaluated in this thesis all could adapt to the changing light conditions from full-sun to shade by adjusting leaf traits to maintain growth (leaf expansion). Despite the limitation noted in rooting volume, woody climbing plant species were quite resilient and adaptable to the different growing conditions evaluated in this thesis. This would likely enable them to maintain their function and performance and thereby continue to deliver ecosystem services under fast-changing urban environments. The findings of this thesis have significant implications for the understanding of how woody climbing plants respond to potentially unfavorable growing conditions, providing insights into plant selection for indirect green façades in urban environments. This thesis further supports the possibility to implement indirect green façades as an integrated approach of sustainable urban greening and water management on high-rise buildings. Further research should be undertaken however with a longer experimental period (beyond six months), the inclusion of more climbing plant species and field studies (i.e., existing indirect green façades on buildings). The research should also be conducted with the inclusion of different types of substrates with the application of on-site greywater and various light intensity levels (e.g., lower or higher).

Declaration

This is to certify that:

1) This thesis comprises only my original work.
2) Due acknowledgement has been made in the text to all other material used.
3) The thesis has been completed during the enrolment period of a PhD degree at The University of Melbourne and has not been used previously for a degree or diploma at any other institution.

Pei-Wen Chung
January 2021

Preface

This PhD thesis consists of five chapters, one of which has been submitted for publication in an international peer-reviewed journal. Citation for the manuscript submitted is:

Chapter 3

CHUNG, P.-W., LIVESLEY S.J., RAYNER J.P. & FARRELL C., Greywater irrigation can support climbing plant growth on building green façades. *Urban Landscape and Urban Greening*. (In revision following peer review by Urban Forestry & Urban Greening)

Acknowledgements

It takes a village to finish a PhD…

Huge thanks to my wonderful supervisors, Dr. Claire Farrell, Assoc. Prof. Stephen Livesley and Assoc. Prof. John Rayner. Their invaluable support, patience, encouragement and positivity through my PhD, particularly during the Coronavirus lockdown period, guide me through to completion of this thesis.

I am grateful to the support of the Melbourne Research Scholarship for my PhD study. I would also like to thank the Frank Keenan Scholarship and Betty Elliot Horticulture Scholarship for the research grants in supporting the nursery and lab work.

Special thanks to Burnley nursery staff Nick Osborne, Sascha Andrusiak and Rowan Berry for the technical assistance. I would also like to thank and acknowledge Lisa Wittick and Chris Szota for assistance with the use of measuring devices.

For all assistance, support and company from fellow students and staffs at the Burnley Campus of the University of Melbourne during these years are much appreciated. I'm particularly appreciative of the hours dedicated and assistance from Peter Somerville, Pengzhen Du, Dean Schrieke, Bihan Guo, as well as amazing interns Audrey Michenaud-Rague and Léa Wirz for all field work and plant harvesting. MB31 is another home of mine in Melbourne, warmest food/snacks and companies at Burnley.

To my family; the consistent support and encouragement from my parents is indescribable. My partner Asif has been incredibly supportive and patient during this journey.

This thesis is dedicated to all the people involved in my PhD. Cheers!

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1- Introduction

Urbanisation and vertical greening

Rapidly rising global urban population has resulted in the expansion and/or densification of urbanised areas, leading to negative impacts, including; raised ambient air temperatures, increased water and energy consumption, increased wastewater discharge, escalated flood risks, and growing heat-related illness and mortality (Oleson et al., 2015; Uttara et al., 2012). To help tackle these negative impacts and to promote sustainable urban development, cities are increasingly adopting urban greening strategies and technologies (Ahern, 2013). In dense cities where ground-level space is limited, one solution to urban greening is growing plants on the vertical surfaces of buildings (James et al., 2009; Russo and Cirella, 2018).

Vertical greening can be categorized into two broad types - living wall systems or façade greening, based on their unique growing methods (Fernández-Cañero et al., 2018; Medl et al., 2017). In general, living walls are modular or fabric-based systems that comprise growing media, vegetation and irrigation (hydroponics) fixed to a wall surface (Manso and Castro-Gomes, 2015) (Fig. 1.1A). In contrast, green façades use climbing plants, such as lianas, vines and scramblers, to cover building walls, offering a flexible and adaptable tool to integrate greening in the cities (Besir and Cuce, 2018; Pérez-Urrestarazu et al., 2015). Green façades are considered more cost-effective and sustainable than living wall systems, because of their simpler design, longevity and lower costs in recurrent maintenance, particularly in fertiliser, irrigation and vegetation maintenance (Chew et al., 2019; Ottelé et al., 2011; Perini and Rosasco, 2013). Green façades can also be further categorised based on the type of climbing plant species in use, particularly traits such as evergreen or deciduous; plant attachment mechanisms; and whether they are grown in ground or in containers (Fernández-Cañero et al., 2018; Manso and Castro-Gomes, 2015). Direct façade greening, the use of self-clinging climbing plants on a building surface, has been prevalent in European towns and cities for centuries (Fig. 1.1B). It has been suggested that self-clinging climbing plants, such as *Hedera helix* and *Parthenocissus tricuspidata*, may adversely affect wall surfaces (Köhler, 2008; Rakhshandehroo et al., 2015), while research also indicated that the vegetation coverage of *Hedera helix* can protect wall surfaces from harsh weather conditions (Sternberg et al., 2011). Climbing plants grown on a building surface and supported by trellis, mesh or steel cables are generally referred to as indirect façades (Fernández-Cañero et al., 2018; Manso and Castro-Gomes, 2015). Indirect green façades tend to be favoured ahead of direct green façades in dense urban areas; partially due to concerns of surface damage and perceptions around maintenance costs, but also because they can be readily fitted into building management systems post construction (Manso and Castro-Gomes, 2015; Perini and Rosasco, 2013; Thottathi et al., 2010). For all façade systems, plant species selection, water availability and the rooting volume are all critical to establish effective façade growth, coverage and ensure they achieve their potential benefits (Bustami et al., 2018; Dunnett and Kingsbury, 2008).

While indirect façades can make use of various materials to support climbing plants such as wood, plastic and even aluminium (Ascione et al., 2020; Manso and Castro-Gomes, 2015), most are based on steel support structures, cables and trellis systems. Climbing plants used in façade greening may be grown in soil at ground level (Fig. 1.1C), or in containers filled with a growing medium (Fig. 1.1D; Dunnett and Kingsbury, 2008; Fernández-Cañero et al., 2018). These containers or planter boxes are often mounted at varying heights across the building elevation, to help facilitate vegetation coverage (Dunnett and Kingsbury, 2008).

Figure 1.1. Examples of different types of vertical greening; (A) Living wall systems on Medibank building, Melbourne, Australia (http://fytogreen.com.au/medibank-docklands-fytowall/), (B) direct green façades at Burnley Campus, University of Melbourne, Australia, (C) indirect green façades in Zurich, Switzerland (https://www.greenroofs.com/2018/05/20/greenroofs-com-project-of-the-week-for-may-21-2018-sihlcity-shopping-centre-living-facade/), (D) indirect green façades with planter boxes at One Central Park, Sydney, Australia (https://www.tensile.com.au/project/one-central-park/).

Benefits of green façades

Green façades can provide a range of ecosystem services in urban environments including environmental, social, and economic benefits (Başdoğan and Çığ, 2016; Ghazalli et al., 2019; Rakhshandehroo et al., 2015; Sheweka and Magdy, 2011). Most research on green façades has focused on the cooling and insulating effects of façade plants (Hoyano, 1988; Peng et al., 2020; Safikhani et al., 2014; Stec et al., 2005). However, green façades have also been studied for their ability to mitigate air pollution (Ottelé et al., 2010; Pandey et al., 2016), create habitat for urban wildlife (Lundholm, 2006), provide aesthetic benefits (Başdoğan and Çığ, 2016), and mitigate noise (Pérez et al., 2016; Wong et al., 2010). In terms of mitigating air pollution, green façades can sequester carbon dioxide (CO_2) (Parhizkar et al., 2020; Tudiwer and Korjenic,

2017) and filter out airborne particles to improve air quality (Köhler, 2008; Sheweka and Magdy, 2011). Boston Ivy (*Parthenocissus tricuspidata*) and English Ivy (*Hedera helix*) have been shown to not only trap airborne particles efficiently but also are well adapted to polluted inner cities (Köhler, 2008; Ottelé et al., 2010). Façade greening can also conserve and support nature in urban environments (Rakhshandehroo et al., 2015; Sheweka and Magdy, 2011). In terms of biodiversity enrichment, façade plants can provide habitat and feeding places for birds, invertebrates and even small mammals (Mayrand et al., 2018). For instance, Climbing Hydrangea (*Hydrangea petiolaris*) and Morning Glory (*Ipomoea purpurea*) can attract butterflies and hummingbirds in urban environments (Elgizawy, 2016).

While many diverse environmental benefits can potentially be achieved with green façades, one of the biggest drivers for their implementation is the cooling benefits they can provide to cities and buildings (Bustami et al., 2018; Hunter et al., 2014). Vegetation can potentially alleviate the urban heat island effect directly through covering heat-absorbing surfaces and reducing solar gain, but also through evapotranspiration which provides latent heat cooling benefits for the plant and the air surrounding it (Hoelscher et al., 2016; Koyama et al., 2013; Vox et al., 2018). The urban heat island effect can also be mitigated by reducing fugitive heat emissions associated with the use of air conditioning because of the insulation benefit provided by green façades (Cameron et al., 2014). In addition, Hoelscher et al. (2016) demonstrated that thermal insulation of the façade greening is effective to decrease the wall surface temperature of buildings by reducing the solar radiation on hot summer days. On the other hand, in winter, evergreen climbing species can offer a degree of insulation by trapping a layer of air against the façade and reducing convectional heat loss by the dense plant foliage, thus prevent building from being cooled down (Bolton et al., 2014; Cameron et al., 2015; Tudiwer and Korjenic, 2017).

In respect to social benefits, green façades can enhance the aesthetic value of the building and urban landscape (Rakhshandehroo et al., 2015). Plants on building surfaces can create livelier and more visually pleasant green spaces to revive urban environments (Sheweka and Magdy, 2011), improving the physical and psychological well-being of urban residents by aiding in the reduction of stress, fear, anger and blood pressure (Elsadek et al., 2019; Farid et al., 2016; Qin et al., 2013). Recovery of patients in hospitals has also showed that patients with rooms facing greenery made quicker recoveries and suffered less pain from their illness compared to patients in rooms facing a building wall (Sadeghian, 2016; Sheweka and Magdy, 2011).

Furthermore, a number of economic benefits can be delivered by green façades. Green façades regulate microclimates of building and their surroundings, reduce energy demand and improving building energy efficiency (Cameron et al., 2015; Fahmy et al., 2020; Pérez et al., 2011). In addition, the insulating effect of indirect green façades has shown not only to significantly decrease heating demand in winter (Tudiwer and Korjenic, 2017), but also increase wall durability by reducing damage caused by excessive UV light, wind, as well as frost and salt deterioration (Sternberg et al., 2011). Accordingly, green façades may increase building market values due to their aesthetic and functional properties (Dunnett and Kingsbury, 2008).

Overall, all the potential benefits of green façades to a large extent depend on their overall design and plant growth outcomes. This is particularly true for containerised indirect green façades that are critically dependent on sufficient rooting volume, irrigation and sunlight to sustain plant growth successfully and enable good vegetation coverage.

Climbing plant species for green façades

Selecting appropriate vegetation for green façade systems is key for successful system performance as plants need to adapt to and thrive under the on-site conditions of exposure (e.g., sun, semi-shade or shade) and weather conditions (e.g., wind, rainfall, heat, drought and frost) (Dunnett and Kingsbury, 2008; Growing Green Guide, 2014). Green façades typically involve herbaceous or woody climbers (lianas) and vines (Köhler, 2008). Historically, woody climbing plants and vines have been more frequently used than herbaceous climbing species, as woody climbing plants can quickly cover walls with little maintenance (Köhler, 2008). Therefore**,** the most commonly used species for green façade research include; *Hedera helix* and *Parthenocissus tricuspidata* for direct green façades; *Parthenocissus quinquefolia*, and *Wisteria sinensis* for indirect green façades, in tropical, subtropical and Mediterranean climates (Hunter et al., 2014). These studies have given insights into the cooling effect of green façades and the contribution of façade climbing plants to building energy savings (Cameron et al., 2014; Hunter et al., 2014).

Façade greening can provide cooling through shading, evapotranspiration cooling, and thermal insulation (Cameron et al., 2014). Therefore, their cooling potential is influenced by plant species, plant vigour, canopy cover, resource availability (moisture, nutrient and sunlight) and also seasonality for deciduous species (Cameron et al., 2014; Koyama et al., 2013). Shading of wall surfaces is generally considered the most important aspect of how green façades provide cooling, with studies suggesting that plants with greater canopy cover, foliage thickness and overlapping leaf layers provide the most effective cooling (Pérez et al., 2011; Tzachanis, 2011; Wong et al., 2010; Li et al., 2019). In addition, Koyama et al. (2013) suggested that percentage coverage is the most critical trait contributing to cooling through intercepting solar radiation due to the strong positive relationships between percentage coverage and wall surface temperature reduction. Evapotranspiration cooling is a beneficial outcome from photosynthesis (Koyama et al., 2013; Wong et al., 2010). During photosynthesis, leaves absorb incoming solar radiance and convert it into latent energy, thus dissipating solar radiation and reducing air temperatures (Allakhverdiev, 2020). In addition, evaporation of water from leaves during transpiration also contributes to cooling (Hoelscher et al., 2016). Therefore, the contribution of evapotransporation to cooling by green façades will vary depending on plant species, local microclimate conditions including wind speed, temperature and humidity, and soil water availability (Koch et al., 2020; Medl et al., 2017).

Most research on the cooling potential of green façades has focused on the reduction of surface wall temperatures and their impact on improving building thermal performance in tropical or mediterranean climates (Hunter et al. 2014). For instance, early research in Fukuoka city in Japan, investigated how *Wisteria sinensis* reduced wall surface temperature and incoming solar radiation (Hoyano, 1988). In tropical Singapore, the maximum differences in surface wall temperatures between a bare wall and a wall covered by green façades were shown to be 11.6°C (Wong et al., 2010). In Mediterranean area, studies showed that surface temperatures of the shaded external wall were significantly reduced when using *Parthenocissus quinquefolia* and *Wisteria sinensis* as façade plants (Pérez, et al., 2011; Tzachanis, 2011). These results have indicated that façade greening can be an effective passive cooling system for thermal control of buildings. On the other hand, it may also be desirable to select species which enable heat gain for parts of the year (Pérez et al., 2017). In climates with cool winters, green façades can provide thermal resistance through trapping solar radiation and delaying heat loss in the evening, which can reduce requirements for heating buildings (Köhler, 2008; Tudiwer and Korjenic, 2017). In the case of Mediterranean-type climates, which have hot and dry summers and mild and rainy winters, deciduous climbing plants such as *Parthenocissus tricuspidata* and

Wisteria sinensis, may be more preferable than evergreen species as. they can provide shade during summer, and allow solar gain in winter (Cameron et al., 2014; Köhler, 2008).

While research into the cooling benefits of green façades has been extensive (Cameron et al., 2014; Hoelscher et al., 2016; Koch et al., 2020; Medl et al., 2017; Wong et al., 2010), there has been very little research done on the horticultural aspects of green façade plants. Given that many of the benefits for cooling rely on good plant cover and appropriate plants selection (Besir and Cuce, 2018; Hoelscher et al., 2016), it is surprising that studies have not evaluated how different plant species respond to different growing conditions such as rooting volume constraints, changing light availability and non-potable irrigation sources. Whilst there is little research in urban environments on green façades, a considerable body of research exists in forests investigating plant traits of woody climbing plants (DeWalt et al., 2006; Gagliano, 2015; Gallagher and Leishman, 2012), which might be useful to inform green façade plant selection.

In their natural habitats, woody climbers or lianas are abundant in tropical forests with seasonal droughts and low annual rainfall, but become scarce in areas with increasing precipitation (Parthasarathy et al., 2004; Schnitzer, 2005). Explanations for distribution of woody climbing species may be due to their unique ecological and physiological adaptations (Schnitzer, 2005). Lianas have deep and wide root systems and efficient vascular transport which can access deeply stored water and nutrients that most plants in tropical forests are unable to access during dry seasons (Andrade et al., 2005; Schnitzer, 2005). With this growth advantage, woody climbers may ensure a constant supply of moisture and nutrients and thus having relatively fast growth compared to trees and shrubs in seasonal forests (Andrade et al., 2005; Schnitzer, 2005). In addition, liana's leaves usually have higher specific leaf area (SLA), higher photosynthetic rates, higher photosynthetic nitrogen and phosphorus use efficiencies, lower leaf construction costs, and shorter leaf life span compared to co-occurring trees, giving lianas a competitive advantage (Cai et al., 2009).

Soils and substrates for green façades

As noted previously climbing plants in green façade systems may be grown at ground level, in existing soil, or in containers or planter boxes filled with a growing substrate (Manso and Castro-Gomes, 2015). For climbing plants growing in the ground, fertile soils are generally preferable (Dunnett and Kingsbury, 2008), as this reflects the fact that most woody climbing species are distributed in natural soils with high soil fertility in forested ecosystems (DeWalt et al., 2006). For soils, high fertility is generally associated with greater amounts of organic matter which will need to be replenished over time, due to decomposition (Haege and Leake, 2014). This is not generally a problem with plantings at ground level, as these can be easily accessed for maintenance (Haege and Leake, 2014). However, for climbing plants growing in containers, especially at height, it is important that substrates are specifically designed to ensure long-term plant growth and performance (Dunnett and Kingsbury, 2008). These situations can also be much harsher than at ground level, as rooting volumes are often limited due to building weight loading constraints and plants experience greater exposure to wind, heat and sunlight at elevation on tall buildings (Manso and Castro-Gomes, 2015). However, very little is currently known about the appropriate design of containerised green façade substrates. It has been suggested that substrate mixes for containerised green façades can be designed based on the principles of green roof substrates (Growing Green Guide, 2014). For green roofs, substrates with a high water holding capacity is preferable as they can retain more water after irrigation events. However, this needs to be balanced with high air-filled porosity to ensure sufficient oxygen availability for plant roots in containers (Growing Green Guide, 2014; Haege and Leake, 2014). Although substrate fertility and water holding capacity can be enhanced by adding organic matter (e.g., coconut fibers) (Fernández-Cañero et al., 2018), high rates of organic matter may result in decomposition and slumping over time, which in turn alters substrate properties and reduces longevity (Bilderback et al., 2005). Thus, substrates for containerised green façades are often designed as mineral substrate mixes, with low amounts of organic matter to ensure their porosity, water holding capacity and bulk density are maintained over time and not altered through decomposition of organic matter (Growing Green Guide, 2014; Xue and Farrell, 2020). This ensures that their porosity, water holding capacity and bulk density are maintained over time and not altered through decomposition of organic matter (Xue and Farrell, 2020). Furthermore, sufficient rooting volume is essential to sustain plant growth, by ensuring there is sufficient space for root growth and adequate supply of moisture and nutrients to maintain effective façade coverage (Dunnett and Kingsbury, 2008; Growing Green Guide, 2014; Haege and Leake, 2014).

Despite these general recommendations, reductions in container volumes and the use of lightweight growing medium are often used in elevated planters on high-rise buildings, to accommodate weight-loading limitations and enable easier installation at different building levels (Haege and Leake, 2014). For example, the green façade planters used at One Central Park in Sydney, used a lightweight growing medium in multiple planters at each level to support façade plant growth and coverage (Haege and Leake, 2014). However, the use of reduced substrate volumes may have significant drawbacks (Poorter et al., 2012). Smaller container volumes directly reduce the available rooting space and as such decrease plant resource availability (water and nutrients), and thereby potentially reduce plant growth rate and size (NeSmith and Duval, 1998; Poorter et al., 2012). Reduced plant growth and development has been observed in woody plants under restricted rooting volume treatments, including trees (Hsu et al., 1996; Ran et al., 1992; Rieger and Marra, 1994), shrubs (Dubik et al., 1992), and vines (Wang et al., 2001). For instance, Wang et al. (2001) studied the effect of reducing the root zone volume and depth of field-grown grapevines (Kyoho grapevine), and showed that plants growing in root restricted spaces had smaller stem diameters, shorter shoots and smaller total leaf areas than those growing in normal field soil conditions, despite sufficient irrigation and fertiliser application. This has also been shown for other woody plant species, where confined rooting volume has led to significant reductions in total biomass, leaf area and stem length (Hsu et al., 1996; Ran et al., 1992). Therefore, as wall coverage and growth are important aspects for determining the cooling benefits of green façades, there is a need to determine how limitations in container size and volume influence green façade plant growth and coverage. Further, it is also important to determine whether reduced volume effects on climbing plant growth are due to space constraints alone, or whether these can be overcome with adequate supply of nutrients and water. Therefore, there is a need to determine how limitations in container size and volume influence green façade plant growth and coverage, particularly over time and whether these can be overcome with adequate supply of nutrients and water.

Water resources for irrigation

For containerised indirect green façades, adequate irrigation is required to prevent the substrate from drying out and to maintain climbing plant performance and canopy coverage, particularly during hot summer months. However, potable water in urban areas is becoming an increasingly precious and valued resource, not only in arid and drought prone cities, but also in abundant precipitation regions due to climate change, rapid population growth and industrial development (Seckler et al., 1999; Vörösmarty et al., 2000). To ensure sustainable water supply, the balance between potable water consumption and disposal of wastewater has become a significant challenge worldwide (Stikker, 1998). Therefore, rainwater harvesting and sewerage wastewater recycling for non-potable use have been developed in many countries, as a means to promote savings of potable water for more essential uses (Furumai, 2008). While rainwater can be harvested from roof surfaces on-site, and stored for irrigation, this is not always possible; considering the variable climate, high cost of construction for collecting water (e.g. constructed reservoirs and dams) and maintenance (Campisano et al., 2017; Paton et al., 2014). In Australia, below-average precipitation has been recorded in southeast Australia in the past two decades due to global warming (Murphy and Timbal, 2008; Van Dijk et al., 2013). Rainfall deficit has caused drought and thus led to water restrictions at certain times in most Australian urban areas, which significantly reduces water availability for irrigation (Heberger, 2012). This has placed a greater pressure on governments making policies for conserving and recycling water (Radcliffe, 2006).

In response to the occasional restriction on potable water use and limitations for rainwater storage, greywater is being considered as a potential alternative resource for uses such as toilet flushing, landscape irrigation, and crop production (Al-Jayyousi, 2003; Friedler, 2004). In arid and semi-arid regions, for example, studies have indicated that household greywater reuse for toilet flushing and irrigation could achieve up to a 50% reduction in potable water use (Jeppesen, 1996).

Greywater is the wastewater from bath tubs, showers, hand wash basins, and laundry, and while it is of lower quality than drinking water, it is usually less polluted than municipal wastewater due to the absence of human excretions (urine and faecal matter) (Eriksson et al., 2002; Friedler, 2004). The main components are soaps and detergents, yet the chemical and physical characteristics of greywater are quite variable due to the type of detergents used, goods being washed and practices followed at household levels (Christova-Boal et al., 1996).

Studies have indicated that long-term application of greywater for irrigation of agriculture crops can adversely impact plant growth due to changes in critical soil properties (Al-Hamaiedeh and Bino, 2010; Hamilton et al., 2007; Pinto et al., 2010). For instance, greywater can increase soil alkalinity and salinity, reducing availability of some micro-nutrients and thereby resulting in reduced plant growth (Christova-Boal et al., 1996; Hamilton et al., 2007). In addition, greywater irrigation may induce phytotoxicity due to high levels of surfactants (Garland et al., 2000).

While greywater irrigation has been applied to urban landscapes (Gori et al., 2000), household gardens and small-scale food production (Faruqui and Al-Jayyousi, 2002; Finley et al., 2009; Rodda et al., 2011), greywater has not been used to support green façades. Greywater has been studied for irrigation of living wall systems (Fowdar et al., 2017), however their research was from the perspective of improving greywater quality rather than supporting healthy plant growth. Fowdar et al. (2017) demonstrated that a living wall system consisting of sand filter media planted with *Lonicera japonica*, *Pandorea jasminoides*, *Parthenocissus tricuspidata* and *Vitis vinifera* could effectively remove > 80% of nitrogen from greywater. This may suggest that it is possible to use greywater to similarly irrigate green façade systems, however, the effects of greywater on climbing plant growth, health and water use need to be understood.

Sunlight availability in urban environments

Urbanisation and densification often results in a dynamic and constantly changing built urban form due to the continual construction and redevelopment of building sites in cities (Dadashpoor et al., 2019). While indirect green façade plants are selected for their tolerance of on-site conditions including radiation exposure (e.g., full sun, semi-shade or shade) and weather conditions (e.g., wind, rainfall, heat, drought and frost) (Fernández-Cañero et al., 2018; Growing Green Guide, 2014), the existing green façade plants may fail to adapt to different on-site conditions which occur due to redevelopment. For instance, a newly constructed building neighboring an existing green façade may alter the growing conditions that façade experiences from full sun to shade.

In forests, many understorey woody plants (e.g., trees, shrubs, climbing plants) exhibit considerable plasticity of response to continual spatio-temporal changes in the light environment due to crown canopy developments (Cai et al., 2009; Grassi and Bagnaresi, 2001; Jarvis, 1964). To acclimatise to reduced sunlight, plants tend to increase specific leaf area (SLA) and leaf chlorophyll concentrations to enhance their light capture capacity, whilst lower their light compensation points to photosynthesise at low light levels (Lambers et al., 2008; Lei et al., 1996; Lichtenthaler and Babani, 2004; Wittmann et al., 2001). In addition, reduced sunlight is likely to adversely affect leaf biomass growth due to decreased photosynthetic capacity (Lambers et al., 2008; Lichtenthaler et al., 1981). Research in forest ecosystems has also indicated heavy shade may reduce leaf tolerance to high ambient air temperatures that occur during summer heatwaves (Santamouris, 2020; Slot et al., 2018).

Similarly, variable light conditions also occur in urban environments, although the spectral and temporal characteristics of light regimes are quite distinct in urban canyons from forests. Climbing plants in forests often experience relatively high proportions of penumbral light, a significant proportion of solar radiation is being intercepted and absorbed through the crown canopy (Lambers et al., 2008; Valladares et al., 2002). In contrast, urban environments are usually noted by an alternation between extremely intense solar radiation and deep shade cast by buildings, as well as harsh microclimatic conditions including high air temperatures and wind speed (Rayner et al., 2010; Xue et al., 2020).

However, there have been no previous studies evaluating the effects of the light condition on the morphological and physiological traits of climbing plants in urban environments. Hence, there is a need to determine how climbing plants will adjust to changed light conditions to ensure that green façades can maintain ecosystem provision in dynamic cities.

Relevance and importance of study

Most countries have relatively shorter histories of the use of indirect green façades as compared to direct green façades (Köhler, 2008; Manso and Castro-Gomes, 2015), but are now embracing their use as a green infrastructure technology. In Australia, some cities like Melbourne are incentivising their uptake and it is important to ensure that indirect green façades can be successfully developed for Australian conditions (Growing Green Guide, 2014). For indirect green façades to effectively provide ecosystem services they need to have great coverage with healthy leaves and/or high rates of evapotranspiration (Cameron et al., 2014; Hoelscher et al., 2016; Koyama et al., 2013). However, both characteristics will be affected by unfavorable growing conditions such as small rooting volumes, lack of potable water availability and changing light conditions due to site limitations and weight loading of containers on elevated structures (Dunnett and Kingsbury, 2008; Growing Green Guide, 2014; Wolter et al., 2011).

On reviewing the literature, I found that some important concepts were lacking from the available green façade research. Most studies have emphasised the cooling benefits (Cameron et al., 2014; Hoelscher et al., 2016; Li et al., 2019) and the resulting energy savings (Bustami et al., 2018; Hunter et al., 2014). However, differences in how resources (e.g. soil volumes, water quality, sunlight variability) affect climbing plant growth and coverage have not yet been determined. We know from natural habitats that climbing plants can adjust their morphological and physiological traits in response to resource availability, and it is likely that green façade climbing plants can do the same in urban environments. However, investigating how this relates to climbing plant species differences in growth rate, leaf habit (deciduous vs evergreen) and traits will be important to select green façade plants which can transform our cities and provide the most benefits.

Therefore, the objective of my PhD is to investigate how woody climbing plants respond to different growing conditions by evaluating their morphological and physiological traits. My PhD consists of three experimental chapters, with a general introduction and concluding synthesis chapter (Figure 1.2). The three objectives of three experimental chapters of my thesis are:

1. To evaluate the effects of rooting volume on climbing plant growth, coverage and thermal tolerance in the first growing season (Ch 2);

2. To investigate whether greywater is a viable irrigation resource for indirect green façades in cities by assessing changes in substrate chemical properties and the growth response of six climbing plant species after 18-weeks of irrigation (Ch 3);

3. To explore how shade affects leaf traits and thermal tolerance of climbing plant species assisting green façade design and plant selection (Ch 4).

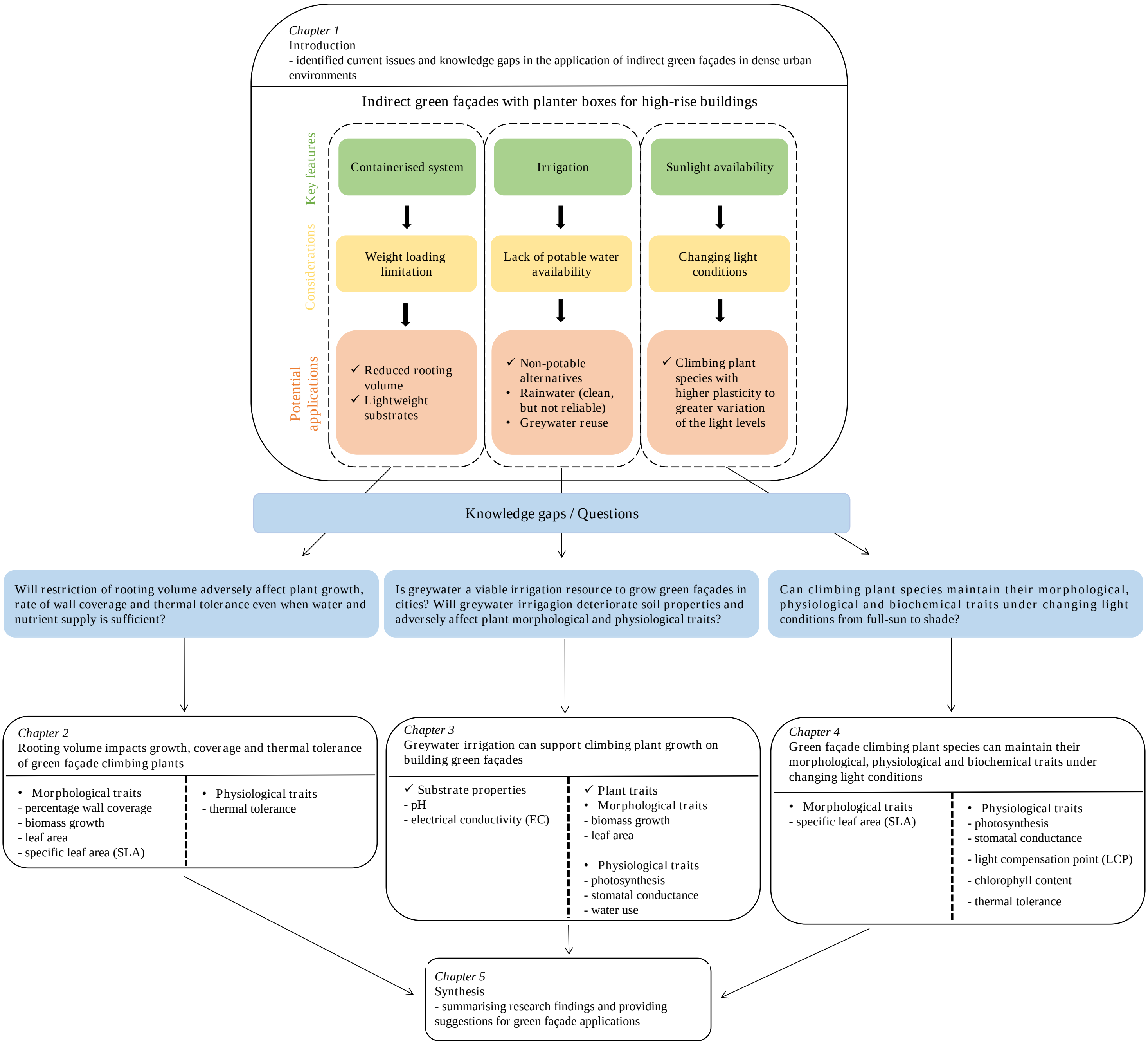

Figure 1.2. The conceptual framework of my PhD research

Three key features and associated considerations for installation and management of containerised indirect green façades for high-rise buildings. Items in yellow rectangles are further explored in this thesis. This thesis comprises three experimental chapters to investigate how climbing plants respond to different growing conditions including the effects of rooting volume, greywater irrigation and changing light conditions from full-sun to shade on climbing plant species. This thesis mainly focuses on the assessment of changes in morphological and physiological traits of climbing plant species under the three growing conditions.

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2 - Rooting volume impacts growth, coverage and thermal tolerance of green façade climbing plants

Abstract

Green façades can improve ecosystem services in dense urban centres deprived of greenery by providing vertically built surfaces. To grow green façades on high-rise buildings, climbing plant growth, performance and resilience to stress can be constrained by container rooting volume provided due to engineering weight constraints and cost.

We assessed the effects of three different rooting volumes (21, 42 and 63 L), on the early growth and coverage of two climbing plant species (*Akebia quinata* and *Pandorea pandorana*). We measured plant physiological responses to air temperatures and solar radiation differences on east- and west-facing aspects.

Larger rooting volumes (42 and 63 L) did significantly increase plant biomass growth and percentage wall coverage for both species over the measured six-month period. Also, the specific leaf area of both species was significantly greater when grown in 63 L as compared to 21 L. However, the shoot:root ratio of climbing plant species did not change with different rooting volumes. Regardless of rooting volume, higher ambient air temperatures and solar radiance on west-facing aspects led to leaf stress in the late afternoon as measured by chlorophyll fluorescence.

In this study, for a wall area of 2.8 m^2, it was suggested that 42 L rooting volume is adequate to support plant growth and coverage of green façades during the first growing season. In practice, approximately 21.3 m^2 of canopy coverage for *P. pandorana* and 10.2 m^2 for *A. quinata* can be expected within 6 months per cubic rooting metre. Further studies are needed

to assess the climbing plant growth and cover in different rooting volumes beyond the first growing season.

Introduction

Worldwide, increasing urbanization and densification continues to have negative environmental impacts in cities, including modification of the urban climate which leading to urban heat island effects, increased water and energy consumption, and heat-related illness and mortality (Bambrick et al., 2011; Oleson et al., 2015). Urban greening is being used in cities to mitigate the adverse effects of increasing urbanization and to improve the environment and residents' health (Lundholm, 2006; Pérez-Urrestarazu et al., 2015). However, with urban densification, there are limited ground-level green space such that vertical or roof-top urban greening approaches, such as green roofs and green walls are being used (Haaland and van Den Bosch, 2015; Lundholm, 2006). Vertical greening of building walls can incorporate more vegetation into our cities (Hunter et al., 2014; Perini et al., 2011). Vertical greening can potentially provide numerous benefits, including improved air quality, increased plant diversity and habitat provision for wildlife, noise reduction, microclimate improvement, urban heat island mitigation, building thermal performance improvement and lower energy costs, as well as improved aesthetics and psychological well-being of urban residents (Medl et al., 2017; Tudiwer and Korjenic, 2017). Moreover, as many of these benefits are linked to the amount of vegetation cover on buildings, as coverage increases, the positive effects of vertical greening are also increased (Cameron et al., 2014).

Vertical greening can be categorized as either façade greening or living walls based on their methods of growing (Besir and Cuce, 2018; Fernández-Cañero et al., 2018). Living wall systems grow directly into substrate attached to a wall surface, whereas green façades use climbing plants such as vines, lianas and scramblers, that grow in either ground-level substrate or in a containerised substrate, to grow up and cover a wall (Fernández-Cañero et al., 2018; Manso and Castro-Gomes, 2015). Green façade systems are further classified according to whether the climbing plant species are directly attached onto walls (direct façade) or indirectly supported by trellises, mesh or steel cables (indirect façade) (Jim, 2015; Manso and Castro-Gomes, 2015). For multi-storey buildings, however, direct façades are not viable as the materials of building wall do not provide adequate support for climbing plants to anchor or to attach (Manso and Castro-Gomes, 2015). Therefore, indirect green façade systems are considered better for long-term multi-storey or high-rise urban greening (Perini and Rosasco, 2013; Thottathi et al., 2010). Indirect green façades have long been used in Europe since the 1880s, and has been more recently introduced to North America and Asian countries, such as Japan and Singapore (Köhler, 2008). It is important to research how indirect green façades should be designed to ensure they achieve their benefits and have widespread uptake in these new regions (Ascione et al., 2020).

To support façade plant growth and coverage when grown in containers, it is important that there is an adequate substrate rooting volume (growing media) to supply water and nutrients and provide sufficient space for root growth (Dunnett and Kingsbury, 2008; Growing Green Guide, 2014). Inadequate rooting volumes can lead to reduced plant growth and development for woody plants such as wax-apple (*Syzygium samarangense* (Bl.) Merr. & Perry (Hsu et al., 1996) and euonymus (*E. kiautschovica* Loes. 'Sieboldiana') (Dubik et al., 1992). Studies in natural ecosystems have shown that some woody climbing plants have deep and wide root systems to access deeply stored water (Andrade et al., 2005; Schnitzer, 2005). However, in urban ecosystems to grow woody climbing plants at successive levels of high-rise buildings, they are planted into lightweight containers with reduced rooting volumes or with lightweight substrates due to constrained load-bearing limits and costs (Haege and Leake, 2014;

Handreck and Black, 2010). Therefore, by limiting substrate rooting volumes, there may be insufficient resources for climbing plants to grow well and achieve good wall coverage (NeSmith and Duval, 1998). In addition, under the same growing condition, climbing plant species with different growth strategies may respond differently due to differences in growth rate (Lambers and Poorter, 1992). To sustain rapid growth, fast-growing species have a greater capacity for resource acquisition as compared to slower-growing species (Lambers and Poorter, 1992).

Few studies have evaluated the effect of substrate rooting volume on climbing plant growth. Grapevines grown in isolated buried beds with restricted rooting volumes produced smaller trunks, shorter shoots and lower leaf areas than those growing in field soil; despite irrigation and fertilisers provided being sufficient for their growth (Wang et al., 2001). The effects of reduced substrate volume on plant growth and coverage may also be more pronounced during hot summer periods, where façade plants are subject to prolonged sunlight and extreme heat. Studies have suggested that plants grown in the larger rooting volume outperform plants in the smaller rooting volume under abiotic stress (Matthew et al., 2014; Villar-Salvador et al. 2012). For climbing plants to provide effective cooling, they need to develop a significant vegetation coverage and volume of foliage (Cameron et al., 2014; Hoelscher et al., 2016). However, high temperature might adversely affect plant development and could lead to foliage loss, even when water is not limiting; for example, birch trees dropped 33% of leaves under heat stress (Darbah et al., 2010). Therefore, to determine the effects of rooting volume on façade growth and coverage in the first growing season, we undertook an experiment where two climbing plant species with different growth strategies, were grown in three different rooting volumes (the only differentiator being container depth and not container surface area). We designed a study to answer two hypotheses: (1) restriction of rooting volume will limit plant biomass growth and rate of wall coverage even when water and nutrients are not limiting, and (2) climbing plants grown in greater rooting volumes will exhibit higher Fv/Fm than the ones in the smaller rooting volumes under heat stress.

Materials and Methods

Plant materials and growing conditions

Two widely-used green façade climbing plant species with different growth rates were selected for this study; *Akebia quinata* (Chocolate Vine) and *Pandorea pandorana* (Wonga Wonga Vine). *Akebia quinata* is a semi-deciduous twining climber with a medium growth rate that can reach up to 12 m in height by climbing trees (Brunel et al., 2010; Zimmerman, 2013), and is widely distributed throughout East Asia, including China, Japan and Korea (Yen et al., 2014). In contrast, the second species, *Pandorea pandorana* is a fast-growing evergreen twining climber (Andresen, 2012) that can reach up to 10 m in height at maturity (Moreland City Council 2004), and is widespread throughout Australia (James and Knox, 1993). There is no published information on the root growth habits of either of these climbing plant species.

Twelve uniform seedlings of both species (24 plants in total) were purchased from a commercial nursery (140 mm pot size, Evergreen Nursery, South Silvan, Victoria, Australia) and transplanted into containers (Heavy duty crates, Ki-tab, Bell Dies Sydney, Australia) with three different volumes in September 2017. These containers had the same length and width (370 mm long x 550 mm wide) but different depths and as such different volumes; 21 L (103 mm deep), 42 L (206 mm deep), and 63 L (309 mm deep). A scoria-based substrate was used in this experiment, which comprised of Scoria 7mm aggregate (20% v/v), Scoria 8 mm minus which includes fines (60% v/v), and horticultural grade coir (20% v/v). The water holding capacity and the bulk density of the substrate are 46 % and 1.26 g cm^{-3}, respectively (Farrell et al., 2013).

In the nursery at the Burnley Campus, University of Melbourne, 24 climbing plants were grown on stainless steel, façade supports on a north-south orientated wall. While these species can reach 10-12 m high at maturity, this height was not expected in the first growing season, especially when growing in limited rooting volumes. The supporting wall comprises three sections, each 6 m (W) by 2.4 m (H). The height of the existing wall is 2.4 m, which is the standard height of a building storey according to the Australian Building Code. On each side of the wall (east and west aspect), twelve sections of stainless-steel mesh (1.45 m (W) by 1.95 m (H); mesh size: 200 mm x 340 mm), were attached at the distance of 0.1 m from the wall and 0.45 m above the ground. This meant plants had a 1.95 m structure to climb, which was sufficient during the establishment period, as plants did not exceed the height of the structure. The experiment was a fully-randomized design, with six climbing plants of both species on east-facing and west-facing aspects of the same wall (n = 12, per species). Each species had at least three replicates for each rooting volume treatment. Two climate stations with data loggers (CR1000, Campbell Scientific Inc., Queensland, Australia) were used, one on the eastern aspect and one on the western aspect. These climate stations continuously recorded, irradiance (CS300 Pyranometer, Apogee Instruments Inc., Utah, USA), ambient air temperature and humidity (HMP60 Temperature and Relative Humidity Sensor, Vaisala, Helsinki, Finland). Mean values for one -hour intervals were calculated.

Fertiliser and irrigation amount were applied according to the rooting volume of each container, based on the standardised approach to grow containerised plants (Wilkinson et al., 2014). In other words, the fertiliser and irrigation amount were applied to the 21 L rooting volume was one-third of that applied to the 63 L rooting volume. Slow release fertiliser (NPK 15.4-3.5-8.9 + trace element) (Macracote Coloniser Plus, Langley Fertilizers, Western

Australia, Australia) was applied at a rate 3 g L^{-1} to all rooting volume treatments at the beginning of the experiment. Irrigation was applied using drippers, with flow rate at 0.5 L min^{-1} for each dripper. Two, four and six drippers were installed for 21 L, 42 L and 63 L rooting volumes, respectively. To maintain well-watered conditions, irrigation was applied twice daily (7:00 and 17:00 h of the day) from September to November (spring) and three times daily (7:30, 14:30 and 19:30 h of the day) from December to March (summer), for two minutes each time. Daily irrigation supplies were 4 L, 8 L and 12 L during spring, and 6 L, 12 L and 18 L during summer for the three rooting volumes, respectively (21 L, 42 L and 63 L). These irrigation volumes ensured all three different rooting volumes were irrigated to field capacity. All the climbing plants were well-watered throughout the experiment and did not show signs of water deficit (as indicated by high leaf water potentials – Appendix 2.1).

Plant growth and canopy cover measurements

Canopy cover was measured every two months (two, four and six months) using a high-resolution (2760 x 4912 pixels) digital camera to take photographs of each green façade at a fixed distance (1.9 m) and a fixed height (0.9 m) using a tripod. Photographs were taken on cloudy days with diffuse light conditions. Digital image analysis was performed to calculate percentage wall coverage. The background wall and climbing support were removed from images manually using PhotoFiltre 7 (freeware, Antonio Da Cruz) and colour images of plants were then converted into binary images (black pixels only) to calculate the area of canopy cover in pixels with Image J (free software for image processing and analysis in Java) (Fig. 2.1). Percentage coverage was the percentage of the area covered by plants to the area of wall (1.45 m (W) x 1.95 m (H)).

Climbing plants in all rooting volume treatments were harvested in March 2018 (6 months after the experiment commenced), and all plants were separated into leaves, stems and roots.

Leaf area was measured on a sub-sample of leaves (10% of total fresh weight). The projected leaf area (one side of the leaves) was measured using a leaf area meter (LI-3100C Area Meter, LI-COR Inc.). The whole root system was excavated and cut into smaller pieces whilst washing away the substrate. Plant parts were oven dried (70°C) for a week to obtain dry total biomass. From these measurements, mean values of specific leaf area (SLA, the ratio of leaf area to leaf dry mass) and shoot:root ratio were calculated.

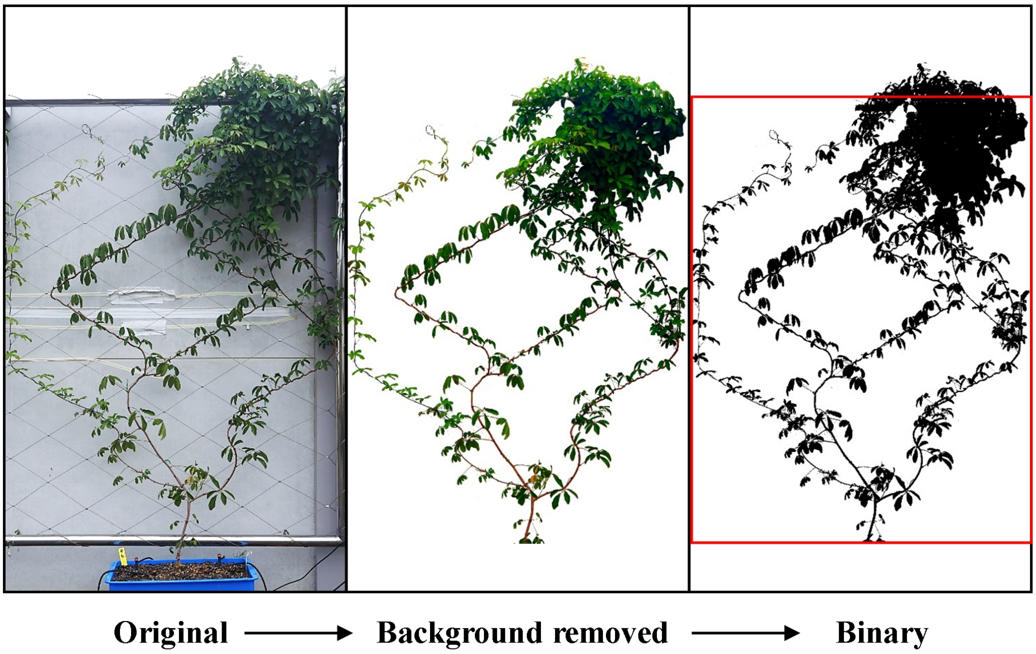

Figure 2.1. Image processed for measuring percentage wall coverage. The binary image (black pixels) of the same individual was used to calculate percentage wall coverage. Red outline indicates the area of the wall (1.45 m (W) x 1.95 m (H)) that was examined to determine percentage wall coverage.

Chlorophyll fluorescence

Chlorophyll fluorescence is a commonly used indicator of plant stress, including extremes of temperature and light intensity (Dias and Marenco, 2007; Yamane et al., 1997). The ratio of variable (Fv = Fm – Fo) to maximal (Fm) fluorescence of dark-adapted leaves was measured with a portable chlorophyll fluorometer (FMS2+ Field Fluorescence Monitoring System, Hansatech Instruments), because it is a rapid determination of changes in the maximum quantum efficiency of PSII photochemistry when plants are under stress (Maxwell and Johnson, 2000). The ratio of variable to maximum fluorescence (Fv/Fm) was recorded on three fully expanded mature leaves after dark adaption for 20 minutes. Measurements were conducted at 10:00, 13:00 and 16:00 hr of the day, in a hot sunny summer day.

Statistical analysis

Back-transformation was applied to percentage wall coverage, total biomass and total leaf area to ensure univariate normality before data analysis. Linear mixed models were performed in GenStat 16 (2013, VSN International Ltd., England, UK), and the least significant difference (LSD at $p \leq 0.05$) was applied to assess the differences among pairs of treatment means within both climbing plant species. We also tested significant differences between east and west-facing aspects for all variables. If the differences were insignificant, data was combined from both aspects; if significant, data was kept separate (e.g., chlorophyll fluorescence).

Results

Climbing plant coverage and growth response to different rooting volumes

Rooting volume had no significant effect on percentage wall coverage of either climbing plant species in the first two months after transplanting (Fig. 2.2). However, after four-month growth, the percentage wall coverage of *P. pandorana* was significantly greater for plants with a volume of 42 L (+267.4%) or 63 L (+246.7%) as compared to 21 L ($p < 0.05$). In contrast, there was no significant difference in percentage wall coverage of *A. quinata* amongst the three rooting volume treatments after four-month growth. It was not until after six-month growth, that the percentage wall coverage of *A. quinata* was significantly greater (+85.7% and +105.6%) in 42 L or 63 L rooting volumes as compared to 21 L ($p < 0.05$). Similarly, percentage wall coverage of *P. pandorana* was significantly greater when grown in 42 (+104.2%) or 63 L (+138.3%) as compared to 21 L ($p < 0.001$).

On the other hand, *P. pandorana* had approximately double the wall coverage of *A. quinata* across all rooting volume treatments at the end of this experiment. Interestingly, there was no significantly difference in percentage wall coverage of either climbing plant species between plants grown in 42 L and 63 L rooting volumes.

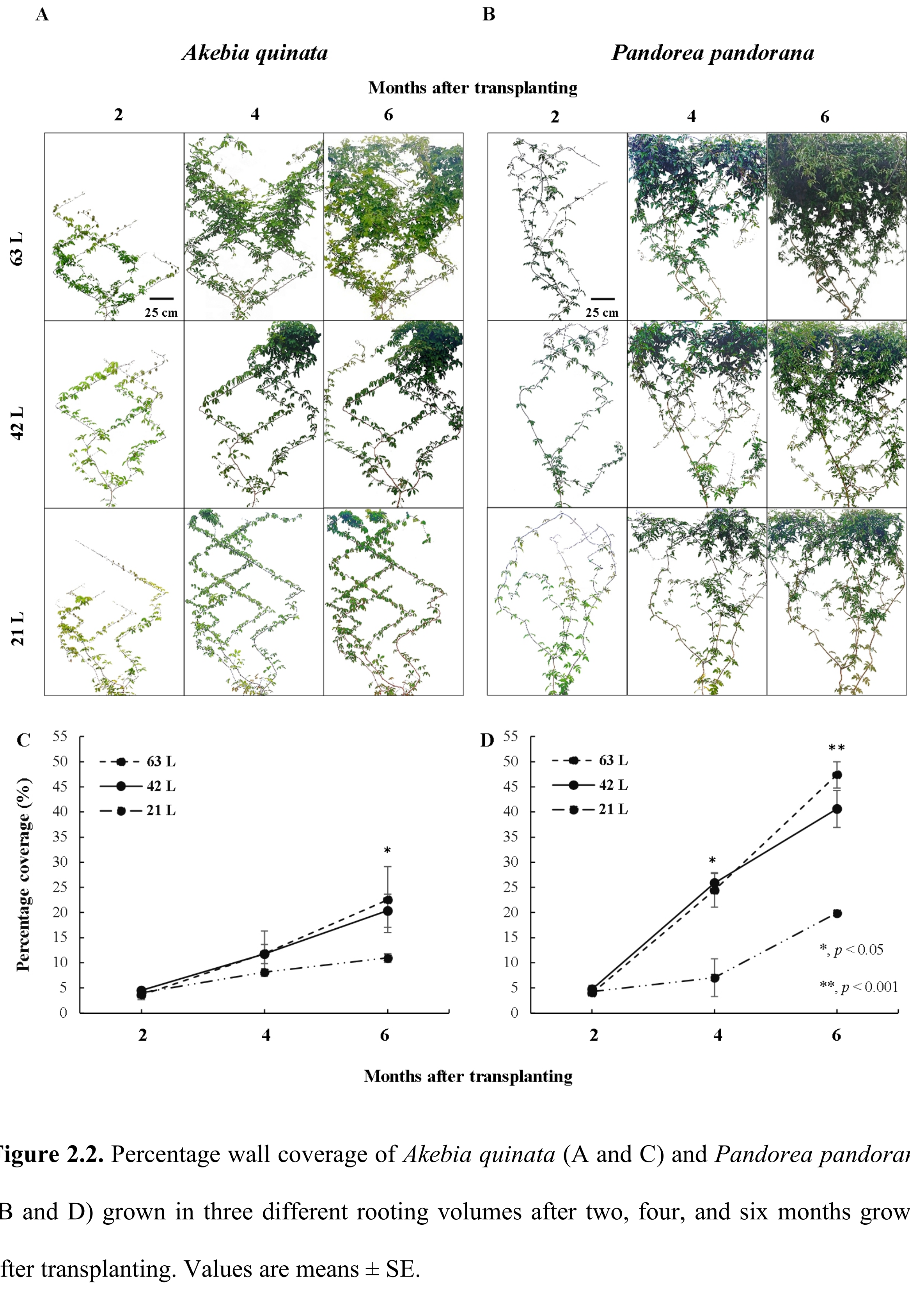

Figure 2.2. Percentage wall coverage of *Akebia quinata* (A and C) and *Pandorea pandorana* (B and D) grown in three different rooting volumes after two, four, and six months growth after transplanting. Values are means ± SE.

The biomass growth of both climbing plant was strongly affected by rooting volume ($p < 0.001$) (Fig. 2.3A). Total biomass of *Akebia quinata* and *Pandorea pandorana* more than doubled (+107% and +141%, respectively) when grown in 42 L or 63 L as compared to 21 L. However, there was no significant difference in biomass of either climbing plant species between plants grown in 42 L and 63 L rooting volumes. After six months growth, *P. pandorana* had more than double the biomass of *A. quinata* under the same rooting volume treatments.

Shoot:root biomass allocation was unaffected by rooting volume, with no significant differences for either climbing plant species (Fig. 2.3B). However, between the two species, *P. pandorana* allocated more biomass to shoots than *A. quinata* in all rooting volume treatments.

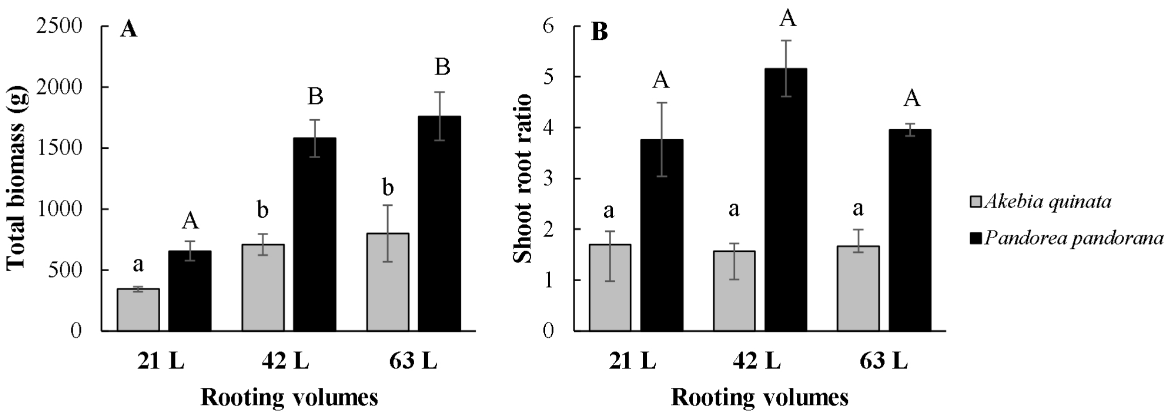

Figure 2.3. Average total biomass (A) and average shoot:root ratio (B) of *Akebia quinata* and *Pandorea pandorana* plants grown in three different rooting volumes after six months growth. Values are means ± SE. Different letters represent significant differences within species, $p < 0.001$; lowercase, *Akebia quinata*; capital, *Pandorea pandorana*.

Total leaf areas of *A. quinata* ($p < 0.01$) and *P. pandorana* ($p < 0.001$) were also significantly affected by rooting volume (Fig. 2.4A). Both climbing plant species at least doubled their leaf area when grown in 42 L as compared to 21 L. However, there was no significant increase in leaf area of either climbing plant species between plants grown in 42 L and 63 L rooting volumes. *P. pandorana* had a two or three-fold greater leaf area than *A. quinata*, regardless of rooting volume. Similarly, SLA increased significantly with increasing rooting volume for both species ($p < 0.001$) (Fig. 2.4B). SLA increased from 74 to 95 $cm^2 g^{-1}$ for *A. quinata* and from 69 to 98 $cm^2 g^{-1}$ for *P. pandorana* was grown in 21 L and 63 L, respectively.

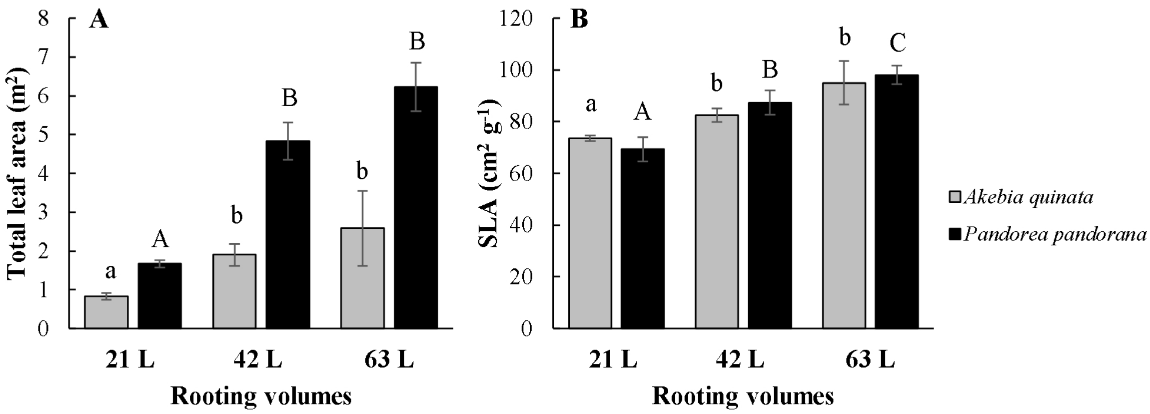

Figure 2.4. Average total leaf area (A) and average specific leaf area (SLA) (B) of *Akebia quinata* and *Pandorea pandorana* grown in three different rooting volumes after six months growth. Values are means ± SE. Different letters represent significant differences within species, $p < 0.001$; lowercase, *Akebia quinata*; capital, *Pandorea pandorana*.

Effects of air temperature and solar radiation on the Fv/Fm ratio of climbing plants

During a hot sunny day, on the west-facing aspect, air temperature peaked at approximately 40°C in the late afternoon and solar radiation peaked above 900 W m^{-2} (14:00 hr; Fig. 2.5B).

Whereas on the east-facing aspect, ambient air temperature reached a maximum of 37^{o}C at midday and solar radiation reached a maximum of 757 W m^{-2} (Fig. 2.5A).

For both climbing plant species, there was no significant effect of rooting volume on the Fv/Fm ratio (maximum potential quantum yield of PSII) on either side of the wall at any time of day (Figs. 2.5C-5F). However, on the hotter west-facing side, Fv/Fm ratios of both climbing plant species were significantly lower in the late afternoon (16:00 hr; Figs. 2.5D and 2.5F) ($p < 0.001$). On the west side of the wall, Fv/Fm ratio decreased to a greater extent between 10:00 and 16:00 hr in *P. pandorana* (+49% to +64%) (Fig. 2.5F) as compared with *A. quinata* (+40 to +46%) (Fig. 2.5D). In contrast, on the east-facing aspect, the Fv/Fm ratio did not change significantly during the day as air temperature and solar radiation changed.

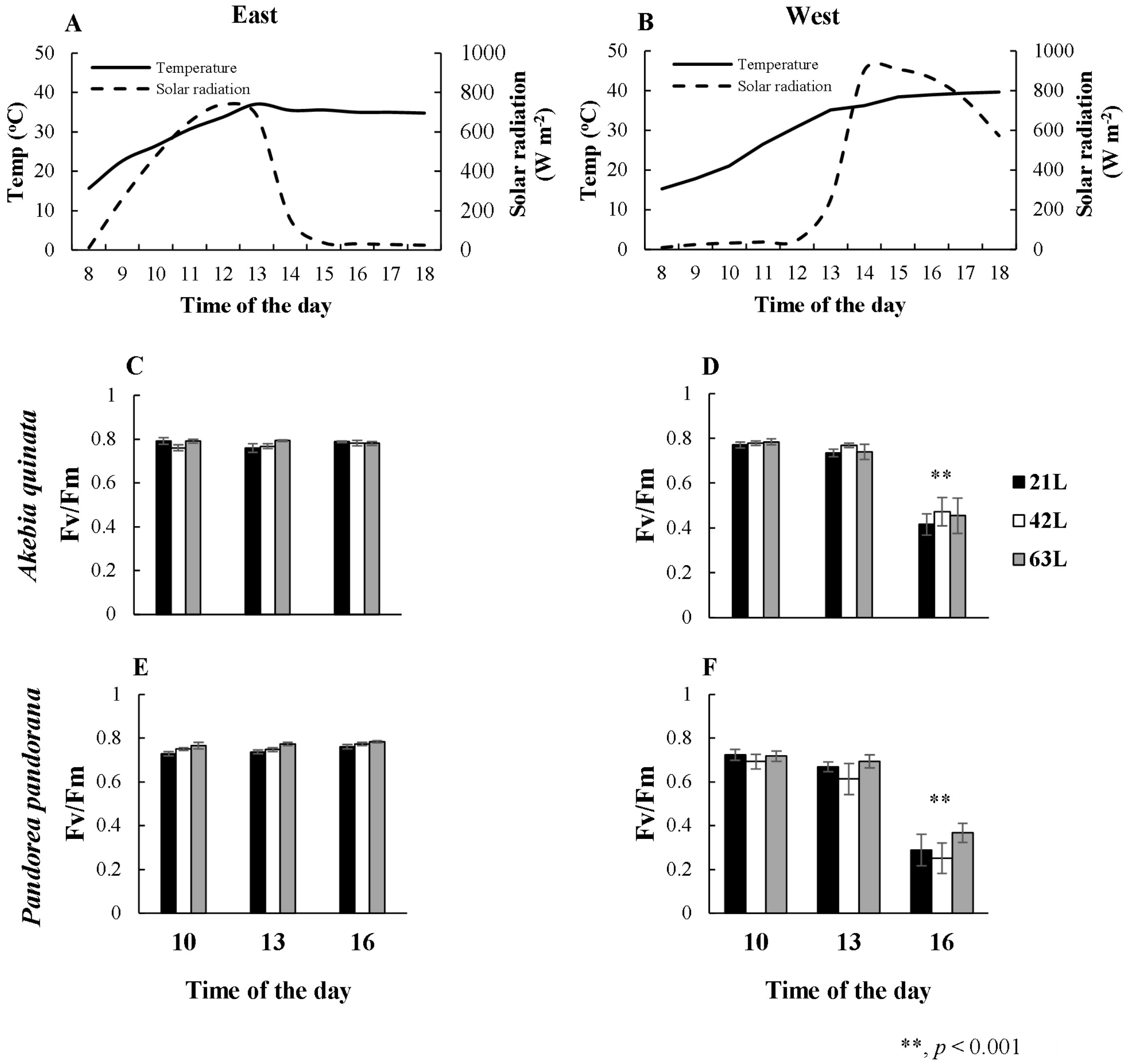

Figure 2.5. Daytime changes of air temperature and solar radiation of east-facing (A) and west-facing façades (B) in a clear summer day in March 2018. Variation of Fv/Fm ratio of *Akebia quinata* (C and D) and *Pandorea pandorana* (E and F) grown in different rooting volumes in response to the changes of air temperature and solar radiation. Values are means ± SE for three different leaves.

Discussion

We evaluated the effects of rooting volume (the only differentiator being container depth and not container surface area) on the growth, coverage and thermal tolerance of two climbing plant species in their first growing season. As container surface area was constant among all three rooting volumes in this study, the morphological and physiological responses of climbing to rooting volume differences indicated the sensitivity to changes in rooting depth. We hypothesised that restriction of rooting volume will limit plant biomass growth and rate of wall coverage while supply of water and nutrients are not limited. Our study showed that plant growth and coverage of *A. quinata* and *P. pandorana* can be significantly reduced by small rooting volumes. In general, plants grown in restricted rooting volumes have reduced shoot and root growth compared with unrestricted plants (NeSmith and Duval, 1998). As woody climbing species tend to develop deep root systems (Andrade et al., 2005; Schnitzer, 2005), the small rooting volume (21 L, 103 mm deep) may impose physical limitations on the growth of roots and subsequently restrict the shoot growth (McConnaughay and Bazzaz, 1991).

Providing a greater rooting volume led to a significant increase in biomass and area coverage increase in both climbing plant species, which is likely due to higher water and nutrient availability in greater rooting volumes to facilitate plant growth (Kostopoulou et al., 2011). Although a clustering habit of branches and foliage was observed in this study, this did not hinder plant growth. At the top of the supporting wall, branches of climbing plants spread horizontally and developed thicker foliage layers. Therefore, the biomass growth and foliage development were not suppressed during the first growing season. Interestingly, the significant wall coverage response to greater rooting volumes was evident earlier for the faster growing species *P. pandorana* than the slower growing species, *A. quinata*. Faster growing species can exhibit a more effective forage behaviour to acquire more water and nutrients for plant growth and thus achieve higher growth rate (Fransen et al., 1999). It can therefore be assumed that both 42 L and 63 L rooting volumes provided sufficient resources to support the growth of these two climbing plant species for six months after transplanting. On the other hand, our results suggested that the smallest rooting volume was considered not suitable to grow climbing plants for indirect green façades due to the restriction of plant growth and cover.

Percentage wall coverage of façade plants has been reported as the key trait to determine the cooling effect that contributes to thermal performance and energy savings of buildings (Koyama et al., 2013; Pérez et al., 2011). At the end of this experiment, approximately one quarter and half of the wall areas were covered by *A. quinata* and *P. pandorana*, respectively, in both 42 L and 63 L rooting volumes. According to the relationship between the percent wall coverage and wall surface temperature reduction (WTR) investigated by Koyama et al. (2013), we could estimate that WTRs were 3.7 and 4.3°C for *A. quinata*, and 8.3 and 9.6°C for *P. pandorana* with 42 L and 63 L rooting volumes, respectively. Therefore, in this study, the rooting volume of 42 L appeared to be adequate for wall coverage and growth in the first growing season, considering there was no significant additional benefit from providing a 63 L rooting volume and associate economic costs and benefits of indirect green façades.

In our study, the approximate vegetation wall coverage was 1.3 m^2 for *P. pandorana* and 0.6 m^2 for *A. quinata* at the end of six months, when grown in the larger 63 L rooting volume (data not shown). Therefore, for every cubic metre of rooting volume, *P. pandorana* could achieve 21.3 m^2 of wall coverage in six months. Whereas, *A. quinata* could achieve 10.2 m^2 of wall coverage in six months per cubic metre of rooting volume. Nevertheless, these expectations are subject to the vigour and growth of the species and will change over time. This does not indicate the final coverage of these species. The ratio of rooting volume to canopy coverage for planting trees in urban areas has been studied (Kent et al., 2006). However, for green façade climbing plants, no previous research has investigated the ratio of rooting volume to vegetation wall coverage.

For both climbing plant species, SLA increased with increasing rooting volume, which is consistent with that of a previous study. Research showed that euonymus (*E. kiautschovica* Loes. 'Sieboldiana') plants maintained lower SLA than those in larger containers (Dubik et al., 1992). This is likely due to increased resource availability in greater rooting volumes as plants from higher rainfall or nutrient-rich soils tend to have higher SLA (Wright et al., 2001). On the other hand, SLA is positively correlated to relative growth rate (RGR) in woody plants (Antúnez et al., 2001). This may help explain the result that both climbing plant species grown in the smallest rooting volume had relatively lower growth rate and SLA compared with those in the greater rooting volumes.

Despite increased percentage wall coverage, biomass, and leaf area with increased rooting volume, the allocation of biomass to shoots and roots (shoot: root ratio) was unaffected by rooting volume. When roots are confined in a container that restrains their development, the roots compete for essential resources (NeSmith and Duval, 1998). With respect to shoot:root ratio, increases of top biomass were linearly correlated with increasing pot sizes were found in some woody plants, including *Ilex cornuta* Lindl. & Paxton, *Euonymus japonica* Thunb., *Rhododendron* x sp. (Keever et al., 1985), and *Syzygium samarangense* (Bl.) Merr. & Perry (Hsu et al., 1996). In contrast, other studies have demonstrated that shoot: root ratio remains constant as rooting volume changed for various plant species, such as peach (*Prunus persica*) (Rieger and Marra, 1994), cucumber (*Cucumis sativus*) (Robbins and Pharr, 1988), soybean (*Glycine max*) (Krizek et al., 1985), salvia (*Salvia splendens*) (Van Iersel, 1997), and euonymus (*Euonymus kiautschovica* Loes. 'Sieboldiana') (Dubik et al., 1992). In addition, it has been reported that woody plants might be able to maintain a balance in root and shoot growth in stable environments as an adaptive mechanism to varying rooting volumes (Dubik et al., 1992; Kramer and Kozlowski, 1979). This is consistent with our study and may be because the provision of water and nutrients were not limited in our study, this may be the reason biomass allocation did not change among the different rooting volumes.

In contradiction to our second hypothesis, climbing plants grown in the greater rooting volume did not show higher Fv/Fm than the ones in the smaller rooting volumes under heat stress. Regardless of rooting volume, there was a significant decrease in Fv/Fm observed in both climbing plants exposed to high ambient air temperature and high solar radiance on the west-facing aspect. During our study period, the highest ambient air temperature recorded was around 40°C in the late afternoon. Presumably, the decline in Fv/Fm ratio under heat stress might be a result of partly reversible inactivation of the PS II reaction centre (Camejo et al., 2005; Yamane et al., 1997). A decrease in the Fv/Fm ratio of oak trees was observed when air temperature rose above 36°C, which caused a temporary heat-stress-dependent reduction of leaf photosynthesis (Haldimann and Feller, 2004). In addition, research has indicated that water bath temperature around 60°C led to almost complete inactivation of the PS II reaction centre in spinach and rice chloroplasts (Yamane et al., 1997). However, the decline in the Fv/Fm ratio recorded was not associated with a decrease in either plant biomass or leaf development. Although we did not measure the Fv/Fm ratio of the same leaves the next day, it is likely that both climbing species on the west-facing aspect had recovered from heat stress after temperature dropped at night.

This study focused mainly on the influence of rooting volume on above-ground growth and coverage of the two climbing plant species. Although rooting behaviour of the two climbing plant species was not investigated in our study, it could be suggested that root growth of the two climbing plant species was not limited by 42 L (370 mm (L) x 550 mm (W) x 206 mm (D)) or 63 L (370 mm (L) x 550 mm (W) x 309 mm (D)) rooting volumes during the first growing season. As according to Wang et al. (2014), during the first year, root growth of Kyoho grapevines were not adversely affected by the restricted rooting volume, i.e. 50 L (500 mm long, 500 mm wide, and 200 mm deep). While we investigated shoot: root allocation, further studies should be undertaken to assess the influence of limited rooting volume on root development (e.g., root density, rooting depth and root length), as well as the long-term effects of several years on the growth and coverage of climbing plant species.

Conclusions

The aim of this study was to evaluate the effect of rooting volume on initial growth, coverage and thermal tolerance of climbing plants for indirect green façades. Our study showed that rooting volume did affect the development of *A. quinata* and *P. pandorana* over the six-month period of the experiment. Significant increases in percentage wall coverage, total biomass and leaf area of both plant species were observed in 42 L or 63 L rooting volume treatments as compared to those planted in 21 L rooting volume. Interestingly, the significant wall coverage response to greater rooting volumes was evident earlier for the faster growing species *P. pandorana* (after four-month growth) than the slower growing species, *A. quinata* (after six-month growth). In addition, the building wall area that these climbing plant species can cover when planted in to 1.0 m^3 rooting volume was identified. During the first growing season, *P. pandoran*a could provide 21.3 m^2 of wall coverage, and *A. quinata* could achieve 10.2 m^2 of wall coverage per cubic metre of rooting volume.

SLA of both climbing plant species increased as rooting volume increased; yet no significant difference was evident in shoot:root ratio of either species across all rooting volume treatments. However, there were no significant differences in plant growth and coverage of either climbing plant species planted in 42 L and 63 L rooting volumes. On the other hand, the greater rooting volume did not further assist either climbing plant species under heat stress condition. A significant decline in Fv/Fm observed in both climbing plants across all rooting volume treatments when exposed to high ambient air temperature and high solar radiance on the west-facing aspect in the late afternoon on a hot and dry summer day. Taken together, in this study, these results suggested that, for a wall area of 2.8 m^2, 42 L rooting volume could be enough to obtain normal plant growth and great wall coverage during the early stages of façade growth and performance. While it could be expected that shallow rooting volumes would restrict climbing plant growth in this study, the effects differed according to plant growth strategies. As both *A. quinata* (slower-growing) and *P. pandorana* (faster-growing) are commonly used climber species for green façades, their growth responses to different rooting volumes reflect different resource-use strategies, and are useful for assisting green façade design and plant selection for species with similar growth rates.

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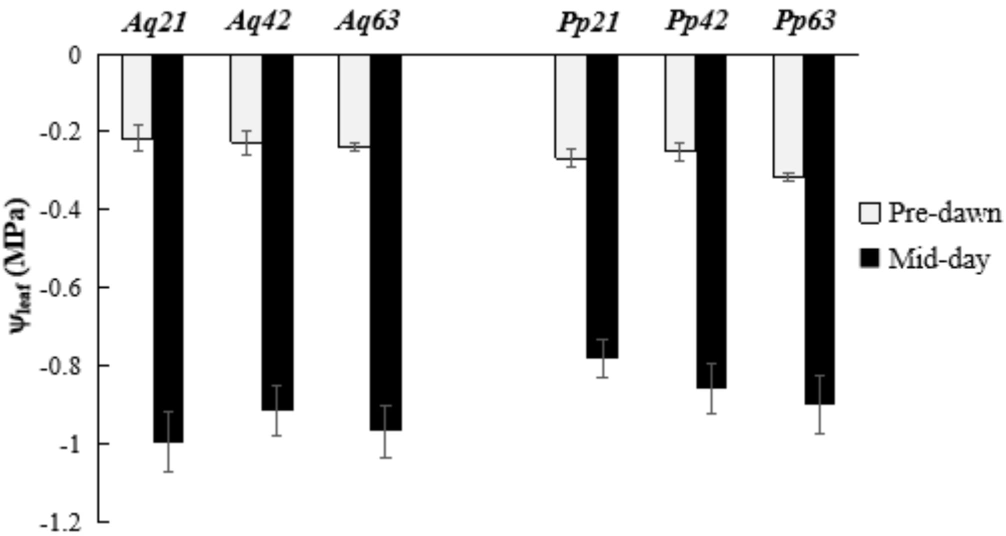
Appendices Chapter 2

Appendix 2.1. Pre-dawn and mid-day leaf water potential (ψ_{leaf}) of the *Akebia quinata* (*Aq*) and *Pandorea pandorana* (*Pp*) grown in three rooting volumes (21, 42, and 63 L). Healthy and young fully-expanded leaves were collected before dawn and at mid-day at the end of the experiment. Values are means ± SE (n=3-5).



3 - Greywater irrigation can support climbing plant growth on building green façades

This chapter has been submitted for publication as:

CHUNG, P.-W., LIVESLEY S.J., RAYNER J.P. & FARRELL C., Greywater irrigation can support climbing plant growth on building green façades. *Urban Landscape and Urban Greening*. (In revision following peer review by Urban Forestry & Urban Greening)

Abstract

Indirect green façades can help to cool cities and buildings best when they are irrigated to provide good canopy growth and transpiration cooling. Irrigating indirect green façades with greywater can reduce potable water demand and make use of building greywater supplies, however, greywater may negatively impact climbing plant growth through salt accumulation. We evaluated the effects of greywater irrigation on substrate properties (pH and EC) and the growth of six climbing plant species in an 18-week glasshouse experiment. Three irrigation treatments were applied to climbing plants: (1) potable water, (2) greywater, and (3) greywater flushed with potable water once every three weeks. Substrate pH did not differ among treatments, but substrate EC was the greatest when irrigated with greywater flushed with potable water once every three weeks. Greywater irrigation was not detrimental to climbing plant growth, demonstrating that domestic greywater could replace potable water irrigation of building indirect green façades in urban environments.

Introduction

Growth of urban population worldwide has led to land use changes and reduced vegetation cover (Dadashpoor et al., 2019), which have negatively impacted urban environments resulting in warming of the urban climate, high water and energy consumption, localised water and soil pollution and heat-related illness and mortality (Bambrick et al., 2011; Madlener and Sunak, 2011; Vörösmarty et al., 2000). To counteract these impacts, there has been greater focus on increasing vegetated area in our cities through urban greening, and vertical greening of buildings is one strategy to achieve this where ground space is limited (Köhler, 2008; Sheweka and Magdy, 2011). Green façades use climbing plants and vines to cover building walls and provide passive cooling (e.g., shading and evapotranspiration) to improve building energy saving (Hunter et al., 2014; Perez et al., 2011). For green façades to provide optimal cooling, it has been estimated that façade vegetation can require additional daily irrigation of up to 2.5 L m^{-2} (wall area) to maintain transpiration (Hoelscher et al., 2016). However, this is considered unsustainable as the availability of fresh or potable water for irrigation is of low priority due to urbanisation, particularly in water-scarce arid areas that are most in need of cooling benefits provided by urban greening (Shashua-Bar et al., 2009; Stikker, 1998). Growing urbanisation is also placing increased pressure on sewerage systems with a likely increase in wastewater discharge over coming decades' urbanisation (Uttara et al., 2012).

As the balance between potable water consumption and wastewater disposal becomes a crucial challenge worldwide (Seckler et al., 1999), alternative and sustainable water sources for urban irrigation are needed. On-site wastewater reuse shows promise as it tends to conserve valuable potable water sources whilst reducing its discharge to the environment and overall supply costs (Behzadian and Kapelan, 2015). High volumes of daily domestic greywater production (between 14 and 225 L per person) worldwide has resulted in the rapid development of greywater reuse in many countries (Boyjoo et al., 2013), including Australia (Jeppesen, 1996; Radcliffe, 2006), UK (Diaper et al., 2001), Germany (Nolde, 2000), Jordan (Faruqui and Al-Jayyousi, 2002), and Brazil (Ghisi and Ferreira, 2007). Greywater comes from bath tubs, showers, hand wash basins and laundry, and while it is of lower quality than drinking water, it is generally less polluted than blackwater owing to the absence of faecal contamination (Boyjoo et al., 2013). Hence, greywater is considered as an alternative resource for non-potable purposes such as toilet flushing, landscape irrigation, and crop production (Finley et al., 2009; Friedler, 2004; Nolde, 2000). However, greywater reuse can be associated with increased environmental risk of pollutant distribution into soils and water sources (e.g., nitrogen, phosphorus, chemicals, pathogens) (Gross et al., 2005). In dense urban areas, container-based green façades and green walls are among recent trends in greywater treatment, as the selected plants and substrates can contribute to pollutant removal from greywater (Masi et al., 2016; Prodanovic et al., 2017; Prodanovic et al., 2018; Prodanovic et al., 2019). Simultaneously, the plants in these systems are irrigated by greywater. Greywater may be an alternative irrigation source for green façades in the long-term, as green façades require sufficient water supply to sustain growth and provide effective cooling (Pradhan et al., 2019). Thus, greywater irrigation has potential to increase the applicability and sustainability of indirect green façades; but has not yet been widely used to support climbing plant growth and survival.

To date, in order to determine whether green façades/walls could become effective on-site greywater treatment solutions, research has only focused on the pollutant removal performance of plant species or substrates (Pradhan et al., 2019). For instance, green façade climbing plants (i.e., *Lonicera japonica*, *Pandorea jasminoides*, *Parthenocissus tricuspidata* and *Vitis vinifera*) (Fowdar et al., 2017) and green wall plants (i.e., *Carex appressa*, *Nadina obliterate, Myoporum parvifolium* and *Liriope muscari*) (Prodanovic et al., 2019), have been shown to effectively remove over 80% of nitrogen from greywater. Pollutant removal performance of substrates varies according to substrate permeability with greater removal from slower draining substrates (e.g., coir, rock wool and fyto-foam) compared to hydraulically faster substrates (e.g., perlite, growstone and river sand) (Prodanovic et al., 2017). However, while some mature climbing plants showed high pollutant removal performance in the Fowdar et al. (2017) study, it remains uncertain whether greywater treatment will adversely impact on the health and growth of climbing plants.

Research has shown that greywater irrigation reduces plant growth in trees (Nackley et al., 2015), shrubs (Bañón et al., 2011), and leafy vegetables (Lubbe et al., 2016) as a result of elevated pH and EC of greywater or changes in soil properties. In addition, the significant decrease in plant photosynthesis under greywater irrigation was reported in *Sequoia sempervirens* (Nackley et al., 2015). The increased soil electrical conductivity (EC) can lead to the reduced water and nutrient availability in the soil (Boyjoo et al., 2013), in turn reducing plant water uptake, decreasing photosynthesis, and eventually adversely affecting plant growth (Cabot et al., 2014). For green façades, these plant traits are associated with the passive cooling (Cameron et al., 2014; Yazdanseta, 2017), and therefore the cooling potential of green façades may be adversely affected by greywater irrigation. Therefore, we investigated whether greywater is a viable irrigation resource to grow climbing plant species during the early establishment phase of green façades by assessing changes in substrate chemical properties and the growth of six climbing plant species after 18-weeks of irrigation. We undertook a glasshouse experiment with six climbing plant species and examined changes in (1) substrate pH and EC as well as (2) plant biomass, morphology, physiology and water use over an 18-week experiment when plants were irrigated with (1) potable water irrigation, (2) greywater irrigation, and (3) greywater irrigation with a potable water ‘flushing’ once every three weeks (greywater & potable water).

Materials and Methods

Plant materials and growing conditions

This experiment was conducted in a glasshouse under controlled environmental conditions, with mean daily temperature ranging from 20ºC to 35ºC, average relative humidity 45% to 50%, at the Burnley Campus, University of Melbourne, Australia. This glasshouse experiment ran for 18 weeks from August to December (late winter to early summer) in 2017. The three irrigation treatments included in the 18-week glasshouse study were: (1) potable water irrigation, (2) greywater irrigation, and (3) greywater irrigation with a potable water 'flushing' once every three weeks (greywater & potable water). The synthetic greywater mix was utilised in this experiment to ensure that greywater quality remained consistent throughout the experiment. Synthetic greywater mix was formulated to conform with Australian office building and household bathroom wastewater, which does not involve laundry wastewater accrording to Christova-Boal et al. (1996). Bathroom greywater is considered 'light greywater', therefore the pH and EC of the synthetic greywater in my study were lower as compared to the general domestic greywater (Appendix 3.1). The synthetic greywater recipe in this experiment was modified based on the greywater recipe (Table 2 and Appendix 5 in Diaper et al., 2008) developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Australia) (Diaper et al., 2008; Table 3.1).

Six climbing plant species from various natural habitats were measured in this 18-week glasshouse study (Table 3.2): *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Trachelospermum jasminoides* (*Tj*), *Pandorea pandorana* (*Pp*), and *Vitis* 'Ganzin Glory' (*Vs*). In mid-June, 15 healthy and uniform seedlings of each species were purchased from a commercial nursery (Evergreen Nursery, South Silvan, Victoria, Australia), and each seedling was transplanted into a 4 L pot then grown-on for six-week establishment before the experiment commenced. During the six-week establishment period, all seedlings were irrigated daily with potable water, and slow release fertiliser (Macracote Coloniser Plus; NPK 15.4-3.5-8.9 + trace element) (Macracote Coloniser Plus, Langley Fertilizers, Western Australia, Australia) was applied at a rate 3 g L^{-1} each pot after transplantation. A scoria-based substrate was used in this experiment, which comprised of Scoria 7mm aggregate (20% v/v), Scoria 8 mm minus which includes fines (60% v/v), and horticultural grade coir (20% v/v). The substrate had a pH of 6.9 and an electrical conductivity of 140 µs cm^{-1} (Farrell et al., 2012). The water holding capacity and the bulk density of the substrate were 46 % and 1.26 g cm^{-3}, respectively (Farrell et al., 2013). The mineral-based scoria-based substrate used in this experiment was chosen due to its' widespread use in Australian containerized façades, which is due to the availability of components, consistency in composition, and the stability of physical and chemical properties (Cao et al., 2014; Growing Green Guide, 2008). The experiment was a fully-randomized design, and each treatment had five replicates of each species.

Water samples (two potable water and four synthetic greywater) were sent to TrACEES (Trace Analysis for Chemical, Earth and Environmental Science, University of Melbourne, Australia) for analysis. The general water qualities of potable water and synthetic greywater are given in Table 3.3. All pots were hand-watered twice a week (Mondays and Thursdays) from the 1^{st} week to the 9^{th} week, and three times a week (Mondays, Wednesdays and Fridays) from the 10^{th} week to the 18^{th} week; with 1 L water supplied each time.

Table 3.1. Synthetic greywater recipe (formulated to conform to office building/household bathroom wastewater).

Ingredients used for synthetic greywater	
Ingredients (Product used)	Concentration (mg L^{-1})
Moisturiser (Dove)	10
Toothpaste (Colgate Cavity Protection)	32.5
Deodorant (Mum)	10
Hand wash (Palmolive Softwash)	720
Vegetable oil (Coles Own brand)	7
Antifoam (Silfoam SEA39)	0.075 (ml L^{-1})
Sodium hydrogen carbonate ($NaHCO_3$)	25
Urea	7.5

Table 3.2. The six climbing plant species (and abbreviations) used in this experiment and their origin, climate, habitat, leaf habit, climbing mechanism, growth rate, mature height and time to reach maturity.

Species	Family	Origin/ climate	Habitat	Leaf habit	Climbing mechanism	Growth rate	Mature height / time to achieve the mature height
Akebia quinata (*Aq*)	Lardizabalaceae[2,3]	East Asia[2,3]/ temperate climate[5]	Forest margins and mountain slopes[3]	Deciduous[2,3]	Stem twining[2]	Medium[2]	12 m / 5-10 years[2,3]
Gelsemium sempervirens (*Gs*)	Loganiaceae[2,3]	South-eastern parts of North America[2,3]/ temperate climate[5]	Thickets, woodlands, and pine forests[2,3]	Evergreen[2,3]	Stem twining[2]	Medium[2]	6 m / 5-10 years[2]
Jasminum azoricum (*Ja*)	Oleaceae[2]	Madeira Island[3]/ Mediterranean climate[5]	Coastal area[3]	Evergreen[2]	Stem twining[2]	Fast[2]	4 m / 5-10 years[2]
Trachelospermum jasminoides (*Tj*)	Apocynaceae[2]	East and southeast Asia[2,3]/subtropical and temperate climate[4,5]	Sunny edges of forests, and brushwoods (at elevation from 200-1300 m)[2]	Evergreen[2]	Stem twining[2]	Medium[2]	8-15 m / 10-20 years[2]
Vitis ‘Ganzin Glory’ (*Vs*)	Vitaceae[2]	France[1]/ temperate climate[5]	N/A[a]	Deciduous[2]	Tendrils[2]	Fast[2]	15-20 m / 10 years[2]

Pandorea pandorana (*Pp*)	Bignoniaceae[2,4]	QLD, NSW, VIC, SA, WA, Lord Howe[4]/ temperate climate[4,5]	Rainforest, dry sclerophyll forest, dry scrub and rocky outcrops in arid regions[4]	Evergreen[2,4]	Stem twining[2]	Fast[2]	20 m / 5-10 years[2,4]

[a] *Vitis* 'Ganzin Glory' (*Vs*) was the cultivar name for the ornamental vine derived from the cross *Vitis vinifera* x *V. rupestris*, no natural habitat.

References of Table 3.2.

[1]Armitage, A.M. 2010, *Armitage's Vines And Climbers: A Gardener's Guide To The Best Vertical Pla*nts, Timber Press, Portland.

[2]Dunnett, N. & Kingsbury, N. 2008, *Planting Green Roofs And Living Walls*, Timber Press, Portland.

[3]Harrison, R.E. 1973, *Climbers And Trailers*, A. H. & A. W. Reed, Sydney.

[4]Jones, D. & Gray, B. 1988, *Climbing Plants In Australia: With Emphasis On Australian Native Species*, Reed Books, N.S.W., Australia.

[5]Peel, M.C., Finlayson, B.L. & Mcmahon, T.A. 2007, 'Updated world map of the Köppen-Geiger climate classification', *Hydrology and Earth System Sciences*, vol. 11, no. 5, pp.1633-1644.

Table 3.3. Key chemical properties of greywater and potable water used to irrigate the six climbing plant species in the 18-week glasshouse experiment.

Water Quality Characteristics					
	pH	EC ($\mu s\ cm^{-1}$)	P-Phosphate ($mg\ L^{-1}$)	TC ($mg\ L^{-1}$)	TN ($mg\ L^{-1}$)
Greywater	7.5 ± 0.1	138.0 ± 3.0	1.0 ± 0.1	48.2 ± 0.2	5.6 ± 0.1
Potable water	7.1 ± 0.1	63.2 ± 1.4	< 0.2	3.5 ± 0.1	0.4 ± 0.1

Substrate pH and EC

Substrate samples were collected from each pot at the end of experiment after final harvest. These were air dried and sieved through 2 mm sieve to remove non-soil material, and were prepared for analysis using the 1:5 soil:water suspension method, according to procedures described by the Australian Standard (AS 4419) (Standards Australia Committee, 2003). Substrate pH and electrical conductivity (EC) were measured by a benchtop Conductivity-TDS-pH-mV Meter (labCHEM-CP, TPS, Queensland, Australia).

Plant biomass and leaf area measurements

Plants in all treatments were harvested at the end of the irrigation experiment (18 weeks), and separated into leaves, stems and roots and oven dried (80°C) for a week to obtain dry total biomass. The projected leaf area (one side of the leaves) was measured using a LI-3100C Area Meter (LI-COR, Lincoln, NE, USA).

Photosynthesis and stomatal conductance

Leaf photosynthesis and stomatal conductance were measured using a portable photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA) between 8:30 am and 12:00 pm on the 7th December 2017. Four measurements were taken on a fully expanded mature leaf at saturated light intensity of 1500 µmol m^{-2} s^{-1}, with an air flow rate of 500 µmol s^{-1} and at a CO_2 concentration of 400 ppm. Leaves measured on four individuals per species for each treatment.

Plant water use

Daily water use (evapotranspiration) of plants is the water loss from plant transpiration and substrate evaporation (Yeager, 2003), and this was determined by measuring the loss of water from the pots. Net amount of irrigation water retained in soil during a watering event was measured by weighing each pot before irrigation and 2 hours after irrigation (after drainage ceased) with an electronic balance of 35 kg capacity (± 0.01 g) (Adam Equipment PTY Ltd, Perth, WA, Australia). Daily water use was measured and calculated in the week 18th on a pot basis before final harvest, and also expressed on a leaf area basis using measured leaf area at the final harvest.

Statistical analyses

Inter-treatment differences in the substrate chemical properties (pH and EC) were tested by one-way analysis of variance (ANOVA). The effect of greywater irrigation on climbing plant biomass, shoot:root ratio, photosynthetic rate, stomatal conductance, and plant daily water use were analysed using two-way analysis of variance (ANOVA). Significant differences were determined by Tukey’s post hoc test ($p < 0.05$) to assess the differences among pairs of treatment means as well as the interactions with plant performance. All data analyses were performed in GenStat 16 (2013, VSN International Ltd., England, UK).

Results

Substrate chemical properties

There was no significant increase in substrate pH with greywater irrigation treatments (greywater and greywater & potable water) after 18 weeks (Fig. 3.1A). Substrate pH values ranged from 5.8 to 6.3 across three irrigation treatments at the end of this experiment. However, there was a significant increase (+39%) in average substrate EC value in pots with the greywater & potable treatment (154.6 µs cm^{-1}) as compared to potable water irrigation (111.1 µs cm^{-1}; $p < 0.001$; Fig. 3.1B).

Climbing plant growth and biomass allocation

Greywater irrigation treatments (greywater and greywater & potable water) did not significantly reduce the growth of the six climbing plant species (Fig. 3.2). However, unsurprisingly there were significant species differences ($p < 0.001$) in total biomass, shoot:root ratio and total leaf area. *P. pandorana* had the greatest total biomass, and *A. quinata*, *J. azoricum* and *T. jasminoides* produced the least total biomass regardless of irrigation treatments ($p < 0.001$; Fig. 3.2A). With respect to shoot:root allocation, *G. sempervirens* allocated a greater proportion of total biomass to shoots than the other five climbing plant species regardless of irrigation treatments (Fig. 3.2B). The greatest leaf area was produced by *V.* 'Ganzin Glory' as compared with the other five climbing plant species regardless of irrigation treatments ($p < 0.001$; Fig 3.2C). Interestingly, *V.* 'Ganzin Glory' developed greater (but not significant) leaf area when in the greywater & potable irrigation as compared to greywater only (+18%) or potable water (+49%).

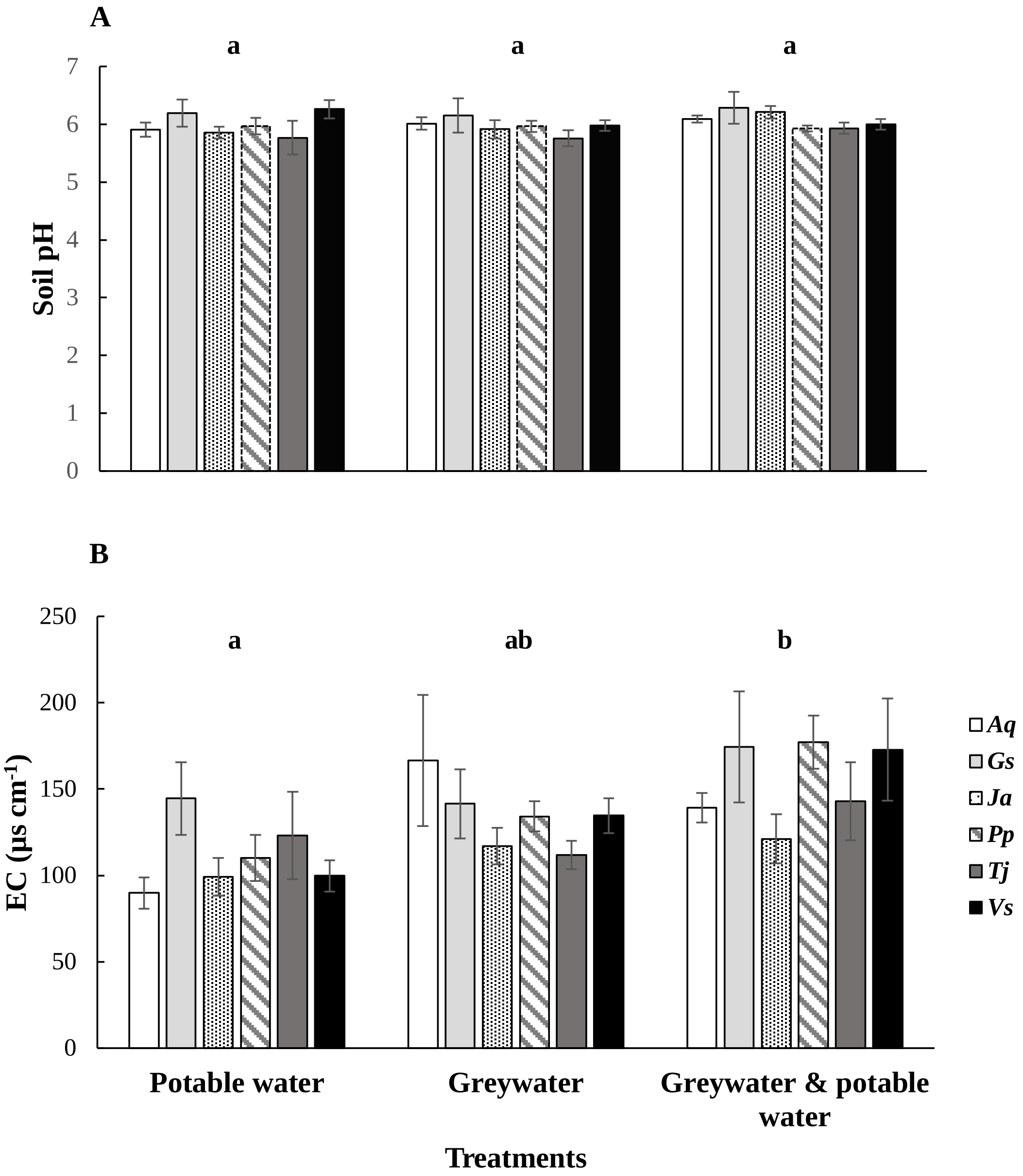


Figure 3.1. Effects of three irrigation treatments on substrate pH (A) and electrical conductivity (EC) (B) at the end of 18 weeks after final plant harvest. Substrates collected from each pot containing a climbing plant of six climbing plant species: *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Pandorea pandorana* (*Pp*), *Trachelospermum jasminoides* (*Tj*), and *Vitis* 'Ganzin Glory' (*Vs*). Different letters represent significant differences among treatments, $p < 0.001$. Values are means ± SE, n=5.

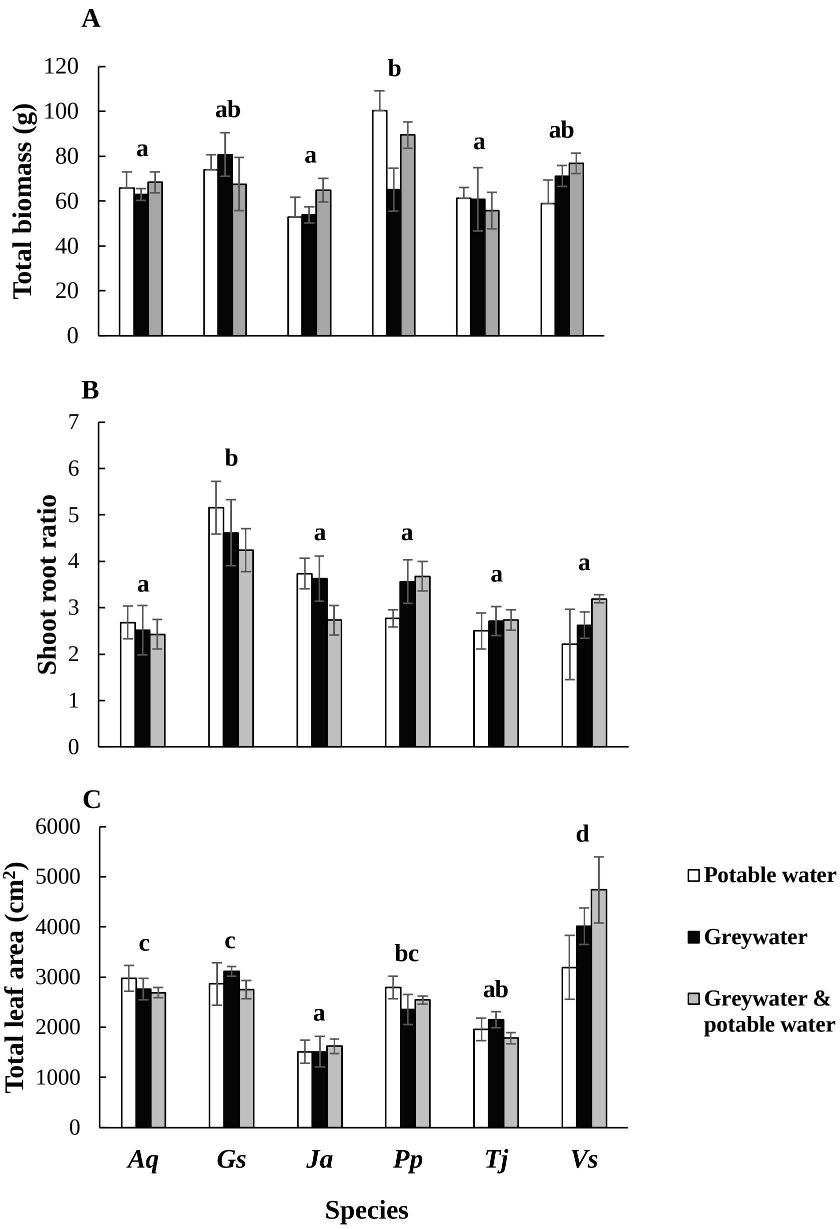


Figure 3.2. Greywater irrigation treatments (greywater and greywater & potable water) did not significantly affect total biomass, shoot:root ratio, and total leaf area of the six climbing plant species: *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Pandorea pandorana* (*Pp*), *Trachelospermum jasminoides* (*Tj*), and *Vitis* 'Ganzin Glory' (*Vs*). However, there were significant species differences ($p < 0.001$) in average total biomass (A), shoot root ratio (B) and total leaf area (C) of six climbing plant species grown under different irrigation treatments after 18 weeks. Different letters represent significant differences among species, $p < 0.001$. Values are means ± SE, n=5.

Photosynthesis and stomatal conductance

Greywater irrigation treatments (greywater and greywater & potable water) did not significantly reduce gas exchange rates of the six climbing plant species (Fig. 3.3). Significant species differences ($p < 0.001$) were evident in photosynthesis and stomatal conductance. *G. sempervirens* had the lowest photosynthetic rate and stomatal conductance among the six climbing plant species across all three irrigation treatments.

Water use of climbing plants

In the week 18th, total daily water use and daily water use per unit leaf area of the six climbing plant species did not differ among the three irrigation treatments (Fig. 3.4). However, total daily water use and daily water use per unit leaf area differed significantly among the six species ($p < 0.001$). In the week 18th, *P. pandorana* used the greatest amount of water and *G. sempervirens* used the least amount of water per day (Fig. 3.4A). On a leaf area basis, *J. azoricum* used the most water and *G. semepervirens* used the least (Fig. 3.4B).

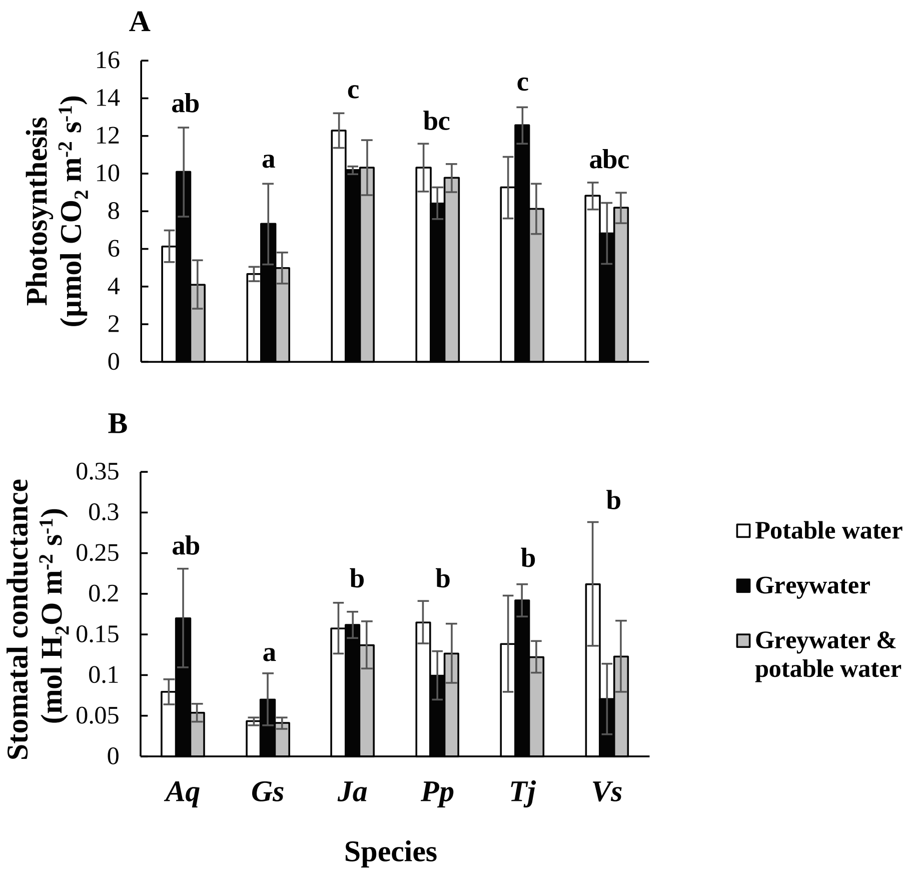


Figure 3.3. Greywater irrigation treatments (greywater and greywater & potable water) did not significantly affect photosynthesis and stomatal conductance of the six climbing plant species: *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Pandorea pandorana* (*Pp*), *Trachelospermum jasminoides* (*Tj*), and *Vitis* 'Ganzin Glory' (*Vs*). However, there were significant species differences ($p < 0.001$) in photosynthesis (A) and stomatal conductance (B) of six climbing plant species under different irrigation treatments after 18 weeks. Different letters represent significant differences among species, $p < 0.001$. Values are means ± SE, n=5.

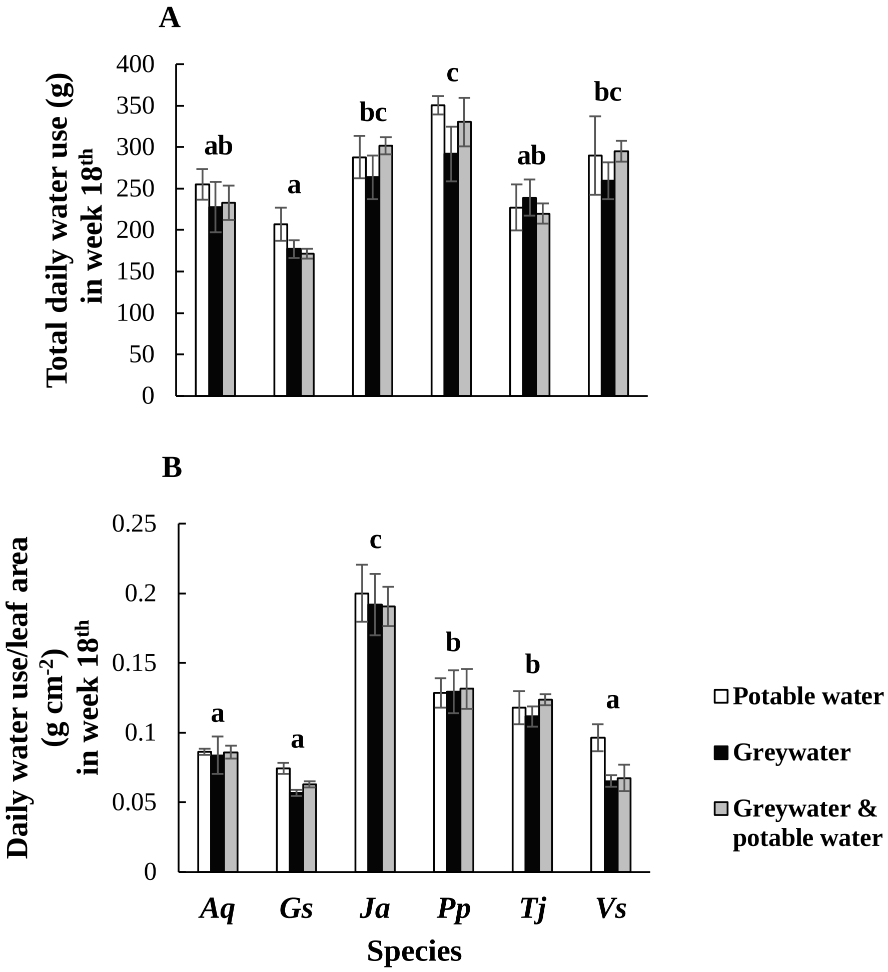


Figure 3.4. Greywater irrigation treatments (greywater and greywater & potable water) did not significantly affect total daily water use and daily water use per unit leaf area of the six climbing plant species: *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Pandorea pandorana* (*Pp*), *Trachelospermum jasminoides* (*Tj*), and *Vitis* 'Ganzin Glory' (*Vs*). However, there were significant species differences ($p < 0.001$) in average total daily water use (A) and average daily water use per unit leaf area (B) of six climbing plant species under different irrigation treatments after 18 weeks. Different letters represent significant differences among species, $p < 0.001$. Values are means ± SE, n=5.

Discussion

We investigated whether greywater is a viable irrigation resource to grow climbing plant species during the early establishment phase of green façades by assessing changes in substrate chemical properties and the growth of six climbing plant species after 18-weeks of irrigation. In terms of substrate properties, substrate pH did not change during this study which agrees with previous studies of domestic greywater-irrigated substrates (Agra et al., 2018; Mzini and Winter, 2015). After one year of greywater irrigation, Agra et al. (2018) showed that the pH of two different green roof substrates, coal ash or perlite based (80% v/v), planted with *Phyla nodiflora* or *Convolvulus mauritanicus*, were not significantly affected (Agra et al., 2018). Similarly, the pH value of a sandy loam soil planted with five different vegetables (cabbage, onion, spinach, beetroot, and lettuce) remained unchanged when irrigated with greywater after four growing seasons (Mzini and Winter, 2015). However, Pinto et al. (2010) did measure an increase in substrate pH with laundry greywater irrigation after 60 days. Research indicates that the pH of laundry greywater ranges between 8 to 10, which is higher than that of other domestic greywaters that typically range between 5 to 8.7 (e.g., hand basin and shower) (Eriksson et al., 2002). The synthetic greywater used in our study was designed to mimic greywater generated by domestic hand basins and showers, and was not highly alkaline (pH was 7.5), and this may explain why there was no increase in substrate pH after 18 weeks in our study. However, substrate EC in our study did increase when irrigated with greywater flushed with potable water once every three weeks. This unexpected outcome is contrary to previous studies which have suggested that irrigating alternately with greywater and potable water or rainwater can moderate any adverse impact on soil chemistry (Faruqui and Al-Jayyousi, 2002; Pinto et al., 2010). It should be noted that the greywater-irrigated substrates in our study remained within the recommended range of 0 - 1500 µs cm^{-1} for growth of most plant types (Bernstein, 1975). As such, the synthetic greywater used in our study did not lead to an excessive or deleterious change in substrate salinity. These findings do however highlight the need to understand the source of greywater for irrigation as this can influence the subsequent greywater characteristics, composition and concentration of chemicals.

In terms of plant growth, the total biomass of each climbing plant species did not differ significantly across the three irrigation treatments. This is consistent with previous research that has shown domestic greywater irrigation does not decrease the total biomass of various edible plants, including silverbeets (Pinto et al., 2010), carrots, peppers and lettuce (Finley et al., 2009; Travis et al., 2010). In comparison, other studies have shown that domestic greywater irrigation can lead to either a reduction in plant biomass growth in response to increased soil EC/salinity (Boland et al., 1993; Lubbe et al., 2016), or an increase in plant biomass growth as compared to potable water (Reichman and Wightwick, 2013; Rodda et al., 2011). Again, this implies that plant growth may or may not decrease when irrigated with greywater, depending on the plant species, soil/substrate used, as well as greywater quality and source (Travis et al., 2010). In our study, the EC of synthetic greywater was 138 µs cm^{-1}, but there was no accumulation of salts in the coarse mineral-based substrate used in our study as it was fast-draining with a low cation exchange capacity (Collison and Grismer, 2014; Prodanovic et al., 2017). In addition, the presence of coarse scoria particles (7-8 mm) in the substrate would increase soil porosity, hydraulic conductivity and drainage, therefore it is likely to prevent salt accumulation with greywater irrigation (Da Silva et al., 1993; Raviv et al., 2002; Wallach et al., 1992). Despite the coarse mineral-based substrate used in this study, it should be noted that all the climbing plants were well-watered throughout the experiment and did not show signs of water deficit (as indicated by high leaf water potentials – Appendix 3.2).

The morphological traits, shoot:root ratio and leaf area, were not affected by greywater irrigation in this study. This is beneficial as façade climbing plants need to maintain great canopy cover to provide shade and evapotranspiration for effective cooling (Cameron et al., 2014; Hoelscher et al., 2016). In comparison, laundry greywater-irrigated tomatoes (*Lycopersicon esculentum* Mill. cv. Grosse Lisse) showed unchanged biomass allocation but had significantly higher leaf area (Misra et al., 2010), while increases in shoot:root ratio and leaf area were reported in container-grown Laurustinus (*Viburnum tinus* ‘French White’) and Weigela (*Weigela florida* ‘Bouquet Rose’) irrigated with greywater (Gori et al., 2000). However, in both the abovementioned studies the greywater EC was 635-655 µs cm^{-1}, which is much higher than the EC in this study (138 µs cm^{-1}). Thus, the unaffected biomass allocation and leaf area in our results could be attributed to low salinity of the synthetic greywater used. On the other hand, reduced leaf area was recorded in *Amaranthus dubius* and *Solanum nigrum* that were irrigated with greywater leading to a significant increase in soil EC (from 130 to 270 µs cm^{-1}) (Lubbe et al., 2016). However, the low salinity of the scoria-based substrate at the end of our study (after 18-weeks greywater irrigation) explains why there was no deleterious effect in leaf area for any of the species. As, total dry mass and biomass allocation can indicate overall plant health (Abrahamson and Caswell, 1982; Gedroc et al., 1996), we could therefore assume that the synthetic greywater in our study did not adversely affect climbing plant growth.

In this experiment, the physiological traits, i.e. gas exchange rates, were unaffected by greywater irrigation. Other research showed that photosynthesis and stomatal conductance of *A. dubius* and S. nigrum were not affected by greywater treatments even with an increase in soil EC (Lubbe et al., 2016). In this study, it was assumed that the greywater-induced increase in soil EC did not cause salinity stress in both plant species, which contributed to the unaffected gas exchange rates (Lubbe et al., 2016). Therefore, this may explain that the differences of gas exchange rates among irrigation treatments were non-significant in our study. This further supports plant growth responses to greywater irrigation in our study since gas exchange accounts for plant growth and development that determines biomass production.

In the final week of the experiment, after 18 weeks of irrigation there were no differences in total daily water use and daily water use per unit leaf area of six climbing plant species under three irrigation treatments. This result supports other studies where the water use of silverbeets (Pinto et al., 2010) and tomatoes (*Lycopersicon esculentum* Mill. cv. Grosse Lisse) (Misra et al., 2010) was unaffected by domestic greywater irrigation over short periods (eight to nine weeks). Whilst our study period was much longer, the substrate EC with greywater irrigation remained low at the end of the 18 week, and this would not hinder plant water uptake (Kotuby-Amacher et al., 2000). We have attributed this to the substrate properties in our experiment but it may also be due to the comparatively short experimental period of our study that there were non-significant effects of greywater on plant daily water use.

Our 18-week timeline is designed to investigate whether greywater can successfully support the growth of climbing plant species during the early establishment phase of green façades. Our results showed that greywater treatments did not detrimentally change the substrate properties (pH and EC) as salts did not accumulate in the coarse mineral-based substrate after 18-week greywater irrigation treatments. However, in this study, only one type of substrate (fast-draining) was used to grow six climbing plant species for 18 weeks, and the synthetic greywater was formulated to conform to Australian office building and household bathroom wastewater (Diaper et al., 2008), which is considered 'light greywater' (Boyjoo et al., 2013). Although the synthetic greywater in our study might be relatively simple and less variable compared with the composition and quality of greywater, synthetic greywater application is a common approach in plant studies and helps to generalise the results to different building applications in different regions and climates (Fowdar et al., 2017; Lubbe et al., 2016; Pinto et al., 2010; Prodanovic et al., 2020). We acknowledge that further research should be undertaken to investigate the impact of different types of greywater and substrates (e.g., slow-draining) on the growth and performance of green façade climbing plants. It would also be beneficial to evaluate the effects of greywater irrigation on green façades planted on buildings where the greywater is directly sourced from the building occupants. This would enable testing of plant growth and performance in a situation where water quality is variable and more realistic.

Conclusions

This study revealed that for six climbing plant species examined, greywater and alternate (greywater & potable water) irrigation treatments had no significant, detrimental effects on substrate chemical properties (pH and EC), plant growth (biomass, shoot root allocation and leaf area) and daily water use after 18 weeks. These results suggest that domestic greywater has potential for reuse as an irrigation source for green façades, which could potentially reduce the demand of potable water. Therefore, our study has implications for the expansion of green façades in urban areas as a climate mitigation and water sensitive strategy. However, further long-term research, beyond establishment, is needed to determine if domestic greywater will adversely affect morphological and physiological traits of green façade climbing plants as well as substrate properties.

Acknowledgements

We are grateful to the research scholarship Betty Elliott Horticulture Scholarship for funding this study. We thank Peter Somerville, Pengzhen Du and Zheng Zhang for assistance in harvesting plants, and assiatance from Audrey Michenaud-Rague through the entire experimental period. We also thank Burnley nursery staff Nick Osborne and Sascha Andrusiak for the technical assistance. Pei-Wen was supported by the postgraduate scholarship from The University of Melbourne.

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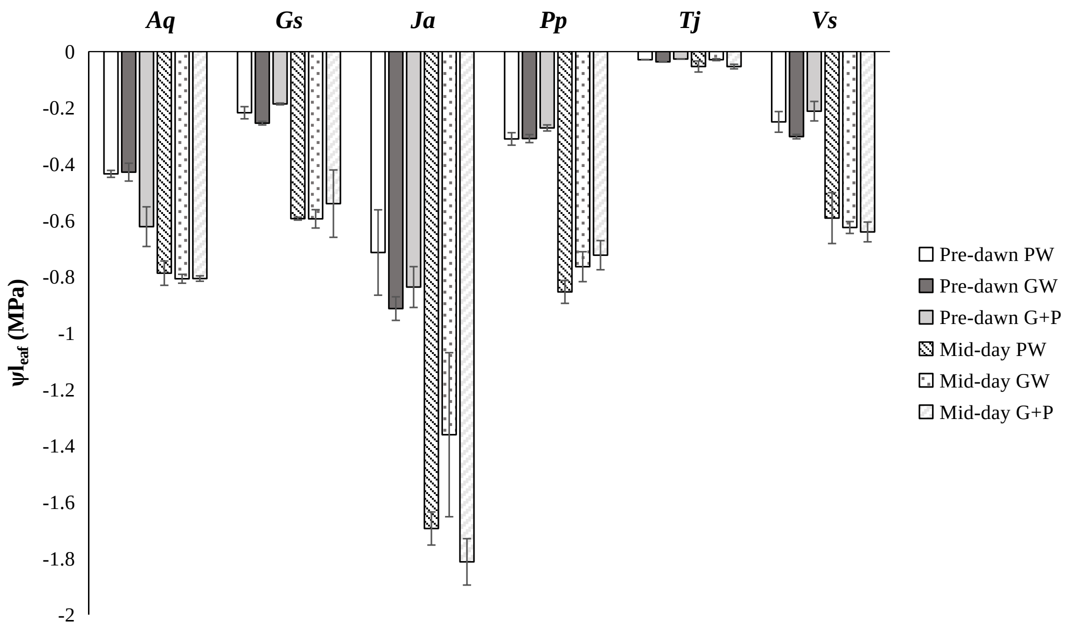
Appendices Chapter 3

Appendix 3.1. Comparison of water quality characteristics between synthetic greywater used in this study and bathroom greywater in Christova-Boal et al. (1996) study.

Water Quality Characteristics					
	pH	EC (µs cm^{-1})	P-Phosphate (mg L^{-1})	TC (mg L^{-1})	TN (mg L^{-1})
Synthetic greywater (average)	7.5 ± 0.1	138.0 ± 3.0	1.0 ± 0.1	48.2 ± 0.2	5.6 ± 0.1
Bathroom greywater (range) (Christova-Boal et al., 1996)	6.4 - 8.1	82 - 250	0.11 - 1.8	N/A	4.6 - 20

Appendix 3.2. Pre-dawn and mid-day leaf water potential (ψ_{leaf}) of the six climbing plant species: *Akebia quinata* (*Aq*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Pandorea pandorana* (*Pp*), *Trachelospermum jasminoides* (*Tj*), and *Vitis* 'Ganzin Glory' (*Vs*). Healthy and young fully-expanded leaves were collected before dawn and at mid-day after the 18-week treatment. Values are means ± SE (n=3-5).



Green façade climbing plant species can maintain their morphological and physiological traits under changing light conditions

Abstract

Indirect green façades can provide ecosystem service benefits (e.g., cooling, biodiversity, amenity) in dense urban areas as they can cover vertical built surfaces, offering shade and reducing reflectivity of wall surfaces, and bearing greenery where ground space is limited. However, as urban form is constantly changes with development and redevelopment, resulting in changing light availability, growth and stress resilience of green façade climbing plants to both full-sun and shaded conditions will be key to maintaining building cover. While there is little research on how façades respond to increased shade, based on research in forests we hypothesised that (1) a change from sun to shade will increase specific leaf area (SLA), leaf expansion rate, and chlorophyll content of climbing plants; whilst decreasing leaf photosynthetic rate (A_{1500}), stomatal conductance (g_s), light compensation point (LCP) and thermal tolerance (T_{50}), and (2) deciduous climbing plant species will be more plastic (greater changes in morphology and physiology) in response to shade than evergreen species.

To test our hypotheses, we evaluated trait responses of seven climbing species to changing light levels from full-sun to shade in a 15-week field experiment with mature plants. Traits measured, included leaf morphology (SLA, leaf expansion rate), physiology (A_{1500}, g_s, LCP, Chl a+b), and thermal tolerance (T_{50}). We also assessed differences in trait response to shade between deciduous and evergreen climbing plants.

All seven climbing species responded similarly to shade by significantly increasing SLA; whilst g_s, LCPs and T_{50} remained unchanged between full-sun and shaded conditions. However, only two evergreen climbing species (*Gelsemium sempervirens* and *Jasminum azoricum*) significantly increased leaf expansion rates in response to shade. Moreover, deciduous *Ampelopsis brevipedunculata* and *Vitis* 'Ganzin Glory' as well as evergreen *Pandorea pandorana* significantly decreased leaf A_{1500} under the shade treatment. The shade treatment significantly increased leaf Chl a+b in most of the climbing plant species, except for deciduous *V.* 'Ganzin Glory' and evergreen *G. sempervirens*.

Overall, deciduous climbing plant species exhibited significantly greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl a+b, while lower LCP than evergreen species regardless of light treatments in this study. Contrarily, in response to shade, deciduous and evergreen climbing plant species did not differ in plasticity; however, deciduous species had greater reduction in A_{1500} than evergreen species. Our results suggested that all seven climbing species in this study can maintain growth and physiological function when exposed to changing light conditions from full-sun to shade, and are likely to maintain building wall coverage and delivery of ecosystem service benefits in cities. This finding improves our understanding of plant selection for indirect green façades in cities with changing light conditions.

Introduction

Indirect green façades use climbing plants to cover building walls, offering a flexible and adaptable approach to increase vegetation covers in cities (Perini et al., 2011). Indirect green façades are relevant to cities where building walls comprise a large proportion of the surface area, reducing opportunities for urban greening at ground level (Abdullahi and Alibaba, 2016; Besir and Cuce, 2018). Indirect green façades can be retrofitted onto existing building walls or integrated into new built constructions to provide ecosystem service benefits, including the improvement of physical and psychological well-being of urban residents (Rakhshandehroo et al., 2015), aesthetical enhancement (Başdoğan and Çığ, 2016; Melo et al., 2020), urban biodiversity provision (Collins et al., 2017), air pollution mitigation (Farid et al., 2016), noise reduction (Pérez et al., 2016), and amelioration of the urban heat-island effect (Pigliautile et al., 2020; Sheweka and Magdy, 2011). However, the potential benefits of indirect green façades depend upon good plant coverage of wall surfaces, which relates to appropriate plant selection, the design of supporting structures, and the availability of essential resources to promote plant growth, including water and nutrients below ground, and access to sunlight (solar radiation) aboveground (Ottelé et al., 2011).

Indirect green façades typically involve herbaceous or woody climbing plants (lianas) and vines (Köhler, 2008), which are selected according to their tolerance of on-site conditions of radiation exposure (e.g., full sun, semi-shade or shade) and weather conditions (e.g., wind, rainfall, heat, drought and frost) (Growing Green Guide, 2014; Fernández-Cañero et al., 2018). However, in many cities, urban populations are increasing in number, often through a policy of urban densification such that continual processes of construction and redevelopment results in a dynamic and constantly changing built urban form (Dadashpoor et al., 2019; Uttara et al., 2012). This can mean that green façades designed for a certain site conditions, including aspect exposure to weather and sunlight conditions; may be negatively impacted by a change in these conditions, as existing climbing plants may fail to adapt to the new environment and to maintain their growth and performance. In terms of light conditions, newly constructed buildings may change the growing conditions that an existing green façade experiences from full sun to shade. These conditions are likely to affect growth and morphological traits such as leaf area and expansion due to changes in photosynthesis, however, heavy shade may also reduce leaf tolerance to high ambient air temperatures that occur during summer heatwaves

(Santamouris, 2020). Therefore, it is important to determine how climbing plants will adjust to changed light conditions to maintain ecosystem provision in dynamic cities.

Research to date has not yet investigated the influence of light intensity on the growth of climbing plant of green façades in cities. However, research in forest ecosystems has indicated that some woody plants can adjust to changing light conditions through plasticity, in morphology, physiology and leaf biochemistry (Lichtenthaler et al., 1981; Valladares et al., 2011; Zhu and Cao, 2010). Shade conditions can impose stress on plants because the low light intensities adversely affect physiological activities such as photosynthesis and stomatal conductance (Givnish, 1988; Lambers et al., 2008). To adjust to shaded conditions, plants tend to produce thinner leaves with fewer chloroplasts for light capture in shady environments as compared with full sun conditions (Lambers et al., 2008). For instance, the morphological trait, specific leaf area (SLA), is negatively correlated to light intensity in most mature woody plant species (Jarvis, 1964; Niinemets, 2001), which enables efficient light interception and use by plants when sunlight is limited to maintain growth and a positive carbon budget (Wright et al., 2004). Some plants are able to photosynthesise more effectively at low light levels by lowering their light compensation points (LCPs) in shade (Slot et al., 2018; Sterck et al., 2013). Plants tend to produce thinner leaves with fewer chloroplasts for light capture in shady environments as compared with full sun conditions (Lambers et al., 2008). Hence, to enhance lost light capture capacity under shade conditions leaf biochemical traits can change and adapt, by increasing chlorophyll concentrations per unit dry leaf mass (Lambers et al., 2008).

Biochemical changes in chlorophyll content in response to shade may also affect the thermal tolerance of green façades to heatwaves (Allakhverdiev et al., 2008). As global warming can exacerbate warmer temperatures associated with the urban heat island in large urban centers (Holderness et al., 2013), urban plantings are likely to be exposed to higher ambient air temperatures along with increases in the frequency and intensity of heat waves (Gregg et al., 2003; Kim et al., 2020). Elevated air temperatures can suppress plant biomass growth (Bauweraerts et al., 2013; MacAlister et al., 2020) and photosynthesis (Allakhverdiev et al., 2008; Tan et al., 2020). However, shade leaves may downregulate their thermal tolerance as it would be costly to maintain high thermal tolerance (Wahid et al., 2007). Hence, climbing plants that develop under shade conditions may be more vulnerable to heat stress during hot summer periods. In order for indirect green façades to continue supplying the many ecosystem services they provide, it is crucial that they maintain good wall cover with healthy foliage that can withstand heat stresses in cities (Cameron et al., 2014).

The ability of a climbing plant to continue healthy growth and coverage under shade conditions, as well as tolerate high air temperature events in summer, will obviously vary from species to species, but may also differ significantly between deciduous and evergreen plant types, because of distinct differences in their leaf structural traits (Böhnke and Bruelheide, 2013). Evergreen woody plants exhibit resource-conservative leaf traits, while deciduous species demonstrate an acquisitive resource-use strategy (Álvarez-Yépiz et al., 2017; Zhao et al., 2017). Compared with deciduous species, evergreen woody plants generally have thicker and/or denser leaves (lower SLA) with lower photosynthetic capacity and respiration rates per unit leaf mass, as well as lower nitrogen contents, greater leaf durability, and longer leaf lifespan (Wright et al., 2004; Zhao et al., 2017). Regarding plasticity to changing light levels, the conservative resource-use strategy of evergreen climbing plants could be a disadvantage as evergreen trees leaves show little plasticity to changing light levels (Valladares et al., 2000). On the other hand, it could be assumed that deciduous woody plants will exhibit greater plasticity in their response to a change in light levels than evergreen species (Böhnke and Bruelheide, 2013; Lusk et al., 2008).

As it is unknown how woody climbing plants will respond to changing light conditions from full sun to shade in urban areas where building constructions can rapidly change light growth environments. As they may also experience extreme air temperature in cities (Taha, 1997), it is important to understand the shading effects on leaf traits and thermal tolerance of climbing plant species to gain a comprehensive perspective in green façade design. Therefore, this study aims to test two hypotheses: (1) a change from sun to shade will increase climbing plant SLA, leaf expansion rate, and Chl *a*+*b*; whilst decreasing leaf photosynthetic rate (A_{1500}), stomatal conductance (g_s), light compensation point (LCP) and thermal tolerance (T_{50}), and (2) deciduous climbing plant species will be more plastic (greater changes in morphology and physiology) in their response to shade than evergreen climbing plant species.

Materials and Methods

Plant materials and experimental design

This study was undertaken in the Field Station at the University of Melbourne, Burnley campus, from mid December 2018 to end of March 2019, approximately 15 weeks. Existing green façade with seven climbing plant species across different habitats were ustilised in this study, including three deciduous climbing plant species (*Ampelopsis brevipedunculata*, *Akebia quinata*, *Vitis* 'Ganzin Glory'), and four evergreen species (*Gelsemium sempervirens*, *Jasminum azoricum*, *Trachelospermum jasminoides*, *Pandorea pandorana*) (Table 4.1). Light requirements of the seven climber species range from full sun to semi-shade (Table 4.1), as the seven species were originally selected for growing under full-sun conditions and we were interested in how these species which established under full sun conditions would then adapt to lower light conditions. Seventy seedlings were transplanted into 90 L pots in March 2013, and randomly placed on five east/west-orientated freestanding trellis rows in the Field Station. Each trellis was constructed of fourteen sections of reinforcing steel mesh (1500 mm (W) by

1950 mm (H); mesh size: 210 mm x 210 mm). We lost one *A. quinata* before the commencement of this experiment.

In December 2019, in each row of climbing plants, two layers of neutral density white shade cloth (70%) were placed over half a row of the freestanding trellis to shade one replicate of each of the seven climbing plant species (Fig. 4.1). All climbing plants were tip pruned before installing shade cloths. Five star pickets were placed 1.5 m from freestanding trellis and triangle support brackets were installed at the top of the freestanding trellis to support the shade cloth without disturbing the growth of climbing plants. The shade cloth extended down each side of the trellis by 1.2 m for some early morning to late afternoon cover. There were five replicates of each species used for sun and shade treatments, except for *A. quinata* in sun (n = 4). To investigate the plasticity of the existing climbing plant species to the changed light condition from full-sun to shade, leaf-level measurements of traits were used in this study. To ensure that only newly grown leaves were assessed for plant traits, at least ten newly formed shoots were selected and labeled on each plant, including full-sun and shaded treatments, one month after the shade cloth was installed.

A scoria-based substrate was utilised in this experiment, which comprised of Scoria 7 mm aggregate (20% v/v), Scoria 8 mm minus which includes fines (60% v/v), and horticultural grade coir (20% v/v). The water holding capacity and the bulk density of the substrate are 46 % and 1.26 g cm^{-3}, respectively (Farrell et al., 2013). Slow release fertiliser (NPK 15.4:3.5:8.9 + trace elements) (Macracote Coloniser Plus, Langley Fertilizers, Western Australia, Australia) was applied at a rate 3 g L^{-1} to each pot at the beginning of the experiment. Three drippers were installed for irrigation supply to each pot, with flow rate at 0.5 L min^{-1} for each dripper. To maintain well-watered conditions, irrigation was applied three times daily (07:00, 13:00 and 19:00) from December to March (summer), for four minutes each time. Daily irrigation supplies were 18 L per pot during summer.

Two climate stations with data loggers (CR1000, Campbell Scientific Inc., Queensland, Australia) were placed along freestanding trellis outside and under shade cloth. At both outside and inside the shaded structures, ambient air temperature and humidity (HMP60 Temperature and Relative Humidity Sensor, Vaisala, Helsinki, Finland) were measured at a height of 1.65 m above ground surface; solar irradiance (CS300 Pyranometer, Apogee Instruments Inc., Utah, USA) was measured at a height of 1.45 m above ground surface. Weather data were logged at 30 minute intervals throughout the study period. Daily ambient air temperatures and relative humidity were similar between outside and under the shade, ranged from 17 to 34^{o}C and from 40 to 90%, respectively. Maximum solar irradiation was recorded between 12:30 to 16:00 hr of a day ranged from 700 to 900 W m^{-2} outside the shade, which was three times more than that under the shade (220 to 300 W m^{-2}). The solar radiation level of shade treatment in this study reflects light levels measured in a dense downtown area where the average solar radiation received is approximately 295 W m^{-2} (Yu et al. 2009).

Table 4.1. The seven climbing plant species (and abbreviations) used in this experiment and origin, climate, habitat, leaf habit, climbing mechanism, growth rate, and light requirements.

Species	Family	Origin/ climate	Habitat	Leaf habit	Climbing mechanism	Growth rate	Light requirements
Ampelopsis brevipedunculata (*Ab*)	Vitaceae[2,3]	North-east Asia[2,3]/ temperate climate[5]	Forest edges and riparian area[2,3]	Deciduous[2,3]	Tendrils[2]	Medium[2]	Full sun[2,3]
Akebia quinata (*Aq*)	Lardizabalaceae[2,3]	East Asia[2,3]/ temperate climate[5]	Forest margins and mountain slopes[3]	Deciduous[2,3]	Stem twining[2]	Medium[2]	Semi-shade to full sun[2,3]
Vitis 'Ganzin Glory' (*Vs*)	Vitaceae[2]	France[1]/ temperate climate[5]	N/A[a]	Deciduous[2]	Tendrils[2]	Fast[2]	Full sun[1,2]
Gelsemium sempervirens (*Gs*)	Loganiaceae[2,3]	South-eastern parts of North America[2,3]/ temperate climate[5]	Thickets, woodlands, and pine forests[2,3]	Evergreen[2,3]	Stem twining[2]	Medium[2]	Semi-shade to full sun[2,3]
Jasminum azoricum (*Ja*)	Oleaceae[2]	Madeira Island[3]/ Mediterranean climate[5]	Coastal area[3]	Evergreen[2]	Stem twining[2]	Fast[2]	Semi-shade to full sun[2,3]
Pandorea pandorana (*Pp*)	Bignoniaceae[2,4]	QLD, NSW, VIC, SA, WA, Lord Howe[4]/	Rainforest, dry sclerophyll forest, dry	Evergreen[2,4]	Stem twining[2]	Fast[2]	Semi-shade to full sun[2,3]

		temperate climate[4,5]	scrub and rocky outcrops in arid regions[4]				
Trachelospermum jasminoides (*Tj*)	Apocynaceae[2]	East and southeast Asia[2,3]/subtropical and temperate climate[4,5]	Sunny edges of forests, and brushwoods (at elevation from 200-1300 m)[2]	Evergreen[2]	Stem twining[2]	Medium[2]	Semi-shade to full sun[2,3]

[a] *Vitis* 'Ganzin Glory' (*Vs*) was the cultivar name for the ornamental vine derived from the cross *Vitis vinifera* x *V. rupestris*, no natural habitat

References of Table 4.1.

[1]Armitage, A.M. 2010, *Armitage's Vines And Climbers: A Gardener's Guide To The Best Vertical Plants*, Timber Press, Portland.

[2]Dunnett, N. & Kingsbury, N. 2008, *Planting Green Roofs And Living Walls*, Timber Press, Portland.

[3]Harrison, R.E. 1973, *Climbers And Trailers*, A. H. & A. W. Reed, Sydney.

[4]Jones, D. & Gray, B. 1988, *Climbing Plants In Australia: With Emphasis On Australian Native Species*, Reed Books, N.S.W., Australia.

[5]Peel, M.C., Finlayson, B.L. & Mcmahon, T.A. 2007, 'Updated world map of the Köppen-Geiger climate classification', *Hydrology and Earth System Sciences*, vol. 11, no. 5, pp.1633-1644.

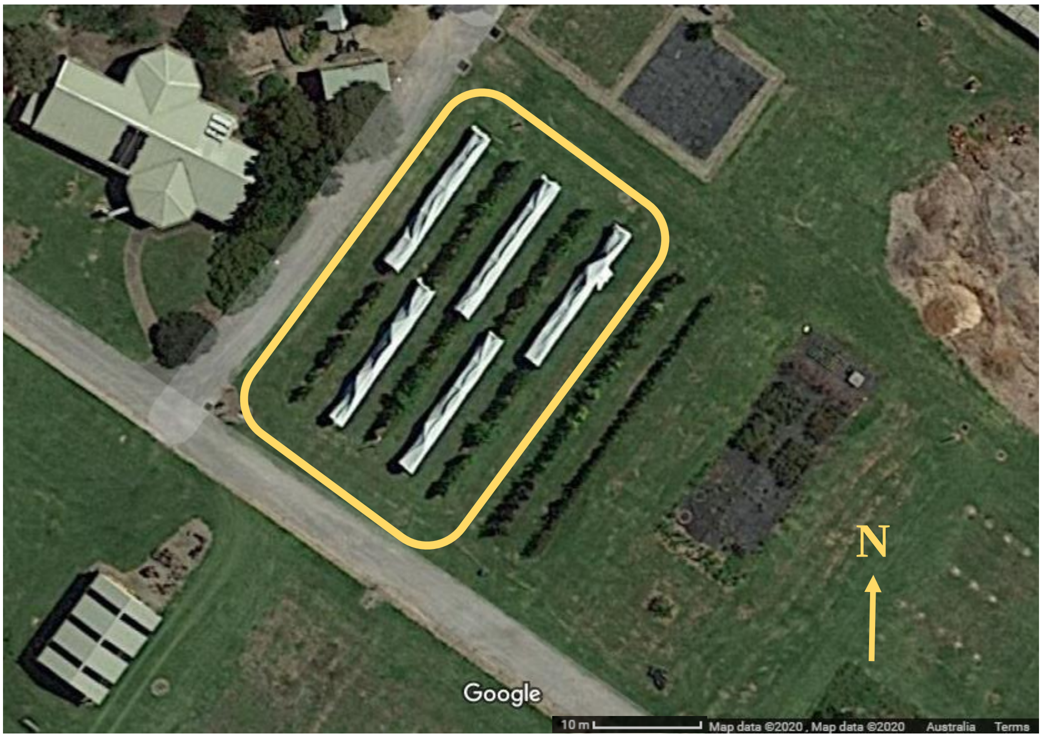


Figure 4.1. Top-down view of the five east/west-orientated freestanding trellis rows (within the yellow rectangle) used in the climbing plant field experiment, showing the half-row covered with shade-cloth to ensure sun and shaded treatments in each replicate row (n = 4-5), with the arrow indicating compass North. (Source: Google Maps).

Leaf specific area and leaf expansion rate

Ten healthy and young fully-expanded leaves (from the 3rd to the 5th nodes of each shoot) of each climbing plant under sun and shade treatments were randomly selected from the top, middle and bottom sections of each plant and harvested at the end of the experiment (15 weeks after the experiment commenced). All the leaves measured were newly formed after the shade treatment was installed. The projected leaf area (one side of the leaves) was measured using a LI-3100C Area Meter (LI-COR, Lincoln, NE, USA). Leaves were oven dried (70°C) for a week to obtain dry biomass. From these measurements, mean values of specific leaf area (SLA, the ratio of leaf area to leaf dry mass) were calculated. An SLA would be averaged for each plant replicate from ten of its leaf samples.

Leaf expansion rate, determined as the difference between measurements of leaf area at the beginning and at the end of measurement. To determine the average leaf expansion rate, three newly produced young leaves of each climbing plant were randomly selected for this measurement. However, some *A. quinata* and *J. azoricum* plants lost leaves to pests, reducing the usable sample to only one or two leaves per plant. The projected leaf area (one side of the leaves) of each newly produced leaf was recorded by taking photographs at the beginning (Day 0) and the end (Day 27-34) of this measurement, and was measured using Image J (free software for image processing and analysis in Java).

Gas exchange rates and photosynthetic light response curves

Gas exchange rates and photosynthetic capacity were measured using a portable photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA) between 09:00 hr and 12:30 hr on sunny days in March 2019. To obtain the rate of photosynthesis in situ (A) and the stomatal conductance (g_s) for sun and shade leaves, four measurements were taken on a healthy fully expanded leaf using a saturated light intensity of 1500 μmol m^{-2} s^{-1}, block temperature of 25^{o}C, air flow rate of 500 μmol s^{-1} and a CO_2 concentration of 400 ppm. Leaves measured on five individuals per species for each treatment. All the leaves measured were newly formed after the shade treatment was installed.

Photosynthetic capacity was measured at nine different photosynthetic photon flux density (PPFD) to develop the photosynthetic light response curve. PPFD was decreased in steps from 1500 to 0 μmol m^{-2} s^{-1} (1500, 1200, 900, 600, 300, 200, 100, 50, 0 μmol m^{-2} s^{-1}). Each step lasted 5 to 15 minutes to reach a steady state, and rates of photosynthesis were recorded. The light compensation point (LCP) was determined from the x-axis intercept of the photosynthetic light response curve, where photosynthesis equals zero (Lambers et al., 2008; Valladares et al., 2000). Three replications were used for each climbing plant species for each treatment.

Photochemical efficiency

Chlorophyll fluorescence serves as a widely-used indicator to monitor the photochemical efficiency of leaves under environmental stress, such as extremes of temperature and light intensity (Dias and Marenco, 2007; Yamane et al., 1997). The ratio of variable (Fv = Fm – Fo) to maximal (Fm) fluorescence of dark-adapted leaves was measured with a portable chlorophyll fluorometer (FMS2+ Field Fluorescence Monitoring System, Hansatech Instruments, United Kingdom), because it is a rapid determination of changes in the maximum quantum efficiency of PSII phytochemistry when plants are under stress (Maxwell and Johnson, 2000). Fully expanded and healthy leaves (newly formed after the shade treatment was installed) were collected in the morning predawn before exposure to daylight and day temperatures, and placed in sealed bags with moistened paper towels before measurements. Leaf discs (diameter 10 mm) from each species replicates under sun and shade treatment (n = 4-5) were separately attached to a paper cardboard strip. Placed four strips (two species under sun and shade treatment) in a bag and ensured strips not overlapping and leaves facing up, then sealed bags and removed as much air as possible. Leaf samples were incubated in water baths in three steps: (1) in the control water bath (23^{o}C) for 15 minutes, (2) in a water bath with the target temperature (23, 45, 47.5, 50, 52.5, 55^{o}C) for heat stress treatments, (3) in the control water bath for a recovery period of 90 minutes. Leaf samples were then removed from water baths and dark-adapted for 30 minutes. The ratio of variable to maximum fluorescence (Fv/Fm) was recorded with a portable chlorophyll fluorometer (FMS2+ Field Fluorescence Monitoring System, Hansatech Instruments, United Kingdom) on five sun/shade leaf disks of each species after dark adaption. The responses of Fv/Fm with increasing temperature were analysed by a sigmoidal fit model in GenStat 16 (2013, VSN International Ltd., England, UK) using the data obtained for each leaf disc (Appendix 4.2). The reduction 50% of initial Fv/Fm values (T_{50}), indicating the thermal tolerance of leaves, was determined from each curve.

Leaf chlorophyll contents

Chlorophyll extraction from leaves was determined following the protocol described by Hiscox & Israelstam (1979). Five leaf discs (30 mm) were cut from fresh leaves (newly formed after the shade treatment was installed) collected from each climbing plants. Measured the fresh weight of individual leaf disc and put the disc in a glass centrifuge vial containing 7 ml dimethyl sulphoxide (DMSO). Incubated vials in a water bath at 65°C for 15 mins to an hour, depending on the thickness of a leaf, till chlorophylls were completely extracted into the fluid. Removed glass vials from water bath after extractions were complete, and transferred 3 ml of each extract to disposable polystyrene cuvettes for absorbance measurements. The UV-Vis spectrophotometer (UV-1800 Spectrophotometer, Shimadzu Scientific Instruments Inc., American) was calibrated to zero absorbance using a blank of pure DMSO. Absorbance of both blank and sample were measured at 649 and 665 nm. The chlorophyll concentration of the extract was determined using Wellburn (1994) equation:

$$\text{Chl } a \ (\mu\text{g ml}^{-1}) = 12.19*A_{665} - 3.45*A_{649};$$

$$\text{Chl } b \ (\mu\text{g ml}^{-1}) = 21.99*A_{649} - 5.32*A_{665},$$

and was converted to leaf Chl content on a fresh leaf mass basis (µg Chl g^{-1} leaf mass).

Phenotypic plasticity index

Phenotypic plasticity index (PPI) for seven variables was determined by the formula given by Valladares et al. (2000): PPI = (maximum − minimum)/maximum, with maximum and minimum corresponding to the highest and the lowest values, respectively, among the mean values of each variable. Theoretically, PPI ranges from 0 to 1, representing the plasticity of a trait to the environmental stress-gradient (Valladares et al., 2000).

Statistical analyses

The effects of light exposure (sun and shade) on SLA, leaf expansion rate, A_{1500}, g_s, LCP, Chl a+b and T_{50} were tested using generalized linear models performed in GenStat 16 (2013, VSN International Ltd., England, UK), and the least significant difference (LSD at $p \leq 0.05$) was applied to assess the differences among seven climbing plant species. Generalized linear models were also applied to assess the differences of seven traits between deciduous and evergreen plant types. For phenotypic plasticity, two-sample t-tests was used to assess the differences between deciduous and evergreen plant types the phenotypic plasticity of each measured traits.

Results

Leaf morphology

Average specific leaf area (SLA) of all seven climbing plant species was significantly increased by the 15-week shade treatment ($p < 0.001$) (Fig. 4.2A). *Akebia quinata* and *P. pandorana* had greater increases in SLA from 10 to 19 cm^2 g^{-1} (+90%; PPI = 0.47) and 9 to 20 cm^2 g^{-1} (+107%; PPI = 0.51), respectively, as compared to the other five climbing species (between +41% and +54%) (Figs. 4.2A and 4.3A). Regardless of light treatments, *Vitis* 'Ganzin Glory' had the greatest SLA (sun, 18.5 cm^2 g^{-1} in sun; 26.2 cm^2 g^{-1} in shade), and *J. azoricum* had the lowest SLA (7.1 cm^2 g^{-1} in sun; 10.7 cm^2 g^{-1} in shade) SLA among the seven climbing plant species ($p < 0.001$; Fig. 4.2A). Deciduous climbing species had significantly greater SLA (+77%) than evergreen climbing species ($p < 0.001$), regardless of the light conditions (Fig. 4.2A). There were no significant differences in phenotypic plasticity for SLA between evergreen (PPI = 0.38) and deciduous climbing plant species (PPI = 0.37) in response to shade (Fig. 4.3A).

Leaf expansion rates did not differ between sun and shade treatments for most of the climbing plant species (Fig. 4.2B). However, there were significant increases in leaf expansion rate for *G. sempervirens* (+82%; $p < 0.01$; PPI = 0.45) and *J. azoricum* (+75%; $p < 0.05$; PPI = 0.43) in shade as compared to full sun (Figs. 4.1B and 4.2B). Regardless of light treatments, *Vitis* 'Ganzin Glory' had the greatest leaf expansion rate (222% in sun; 215% in shade), and *G. sempervirens* had the lowest leaf expansion rate (4.3% in sun; 7.8% in shade) among the seven climbing plant species ($p < 0.001$) (Fig. 4.2B). Deciduous climbing species had significantly greater leaf expansion rate than evergreen climbing species (+203%) ($p < 0.001$), regardless of the light conditions (Fig. 4.2B). There were no significant differences in phenotypic plasticity for leaf expansion rate between evergreen (PPI = 0.31) and deciduous climbing species in response to shade (PPI = 0.1) (Fig. 4.3B).

Gas exchange, light response and chlorophyll contents

Photosynthetic rate (A_{1500}) in three of the climbing plant species were significantly decreased by the shade treatment after 15 weeks ($p < 0.01$) (Fig. 4.2C). Under the shade treatment, significant decreases in A_{1500} were evident in *A. brevipedunculata* (-15%; PPI = 0.15), *V.* 'Ganzin Glory' (-30%; PPI = 0.3), and *P. pandorana* (-10%; PPI = 0.1) ($p < 0.05$) (Figs. 4.2C and 4.3C). Regardless of light treatment, *A. quinata* had the greatest A_{1500} (15.7 µmol CO_2 m^{-2} s^{-1} in sun; 14 µmol CO_2 m^{-2} s^{-1} in shade) and, *G. sempervirens* had the lowest A_{1500} (5 µmol CO_2 m^{-2} s^{-1} in sun; 5.2 µmol CO_2 m^{-2} s^{-1} in shade) among the seven climbing plant species ($p < 0.001$) (Fig. 4.2C). Deciduous climbing species had significantly higher A_{1500} than evergreen climbing species (+50%; $p < 0.001$), regardless of the light conditions (Fig. 4.2C). Similarly, deciduous climbing plant species also exhibited significantly greater decreases in A_{1500} (+17%;

PPI = 0.19) than evergreen species (+3%; PPI = 0.05) in response to shade ($p < 0.05$; Figs. 4.2C and 4.3C).

In contrast, stomatal conductance (g_s) was not affected by the 10-week shade treatment (Fig. 4.2D). Under the sun treatment, *A. brevipedunculata* had the greatest g_s (0.37 mol H_2O m^{-2} s^{-1}) and, *G. sempervirens* and the lowest g_s (0.08 mol H_2O m^{-2} s^{-1}) among the seven climbing plant species ($p < 0.001$). Whilst under the shade treatment, *A. quinata* had the greatest g_s (0.36 mol H_2O m^{-2} s^{-1}) and *T. jasminoides* had the lowest g_s (0.09 mol H_2O m^{-2} s^{-1}) g_s among the seven climbing plant species ($p < 0.001$). Deciduous climbing species had significantly higher g_s than evergreen climbing species (+111%) ($p < 0.001$), regardless of the light conditions (Fig. 4.2D). There were no significant differences in phenotypic plasticity for g_s between evergreen (PPI = 0.13) and deciduous climbing plant species (PPI = 0.14) in response to shade (Fig. 4.3D).

The average light compensation point (LCP) was significantly lower (-31%) under the shade treatment ($p < 0.01$) (Fig. 4.2E). Both *G. sempervirens* and *J. azoricum* showed the greatest changes (-54%; PPI = 0.54) in LCP among the seven climbing plant species in response to shade (Figs. 4.2E and 4.3E). Regardless of light treatment, *T. jasminoides* had the greatest LCP (32.7 µmol m^{-2} in sun; 27.5 µmol m^{-2} in shade) and, *G. sempervirens* had the lowest LCP (6.9 µmol m^{-2} in sun; 3.2 µmol m^{-2} in shade) among the seven climbing plant species ($p < 0.001$). Deciduous climbing species had significantly lower LCP (-37%) than evergreen climbing species ($p < 0.05$), regardless of the light conditions (Fig. 4.2E). There were no significant differences in phenotypic plasticity for LCP between evergreen (PPI = 0.4) and deciduous climbing plant species (PPI = 0.23) in response to shade (Fig. 4.3E).

Leaf chlorophyll content (Chl *a*+*b*) in five climbing plant species was significantly increased by the shade treatment (Fig. 4.2F). There were significant increases in Chl *a*+*b* in *A. brevipedunculata* (+86%; PPI = 0.46), *A. quinata* (+30%; PPI = 0.23), *P. pandorana* (+101%;

PPI = 0.5), *T. jasminoides* (+51%; PPI = 0.34) ($p < 0.01$) and *J. azoricum* (+34%; PPI = 0.25) ($p < 0.05$) (Figs. 4.2F and 4.3F). Regardless of light treatments, *A. quinata* had the greatest Chl *a*+*b* (2.2 mg g^{-1} in sun; 2.8 mg g^{-1} in shade) and, *T. jasminoides* had the lowest Chl *a*+*b* (0.6 mg g^{-1} in sun; 0.8 mg g^{-1} in shade) among the seven climbing plant species ($p < 0.001$) (Fig. 4.2F). Leaves of deciduous climbing species had significantly higher Chl *a*+*b* (+63%) than leaves of evergreen climbing species irrespective of the light conditions ($p < 0.001$) (Fig. 4.2F). There were no significant differences in phenotypic plasticity for Chl *a*+*b* between evergreen (PPI = 0.33) and deciduous climbing plant species (PPI = 0.25) in response to shade (Fig. 4.3F).

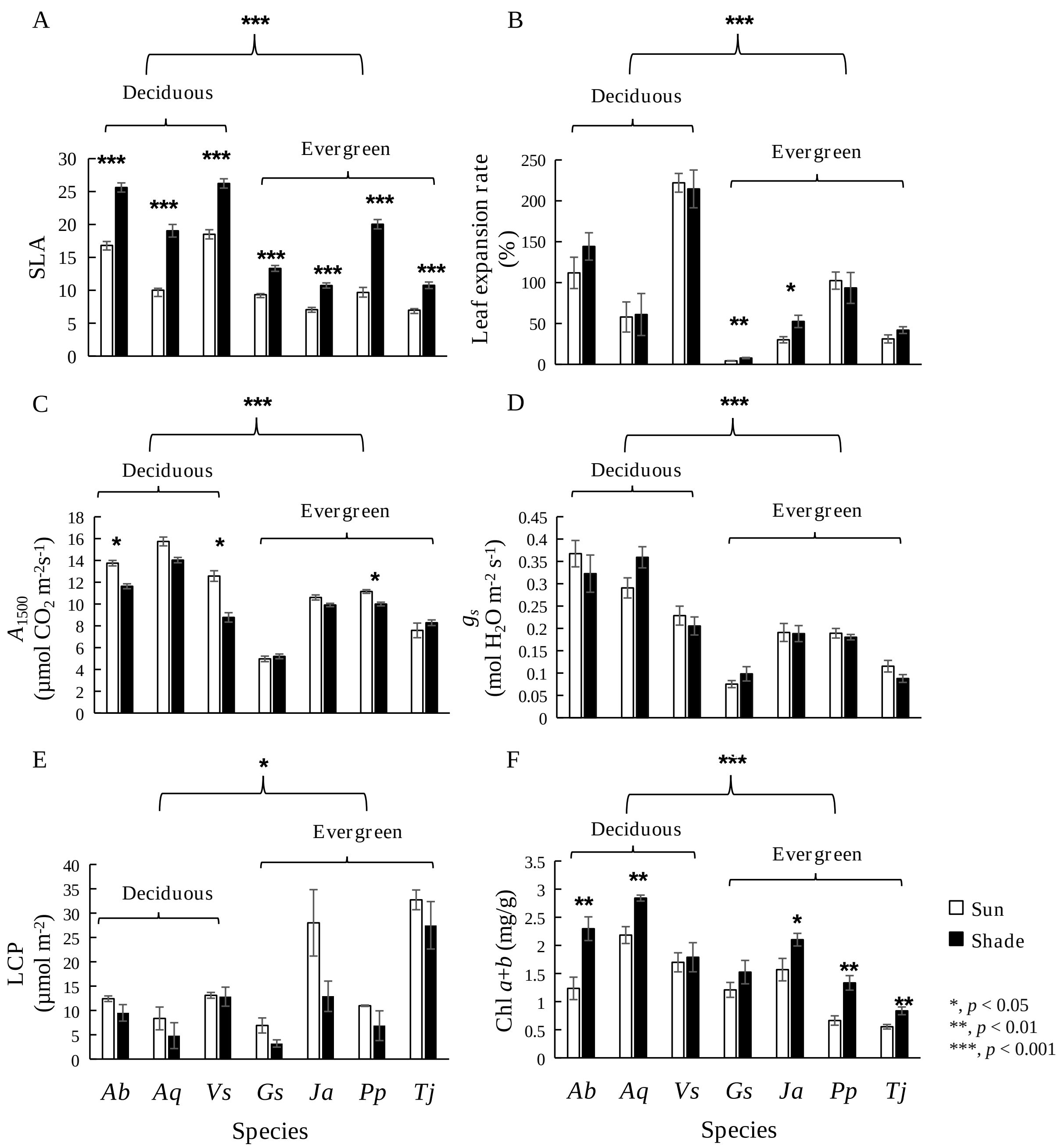


Figure 4.2. Specific leaf area (SLA) (A), leaf expansion rate (B), leaf photosynthetic rate at saturated light intensity of 1500 µmol m^{-2} s^{-1} (A_{1500}) (C), stomatal conductance (g_s) (D), light compensation point (LCP) (E), and leaf chlorophyll content (Chl *a*+*b*) (F) of seven mature climbing plant species; *Ampelopsis brevipedunculata* (*Ab*), *Akebia quinata* (*Aq*), *Vitis* 'Ganzin Glory' (*Vs*), *Gelsemium sempervirens* (*Gs*), *Jasminum azoricum* (*Ja*), *Trachelospermum jasminoides* (*Tj*) and *Pandorea pandorana* (*Pp*), grown in a field experiment after 15 weeks

exposed to sun (control) and shade conditions. Significant shade-sun differences are indicated: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Values are means ± SE.

Table 4.2. Leaf thermal tolerance according to T_{50} measurements (the temperature caused a 50% decrease in the fluorescence Fv/Fm ratio) for sun and shade leaves of seven mature climbing plant species; *A. brevipedunculata* (*Ab*), *A. quinata* (*Aq*), *V.* 'Ganzin Glory' (*Vs*), *G. sempervirens* (*Gs*), *J. azoricum* (*Ja*), *T. jasminoides* (*Tj*) and *P. pandorana* (*Pp*), grown in a field experiment after 15 weeks exposed to sun (control) and shade conditions. Standard errors (± SE) are presented.

T_{50}							
	Deciduous			Evergreen			
Treatment	*Ab*	*Aq*	*Vs*	*Gs*	*Ja*	*Pp*	*Tj*
Sun	49.3 ± 0.4	50.6 ± 0.1	48.3 ± 0.4	50 ± 0.3	49.2 ± 0.3	49.3 ± 0.3	49.9 ± 0.8
Shade	49.3 ± 0.4	50.7 ± 0.2	48.4 ± 0.6	48.8 ± 0.1	48.6 ± 0.2	49.4 ± 0.3	48.8 ± 0.2

Leaf thermal tolerance

There was no significant effect of shade treatment on T_{50}, i.e., the temperature caused a 50% decrease in the fluorescence Fv/Fm ratio, across the seven climbing plant species (Table 4.2). However, significant species differences were evident in T_{50} ($p < 0.001$), with *A. quinata* having the highest T_{50} (50.6ºC in sun; 50.7ºC in shade) and *V.* 'Ganzin Glory' had the lowest T_{50} (48.3ºC in sun; 48.4ºC in shade). There was no significant difference in T_{50} between deciduous and evergreen climbing species regardless of the light conditions. There were no significant differences in phenotypic plasticity for T_{50} between evergreen (PPI = 0.009) and deciduous climbing plant species (PPI = 0.001) in response to shade (Fig. 4.3G).

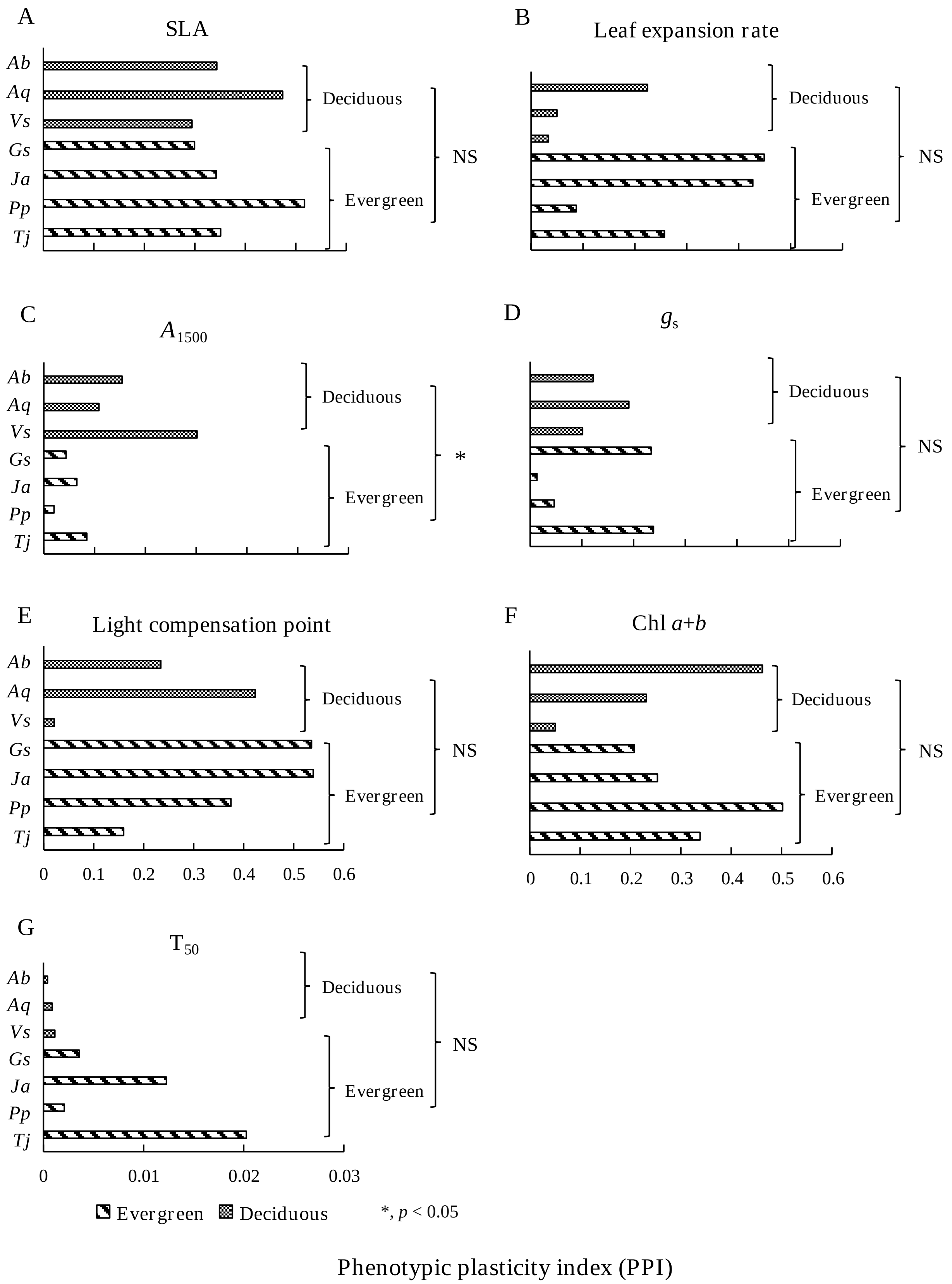


Figure 4.3. Phenotypic plasticity index (PPI), (maximum - minimum)/ maximum, for specific leaf area (SLA) (A), leaf expansion rate (B), leaf photosynthetic rate at saturated light intensity

of 1500 μmol m^{-2} s^{-1} (A_{1500}) (C), stomatal conductance (g_s) (D), light compensation point (LCP) (E), Chl *a+b* (F), and T_{50} (G) of seven climbing plant species, *A. brevipedunculata* (*Ab*), *A. quinata* (*Aq*), *V.* 'Ganzin Glory' (*Vs*), *G. sempervirens* (*Gs*), *J. azoricum* (*Ja*), *T. jasminoides* (*Tj*) and *P. pandorana* (*Pp*), grown in a field experiment after 15 weeks exposed to sun (control) and shade conditions. Dotted bars represent deciduous climbing species and hatched bars represent evergreen species. Star symbols represent significant differences; *, $p < 0.05$. NS represents no significant difference.

Discussion

In order to inform plant selection for green façades in cities, we evaluated the trait responses of seven climbing species to increased shade, including plant morphology (SLA and leaf expansion rate), physiology (gas exchange, light response and chlorophyll contents), and thermal tolerance (T_{50}). Our first hypothesis was that reducing light intensity for established climbing plants would increase specific leaf area (SLA), leaf expansion rate, and leaf chlorophyll content (Chl *a+b*), and decrease their leaf photosynthetic rate (A_{1500}), stomatal conductance (g_s), light compensation point (LCP) and thermal tolerance (T_{50}) in climbing plant species. Our second hypothesis was that deciduous climbing plant species would be more plastic (greater changes in morphology and physiology) in their responses to shade than evergreen climbing plant species.

While we hypothesized that climbing plants would increase both their SLA and leaf expansion rate in response to the shade treatment, this was not the case. We observed that the all the seven climbing species responded to the shade condition similarly by significantly increasing SLA; whilst keeping their g_s LCP, and thermal tolerance (T_{50}) unchanged. However, leaf expansion rates, A_{1500} and Chl *a*+*b* were significantly affected by the shade treatment in only some of the climbing plant species in our study.

The significant increases in SLA of all seven climbing plant species, is in accordance with previous studies in shade leaves across a range of life-forms, including climbing species (Greer et al., 2010; Lei et al., 1996), trees (Lichtenthaler et al., 2007; Pilegaard et al., 2003; Lei et al., 1996; Slot et al., 2018), and herbs (Lei et al., 1996). However, contrary to our expectations, leaf expansion rate did not increase under the shade treatment for most climbing plant species. The exception was, for two evergreen species *G. sempervirens* and *J. azoricum*, which increased leaf expansion in shade. Other studies have also shown variable results for leaf expansion in response to shade, for example the evergreen climbing plant species *Stigmaphyllon lindenianum* did not change leaf expansion rates in shade (63% greenhouse shade cloth) (Avalos and Mulkey, 1999); whereas the deciduous *Vitis vinifera* L. exhibited significantly increased leaf expansion rates under 70% shade cloth (Greer et al., 2010). This indicates that shade-sun differences in leaf expansion of climbing species may be species specific.

For leaf physiological traits, including A_{1500}, g_s and LCP, species also responded differently to the shade treatment. Leaf A_{1500} was significantly lower in two deciduous climbing species (*A. brevipedunculata* and *V.* 'Ganzin Glory') and one evergreen species (*P. pandorana*). However, g_s and LCP were unaffected by increased shade for all seven climbing plant species. In comparable studies across life-forms, both leaf photosynthesis and g_s significantly decreased for oaks (*Quercus robur* and *Quercus petraea*) (Gross et al., 1996) and the liana *Stigmaphyllon lindenianum* (Avalos and Mulkey, 1999) under increased shade. In our study, A_{1500} or g_s in *A. quinata*, *G. sempervirens*, *J. azoricum* and *T. jasminoides*, was not affected by the shade treatment, which indicates that these four climbing species are still productive in shade. On the other hand, although the shade treatment significantly decreased A_{1500} in *A. brevipedunculata*, *V.* 'Ganzin Glory' and *P. pandorana*, g_s in these three climbing species was not affected by the shade treatment. It was also noted that these three climbing plant species developed relatively larger leaf areas in shade among the seven species (data not shown). An outcome of the optimization of resource allocation is leaf size (i.e., lamina area) variation with respect to environmental conditions, for instance the proportion of resources available for photosynthesis is relatively less in larger leaves than the other activities (Niinemets et al., 2007). This may explain our results that leaf A_{1500} and g_s in three climbing plant species were decoupled.

The light response curves of the seven climbing plant species showed a slight, but not significant, reduction in LCP in shade as compared with in full sun. Light response curves from studies have demonstrate a significant reduction in leaf LCP in shade conditions for tree species (e.g., *Fagus sylvatica, Calophyllum inophyllum* and *Ormosia macrocalyx*) (Lichtenthaler et al., 1981; Slot et al., 2018), climbing plant species (e.g., *Mikania micrantha*), and herbs (e.g., *Centella asiatica* and *Tridax procumbens*) (Idris et al., 2019). Moreover, these plant species also showed significantly lower rates of net CO_2 uptake in shade compared to in sun (Idris et al., 2019; Lichtenthaler et al., 1981; Slot et al., 2018). This contrasts with the light response curves of most climbing plants in our study, which exhibited either slightly lower or unchanged rates of net CO_2 uptake in response to the shade treatment (Appendix 4.1). The one comparable study of LCP under shade for a climbing plant species (*S. lindenianum*) supports our findings as there was no significant change in LCP for *S. lindenianum* under shade conditions (Avalos and Mulkey, 1999). However, in their study, the *S. lindenianum* leaves were fully developed before being transferred to shade, whereas in our study we measured leaves which had grown under the shade treatment. Therefore, it may be that LCP in developed leaves are not plastic and are determined by initial light conditions at leaf establishment.

Consistent with our hypothesis, the shade treatment significantly increased the total leaf chlorophyll content (Chl *a*+*b*), at least for five of the seven species. Our results are supported by previous observations on climbing plant species (Lei et al., 1996; Dai et al., 2009), trees (Lichtenthaler et al., 1981; Lei et al., 1996; Gross et al., 1996), herbs (Lei et al., 1996) and an orchid (Shao et al., 2014). It has been noted that leaf chlorophyll content is of importance in determining the photosynthetic activity and dry matter production in plants (Mao et al., 2007; Ghosh et al., 2004). Moreover, the increase in leaf chlorophyll content under shade demonstrates the ability of a plant to optimize light harvesting in low-light environments (Lei et al., 1996; Wittmann et al., 2001). It could therefore be assumed that the climbing plants in our study could still continue their photosynthetic activity and dry matter production to a certain extent by increasing leaf Chl *a*+*b* in shade. This is further supported by the minimal changes in A_{1500} under shade conditions in three climbing plant species in our study.

Surprisingly, leaf thermal tolerance (T_{50}) did not change between shade and sun conditions among the seven climbing species in our study. Other studies have shown differences among tree species differences in T_{50} among shade and sun leaves (Slot et al., 2018). For example, Slot et al. (2018) suggested that among three tree species, only the two species with greater thermal tolerance (*C. inophyllum* and *I. spectabilis*) reduced their leaf thermal tolerance between sun and shade leaves (Slot et al., 2018). In our study, the mean Fv/Fm ratios in sun leaves of all seven climbing species ranged from 0.19 to 0.53 at 50°C (Appendix 4.2), which is much lower than the sun leaves of the two tree species (approximately 0.6 and above) in the Slot et al. (2018) study. It could therefore be presumed that the seven climbing plants in our study may be with low inherent thermal tolerance and thus exhibited reduced plasticity in thermal tolerance in response to shade.

Contrary to our second hypothesis, deciduous climbing plant species were not more plastic than evergreen species in their overall responses (morphology and physiology) to shade. Only plasticity in A_{1500} differed with respect to leaf habit (deciduous and evergreen), in that deciduous climbing plant species were more plastic than evergreen species. The findings of our study do not support previous research showing that deciduous woody plants are more plastic than evergreen species in their responses to light heterogeneity in forested ecosystems (Böhnke et al., 2013). However, among the seven climbing species in our experiment there was a large amount of variation in response to shade for leaf expansion rate, A_{1500}, and Chl $a+b$. This might indicate that variations in response to light exist on a species level, regardless of leaf habit. While deciduous climbing plant species were not more plastic than evergreen species, deciduous species had significantly greater SLA, leaf expansion rate, A_{1500} and g_s, which agrees with other studies (Kröber et al., 2015; Lusk et al., 2008). However, thermal tolerance (T_{50}) did not differ between deciduous and evergreen climbing plant species. Therefore, our results may be partially explained by the slow-fast continuum of resource acquisition strategies (Reich, 2014), which suggests that species with fast resource acquisition (light and water) generally exhibit greater SLA and gas exchange rates, and lower thermal tolerance than resource conservative species (Reich, 2014; Sastry and Barua, 2017; Wright et al., 2004).

In this study, only one level of shade was applied to test the effects of reduced sunlight on seven climbing plant species placed on east/west-orientated freestanding trellis rows at the ground-level for 15 weeks. The solar radiation level of the shade treatment in this study is a realistic reflection of shading in cities, for example, the average solar radiation measured in downtown Houston has been reported as 295 W m^{-2} (Yu et al., 2009). However, more varied light conditions should be evaluated in future studies as solar radiation level varies due to differences in building orientation, building height and surrounding environments (Freitas et al., 2015; Shishegar, 2013). Moreover, climbing plants grown at different façade orientations and façade elevations also need to be further assessed considering variations in hours of solar exposure, air temperature, relative humidity and wind speed. For instance, during summer months in Toronto, the west-facing orientation receives higher level of solar radiation and longer hours of sun exposure than south-facing orientation, which results in differences in air temperature and relative humidity (Millward et al., 2014). In addition, real case studies should also be undertaken to test plant growth and performance of green façades on-site, especially those growing on walls, where this may also change light and heat transfer and therefore plant adjustment of growth and traits.

Indirect green façade recommendations

The ecosystem services delivered via indirect green façades are well documented (Abdullahi and Alibaba, 2016; Başdoğan and Çığ, 2016; Rakhshandehroo et al., 2015). To ensure existing indirect green façades continue to provide these benefits, it is essential for climbing plant species to maintain or increase their wall coverage with healthy foliage and/or productivity (photosynthesis) regardless of the light availability as the built form of the urban landscape changes and increases (Koyama et al., 2013; Cameron et al., 2014). In our study, reduced light intensity (heavy shade) resulted in increased SLA for all seven climbing species, whereas leaf expansion rates only increased in *G. sempervirens* and *J. azoricum*. In addition, significant decreases in gas exchange in response to shade were only observed for A_{1500} in *A. brevipedunculata*, *V.* 'Ganzin Glory' and *P. pandorana*, whilst g_s remained unchanged across all seven climbing plant species. Our findings might suggest that *G. sempervirens* and *J. azoricum* were more productive under, and resilient to, an increase in shade, due to maintained photosynthesis and increased growth, as compared to the other five climbing plant species. Hence, both climbing plant species could continue providing cooling efficiently through shading under the shade treatment in our study. Moreover, although A_{1500} decreased or remained unchanged in shade for the other five climbing species, they could compensate with increased Chl a+b and increased SLA to make them able to tolerate shade conditions for 15 weeks. In addition, as thermal tolerance (T_{50}) of the seven climbing species was unaffected by the shade treatment, these species are unlikely to be affected changes in heat which may occur with changes in light availability in cities. In general, seven climbing plant species in our study showed no difference in phenotypic plasticity for the six variables with respect to leaf habit, except for A_{1500}. Given that deciduous climbing plant species had significantly greater decreases in A_{1500} than evergreen species in shade, it would be likely for urban green façades to continue providing efficient cooling in shade by using evergreen climbing species. However, deciduous climbing species may be more resilient to survive through the fast-changing light conditions in cities, as they are more plastic in their photosynthetic activity regarding light heterogeneity. Besides, deciduous climbing plant species exhibited significantly greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl $a+b$, but lower LCP as compare with evergreen species regardless of light intensity levels in this study. These characteristics are advantageous when it comes to supplying ecosystem services in cities. Therefore, deciduous climbing plant species would be more suitable for indirect green façades in urban environments with changing light conditions.

Conclusions

This study presented that for seven climbing plant species examined, their responses to shade partially varied significantly from species to species as well as between deciduous and evergreen plant types. Under shade treatment, the seven climbing species all significantly increased SLA, while remained their g_s, LCP and thermal tolerance (T_{50}) unchanged. In contrast, shade treatment only significantly affected leaf expansion rates, A_{1500}, and Chl $a+b$ in some of the climbing plant species in our study. Irrespective of light intensity levels, significantly greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl $a+b$, as well as lower LCP were observed in deciduous climbing plant species as compare with evergreen species in this study. On the contrary, there was no significant difference in phenotypic plasticity for the six variables between deciduous and evergreen climbing species. Significant greater plastic response to shade was only evident in A_{1500}, in that deciduous climbing plants significantly decreased leaf A_{1500} in shade. Our results suggested that the seven climbing species in this study can maintain their function and performance to certain extents under shade conditions, and are able to deliver some of the ecosystem service benefits. These findings have significant implications for the understanding of how woody climbing plants respond to increased shade and provide insights for plant selection of indirect green façades under dynamic urban light conditions. However, we only evaluated the trait response of seven climbing plant species to the 15-week shade treatment in this study. More broadly, further research is needed to determine the long-term effect of shade on more climbing plant species and evaluate the influence of various light intensity levels upon the development and performance of indirect green façade climbing plants.

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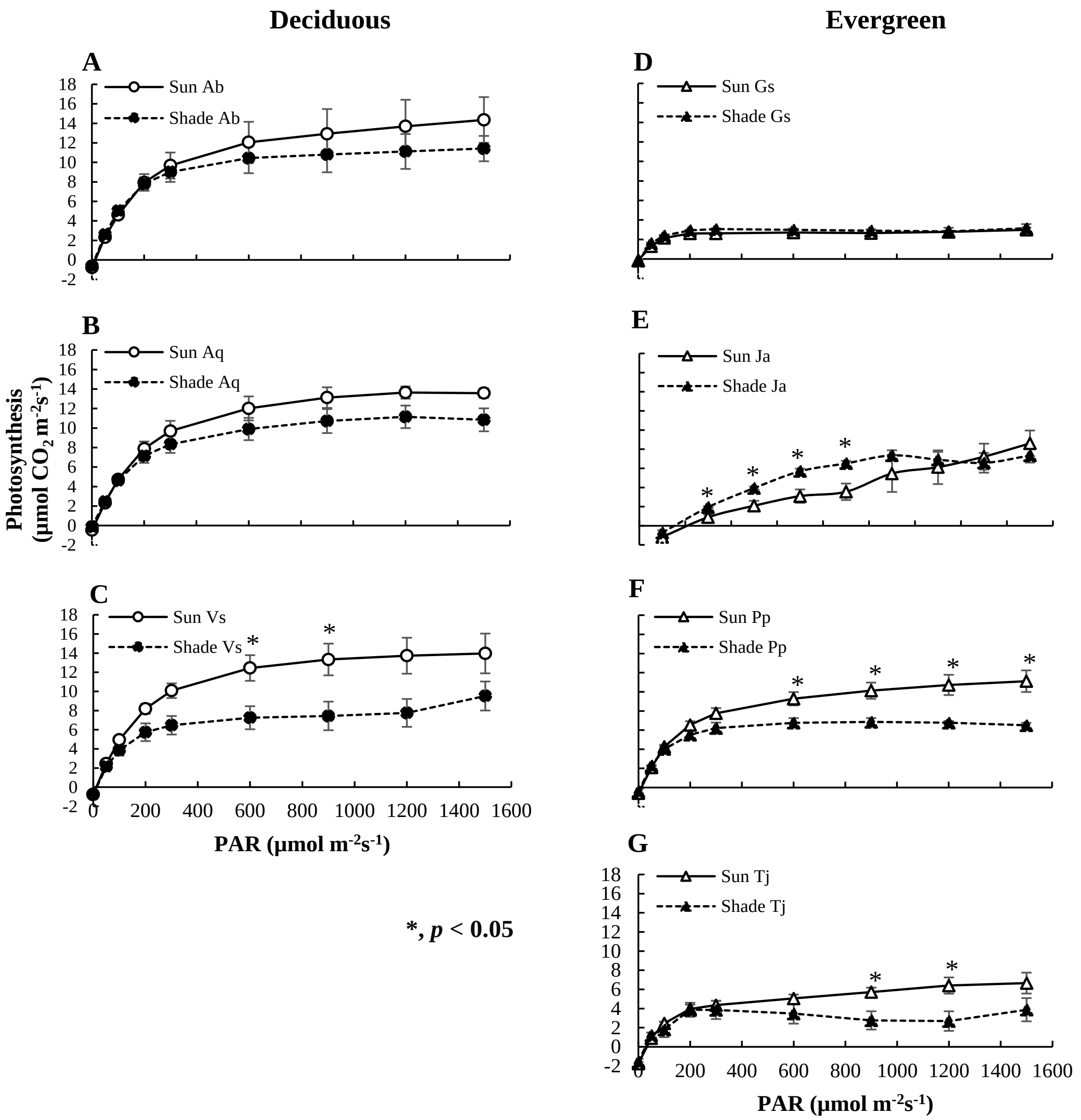
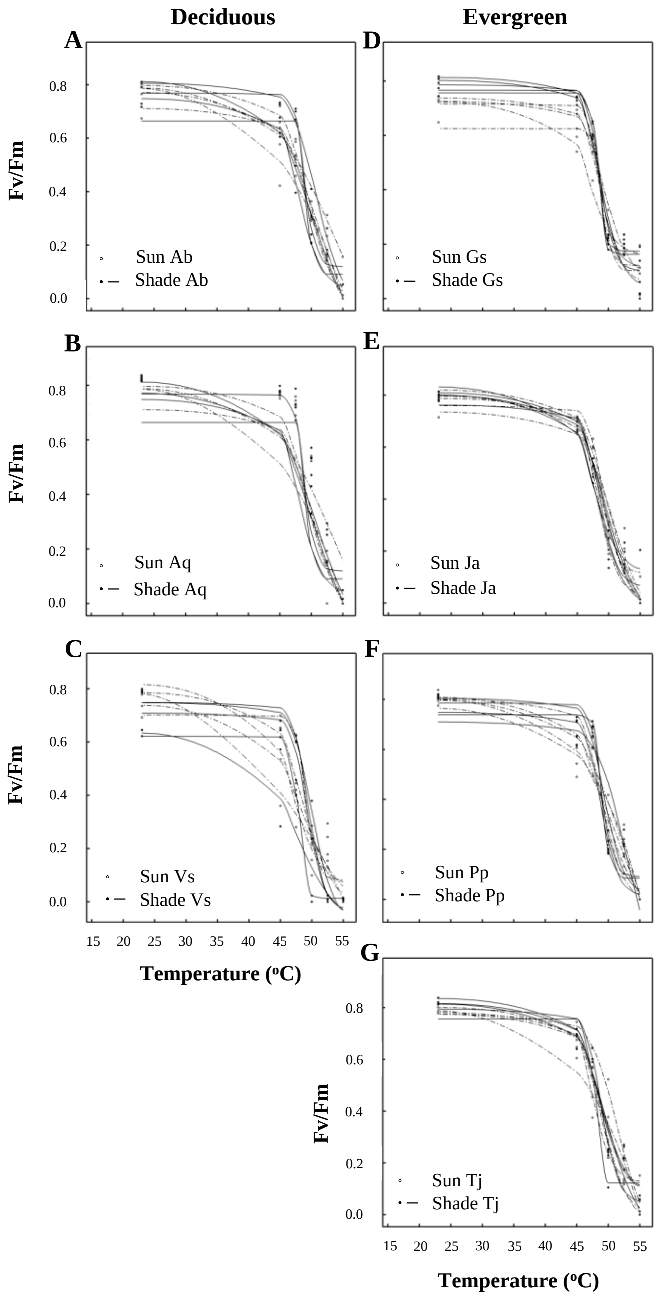
Appendices Chapter 4

Appendix 4.1. Light-response curves from leaves of deciduous climbing species *A. brevipedunculata* (*Ab*) (A), *A. quinata* (*Aq*) (B), and *V.* 'Ganzin Glory' (*Vs*) (C), and evergreen climbing species *G. sempervirens* (*Gs*) (D), *J. azoricum* (*Ja*) (E), *P. pandorana* (*Pp*) (F), and *T. jasminoides* (*Tj*) (G) grown in shade (closed symbols) and sun (open symbols) after 15 weeks. Star symbols represent significant differences; *, $p < 0.05$. Values are means ± SE.



Appendix 4.2. Sigmoidal adjusted curves Fv/Fm ratios in relation to increasing temperature for shade (closed symbols) and sun (open symbols) leaves of deciduous climbing species *A. brevipedunculata* (*Ab*) (A), *A. quinata* (*Aq*) (B), and *V.* 'Ganzin Glory' (*Vs*) (C), and evergreen climbing species *G. sempervirens* (*Gs*) (D), *J. azoricum* (*Ja*) (E), *P. pandorana* (*Pp*) (F), and *T. jasminoides* (*Tj*) (G) as used to determine the critical temperature T_{50} (temperature at which Fv/Fm is reduced by 50%).



5 - Synthesis

To improve sustainable urban development and tackle some of the negative impacts caused by urbanisation, indirect green façades are increasingly being used as a new green infrastructure technology in dense cities (Russo and Cirella, 2018). However, it is challenging to ensure indirect green façades continue to provide ecosystem benefits by maintaining the vegetation growth and coverage in urban environments with potentially disadvantageous conditions (e.g., small containers, limited potable water supply for irrigation, and variable light conditions). Therefore, to improve our understanding of how to manage indirect green façades under unfavorable growing conditions in urban environments, I have investigated how different woody climbing plant species respond to different growing conditions by evaluating their morphological and physiological traits. This thesis had the following three objectives:

1. To evaluate the effect of rooting volume on climbing plant growth, coverage and thermal tolerance in the first growing season;
2. To investigate whether greywater is a viable irrigation resource for indirect green façades in cities by assessing changes in substrate chemical properties and the growth response of six climbing plant species;
3. To explore how shade affects leaf traits and thermal tolerance of climbing plant species assisting green façade design and plant selection.

Empirical findings

1. Effect of rooting volume on façade climbing plant growth, coverage and thermal tolerance in the first growing season

In chapter two, I undertook an experiment to evaluate how rooting volume (21, 42 and 63 L substrate volumes) affected the growth, coverage and thermal tolerance of two climbing plant species with contrasting growth rates (Fig. 5.1). I showed that the smallest rooting volume (21 L) reduced the growth of both climbing plant species in their first growing season. Various types of plants have been shown to have reduced shoot and root growth when grown in restricted rooting volumes as compared with unrestricted ones (NeSmith and Duval, 1998). Inadequate rooting volumes may impose physical limitations on the growth of roots, availability of resources and subsequently restrict the shoot growth (McConnaughay and Bazzaz, 1991). This was also the case in my study as the smallest rooting volume (21 L) significantly reduced plant biomass growth, percentage wall coverage, total leaf area and specific leaf area (SLA) of both the slower-growing species *A. quinata* and the faster-growing species *P. pandorana* over the six-month growing season. However, there were no significant differences between plants grown in 42 L as compared to 63 L rooting volumes. The SLA of both climbing plant species increased as rooting volume increased; whilst shoot:root ratio remained unchanged across all rooting volume treatments There was no change in plant thermal tolerance with rooting volume, however, there was a significant decline in the ratio of variable to maximum fluorescence (Fv/Fm) in both climbing plant species regardless of rooting volume treatments on the west facing façades at 16:00 hr. This was in response to exposure to continuous high ambient air temperature and high solar radiance on the west-facing aspect on a hot and dry summer day. This indicates that greater rooting volume did not aid in thermal tolerance for either climbing plant species, aspect and exposure was more important.

Between the two climbing plant species, I found that the faster-growing species *P. pandorana* had approximately double the wall coverage and biomass growth of the slower-growing *A. quinata* across all rooting volume treatments after the first growing season. *P. pandorana* also allocated more biomass to shoots than *A. quinata* across three rooting volume treatments. Interestingly, the significant wall coverage response to greater rooting volumes was observed earlier for the faster growing species *P. pandorana* than the slower growing species, *A. quinata*. This finding is supported in the literature demonstrating that faster-growing plant species are generally better able to acquire water and nutrients for plant growth and thereby achieve higher growth rates (Fransen et al., 1999). After six-months growth, for a wall area of 2.8 m^2 with stainless-steel mesh (1.45 m (W) by 1.95 m (H); mesh size: 200 mm x 340 mm), the approximate percentage wall coverage was 20-25% for *A. quinata* and 40-50% for *P. pandorana*, in both 42 L and 63 L rooting volumes.

Taken together, with a wall area of 2.8 m^2, these findings indicate that both 42 L and 63 L rooting volumes can provide sufficient resources to support the growth of both slower- and faster-growing climbing plant species during the first growing season for their establishments. Therefore, it could be suggested that, with a wall area of 2.8 m^2, 42 L rooting volume is adequate to support plant growth and coverage of indirect green façades during the first growing season, as 63 L rooting volume did not yield material benefit when factoring in associated economic costs and benefits of indirect green façades. However, to ensure the health and performance of green façade climbing plants in the long-term, larger rooting volume will be recommended.

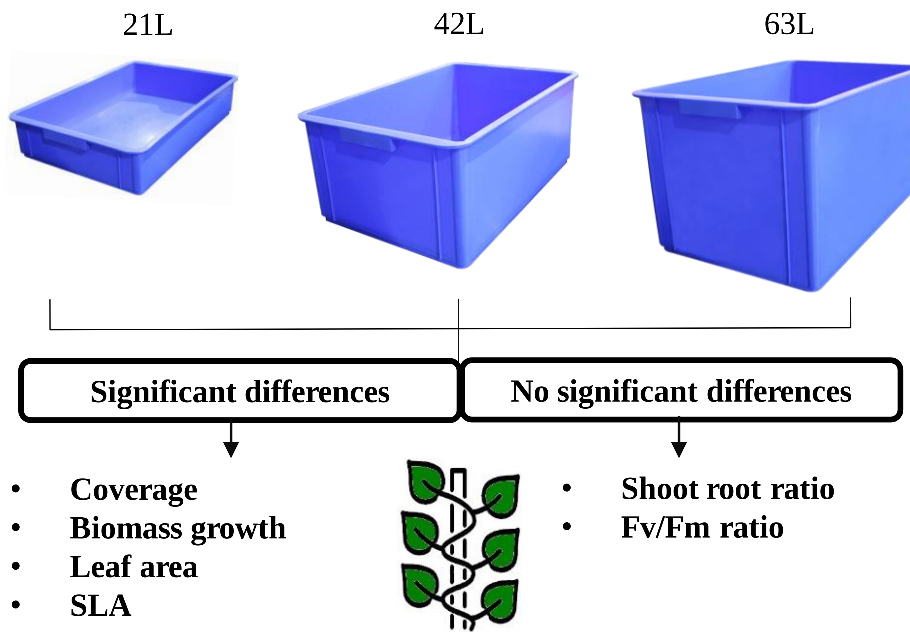


Figure 5.1. Graphical summary of chapter 2 (rooting volume experiment). The smallest rooting volume reduced plant coverage, biomass growth leaf area and SLA in both faster-and slower-growing climbing plant species. In contrast, shoot;root ratio and Fv/Fm ratio were remained unchanged across three rooting volume treatments.

2. **Effect of greywater irrigation on substrate properties and initial growth of façade climbing plants**

In chapter three, I conducted an 18-week glasshouse experiment to investigate the effects of greywater irrigation on climbing plants, by evaluating the substrate properties and plant traits of six climbing plant species (Fig. 5.2). Studies have indicated that greywater irrigation may change substrate properties and thereby adversely affect plant growth and health (Boyjoo et al., 2013; Cabot et al., 2014) However, after 18 weeks in my study, I found that both greywater irrigation treatments, i.e., (1) greywater, and (2) greywater flushed with potable water once every three weeks, did not significantly affect the coarse mineral-based (scoria) substrate pH.

There was a significant difference in substrate EC amongst the three irrigation treatments, however, the increase in EC, remained within the recommended range of 0 - 1500 µs cm^{-1} for the growth of most plant types (Bernstein, 1975). This indicates that there was no significant accumulation of salts in the substrates after 18-week greywater treatments. This is likely due to the coarse mineral-based (scoria) substrate used in this study, as it was fast-draining with a low cation exchange capacity (Collison and Grismer, 2014; Prodanovic et al., 2017).

Greywater irrigation did not reduce the growth of the six climbing plant species in my experiment, despite their differences in biomass, shoot:root allocation and leaf area. Irrespective of irrigation treatments, the greatest total biomass among the six species was observed in *P. pandorana*, the least total biomass was observed in *A. quinata*, *J. azoricum* and *T. jasminoides*. In terms of shoot:root allocation, *G. sempervirens* had a greater shoot:root ratio than the other five climbing plant species. The greatest and the lowest total leaf area was observed in *V.* 'Ganzin Glory' and *J. azoricum*. Surprisingly, *V.* 'Ganzin Glory' produced greater (but not significant) leaf area when irrigated with greywater flushed with potable water once every three weeks than with greywater only or potable water treatments. In terms of gas exchange and water use, the slow-growing *G. sempervirens* had the lowest photosynthetic rate, stomatal conductance and daily water use (in the final week of the experiment). Whereas, the fast-growing species, *P. pandorana* exhibited relatively higher photosynthetic rate and stomatal conductance among the six climbing plant species, and used the greatest amount of water per day.

Overall, the results indicated the greywater did not lead to an excessive or deleterious change in substrate properties, and therefore there was no detrimental effect on climbing plant growth and health. This may reflect the greywater quality and the coarse mineral-based substrate used in this study. Hence, these findings suggested that bathroom greywater (i.e., light greywater) could successfully replace potable water irrigation of building green façades in urban environments.

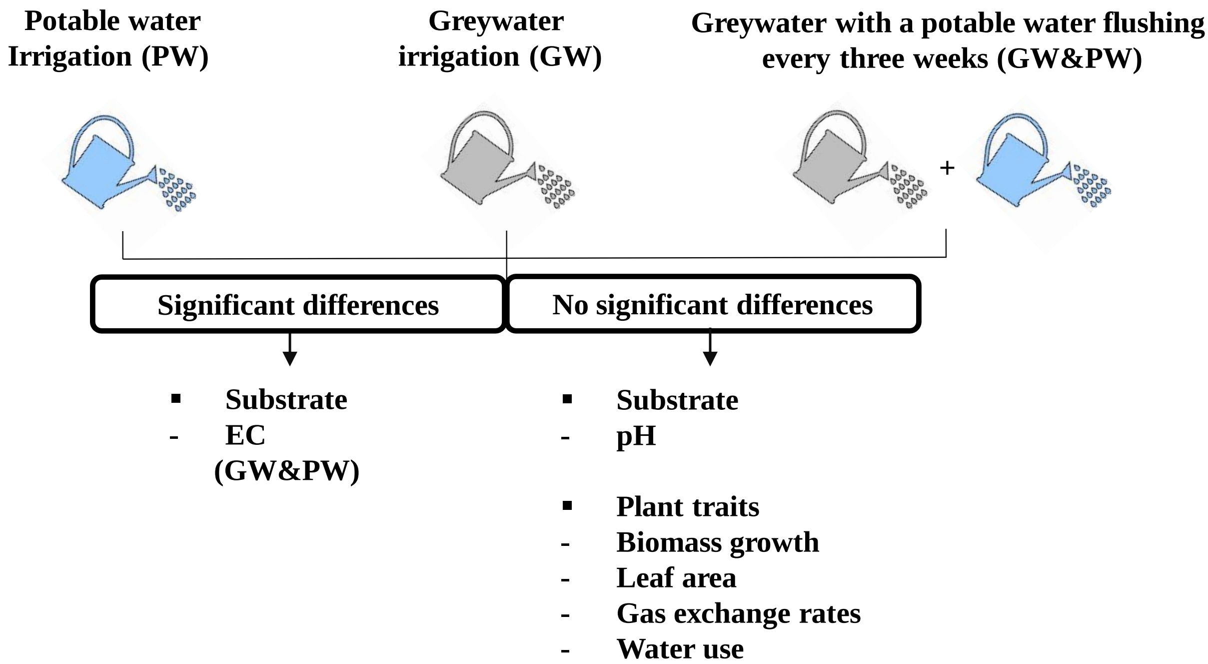


Figure 5.2. Graphical summary of chapter 3 (greywater experiment). Greywater irrigations (GW and GW&PW) did not significantly affect substrate pH or plant biomass growth, leaf area, gas exchange rates and water use. Substrate EC was the greatest under GW&PW treatment.

3. **Mature climbing plants can maintain their morphological and physiological traits under changing light conditions from full-sun to shade**

In chapter four, a 15-week field experiment was conducted to evaluate morphological, physiological and biochemical trait responses of mature climbing plants to changing light conditions from full-sun to shade (Fig. 5.3). Research undertaken in forestry ecosystems has indicated that woody plants can increase specific leaf area (SLA), decrease photosynthesis, lower light compensation points (LCPs), increase leaf chlorophyll contents (Chl *a*+*b*) and decrease thermal tolerance (T_{50}) in response to shade. In addition, studies in forests have suggested that deciduous woody plants are more plastic to changes in light intensity levels than evergreen woody plants (Böhnke and Bruelheide, 2013; Kröber et al., 2015; Lusk et al., 2008). In my study, all seven climbing species responded similarly to shade by significantly increasing SLA; whilst stomatal conductance (g_s), light compensation points (LCPs) and thermal tolerance (T_{50}) remained unchanged under the shade treatment. For other traits, climbing plant species responded differently to the shade treatment. In response to shade, two evergreen climbing species (*G. sempervirens* and *J. azoricum*) increased their leaf expansion rates. In addition, deciduous *A. brevipedunculata* and *V.* 'Ganzin Glory' as well as evergreen *P. pandorana* significantly decreased leaf photosynthetic rate (A_{1500}) in response to shade. The shade treatment significantly increased Chl *a*+*b* in most of the climbing plant species, except for deciduous *V.* 'Ganzin Glory' and evergreen *G. sempervirens*. Among the seven climbing species in this study, the notable variance in response to shade for some plant traits might imply that variations in response to reduced light levels occur on a species level, irrespective of being evergreen or deciduous.

Regardless of light conditions, there were differences in traits between deciduous and evergreen species. Significantly greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl *a*+*b*, and significantly lower LCP were observed in deciduous climbing plant species as compared with evergreen species. However, thermal tolerance (T_{50}) did not differ between deciduous and evergreen climbing plant species. These results can be partially interpreted using the slow-fast continuum of resource acquisition strategies (Reich, 2014). Plant species that rapidly acquire resources (faster), tend to exhibit greater SLA and gas exchange rates, as well as lower thermal tolerance than resource-conservative species (Reich, 2014; Sastry and Barua, 2017; Wright et al., 2004). Generally, evergreen woody plants are more conservative and slower in their resource acquisition and use strategies, due to the longer leaf lifespan. Whereas, deciduous woody plants more rapidly acquire resources and this is reflected in their leaf traits (Álvarez-Yépiz et al., 2017; Zhao et al., 2017). Despite the observed differences in leaf traits between deciduous and evergreen climbing plant species, there was no significant difference in overall phenotypic plasticity (morphology and physiology) to shade. A significant difference was only observed in A_{1500}, in that deciduous climbing plant species exhibited a greater A_{1500} reduction than evergreen species in shade.

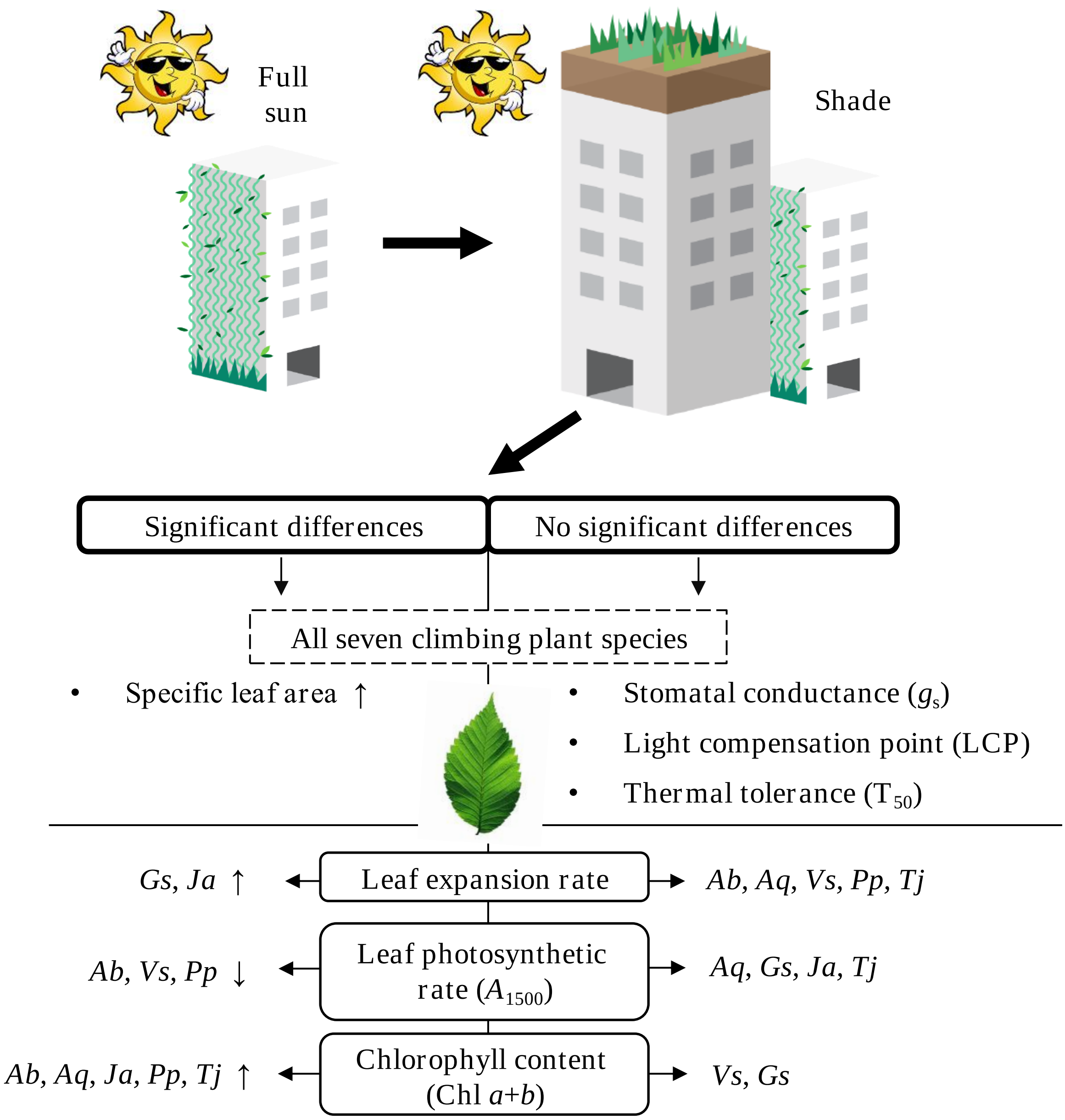


Figure 5.3. Graphical summary of chapter 4 (changing light condition from full-sun to shade). All seven climbing plant species increased SLA, whilst g_s, LCP and thermal T_{50} remained unchanged under the shade treatment. In contrast, there was a significant species difference in the changes of leaf expansion rates, A_{1500}, and Chl *a*+*b*.

Implications for implementing indirect green façade in urban environments

Indirect green façades can help to mitigate many of the adverse impacts of urbanization by integrating greenery and providing shade to areas of high urban density (Abdullahi and Alibaba, 2016; Başdoğan and Çığ, 2016; Rakhshandehroo et al., 2015). However, high urban density poses several challenges to establishing green façades, including reduced rooting volumes, limited potable water supply for irrigation, and variable light conditions. Irrespective of potentially unfavourable growing conditions in urban environments, climbing plant species must maintain or increase their wall coverage with healthy foliage and/or productivity (photosynthesis) to help ensure indirect green façades provide these benefits (Cameron et al., 2014; Hoelscher et al., 2016).

Chapter two of this PhD study indicated that the adequate rooting volume is key to support climbing plant growth and vegetation wall coverage of containerised indirect green façades. The ratio of vegetation wall coverage (m^2) to rooting volume (m^3) for the slower-growing *A. quinata* and the faster-growing *P. pandorana* was identified. Over the first growing season, approximately 10.2 m^2 for *A. quinata* and 21.3 m^2 of canopy coverage for *P. pandorana* and can be expected with each cubic metre of rooting volume supplied. These guidelines for rooting volume to support canopy coverage of green façades are likely to be of interest to landscape architects and building designers. To my knowledge, this is the first study to document the rooting volume required to support climbing plant growth in containers with adequate irrigation and nutrition. While this study showed that the growth of both faster- and slower-growing species could be supported with 42 L or 63 L rooting volumes during the first growing season, this may not be maintained in the long-term. Over time, the adverse impacts of inadequate rooting volumes are likely to be more pronounced in faster-growing plant species, which is due to their fast-resource acquisition capability (Reich, 2014). Hence, species growth rate cannot be overlooked when choosing the right rooting volume for containerised indirect green façades.

Where possible, landscape architects, building engineers and planners should maximise rooting volume to ensure long-term maintenance of plant vegetation coverage.

Chapter three of this PhD study supports the reuse of bathroom greywater (i.e., light greywater) for containerised indirect green façades irrigation. In this study, greywater irrigation did not detrimentally affect substrate chemical properties or growth and health of six climbing plant species. Suggesting that bathroom greywater can be safely used to irrigate climbing plants in indirect green façades, at least for establishment, but likely longer as there was no salt accumulation in the substrate. However, it is important that free-draining mineral substrates are used to avoid salinity problems.The results could be attributed to the quality of the synthetic greywater and characteristics of substrates used in my study. The synthetic greywater was formulated to conformed to office building/domestic bathroom greywater (Diaper et al., 2008). Domestic greywater generated from non-toilet bathroom (wash basins, baths and showers) is considered as the 'light greywater' that is less polluted and have lower environmental impacts than municipal wastewater (Boyjoo et al., 2013). The substrate used in this study was a coarse mineral-based substrate that is fast-draining with a low cation exchange capacity (Collison and Grismer, 2014; Prodanovic et al., 2017), which is less likely to accumulate salts. On the other hand, Fowdar et al. (2017) demonstrated that some climbing plant species exhibited great pollutant removal performance for greywater treatment. Although my study did not assess the pollutant removal performance of climbing plant species, I measured the water use of the six climbing plant species. Regardless of the irrigation treatments, the faster-growing plant species *P. pandorana* and slower-growing *G. sempervirens* consumed the greatest and lowest amount of water, respectively. As greywater did not reduce plant growth or water use, this suggests that greywater discharge could be mitigated by selecting climbing plants with high water requirements, e.g., *P. pandorana*. Therefore, greywater irrigation of containerised indirect green façades can not only reduce potable water consumption but also improve their sustainability.

Chapter four of this PhD study demonstrated that; climbing plants could adapt to changing light levels from full-sun to shade by adjusting their leaf traits measured. As the seven climbing species evaluated originate from various natural habitats, it is therefore assumed that generalisations of adaptability can be made beyond these species. Due to the trait and growth plasticity shown to changing light conditions, all of these species are also likely to maintain their performance in cities with changing light levels from full-sun to shade. For instance, among the seven climbing plant species, *G. sempervirens* and *J. azoricum* were more productive and resilient to reduced light intensity levels, due to increased leaf expansion rates and maintained photosynthesis. Accordingly, both climbing plant species should be able to continue to provide good vegetation coverage even in full shade. In contrast, the other five climbing species either maintained or decreased A_{1500}, however they increased their SLA and chlorophyll contents, making them able to function under shady conditions. On the other hand, as thermal tolerance of the climbing species evaluated was unaffected by the shade treatment or rooting volume treatments (Ch 2), these species are unlikely to be affected by changes in heat which may occur with changes in light availability light or smaller rooting volume on high-rise buildings in cities. The findings support the conceptual premise that climbing plant species are generally quite adaptable to various light conditions.

Although evergreen climbing species continued to maintain productivity (photosynthesis) and growth in shade and deciduous climbing plant species had a greater reduction in A_{1500}, deciduous climbing plant species may outperform evergreen species due to their inherent characteristics, e.g., greater SLA, leaf expansion rates, A_{1500}, g_s, and Chl a+b, as well as lower LCP, irrespective of light intensity levels. Hence, deciduous climbing plant species may be preferential for indirect green façades in urban environments with changing light conditions.

Limitations of the study and recommendations for future research

By necessity, this PhD study had a limited focus on the impacts of three different growing conditions (i.e., rooting volume, greywater irrigation, reduced light availability) on a limited number of climbing plant species (Fig. 5.4), and tackle only a subset of each of the three identified issues. The three main thesis chapters (two, three, and four) mainly investigated the changes in some morphological and physiological traits of climbing plant species to the three growing conditions.

In general, this thesis examined the short-term impacts of three different growth factors on a range of climbing plant species. Studies of more climbing plant species and longer experimental periods would help to establish the broader viability of these findings. Although the establishment of climbing plant species is important for the success of indirect green façade systems, there is a need to assess the long-term influences of unfavourable growing conditions on the growth and performance of climbing plant species to ensure the sustainability of these systems. In chapter two, this study assessed the influence of rooting volume on above-ground growth and performance of two climbing species. While this study evaluated two species of contrasting growth strategies, and provides useful information which is transferable to other species, further studies should be undertaken to evaluate the effects on root development (e.g., root density, root length, and root:container ratio). In chapter three, only one type of substrate (fast-draining) was used to grow six climbing plant species, and synthetic greywater was formulated to conform to the quality of 'light greywater'. Further research should be undertaken to investigate the impact of different types of greywater and substrates (e.g., slow-draining) on indirect green façade climbing plants. It would also be beneficial to evaluate the effects of greywater irrigation on indirect green façade planted on buildings where the greywater is directly sourced from the building occupants. This would enable testing where water quality is variable and more realistic. In chapter four, one level of shade was applied to assess the response of mature climbing plants to reduced light availability. It remains unknown whether young plants would also exhibit similar plasticity to changing light conditions. In addition, various light conditions should be applied in future research as solar radiation levels in cities can vary due to many factors such as building orientation, building height, surrounding environment, and microclimate (Freitas et al., 2015; Shishegar, 2013). Therefore, real case studies should also be conducted to evaluate plant growth and performance of green façades on- in conditions of contrasting light availability. Future studies on these topics are therefore recommended.

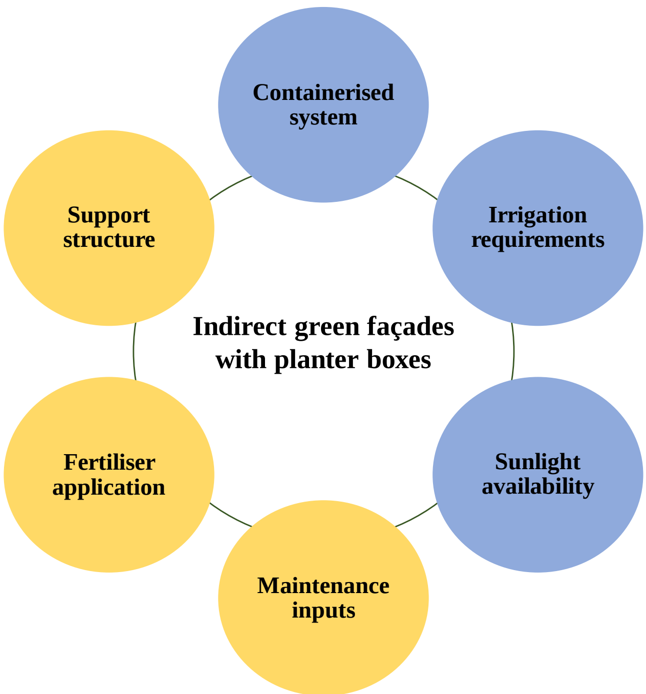


Figure 5.4. Installation and management considerations for indirect green façades with planter boxes. Items in blue ovals are further explored in this thesis. Topics related to the items in yellow ovals need to be investigated in the future.

Conclusions

This PhD study improved our understanding of plant selection for successful indirect green façades in urban environments, particularly under unfavourable growing conditions. This study has indicated that there is a relatively small substrate volume needed to support the early growth and vegetation wall coverage of indirect green façade climbing plants. This study also suggested that domestic greywater could replace potable water irrigation of building indirect green façades in urban environments, as there was no detrimental effect on either substrate properties or climbing plant growth. Lastly, but not least, the seven climbing species in this study could adjust plant traits in response to the changing light conditions from full-sun to shade. This enables them to maintain their function and performance to certain extents under shade conditions, suggesting they can continue to deliver some of ecosystem services under changing urban light environments. This thesis further supports the possibility to implement indirect green façades as an integrated approach of sustainable urban greening and water management on high-rise buildings.

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