CHANGING CONNECTIONS:
Ontogenetic ecophysiology of secondary hemi-epiphytic vines

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ABSTRACT

Secondary hemi-epiphytes start their life as ground-dwelling plants. Like other vines, the plant then climbs the host, but when the plant reaches maturity, the oldest portion of the stem dies. The plant then loses its stem connection to the soil and becomes semi-epiphytic. However, true secondary hemi-epiphytism is probably not as common as thought, since, in most cases semi-epiphytic vines reconnect to the soil through aerial roots. The change in soil connection during the ontogeny of these species may have physiological and anatomical consequences. As they eventually live in the canopy environment, it is feasible that secondary hemi-epiphytes might develop adaptations to cope with the stressful canopy environment, especially water stress during dry periods. However, there is a lack of understanding on the ecophysiology of secondary hemi-epiphytes in rainforests.

There is a paucity of information on the anatomy and physiology of secondary hemi-epiphytes, once they lose their stem connection to the soil, compared with the terrestrial early stage of development. To address this knowledge gap, characteristics of stem water transport, leaf anatomy and physiology, and soil water resource partitioning were examined in this research. Two species were selected for the study: *Freycinetia excelsa* F. Muell (Pandanaceae) and *Rhaphidophora australasica* F.M. Bailey (Araceae), which occur naturally in the Wet Tropics area of north Queensland. The general objective of this research is to better understand

the ecophysiology of secondary hemi-epiphytes during their ontogenetic development.

The capacity of *F. excelsa* and *R. australasica* stems to conduct water differed between plants of different developmental phases. Adult individuals of *F. excelsa* and *R. australasica* had wider vessels than younger plants. Hydraulic architecture parameters, i.e. hydraulic conductivity, stem specific conductivity and leaf specific conductivity, were also higher in adult plants than for intermediate and juvenile individuals. These results indicate that adult plants had a higher capacity to conduct water through the stem to the leaves than did individuals at an earlier stage of development.

As the plants became more mature and longer, they tended to have low hydraulic conductivity at the stem base. This finding is supported by the fact that the size of xylem vessels was found to decrease in the basipetal direction: the base of the stem had narrower vessels than the middle part of the stem. However, the low hydraulic conductivity at the base of the stem may also be related to the fact that monocotyledonous plants lack secondary development. Therefore, the stem base contains the oldest shoot tissues and the vessels might be less functional. Wider vessels and higher hydraulic conductivity in adult individuals of *F. excelsa* and *R. australasica* show that the change in plant-soil connectivity during ontogeny of these species does not physically restrict water transport.

Adult individuals of *F. excelsa* and *R. australasica* had larger stomata than conspecific juveniles. However, adult plants also had more stomata per unit area, which gives them more control of the opening and closing of stomata in certain areas of the leaves. These characteristics of leaf anatomy suggest that secondary hemi-epiphytes are well-adapted to the canopy environment.

Juvenile plants of these two study species appear to be more sensitive to the onset of drought than plants of later developmental stages. Within each dry and wet season, the water potential of leaves from all growth forms were similar but the patterns of daily CO_2 exchange differed, with CO_2 uptake by juvenile plants most affected by dry season conditions. However, the CO_2 exchange rates were similar for adult, intermediate and juvenile plants during the wet season. High water availability in the wet season and relatively low evaporative demands provide excellent conditions for plants to absorb CO_2. The significant down-regulation of CO_2 exchange in the dry season in the juveniles is related to the lower hydraulic conductivity of their stems. Water supply to juveniles may be restricted during the dry season, such that down-regulation of CO_2 uptake and stomatal opening are necessary to diminish water loss and maintain water potential. Water supplied to intermediate and adult plants by aerial roots growing from a number of places along the stem is evidently sufficient to sustain higher rates of CO_2 exchange and water loss.

Plants of different ontogenetic stages had different behaviours towards soil water resources. Based on the hydrogen stable isotopes of water derived from different layers of the soil profile, matched with isotope signatures of the stem water, water

uptake by juvenile individuals was limited to the area near the soil surface; on the other hand, adult plants utilized water from all soil layers studied. This consequently affects the capacity of plants to exploit all available soil water sources across seasons, which influences the performance of individuals of different ontogenetic stages in response to environmental conditions.

Variations in the ecophysiological attributes of the secondary hemi-epiphytes *F. excelsa* and *R. australasica* indicate differences in the ability of these plants to survive during their development. This study showed that smaller size juveniles may have a higher potential susceptibility to stressful environmental conditions compared to larger adult congeners. Based on ecophysiological characters, these two secondary hemi-epiphytes have not adapted especially to the epiphytic habit as they climb the host and live in the canopy. The plants' soil connections through aerial roots provide access to soil, avoid the stem basal hydraulic bottle neck and contribute to more options for soil water resource acquisition.

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LIST OF ABBREVIATIONS AND SYMBOLS

ψ_L	leaf water potential
ψ_{md}	mid-day leaf water potential
ψ_{la}	late-afternoon leaf water potential
ψ_{pd}	pre-dawn leaf water potential
$\sum d^4$	total of the fourth power of vessel diameter
$\delta^{13}C$	the carbon isotope ratio of $^{13}C/^{12}C$
δD	the hydrogen isotope ratio of $^{2}H/^{1}H$
$\delta^{18}O$	the oxygen isotope ratio of $^{18}O/^{16}O$
A	CO_2 assimilation rate
A_{max}	maximum CO_2 assimilation rate
ANOVA	analysis of variance
CAM	crassulacean acid metabolism
CO_2	carbon dioxide
E	transpiration rate
E_{max}	maximum transpiration rate
g_s	stomatal conductance
g_{smax}	maximum stomatal conductance
HV	Huber value
IRGA	infra red gas analysis
KCl	potassium chloride
K_H	stem hydraulic conductivity

K_S	stem specific conductivity
LMA	leaf mass area
LSC	leaf specific conductivity
N	number
NP	National Park
O_2	oxygen
PAR	photosynthetically active radiation
SLA	specific leaf area
V-SMOW	Vienna standard mean ocean water

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CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Tropical rainforests are referred to as forests existing in the tropics which are "evergreen, hygrophilous in character, at least 30 m high, rich in woody climbing plants and epiphytes" (Schimper 1903 *in* Adam 1992). Macroclimates of these ecosystems are greatly governed by tropical climates (Golley 1991). According to Kőppen's climate classification, tropical rainforest occupy areas where the average monthly temperature is higher than 18^0C and minimum precipitation is above 60 mm. Furthermore, the average annual temperature for lowland tropical forests generally exceeds 25^0C. In general, tropical forests are continually moist and warm (Archibold 1995). Outside the tropics, rainforest may occur in temperate regions with high rain intensity and mild winters (Adam 1992). In Australia, the definition of rainforest has progressed from just dense evergreen forests located in areas with abundant rain to include vegetation in seasonally dry environments (Adam 1992).

Tropical rainforests are the most diverse terrestrial ecosystems in the world. Although they only cover about 6 per cent of the Earth's land surface, they contain about 50 per cent of plant and animal species and about 65 per cent of the flowering plants (Crawley 1986). Some 7,900 plant species exist on the Malay Peninsula and about 80,000 plant species live in the Amazon Basin (Whitmore 1998). In primary lowland forest, the number of trees of more than 10 cm diameter at breast height is

usually about 50 – 60 individuals per hectare. In parts of Indo-Malaysia, the number can reach 200 or 300 per hectare (Richards 1996).

Climbing plants and epiphytes are prominent features of tropical rainforests. Climbing plants consist of herbaceous and woody vines (lianas). Woody vines, which can contribute 10 to 40 per cent of woody plant diversity in tropical forests (Schnitzer and Bongers 2002), differentiate tropical rainforests from temperate forests (Gentry 1988; 1991). Lianas are ecologically important as they increase the structural integrity of the forest canopy and provide walkways for arboreal animals, as well as foods (Putz 1995). However, in Australian tropical forests, the density of lianas is lower than in tropical rainforests in other continents (Gentry 1991). In contrast to woody vines, herbaceous vines are more widespread in distribution, spreading from the tropics to the boreal zone.

Vines belong to more than 130 plant families (Gentry 1991). More than 90 seed plant families in the Neotropics contain vines, and most of these families also contain climbing plants in the Palaeotropics. Asclepiadaceae, Asteraceae, Araceae, Bignoniaceae, Convolvulaceae, Leguminoseae and Sapindaceae contain hundreds of climbing species (Gentry 1991). In some families, such as Hippocrateceae and Vitaceae, nearly all the species are climbers (Putz 1984). The climbing plants of more than 30 families of seed plants only occur in the Palaeotropics, including Myrtaceae, Nepenthaceae, Pandanaceae and Rutaceae (Gentry 1991).

Vascular epiphytes, which predominantly inhabit the humid tropics (Benzing 2004), also contribute significantly to the biodiversity of tropical forest ecosystems (Benzing 1990). Vascular epiphytes comprise more than 25,000 species, from 83 families (Kress 1989), which are about 10 per cent of the world's flora (Madison 1977; Benzing 1990). The term "epiphytes" commonly include "true epiphytes", "hemi-epiphytes" and "casual/facultative epiphytes" (Benzing 1989; Kress 1989). True epiphytes spend their entire life cycle as epiphytes and absorb water and nutrients from non-terrestrial sources, while hemi-epiphytes only spend part of their life in epiphytic conditions (Kress 1989). Casual epiphytes are species in which some individuals of a population are true epiphytes while others are terrestrial plants (Kress 1989; Rada and Jaimez 1992).

Epiphytism occurs in a large number of angiosperms and ferns, and some gymnosperms; however, most epiphytes are monocotyledons, with 80 per cent of epiphytes belonging to this group (Kress 1989; Lüttge 1997; Zotz and Hietz 2001). Orchidaceae contains the largest number of epiphytic species (Table 1.1). About two out of three epiphytes are orchids and about 70 per cent of the members of this family are canopy-adapted (Benzing 1990). Araceae and Bromeliaceae are two other prominent monocotyledoneous families containing epiphytic species (Table 1.1). The genera *Anthurium*, *Philodendron* and *Rhaphidophora* contain most epiphytes in the Araceae (Benzing 1990). Dicotyledonous epiphytes are prominent in the Cactaceae, Ericaceae, Gesneriaceae, Melastomataceae and Piperaceae (Table 1.1; Benzing 1990).

Table 1.1 Families of vascular plants containing over 50 epiphytic species, accounting for 98 per cent of vascular epiphytes. Source: Kress (1989).

Family	**Species**		
	Epiphytic	Total	Percent
Orchidaceae	13,951	19,128	73
Araceae	1,349	2,500	54
Bromeliaceae	1,145	2,500	46
Polypodiaceae	1,029	1,100	94
Piperaceae	710	3,100	23
Ericaceae	672	3,500	19
Melastomataceae	648	4,770	14
Gesneriaceae	560	2,500	22
Moraceae	523	1000	52
Aspleniaceae	400	675	59
Hymenophyllaceae	400	600	67
Dryopteridaceae	292	1,920	15
Rubiaceae	223	6,500	3
Lycopodiaceae	200	401	50
Cactaceae	150	1,500	10
Davalliaceae	139	150	93
Asclepiadaceae	137	2,000	7
Vittariaceae	112	112	100
Clusiaceae	92	1,200	8
Marcgraviaceae	89	122	73
Cyclanthaceae	86	200	43
Araliaceae	78	700	11
Solanaceae	56	2,800	2

About 11,000 species of vascular epiphytes in 320 genera exist in the Australo-Asian region. These epiphytes fall into 47 families, with the Orchidaceae having the largest number of epiphytic species (Table 1.2; Benzing 1990; Wallace 1989). The geographic centre of the Australo-Asian diversity appears to be in Borneo, Java, Sumatra and the Malay Peninsula. There are relatively few Australian epiphytes, about 350 species with low endemism (Wallace 1989).

Table 1.2 Synopsis of numbers and groupings of epiphytes occurring in the Australasia region. Source: Wallace (1989).

Taxonomic grouping	No. families	No. genera	No. species
Pteridophytes	14	70	2,200
Dicotyledons	23	72	2,300
Monocotyledons	10	187	5,800
- non-orchid	9	17	300
- orchid	1	170	5,500

Even though secondary hemi-epiphytes are categorized as epiphytes (Benzing 1990; Kress 1989; Nieder *et al*. 2001), their growth habits lie somewhere between vines and epiphytes. As secondary hemi-epiphytic climbers change their growth habit from terrestrial juvenile to semi-epiphytic adult, they may face physiological shifts during their life cycle. As they climb to the top of trees, they also experience heterogeneous environmental conditions. However, to date, only a few studies have investigated their physiological responses to changing growth habits during ontogenetic development and to the environmental conditions in which secondary hemi-epiphytic vines live.

1.2 HEMI-EPIPHYTISM

Hemi-epiphytes are plants that vary their growth habit and spend only a part of their life cycle as epiphytes. Hemi-epiphytes are divided into two categories: primary and secondary epiphytes (Benzing 1990; Kress 1989; Lüttge 1997; Moffet 2000). Around 2,000 species are categorized as either primary or secondary hemi-epiphytes, representing about 8 per cent of vascular epiphytes or 0.8 per cent of vascular plants (Gentry and Dodson 1987; Holbrook and Putz 1996a). Hemi-epiphytism is less systematically widespread than holo-epiphytism. Only about 31 plant families contain hemi-epiphytes, compared with 83 families containing epiphytic species (including hemi-epiphytes) (Nieder *et al*. 2001).

Primary hemi-epiphytes germinate at the top of trees and live as epiphytes in their early life stages (Holbrook and Putz 1996a). After a certain period, adventitious roots reach the ground and become terrestrial roots drawing nutrients from the soil (Holbrook and Putz 1996a; Moffet 2000). The two best studied genera of primary hemi-epiphytes are *Ficus* (Moraceae) and *Clusia* (Clusiaceae) (Lüttge 2006; William-Linera and Lawton 1995).

Secondary hemi-epiphytes, on the other hand, start their life as ground dwelling plants. As do other vines, the plant then climbs the host. When the plant reaches maturity, the oldest portion of the stem dies. The plant then loses its connection to the soil and becomes epiphytic (Benzing 2004). As secondary hemi-epiphytes are vines, they are commonly called semi-epiphytic climbers/vines (Wallace 1989), or tropical nomadic vines (Moffet 2000). However, true secondary hemi-epiphytism is

probably not as common as thought (Lüttge 1997; Moffet 2000). In most cases, semi-epiphytic vines remain connected or reconnect to the soil through aerial roots (Lopez-Portillo *et al.* 2000).

Since primary hemi-epiphytes are more often studied, the term "hemi-epiphyte" is sometimes misleadingly applied solely to primary hemi-epiphytes. Consequently, understanding of the hemi-epiphytic habit is biased towards primary hemi-epiphytes (Lopez-Portillo *et al.* 2000; Meyer and Zotz 2004).

1.3 ECOLOGY AND DISTRIBUTION OF SECONDARY HEMI-EPIPHYTES

As is the case with other vines, secondary hemi-epiphytes climb trees for physical support, which also improves access to light. As they rely on trees for mechanical support, the allocation of resources to supporting tissues is relatively low and more resources (carbon) can be invested in growth from the apical meristem. As a result, secondary hemi-epiphytes usually have substantial vertical growth rates (Mooney and Gartner 1991).

Secondary hemi-epiphytes are root climbers (Wallace 1989), as they climb trees by attaching their roots to the host. They also develop adventitious roots which connect to the soil. These soil connections might reduce the limitations of water and mineral uptake faced by true epiphytes (Williams-Linera and Lawton 1995). Meyer and Zotz (2004), who studied two species of semi-epiphytic Araceae, reported that the growth

and survival of aerial roots of *Philodendron radiatum* and *Anthurium clavigerum* influenced the vertical growth of those species.

The secondary hemi-epiphytic growth habit occurs in several families of climbing plants and is widely spread across tropical regions. Although secondary hemi-epiphytism occurs in some species of Melastomataceae, Vitaceae, Nepenthaceae and Pandanaceae, the Araceae family has the largest number of species with this kind of growth habit (Wallace 1989). As with true vascular epiphytes, secondary hemi-epiphytic vines predominantly inhabit the humid tropics and contribute to the biodiversity in tropical forest ecosystems (Benzing 1990). They are widely spread in the tropics from South America to Indo-Malaya and the Pacific region (Table 1.3; Williams-Linera and Lawton 1995). In Australia, some 12 families contain secondary hemi-epiphytic vines, with Araceae containing the most genera (6) (Wallace 1989).

Table 1.3 Families and genera containing secondary hemi-epiphytes and their distribution. Sources: Wallace (1989) and Williams-Linera and Lawton (1995).

	Semi-epiphytic vine spp. (Generic total)	Distribution
Monocotyledonae		
1. Araceae		
Amydrium	4 (4)	Malaysia, Pacific
Anthurium	200 (550)	Neotropics
Caladiopsis	2 (2)	South America
Epipremnum	15 (15)	Indo-Malaya, Pacific
Monstera	24 (25)	Neotropics
Pedicellarum	1 (1)	Borneo
Philodendron	133 (275)	Neotropics
Porphyrospatha	3 (3)	Neotropics

Pothos	25 (75)	Indo-Malaya, Pacific
Rhaphidophora	100 (100)	Indo-Malaya, Pacific
Syngonium	18 (25)	Neotropics
2. Cyclanthaceae		
Asplundia	20 (82)	Neotropics
Carludovica	1 (3)	Central America
Ludovia	2 (2)	South America
Sphaeradenia	7 (38)	Neotropics
Thoracocarpus	1 (1)	South America
3. Pandanaceae		
Freycinetia	80 (100)	Indo-Malaya, Pacific
Dicotyledonae		
1. Marcgraviaceae		
Caracasia	2 (2)	Venezuela
Marcgravia	50 (55)	Neotropics
Norantea	20 (35)	Neotropics
Souroubea	20 (25)	Neotropics
Ruyschia	2 (10)	Neotropics
2. Melastomataceae		
*Mednilla**	300 (400)	Indo-Malaya, Pacific
3. Vitaceae		
*Pterisanthes**	2 (20)	Indo-Malaya, Pacific
*Tetrastigma**	2 (90)	Indo-Malaya
4. Nepenthaceae		
*Nepenthes**	6 (70)	Indo-Malaya
5. Gesneriaceae		
*Aeschynanthus**	80 (80)	Indo-Malaya, Pacific
*Crytandra**	10 (600)	Indo-Malaya, Pacific
*Dichrotricum**	4 (4)	Indo-Malaya, Pacific
6. Apocynaceae		
*Ceropegia**	3 (160)	Indo-Malaya, Pacific
*Hoya**	100 (200)	Indo-Malaya, Pacific

Note: 1. Pacific includes Australia.
2. * genera containing either semi-epiphytic climbers or facultative/casual epiphytes, i.e. species in which some individuals of a population are true epiphytes while others are terrestrial plants (Kress 1989).

1.4 HETEROBLASTIC GROWTH IN VINES

Many vine species show heteroblastic development. Heteroblastic development is defined as a profound developmental change in a plant's life form from seedling to reproductive age (Lee and Richards 1991); hence, sometimes they are misperceived as different species. This heteroblastic development can include many traits: (a) leaf size, shape and anatomy, (b) internode length, (c) stem thickness, (d) shoot structures, (e) trophic responses, (f) physiology, and (g) regenerative capacity.

Members of the family Araceae show changes in their shoot structures and arrangements. Leaves become larger and often more complex. These changes can be either gradual or abrupt. Such metamorphoses are the result of internally regulated processes, such as in *Syngonium* or *Philodendron*, or changes in environmental conditions and due to loss of contact to the soil, such as in *Monstera* (Ray 1990). In secondary hemi-epiphytic vines, leaves and stems of one individual are exposed to a variety of microhabitats where they grow from the forest floor to the forest canopy. Adaptation of these organs to their immediate environments have been suggested as one explanation as to why metamorphosis in vines is common (Ray 1990; 1992).

Such heteroblastic changes in secondary hemi-epiphytic Araceae emphasize the developmental plasticity in this family (Ray 1992). These metamorphoses create a complex gradation between size and shape of leaves as plants grow and mature. The changes also allow shoots to engage in a variety of development patterns when conditions are not suitable for the species to reach adult and reproductive stages.

The secondary hemi-epiphytic habit itself is a form of heteroblastic development. It is expected that there is a physiological shift when changes occur. As plants lose contact with the soil, or aerial roots connect to the ground, some physiological traits might be affected. The possible ecophysiological consequences that accompany such developmental changes are little investigated.

1.5 THE ECOPHYSIOLOGY OF HEMI-EPIPHYTES

The change in growth habit during the secondary hemi-epiphytic vine life cycle may have physiological and anatomical consequences. The collapse of stem connectivity, which is eventually replaced by aerial root connectivity, may have consequences in relation to water and nutrient availability. True epiphytes generally exhibit multiple mechanisms that cope with drought and the acquisition of essential ions (Benzing 1990). Epiphytes typically exhibit high water use efficiencies (Lüttge 1997). Many epiphytic species develop crassulacean acid metabolism (CAM), a photosynthetic pathway which allows stomata to take up CO_2 in the cool of night, reducing transpirational H_2O loss (Holtum and Winter 1999; Holtum 2002; Lüttge 1997).

The anatomy and physiology of secondary hemi-epiphytes when they have lost stem connections to soil is not well described. The effectiveness of water and mineral transport through the aerial roots is also poorly understood. As secondary hemi-epiphytes climb up trees, they also face environmental gradients of changing light regimes, moisture and perhaps CO_2 levels. Castellanos (1991) noted that heterogeneous light environments are one of the major characteristics of vine microhabitats. Differences in the light climate of the under-storey and upper-storey

are not only due to the light quantity, but also the light quality (Whitmore 1998). The low light environment of the rainforest under-story is likely a selective pressure for the epiphytic and hemi-epiphytic life forms (Lüttge 1997). Organisms need adaptations in behaviour to cope with different environmental conditions, such as variation in CO_2 and light (Ray 1992). Adaptation will direct the growth of plants in a certain orientation rather than in random movements. However, there is limited research on the anatomical and physiological consequences of such changes in environment for secondary hemi-epiphytes.

Although there has been some research on secondary hemi-epiphytic vines, none of the studies have investigated or compared their physiology at different stages of growth. Holbrook and Putz (1996b) noted that secondary hemi-epiphytes might have physiological similarities with lianas. However, what factors contribute to this plastic growth form, and how these species adapt physiologically at different growth stages, are not well-understood. Ray (1990; 1992) studied species in the family Araceae and suggested that, because some semi-epiphytes are plastic in shoot morphology, they might also be plastic in terms of physiology. This theory has not been investigated comprehensively. Therefore, research on secondary hemi-epiphytic climbing plant species is needed.

Unlike secondary hemi-epiphytes, there has been extensive research on primary hemi-epiphytes (Holbrook and Putz 199b; Lüttge 2006; William-Linera and Lawton 1995; Zotz and Meyer 2004). Holbrook and Putz (1996a) studied leaf anatomy, water relations and gas exchange of epiphytic and free-standing stages of several *Ficus*

species. The hydraulic architectures of hemi-epiphytic *Ficus* and *Clusia* in comparison to free-standing individuals have also been investigated (Patiño *et al.* 1995; Zotz *et al.* 1994). Carbon balance and nitrogen use efficiency of *Clusia* have been researched (Zotz and Winter 1994a; 1994b). The photosynthetic flexibility of the genus *Clusia* has also been reviewed extensively (Lüttge 2006).

Leaf anatomy may change during ontogeny in secondary hemi-epiphytic climbers. As they climb to the canopy, adult plants may adapt to the stresses of the canopy environment (Benzing 1990). Secondary hemi-epiphytes are assumed to develop semi-epiphytic characters. Therefore as the plants change from a terrestrial juvenile to a semi-epiphytic adult, they might have greater stomatal control, establishment of water storage tissues, increased number or thickness of epidermal layers and decreased stomatal density. However, there is no research investigating these issues.

Research on casual epiphytes, species in which some individuals of a population are true epiphytes while others are terrestrial plants, has documented different anatomical characteristics in the epiphytic and terrestrial plants (Rada and Jaimez 1992). The epiphyte *Anthurium bredemeyeri* (Araceae) in a tropical cloud forest of La Carbonera, Venezuela, has a significantly lower stomatal density than the terrestrial congener. No other differences, though, were reported between the two growth habits. Leaves of both terrestrial and epiphytic individuals also have large intercellular spaces and about 85 per cent of leaves are occupied by spongy parenchyma.

Anatomical characteristics of several primary hemi-epiphytes differ significantly between epiphytic and free-standing individuals. A study of eight species of Venezuelan primary hemi-epiphytes (*Ficus nymphaeifolia, F. obtusifolia, F. pertusa, F. tuerkheimii, F. aurea, F. trigonata, Clusia minor* and *Coussapoa villosa*) found that epiphytic plants had a significantly higher specific leaf area than terrestrially-rooted individuals in all species except *Coussapoa villosa* (Holbrook and Putz 1996a). In *Ficus* species specific leaf areas were several-fold higher in epiphytic forms, compared with only 30 per cent higher in *Clusia minor*. Leaves of five epiphytic *Ficus* species (*F. nymphaeifolia, F. pertusa, F. obtusifolia, F. pertusa, F. trigonata, F. tuerkheimii*) were also thinner than tree congeners. This thickness difference is the result of differences in the palisade parenchyma and the spongy parenchyma layer. Terrestrial and epiphytic *Ficus* also differ significantly in stomatal density, with free-standing individuals having around 2-4 times more stomata than epiphytic counterparts. In the genus *Clusia*, in contrast, no significant differences in leaf thickness and stomatal density and size were reported between epiphytic and tree individuals (Holbrook and Putz 1996a and Lüttge 2006). In *Clusia,* epiphytism is often accompanied by the development of the CAM photosynthetic pathway (Zotz *et al.* 1994; Zotz and Winter 1994a), and efficient hydraulic architectures (Lüttge 2006).

One possible contrast between leaves of vines and trees is that vines may experience more heterogeneous light environments than trees (Holbrook and Putz 1996b). Vines allocate more biomass to leaf development (Castellanos 1991). In relation to spatially and temporally heterogeneous light environments, vines have developed leaf

characteristics, including sizes and shapes, which might reduce the physiological constraints of a large photosynthetic surface (Givnish and Vermeij 1976).

In general, most vines exhibit C_3 photosynthesis. There are reports, however, of some vine species with crassulacean acid metabolism (CAM) pathway characteristics (Table 1.4). These species have increased nocturnal acid accumulation and strongly negative $\delta^{13}C$ ratios. Interestingly, these CAM vine species do not seem to grow in one type of ecosystem. They are distributed from deserts to tropical rainforests (Castellanos 1991).

Table 1.4 Vine species with CAM metabolism based on $\delta^{13}C$ values, nocturnal acid metabolism and gas exchange (CO_2 or O_2) measurements (From Castellanos 1991).

Species	**$\delta^{13}C$**	**Nocturnal acid metablism** (μeq g^{-1} fresh wt)	**Nocturnal gas exchange** (μmol CO_2 $m^{-2}s^{-1}$)
Cissus antartica			4.1
Cissus quadrangularis	-15.0		
Cissus quadrangularis		35.9	
Cissus quadrangularis		62.6	
Cissus sicyoides			2.5
Cissus trifoliata		27.7	4.5
Clusia flava	-14.8		
Clusia rosea	-17.7		
Clusia witana	-21.2		
Cyphostemma curorii	-12.2		
Cyphostemma quinatum	-14.8		
Hoya australis		12.3	12.2[a]
Hoya australis		20.1	12.0[a]
Hoya nicholsoniae			5.0[a]
Hoya nicholsoniae			2.5[a]
Rhipsalis spp.	-13.3		
Seyrigia humbertii		24.0	
Xerosicyos danguyi		67.0	

Note: [a] μmol O_2 $m^{-2}s^{-1}$

A study of semi-epiphytic *Monstera tenuis* (Araceae) showed that leaf position of juveniles affects the photosynthetic potential carbon gain (Oberbaur and Noudali 1998). Juvenile leaves are vertically arranged and attach to the host's trunk. These leaves absorb less light than if they are horizontal. The differences increase with increasing plant height, ranging from 0.5 to 2 m. This study, however, did not compare photosynthetic differences between juveniles and adult plants.

Leaf physiology of epiphytic and rooted individuals of primary hemi-epiphytic *Ficus* is correlated with water-use strategy. Epiphytic individuals use water more conservatively than free-standing individuals (Holbrook and Putz 1996b). In the dry season, epiphytic *Ficus* only open their stomata for a few hours in the morning and then close them until the next morning. This is not the case for rooted individuals, which open their stomata throughout the day. Epiphytic individuals also have lower stomatal conductance during drought, an indicator of water stress. As epiphytic *Ficus* only open their stomata in the morning, carbon uptake also only occurs at this time. They also have a fleshy thickening at the base of their stems that may store water. Epiphytic individuals also showed a less negative osmotic potential. As a result, leaves of epiphytic individuals have higher leaf water potential (Holbrook and Putz 1996a).

The source of water may change during the life of primary hemi-epiphytes. Different growth stages of *Didymopanax pittieri* (Araliaceae), for example, utilize different

sources of water based on hydrogen stable isotope analysis (Feild and Dawson 1998). Epiphytic *Didymopanax* used water from canopy mats and precipitation (mist, cloud water and fog). Soil water is the only water source for terrestrial individuals. The hemi-epiphytic stage had intermediate characteristics, using all sources of water (Feild and Dawson 1998). The possibility of a shift in water resource utilization has not been investigated in secondary hemi-epiphytes.

In the genus *Clusia*, primary hemi-epiphytes may exhibit an ability to switch between C_3 and CAM photosynthesis (Holbrook and Putz 1996b; Lüttge 2006; Zotz *et al.* 1994; Zotz and Winter 1994a). The capacity to express CAM is exhibited by epiphytic and terrestrial rooted individuals (Zotz and Winter 1994a). *Clusia* is the only truly arborescent species for which CAM has been reported.

Another important aspect in a plant's life is water transport. Water transport through the plant stem depends on the structural characteristics of water conductive tissue, the xylem that distributes water from roots to leaves (Tyree and Zimmerman 2002). This infrastructure, called hydraulic architecture, includes vessel dimension, hydraulic conductivity, leaf specific conductivity, root pressures and vulnerability to the formation of air bubbles inside the xylem due to drought stress (Tyree and Ewers 1996).

The ability of the plant conductive tissue system to transport water dictates the rate at which a plant can take up water and survive. The ability of a plant to maintain water

potential is thus controlled by the efficiencies by which roots take up water from the soil and it is transported to the canopy, and by the ability of a plant to regulate transpirational water loss from the leaves and evaporation across the epidermis (Atwell *et al*. 1999).

Hydraulic conductivity is defined as the rate at which water can be transported through xylem at a given pressure. Aspects of hydraulic conductivity are also determined by the area of both the xylem and leaves supported by a stem (Tyree and Ewers 1996). As hydraulic conductivity may influence water flow from the root to the leaf, it consequently affects leaf physiology and water potential. Therefore, hydraulic architecture may affect plant distribution along environmental gradients and plant height (Tyree and Ewers 1996). Different growth forms, such as trees, vines and epiphytes, have different physiological requirements for moving water, which are often accompanied by appropriately adapted hydraulic architecture.

Hydraulic architecture has been studied in several species of primary hemi-epiphytes (Patiño *et al*. 1995; Zotz *et al*. 1994; Zotz *et al.* 1997; Zotz and Winter 1994b). Only one study so far has investigated hydraulic architecture in a secondary hemi-epiphytic vine, *Monstera acuminata (*Lopez-Portillo *et al*. 2000). Vessels of this species are wider at the top of the stem and narrower towards the stem base. The stem base, based on the hydraulic conductivity and leaf specific conductivity, seems to hydraulically constrict this plant. The root pressures were very high, up to 225 kPa, which is the strongest root pressure so far recorded among plants. This root pressure allows vessels to refill at night when humidity is high. Lopez-Portillo *et al.*

(2000) concluded that hydraulic architecture characteristics of *M. acuminata* may be an evolutionary consequence of an anatomical constraint (lack of vascular cambium and therefore of secondary growth), and the special requirements of the hemi-epiphytic growth form. However, the terrestrially established juvenile stage of the life cycle was not examined in their study. Therefore, studies comparing hydraulic architectures of different ontogenetic stages of secondary hemi-epiphytic vines are needed. The current study provides information on how hydraulic architecture changes with the change of growth form.

Hemi-epiphytes may have intermediate characteristics between terrestrial and epiphytic plants (Holbrook and Putz 1996a). Epiphytes face conditions where water and nutrients are scarce. For secondary hemi-epiphytes, when they have lost connections with the soil through major stems, they overcome the problem by creating adventitious roots in order to take nutrients and water from the ground (Meyer and Zotz 2004). However, how effectively water and minerals are transported through the adventitious roots is unknown.

Physiological adaptations of hemi-epiphytes might not only be expected in relation to the change of growth forms and the dynamics of vertical environmental conditions in forest layers, but also different seasonal environmental conditions. Consequently, physiological features of secondary hemi-epiphytes might be also influenced by seasonality. Schnitzer (2005) noted that, because of their root systems and efficient vascular stems, woody vines can cope with water stress in the drought period. This gives lianas a competitive advantage compared with other plants. However, this

theory has not been investigated in secondary hemi-epiphytic climbers and monocotyledonous vines. Compared with dicotyledonous lianas, monocotyledonous vines have shallower root systems (Cochard *et al.* 1994). Therefore, drought resistance might be different from dicotyledonous lianas and investigation of the responses and adaptations of secondary hemi-epiphytic vines to seasonal changes in water availability is warranted.

1.6 RESEARCH OBJECTIVES

The aim of this research is to better understand the ecophysiology of secondary hemi-epiphytes during their ontogenetic development by investigating relationships between structure and physiological function, and water resource utilization of *Freycinetia excelsa* F. Muell (Pandanaceae) and *Rhaphidophora australasica* F.M. Bailey (Araceae).

In order to achieve the aim, this study was structured to address the following specific objectives:

1. **To establish an understanding of water transport in the secondary hemi-epiphytic vines *F. excelsa* and *R. australasica*.**

The hydraulic architectures of juvenile, intermediate and adult plants of *F. excelsa* and *R. australasica* were studied and compared. Aspects of hydraulic architecture that were studied included stem and root xylem anatomy, stem hydraulic conductivity, stem specific conductivity, leaf specific conductivity and Huber value. It was hypothesized that the shift in growth habit affected the capacity of the plants

to conduct water. This research, which is documented in Chapter 3, addresses the following questions:

a. Are there differences in xylem anatomy between the juvenile (terrestrial), intermediate (climbing) and adult (semi-epiphytic) plants?
b. Are there differences in hydraulic architecture between plants of differing ontogenetic development?
c. Is water transport physically restricted in the secondary hemi-epiphytic life form?

2. To examine leaf anatomy of juvenile, intermediate and adult *F. excelsa* and *R. australasica*.

The hypothesis tested is that the leaf anatomy of the two study species differs during ontogeny. Variables examined included specific leaf area, stomatal distribution and dimension, stomatal density, leaf thickness, the thickness of the palisade parenchyma and spongy mesophyll layers and the thickness of the upper and lower epidermal layers, including, if any, hypodermis, and the number of cell layers in the palisade parenchyma tissue. These quantitative leaf anatomy studies are presented in Chapter 4.

3. To characterize physiology and water relations during the life-cycle of the secondary hemi-epiphytic vines *F. excelsa* and *R. australasica* by comparing plants of different growth stages during a seasonal progression.

Field observations were conducted to study seasonal and diurnal leaf physiology in plants of differing developmental stages. Gas exchange (assimilation, stomatal conductance and transpiration rates) was measured concurrently with leaf water potential. Environmental conditions, including rainfall, soil moisture and photosynthetically active radiation, were also recorded. Chapter 5 covers this work and it addresses the following questions:

a. Are there differences in CO_2 or H_2O exchange between individuals of the juvenile, intermediate and adult stages?
b. Are the physiological attributes influenced by seasonality?
c. How do physiological attributes correlate with each other and correlate to hydraulic architecture?

4. To investigate the shift in the utilization of soil water sources by individuals of differing growth phases of the secondary hemi-epiphytic vines *F. excelsa* and *R. australasica*.

The objective of this study was achieved by studying stable hydrogen and oxygen isotopic compositions of stem water and matching them to soil water isotopic composition. It is hypothesized that there is a shift in water resource utilization with change in developmental stages of secondary hemi-epiphytic vines. Plants of

differing ontogenetic phases may utilize water from different parts of the soil profile. This study, contained in Chapter 6, in conjunction with studies on leaf gas exchange and water potential (Chapter 5), will provide a more detailed understanding of how water resource utilization affects the performance of each growth habit of secondary hemi-epiphytic vines.

CHAPTER 2

STUDY SPECIES AND SITES

2.1 DESCRIPTION OF STUDY SPECIES AND EXPLANATION OF ONTOGENETIC PHASES

Two species of root climbing secondary hemi-epiphytic vines, *Freycinetia excelsa* F. Muell (Pandanaceae) and *Rhaphidophora australasica* F.M. Bailey (Araceae), were studied in this research. These two species occur in the north Queensland Wet Tropics region. Wallace (1989) noted that about 80 out of 100 species of *Freycinetia* and all species of *Rhaphidophora* (100 species) are secondary hemi-epiphytic climbers.

After becoming terrestrially-established, secondary hemi-epiphytes climb a host. Mature individuals of *F. excelsa* and *R. australasica* eventually lose connection to the soil through the main stem. The plant develops adventitious (aerial) roots spreading from the stem which eventually connect to the ground. The continued access of the plant to the soil is then maintained by these adventitious roots (Croat 1988; Meyer and Zotz 2004; Williams-Linera & Lawton 1995). The term "aerial root" used in this thesis refers to these adventitious roots.

As the aim of this research is to study the ecophysiology of secondary hemi-epiphytes during their ontogenetic development, the study species were categorized into three developmental phases. The first is the "**juvenile stage**", the stage at which the plant grows on the ground before climbing the stem of the host (Fig. 2.1a).

However, in *R. australasica* the juvenile stage of the plant appears to directly climb the host soon after germinating. No young plants were found with a clear non-climbing juvenile phase (personal observations). Therefore, the early stage of the climbing habit of *R. australasica* was designated as the juvenile phase (Fig 2.3a). Juvenile individuals were defined as those less than 1 m tall.

The "**intermediate stage"** was defined as when the plant had begun climbing the host tree and was developing aerial roots (Fig. 2.1b and 2.3b). In this study, the range of plant height for the intermediate stage was about 2 m to 3 m.

When the plant has totally lost its connection to the ground via the main stem, it was defined as the "**adult stage**" (Fig. 2.1c and 2.3c). At this stage, aerial roots were further established. The decayed basal part of the stem had moved upward away from the ground and the plant height was above 5 m. The term "ontogenetic/developmental phases" used in this thesis is interchangeable with "growth stages/phases/habits".

2.1.1 *Freycinetia excelsa*

Freycinetia is the only genus of vine in the Pandanaceae (Hegarty 1991). *F. excelsa* is a slender vine with adult stems typically 1-2 cm in diameter (Talley *et al.* 1996). It has adventitious roots spreading from most parts of the vine stems (Hyland *et al.* 2003). A sprawling climber (Harden *et al.* 2007), *F. excelsa* has vigorous multi-branched stems and is characterized by spirally arranged serrulate-spinulose leaves, about 10-20 cm long and less then 2 cm wide. The pale-coloured terminal

inflorescence is surrounded by bracts, which are nearly as long as the leaves (Jones and Gray 1977). It has a red 3-4 cm long syncarpous multiple fruit, in which each individual fruit is a berry (Harden *et al*. 2007). This species occurs in the Northern Territory, Cape York, and north-eastern to south-eastern Queensland (Fig. 2.2), as well as in Papua New Guinea and Southeast Asia (Hyland *et al*. 2003). The Queensland Herbarium has records from the Cook, Moreton, North Kennedy, South Kennedy and Wide Bay districts (Bostock and Holland 2007).

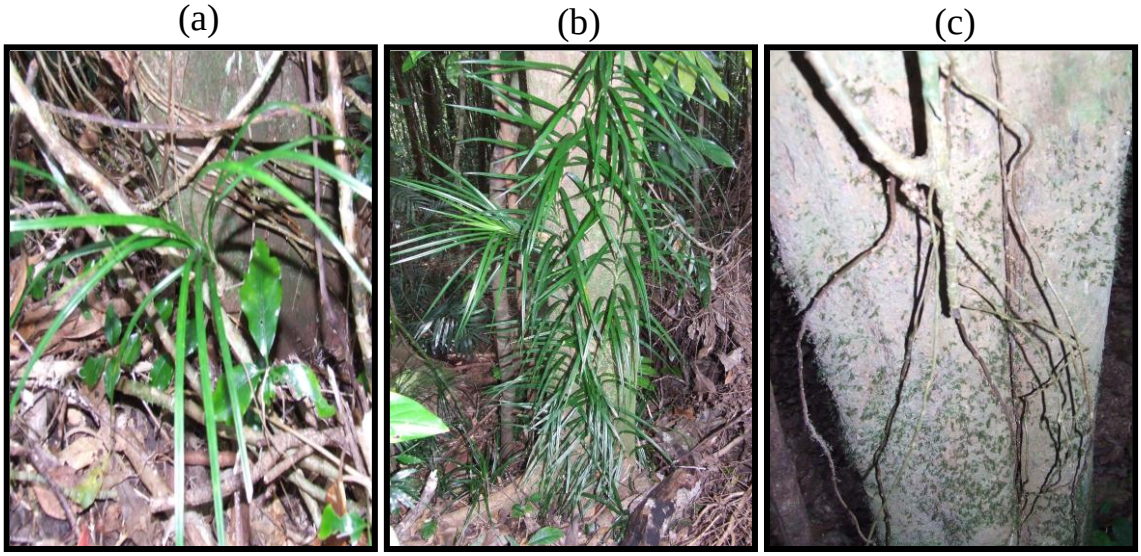


Fig. 2.1 Secondary hemi-epiphytic *F. excelsa* growing in the upper montane rainforest in Paluma Range National Park. The three growth stages of this species are (a) juvenile, (b) intermediate, and (c) adult stages.

Fig. 2.2 *F. excelsa* (●) specimens have been collected from a wide area in Queensland and the Northern Territory. This specimen collection distribution map is generated from the Atlas of Living Australia website (http://www.ala.org.au).

2.1.2 *Rhaphidophora australasica*

R. australasica is a slender vine with a stem diameter not exceeding 2 cm. *R. australasica* has ovate lanceolate leaves up to 30-40 cm long and 15 cm wide (Fig. 2.3; Hyland *et al*. 2003; Jones and Gray 1977). The inflorescence is located at the end of the growing stem as dense, erect spikes of small, crowded flowers. Numerous seeds are contained in a fleshy berry (Jones and Gray 1977). *R. australasica* grows from sea level to 450 m altitude, in wet lowland and upland rainforest (Hyland *et al*. 2003), and is distributed in north Queensland, as well as the Northern Territory (Fig. 2.4). The Queensland Herbarium has records from the Cook and North Kennedy districts (Bostock and Holland 2007).

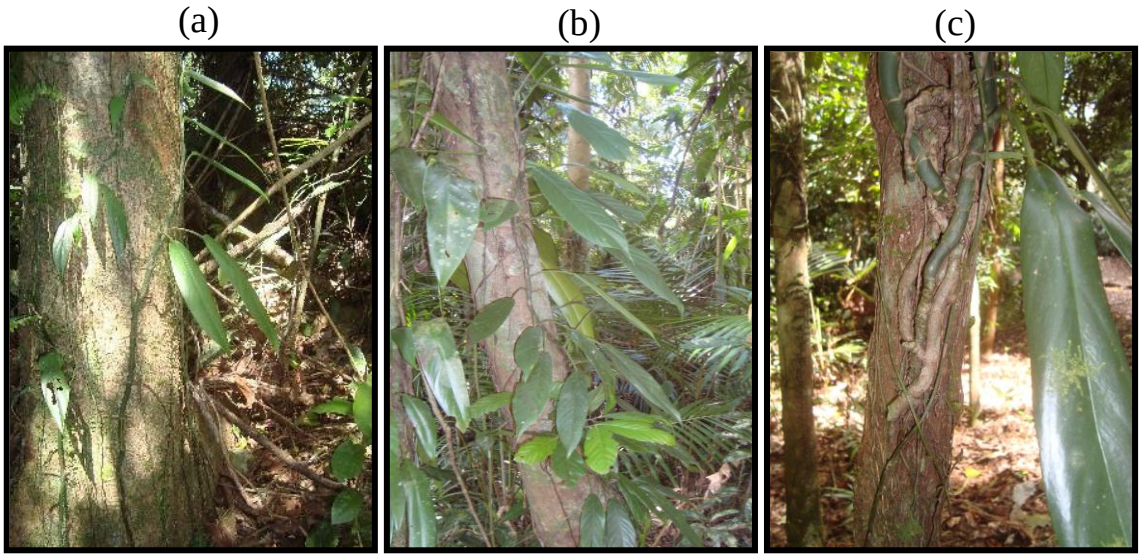


Fig. 2.3 *R. australasica* growing in the rainforest of the Henrietta Creek section, Wooroonooran National Park. The three growth stages of this species are (a) juvenile, (b) intermediate, and (c) adult stages.

Fig. 2.4 *R. australasica* (●) in Australia occurs in Queensland and the Northern Territory, as indicated by the distribution map of specimens collected from a wide area. Data is taken from the Atlas of Living Australia website (http://www.ala.org.au).

2.2 PLANT MATERIAL COLLECTION SITES

Samples of *F. excelsa* were collected in the Mt. Spec section of Paluma Range National Park (18"5 7'S, 1401 1'E, altitude 880-900 m), which is about 80 km north of Townsville, north Queensland (Fig 2.5). Paluma is an upper montane rain forest, classified as a simple notophyll vine forest (Tracey 1982), which is characterized by a relatively even canopy surface, mostly notophyll leaves, commonly slender and wiry vines (few robust woody lianas), and frequent epiphytes in tree crowns (Talley *et al.* 1996). Average annual rainfall in this area is about 3375 mm (Fig. 2.6), in which more rain falls during the wet season (December to April) (Gross 1993 *in* Talley *et al*. 1996). Soils in this area are shallow krasnozems or red friable earths (Congdon and Herbohn 1993). Some of the common trees in the rainforests of the Paluma Range National Park are *Cardwellia sublimis* (Proteaceae), *Citronella smythii* (Icacinaceae), *Xanthophyllum octandrum* (Xanthophyllaceae), and *Balanops australiana* (Balanophoraceae). The Lauraceae are well-represented *(e.g. Beilschmiedia collina, Cvptocarya corrugata,* and *C. densiflora)* (Jackes 2001).

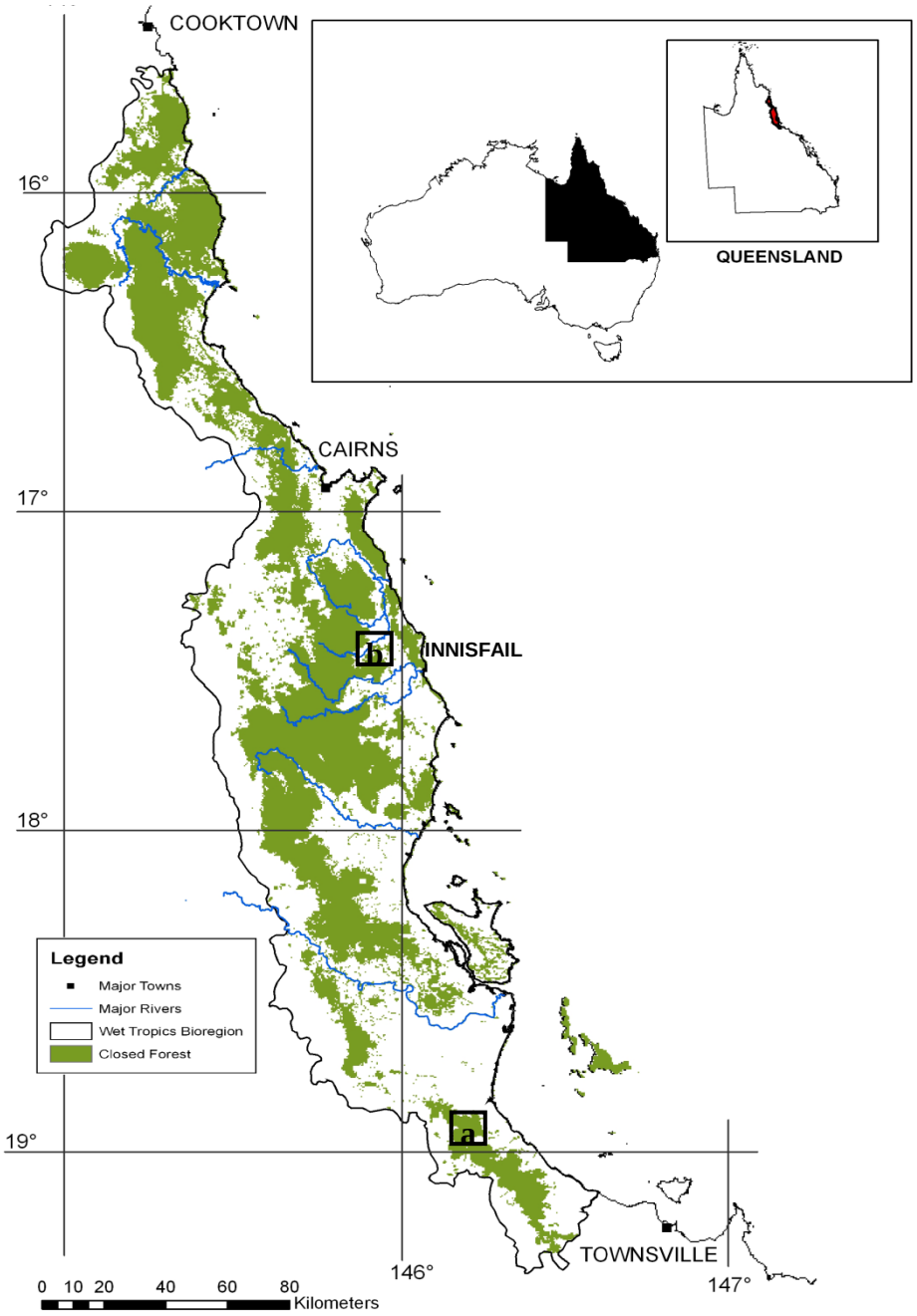


Fig. 2.5 Map of the Wet Tropics area of north Queensland, showing study locations in (a) the Paluma Range National Park and (b) Wooroonooran National Park (Source: Williams 2006).

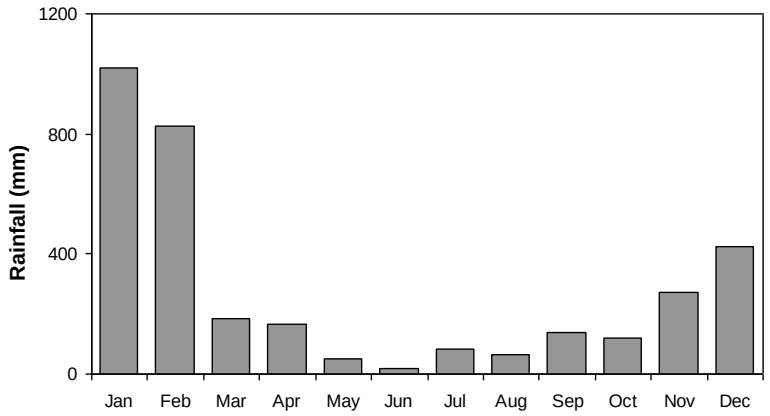


Fig. 2.6 The average monthly rainfall at Paluma Range National Park. These data, which were obtained from the nearest recording station from the study site, i.e. Ivy Cottage Paluma Station (less than a kilometre from the study site), are an average of 2008-2010 climate observations by the Australian Bureau of Meteorology (BOM 2011).

Samples of *R. australasica* were collected in the Henrietta Creek section of Wooroonooran National Park, which is about 30 km west of Innisfail, north Queensland (Fig 2.5). This forest is classified as a complex mesophyll vine forest (Tracey 1982). According to Gleason *et al*. (2008), this ecosystem is classified as sub-tropical wet forest based on Holdridge's (1947) classification system. This area is floristically complex with a high number of species per unit area, approximately 80 species within a 0.25 ha area (Gleason *et al*. 2008). Mean annual rainfall is about 3,625 mm, of which about 70% falls between November and April (Fig. 2.7).

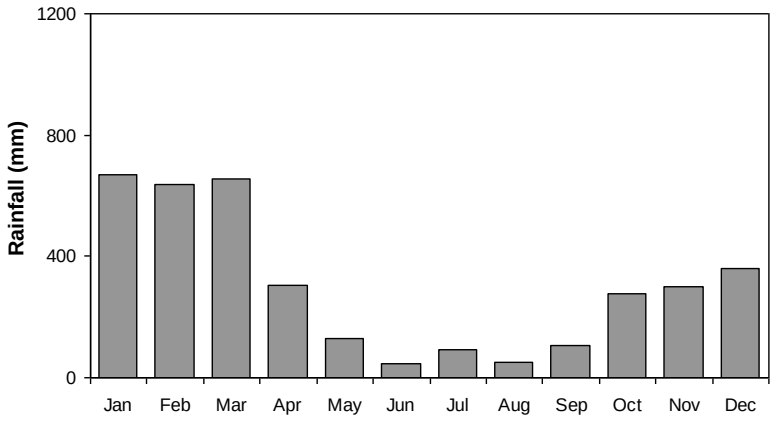


Fig. 2.7 The average monthly rainfall at Wooroonooran National Park. These data, which were obtained from the nearest recording station from the study site, i.e. Crawford's Lookout Station (approximately 4 km from the study site), are an average of 2008-2010 climate observations by the Australian Bureau of Meteorology (BOM 2011).

CHAPTER 3

STEM WATER TRANSPORT IN TWO SPECIES OF SECONDARY HEMI-EPIPHYTIC VINES

3.1 INTRODUCTION

The survival of plants in their environments partly depends upon the efficiency of their water transport mechanisms along the soil-plant-atmosphere continuum. This efficiency is determined by the ability of roots to take up water from the soil, the transport of water to the canopy and the ability of plants to regulate water loss from the leaves through transpiration (Atwell *et al*. 1999). Water transport through the plant stem depends upon the structural design of water conductive tissue, the xylem (Tyree and Zimmerman 2002). Aspects of xylem structure which impinge on water transport efficiency include vessel dimension, hydraulic conductivity and vulnerability to the formation of air bubbles inside the xylem following drought stress.

Hydraulic architecture is the design of the xylem as the water distribution pathway from root to leaves (Tyree and Ewers 1996). This design may influence water flow from the root to the leaf and, consequently, gas exchange, stomatal conductance and water potential. Hydraulic conductivity is the rate at which water can be transported through xylem at a given pressure. Aspects of hydraulic conductivity are also determined by the area of both the xylem and leaves supported by a stem (Tyree and Ewers 1996).

Plant distributions along environmental gradients and the height that plants attain may reflect, at least in part, the efficiency of hydraulic architecture (Tyree and Ewers 1996). Different growth forms, such as trees, vines and epiphytes, have different ecological and physiological adaptations, which result in different hydraulic architecture characteristics. There have been extensive reviews of hydraulic architecture in woody plants, including trees, shrubs, lianas and primary hemi-epiphytes (Drake and Franks 2003; Ewers and Fisher 1989; Ewers *et al*. 1991; Patiño *et al*. 1995; Tyree and Ewers 1991; Tyree and Ewers 1996; Tyree and Zimmerman 2002). Research into non-woody monocot vines included analysis of vessel dimension and hydraulic properties in several species of rattans (Fisher *et al*. 2002; Tomlinson *et al*. 2001) and a semi-epiphytic climbing Araceae, *Monstera acuminata* (Lopez-Portillo *et al*. 2000), and xylem embolism in the palm *Rhapis excelsa* (Sperry 1986) and the vine-like bamboo *Rhipidocladum racemiflorum* (Cochard *et al*. 1994).

In terms of primary hemi-epiphytes, the hydraulic architecture of several woody *Ficus* and *Clusia* have been studied (Patiño *et al*. 1995; Zotz *et al*. 1994), comparing the stem hydraulics of epiphytic and terrestrial stages. In study of seven *Ficus* species, Patiño *et al.* (1995) reported epiphytic individuals have stems which are less conductive per unit leaf area, and invest less wood cross-sectional area per unit leaf area, than free-standing congeners. The hemi-epiphytic C_3/CAM-intermediate *Clusia uvitana*, however, has a larger leaf area per unit of stem cross-sectional area and a lower specific stem conductance than the genus *Ficus*. This means *Clusia* has less effective water transport per unit leaf area (Patiño *et al.* 1995; Zotz *et al.* 1997; Zotz and Winter 1994b). This indicates that the physiological flexibility of the water-

saving CAM strategy in *Clusia* may be associated with morphological characteristics such as hydraulic architecture (Lüttge 2006). The differences in hydraulic properties between *Ficus* and *Clusia* indicate that hydraulic architecture characteristics of hemi-epiphytes are not necessarily similar between different species.

A study by Lopez-Portillo *et al*. (2000) has investigated the hydraulic characteristics of secondary hemi-epiphytic *Monstera acuminata* (Araceae), the only research on hydraulic architecture of secondary hemi-epiphytes so far. This research reported that in reverse to what is commonly found in trees, vessels of *M. acuminata* are wider at the top of the stem and diameters decrease towards the stem base. This plant was also found to have very strong root pressures. The proportion of stem cross sectional area to the distal leaf area (Huber value) tends to increase exponentially along the plant stem. As the hydraulic conductance and leaf specific conductivity were higher at the stem top, the base of the stem which contains narrower vessels may become hydraulically limited. This hydraulically limited stem base may be bypassed by the establishment of aerial roots. Lopez-Portillo *et al*. (2000) concluded that these unique characteristics of hydraulic properties of *M. acuminata*, including the pattern in vessel size along the stem, the exponential increase of Huber value and the stem basal hydraulic limitation, may be special requirements of the secondary hemi-epiphytic growth form. Therefore, studies on more species of secondary hemi-epiphytes will elucidate this conclusion.

The change of growth habit from terrestrially-established juveniles to canopy-occupying adults in the life cycle of a secondary hemi-epiphytic plant may have

consequences in relation to the mechanism of stem water transport. The study of *M. acuminata,* as mentioned above, however, did not examine the juvenile stage of the plant (Lopez-Portillo *et al*. 2000). Consequently, there is limited understanding on how secondary hemi-epiphytic plants respond to the challenge of the shift in growth habit. Therefore, studies comparing hydraulic properties of different growth stages of secondary hemi-epiphytic vines are needed, which will provide information on how hydraulic architecture may change with the shift between growth forms. Studies on hydraulic characteristics may also explain whether or not water availability is restricted in secondary hemi-epiphytic life forms, which consequently may affect the ability of plants to absorb CO_2.

The aim of this study was to establish an understanding of water transport in secondary hemi-epiphytic vines. It is assumed that the change in developmental stages has consequences in terms of the capacity of the plant to conduct water. Questions to be addressed include:

a. Are there differences in xylem anatomy between the juvenile, intermediate and adult stages?
b. Are there differences in hydraulic architecture parameters between the juvenile, intermediate and adult stages?
c. Is water transport physically restricted in the secondary hemi-epiphytic life form?

3.2 MATERIALS AND METHODS

3.2.1 Plant materials and study sites

Freycinetia excelsa samples were collected from Paluma Range National Park and samples of *Rhaphidophora australasica* were collected from the Henrietta Creek Section of Wooroonooran National Park. A description of the study species and growth habits is contained in Section 2.1, and a description of study sites is in Section 2.2.

3.2.2 Stem xylem anatomy and proportion of stellar tissue in the stem

Fifteen individuals of each growth stage were sampled to measure the vessel length, diameter and frequency, and the proportion of stele to stem diameter. As a preliminary study showed that the maximum vessel size was located around the middle of the stem, stem segment samples were taken about half way along each stem.

Vessel length was measured using a modified pneumatic method (Ewers and Fisher 1989; Lopez-Portillo *et al.* 2000). One end of the stem segment was clamped in Tygon tubing and sealed, while the other end was kept under water. A pressure of 20 kPa, produced from the 2 metre height of a reservoir bag containing degassed 10 mM KCL, was then fed through the Tygon tube. Successive segments of about 0.05 m of stem were removed under water using secateurs until a steady stream of air bubbles

emerged from the freshly cut end. The vessel length was estimated from the length of remaining stem segment plus 0.025 m.

After vessel length was measured, the end of the stem segments were transversely sectioned with a razor blade and immediately stained with Alcian blue. Each sample was then photographed using an Olympus digital camera attached to a microscope (Olympus U-CMAD-2, Japan) at x40 magnification at four different locations. Vessel diameters were measured from the micrographs using ImageJ analysis software (National Health Institute, USA). For each sample, the diameter of twenty vessels was measured, making up 300 vessels per growth stage of each species being observed. Vessel density was examined by calculating the number of vessels per square millimetre.

Vessel diameters of stems were divided into 10 μm size classes for each growth habit of each species to obtain frequency distributions. In order to determine the theoretical contribution of each vessel size class to the hydraulic conductance of the stem, vessel diameters were raised to the fourth power (Hagen-Poiseuille Law) (Sperry *et al*. 1993; Tyree and Zimmerman 2002). The Hagen-Poiseuille Law is based on the experiments by Hagen in 1839 and Poiseuille in 1840 which found that water molecules are stationary on the capillary wall and move faster in the centre of the capillary. Therefore, flow rate is proportional to the fourth power of the radius of the capillary (Tyree and Zimmerman 2002). The relative contribution of each diameter class is the proportion of the sum of all the conduits raised to the fourth power. This

sum reflects the hydraulic conductance assuming laminar flow in ideal capillary tubes.

The stele is the central core of tissue in the stem or root of vascular plants, consisting of xylem and phloem together with supporting tissues. The proportions of stele diameter to stem diameter were calculated from the same samples used for xylem anatomy. Stele and stem diameter were measured after the samples were photographed.

3.2.3 Root xylem anatomy

Aerial roots from adult individuals and soil roots from juveniles were also collected in order to measure their vessel diameters. Root segments also were transversely sectioned, stained with Alcian blue and photographed at x40 magnification. ImageJ analysis software was also used to measure the vessel diameters.

3.2.4 Hydraulic conductivity measurements

Parameters used to describe hydraulic architecture were stem hydraulic conductivity (K_H), stem specific conductivity (K_S), leaf specific conductivity (K_L) and Huber value (HV). Explanations of each these parameters are as follows:

a. Stem hydraulic conductivity (K_H)

Hydraulic conductivity is the ratio of water fluxes (F, kg s^{-1}) through an excised stem segment to the pressure gradient (P, MPa) causing the flow across the stem segment (x, m) (Tyree and Ewers 1996):

$$K_H = F / (dP / dx) \quad \text{(Equation 3.1)}$$

b. Stem specific conductivity (K_S)

Specific conductivity is the measure of stem porosity. This is equal to K_H divided by the area of the stem cross section (A_x, m^2) (Lopez Portillo *et al*. 2000):

$$K_S = K_H / A_x \quad \text{(Equation 3.2)}$$

c. Leaf specific conductivity (LSC)

Leaf specific conductivity is equal to K_H divided by the leaf area supported by the segment (A_L, m^2). Leaf specific conductivity is the measure of the hydraulic sufficiency of the segment to supply water to leaves (Tyree and Ewers 1996):

$$LSC = K_H / A_L \quad \text{(Equation 3.3)}$$

d. Huber value (HV)

Huber value is the measure of the investment of stem tissue per unit leaf area fed. HV is equal to the stem cross sectional area (m^2) divided by the distal leaf area of the segment (m^2):

$$HV = Ax / A_L \quad \text{(Equation 3.4)}$$

To study the above four parameters, sixteen plants of the juvenile stage and six plants of each of the intermediate and adult stages were collected for each species. Samples were collected from the field early in the morning by removing the plant carefully from the host tree. The plants were then transported to the laboratory in sealed plastic bags containing a small amount of water. The samples were collected early in the morning because maximum xylem pressures occur before dawn (Sperry *et al*. 1988; Cochard *et al*. 1994). This is due to refilling of vessels with water during the night. *F. excelsa* individuals with stem diameters ranging from 4 mm to 10 mm and *R. australasica* plants with stem diameters ranging from 4 mm to 15 mm were collected for this study during November-December 2008.

Individuals of adult stage are taller than individuals of intermediate and juvenile phases. Therefore, for the purposes of analysis, plants were divided into fractions, i.e. seven fractions for adult individuals, five fractions for intermediate individuals and up to three fractions for juvenile samples. However, because juvenile plants were mainly very short, some juvenile phase samples only consisted of one fraction. Each stem fraction was then cut into 10 cm segments whilst under water. Both ends of each stem segment were shaved with a razor blade and were quickly placed into the "hydraulic conductivity apparatus" (Fig. 3.1). The leaf area was measured using a Paton Electronic Planimeter (Paton Industries Australia). Due to the large number of leaves to be studied, leaf area measurement was estimated by employing linear regression to characterize the relationship between the leaf area and the leaf fresh weight. Twenty-five leaves of each growth stage of each species were measured to

determine their area and fresh weight. The remainder of the leaves were weighed and their fresh weight was converted to area by applying the linear regression formula.

The apparatus for determining the hydraulic conductivity was built by using a variable pressure flow system, described by Sperry *et al*. (1988), as modified by Choat (2003) (Fig. 3.1). Compressed air (1) was pushed to the pressure tank (3) which contained a solution of 10 mM KCl. The pressure of compressed air (1) was set to 100–200 kPa by setting the pressure release valve (2). The solution was then allowed to flow to a secondary supply reservoir (6) through a three way stopcock (5). Two filters of 0.22 μm and 37 mm in diameter (4) were placed either side of the stopcock. The stem segment was then put in the other end of tube. A perfusion solution of KCl was pushed to flow through the stem segment. Hydraulic conductivity was measured by releasing pressure from gravity-induced low pressures (10 kPa produced from the 1 m height difference) from the secondary supply reservoir (6). The stem segment (7) was then flushed for 10 minutes at a maximum of 200 kPa, by allowing the solution from the pressure tank (3) to flow directly through the stem segment. After flushing, tubing and vessels were allowed to relax for about 10 minutes. Hydraulic conductivity was re-measured by allowing the solution from the secondary reservoir (6) to flow. The amount of solution coming out from the stem was weighed and recorded every second by a computer (9) connected to the balance (8).

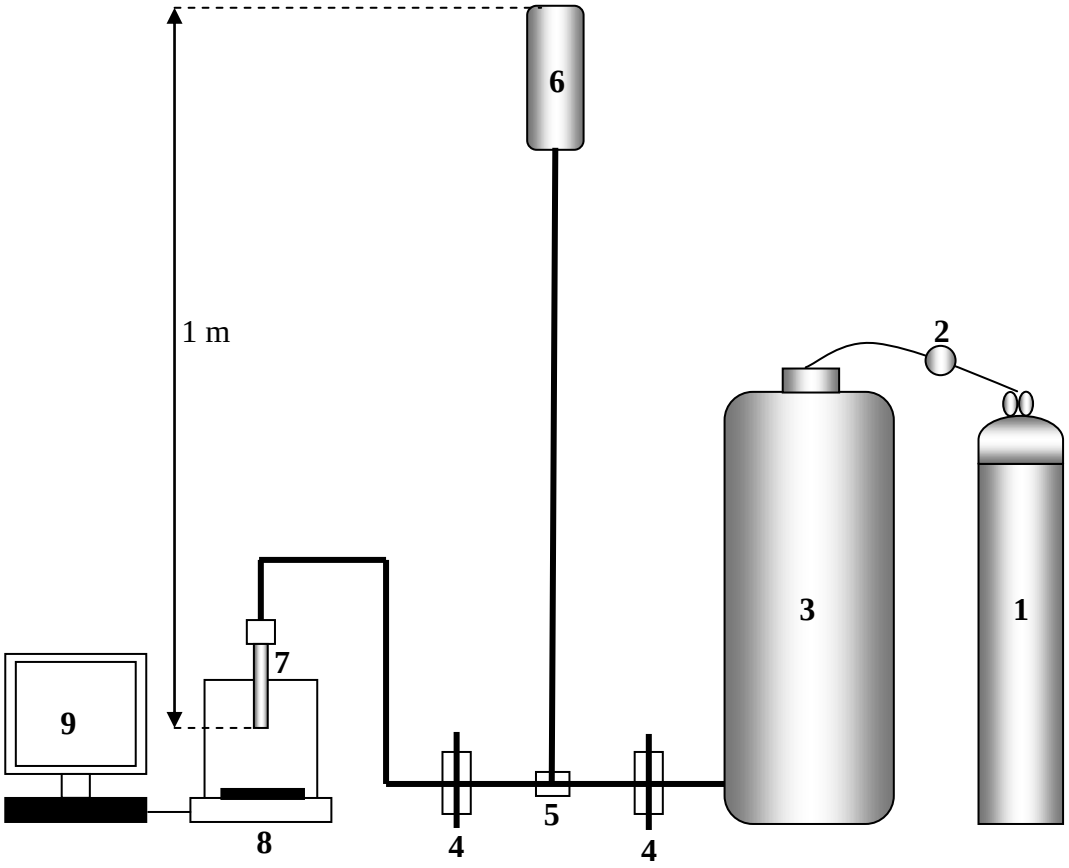


Fig. 3.1 Apparatus for measuring hydraulic conductivity showing the compressed air tank (1), the pressure release valve (2), tank containing degassed 10 mM KCl solution (Signature 2000, Sta-rite) (3), 0.22 μm filters 37 mm in diameter (4), three-way stopcock (5), 1 L intravenous drip bag (Baxter Healthcare) which serves as a secondary supply reservoir (6), a stem segment (7), balance (8) and computer (9). The pressure release valve was set to 200 kPa to ensure the gas pressure in the supply tank never exceeded that limit. The solution tank (3) contains an ethyl-vinyl-acetate bladder which allows gas to be supplied without mixing into solution. This tank was constructed of fibreglass to prevent corrosion (Choat 2003). Silicon tube was used to hold the stem segment. When the stem segment was too small for the tube, a leak proof seal was applied.

Vessel size along the plant stem was also observed for the same segments measured for their hydraulic conductivity parameters. These stem segment transverse sections were cut freehand with a razor blade, immediately stained with Alcian blue, and then photographed using the same procedure as explained in Section 3.2.2.

3.2.5 Statistical analysis

Analyses of variance (ANOVA) and Tukey tests were used to examine differences in xylem size, density and hydraulic architecture parameters between plants of different growth habits. Data of hydraulic architecture parameters were logarithmically transformed before ANOVAs and the Tukey tests were carried out. Linear regression was applied to characterize the relationships between stele and stem area.

3.3 RESULTS

3.3.1 Proportion of stelar tissue in the stem

Both *F. excelsa* and *R. australasica* have cylindrical steles (Figs. 3.2a and 3.3a). Large vessels of *F. excelsa* are located more in the middle of the stele and narrow vessels are commonly found towards the edge (Fig. 3.2b). On the other hand, vessels of different sizes are more randomly distributed in the stele of *R. australasica* (Fig. 3.3b).

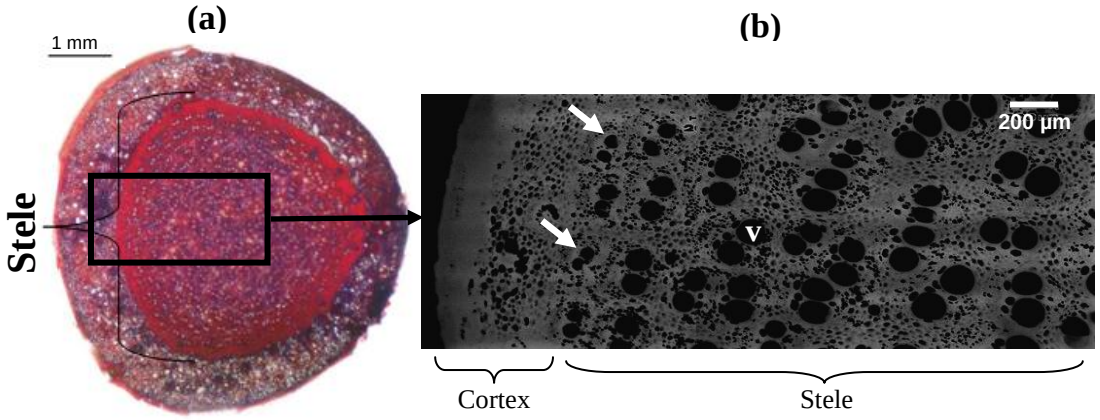


Fig. 3.2 Transverse section of stem of *F. excelsa* showing (a) the stele and (b) distribution of vessels (v). The arrows show examples of smallest vessels.

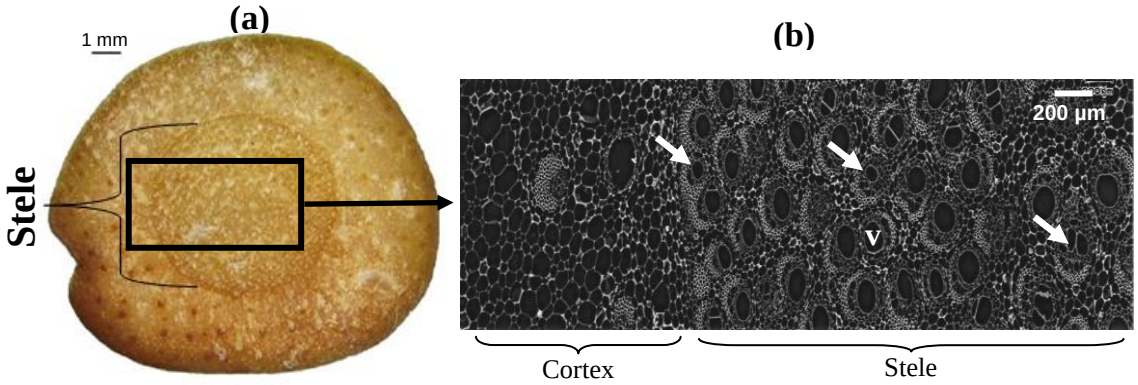


Fig. 3.3 Transverse section of stem of *R. australasica* showing (a) the stele and (b) distribution of vessels (v). The arrows show examples of smallest vessels.

As shown in Fig. 3.4, the proportions of stelar tissue area to the stem area of juvenile, intermediate and adult individuals of *F. excelsa* are 76.43%, 84.7% and 84.2% respectively. By contrast, the stelar areas of *R. australasica* only occupy about a third to a half of the stem area, being 10.42%, 19.96% and 29.40% for juvenile, intermediate and adult plants respectively (Fig. 3.4). The stelar area of adult and intermediate *F. excelsa* occupies more of the stem than in the juvenile stage plants (F= 7.255; P<0.01). Adult individuals of *R. australasica* have a larger proportion of stelar area to stem area than intermediate and juvenile individuals (F= 75.264; P<0.01).

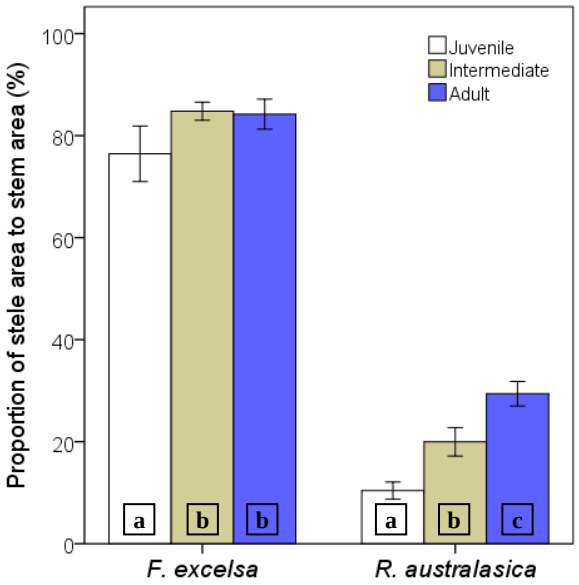


Fig. 3.4 Proportion of stele area (mm^2) to stem area (mm^2) of differing growth habits of *F. excelsa* and *R. australasica* (n= 15 individuals per growth habit). Error bars are 95% confidence intervals. For *F. excelsa*, the same letter indicates that values are not significantly different at $\alpha = 5$ %.

Linear regression analysis indicates that stelar area is significantly related to stem area (Fig. 3.5). The regression analysis results for *F. excelsa* are: juvenile - R^2= 0.926, F= 175.883, P<0.01; intermediate - R^2= 0.989, F= 1310.671, P<0.01; and

adult plants - R^2= 0.90, F= 129.176, P<0.01. The values for *R. australasica* are: juvenile - R^2= 0.821, F= 65.351, P<0.01, intermediate - R^2= 0.816, F= 62.905, P<0.01; and adult individuals - R^2= 0.870, F= 94.930, P<0.01. These values indicate that the stem area is a reliable indicator of stele area.

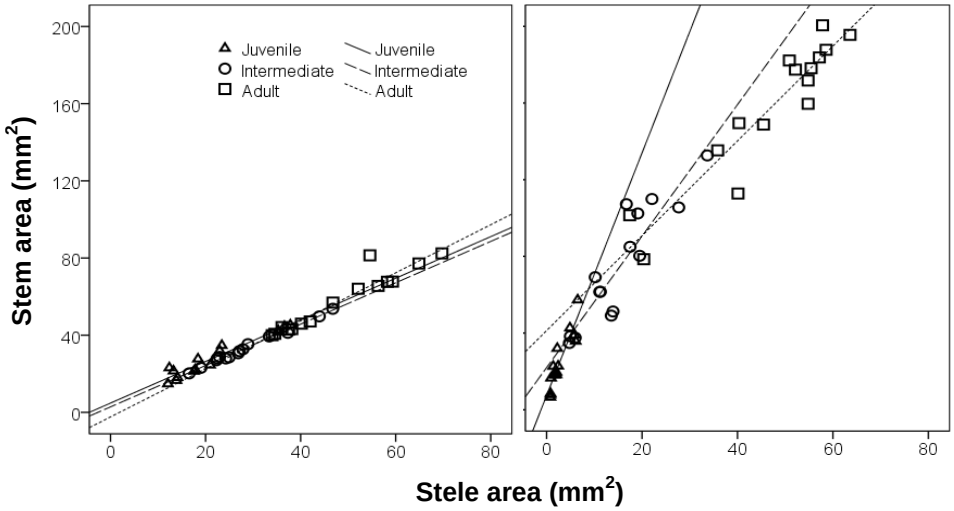


Fig. 3.5 Relationships between stele area and stem area of (a) *F. excelsa,* and (b) *R. australasica,* of differing growth habit.

3.3.2 Xylem anatomy

3.3.2.1 Stem vessel size

The change in growth habits of both species of secondary hemi-epiphytes is associated with differences in vessel size. Adult individuals of *F. excelsa* have wider vessels than intermediates and juveniles (F= 201.190; P<0.01) (Figs. 3.6 and 3.8). A similar pattern occurs in *R. australasica* where adult plants have wider vessels than intermediate and juvenile individuals (F= 914.232; P<0.01) (Figs. 3.7 and 3.8). The development of terrestrial juveniles into climbing adults of both species was also associated with an increase in the abundance of parenchyma (soft) tissues (Figs. 3.6 and 3.7).

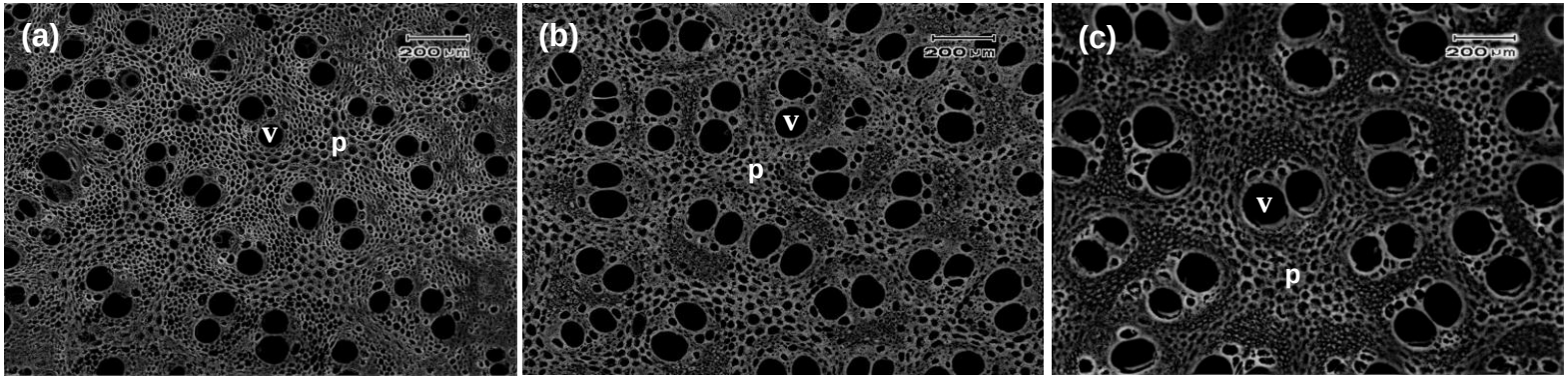


Fig. 3.6 Transverse sections through stem xylem tissue of (a) juvenile, (b) intermediate, and (c) adult *F. excelsa*. Note the size of vessels (v) increases during the plant's developmental stages and the more abundant parenchyma tissues (p) in the stem of the adult plant.

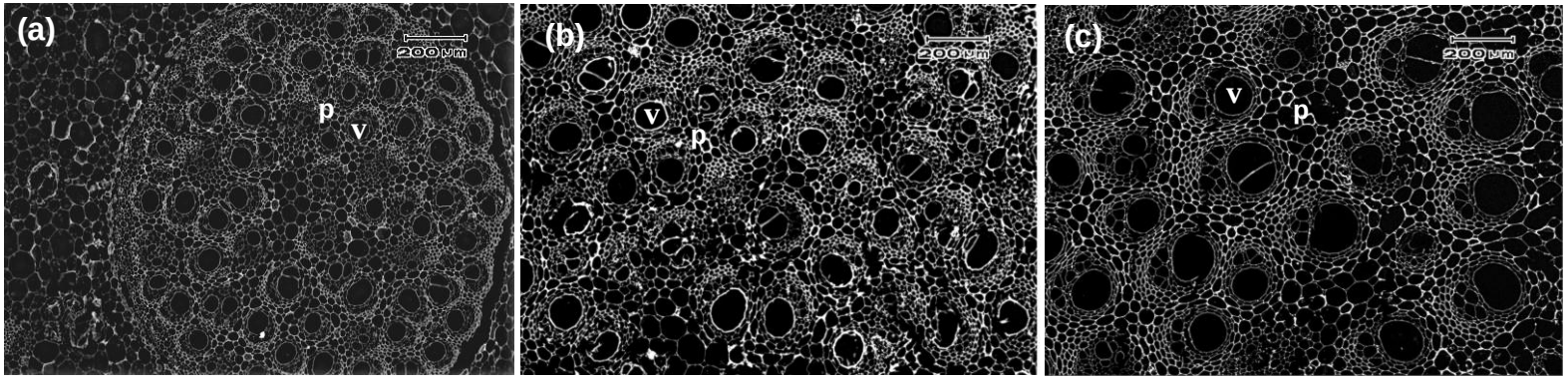


Fig. 3.7 Transverse sections through stem xylem tissue of (a) juvenile, (b) intermediate, and (c) adult *R. australasica*. Note the size of vessels (v) increases during the plant's developmental stages and the more abundant parenchyma tissues (p) in the stem of the adult plant.

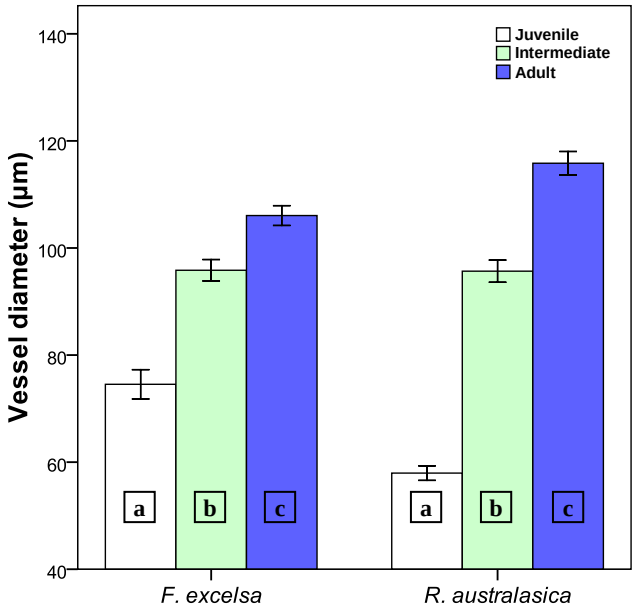


Fig. 3.8 Mean stem vessel diameter of *F. excelsa* and *R. australasica* of differing growth habit (n= 300 vessels of 15 individuals per growth habit). Error bars are 95% confidence intervals. Different letters indicate that values are significantly different between habits of each species at $\alpha = 1$ %.

The vessel size class distribution shows that more than 60 % of vessels in adult individuals of *F. excelsa* have a diameter greater than 100 µm, compared with juvenile and intermediate individuals that have more vessels with diameters below 100 µm (Fig. 3.9). The same pattern can also be seen for the vessels of *R. australasica* (Fig. 3.10). Juvenile and intermediate individuals of both species have fewer large vessels for water transport than adult individuals. For juvenile *F. excelsa*, for example, the 15% of vessels, which are above 100 µm in diameter, would hypothetically provide about 50% of the hydraulic conductance (Fig. 3.9a). For adult plants of both species, the greater abundance of wide vessels provides more than 80% of the hydraulic conductance (Figs. 3.9c and 3.10c).

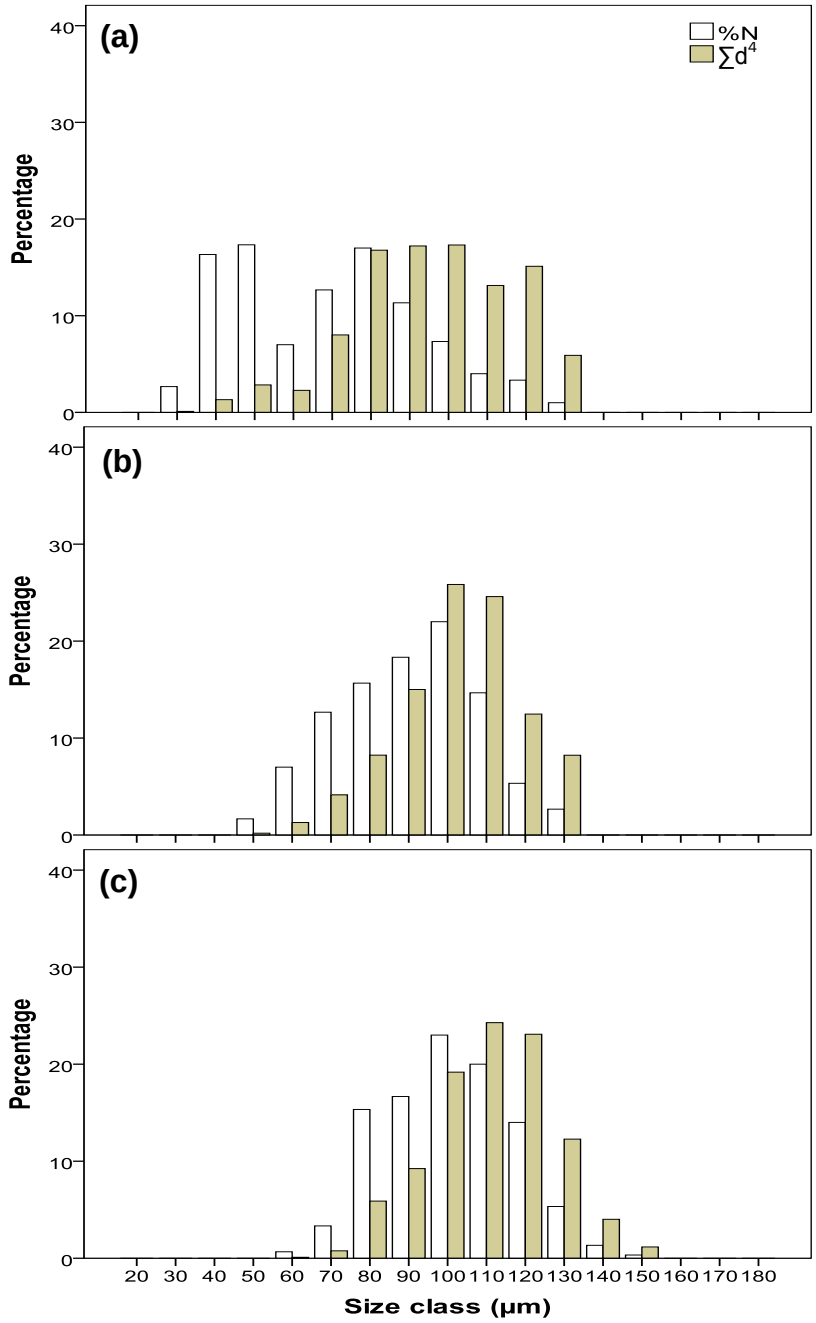


Fig. 3.9 Distributions of stem vessel diameters of (a) juvenile, (b) intermediate, and (c) adult *F. excelsa* in 10 µm size classes. The graphs show the number of vessels of each size class as a percentage of the total vessel number (%N) and the theoretical contribution of each size class to hydraulic conductance (Σd^4) of the stem. The hypothetical contribution to conductance was calculated by summing the diameters of vessels in each size class to the fourth power (Hagen-Poiseuille Law) (Tyree and Zimmerman 2002).

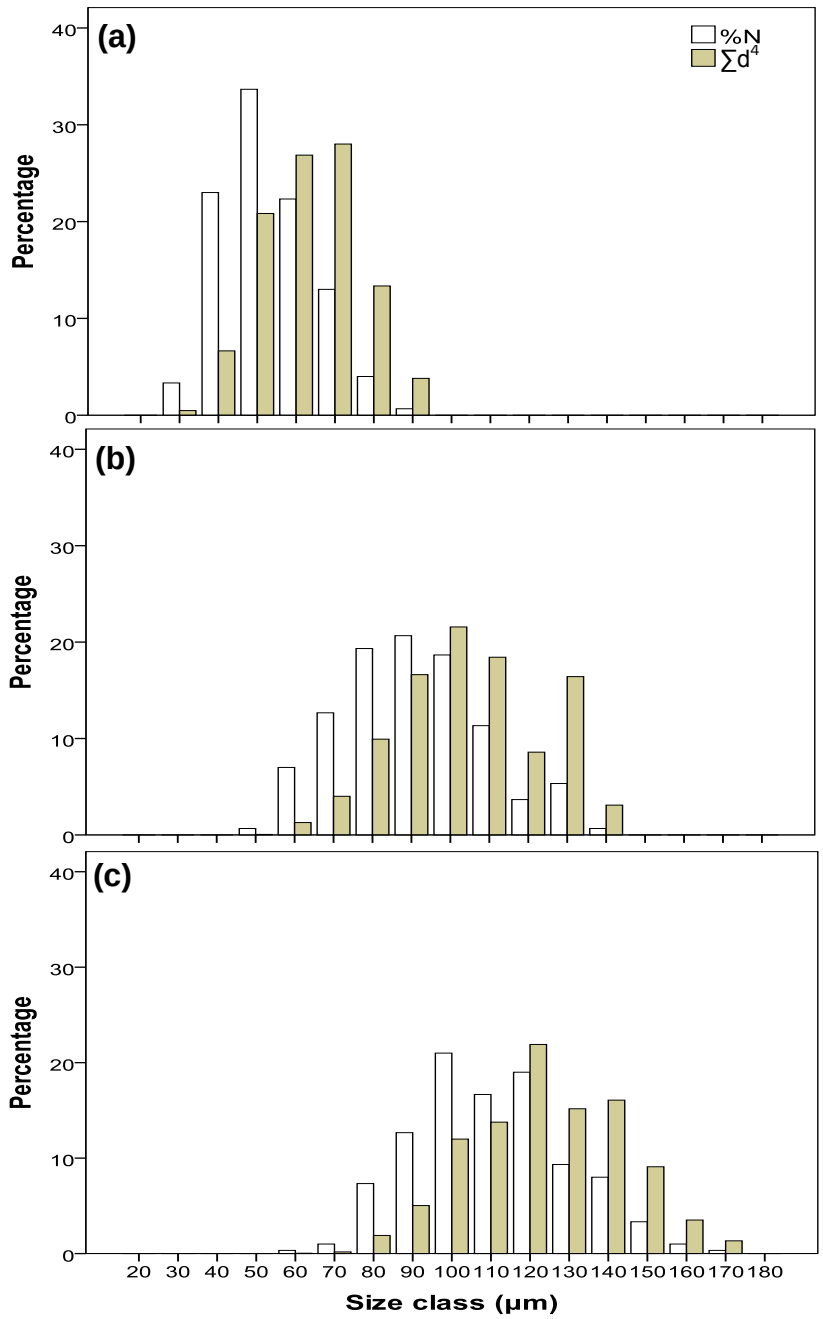


Fig. 3.10 Distribution of stem vessel diameters of (a) juvenile, (b) intermediate, and (c) adult *R. australasica* in 10 µm size classes. The graphs show the number of vessels of each size class as a percentage of the total vessel number (%N) and the theoretical contribution of each size class to hydraulic conductance (Σd^4) of the stem. The hypothetical contribution to conductance was calculated by summing the diameters of vessels in each size class to the fourth power (Hagen-Poiseuille Law) (Tyree and Zimmerman 2002).

3.3.2.2 Stem vessel density

The density of vessels is significantly different in the stems of different growth habits of both species (Fig. 3.11). Adult individuals of both species have fewer vessels per unit area compared with intermediate and juvenile congeners (*F. excelsa*: F= 83.693; P<0.01 and *R. australasica*: F= 234.193; P<0.01). This observation demonstrates that as vessel diameter increases, the vessel frequency per unit area decreases.

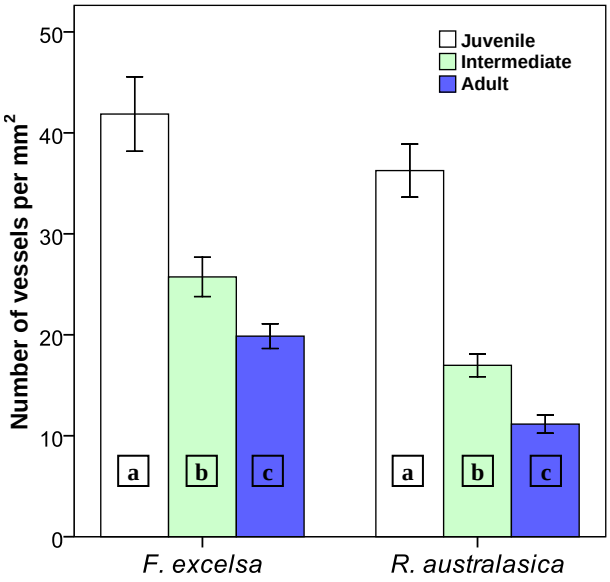


Fig. 3.11 Mean vessel density (the number of vessels per mm^2) of *F. excelsa* and *R. australasica* of differing growth habit. Error bars show 95% confidence intervals. Different letters indicate that values are significantly different between habits of each species at α = 1 %.

3.3.2.3 Vessel length

Individuals of different ontogenetic stages of both study species have different vessel lengths. The vessels of adult individuals of *F. excelsa* are significantly longer than those of the other two growth stages (F= 145.762; P<0.01) (Fig 3.12). Adult individuals of *R. australasica* also have longer vessels, although there was no significant difference in vessel length between adult and intermediate individuals (F= 62.569; P<0.01) (Fig. 3.13).

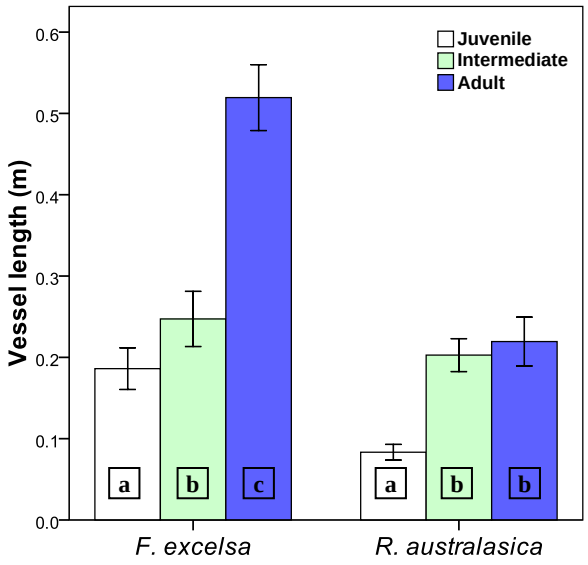


Fig. 3.12 Mean vessel length of *F. excelsa* and *R. australasica* of differing growth habit. Error bars show 95% confidence intervals. Different letters indicate that values are significantly different between habits of each species at α = 1 %. For *R. australasica*, the same letter indicates that values are not significantly different at α = 5 %.

3.3.2.4 Root vessel anatomy

The images of root vessels are shown in Figs. 3.13 and 3.14. Vessels of aerial roots are much larger than vessels of soil roots (*F. excelsa* t= -51.725; P<0.01 and *R. australasica* t= -24.517; P=<0.01) (Fig. 3.15). Even though aerial roots have a smaller diameter, and thus smaller stele area, than the stems, the mean vessel diameters of aerial roots are relatively similar to the mean vessel diameters of stems of intermediate and adult individuals (Figs. 3.7 and 3.15). The parenchyma (soft) tissues in the aerial roots of *F. excelsa* were more abundant than in the soil roots (Fig. 3.13). However, in the aerial roots of *R. australasica*, the parenchyma tissues are less prominent (Fig. 3.14).

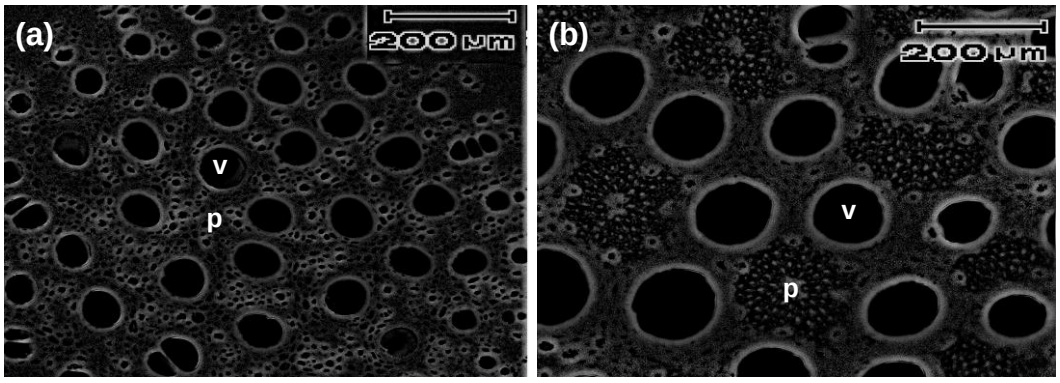


Fig. 3.13 Root xylem tissue of *F. excelsa* showing that vessels (v) of (a) soil roots are narrower than those of (b) aerial roots. The parenchyma tissues (p) between the vessels appear more abundant in the aerial roots than in the soil roots.

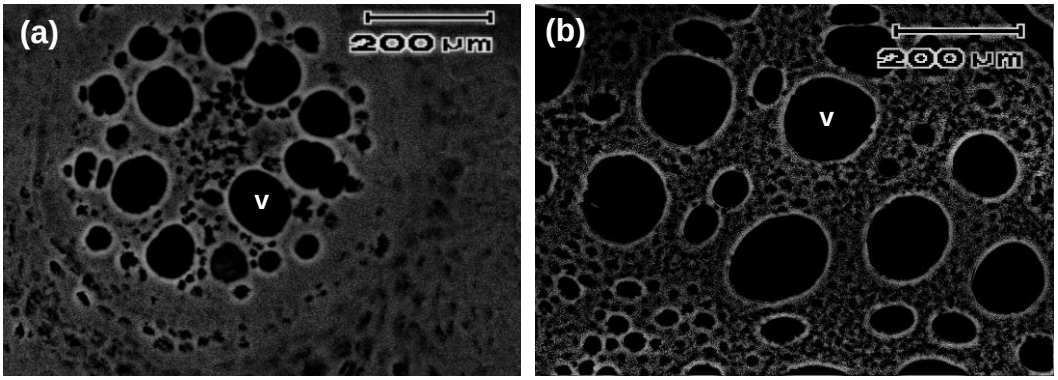


Fig. 3.14 Root xylem tissue of *R. australasica* showing that vessels (v) of (a) soil roots are narrower than those of (b) aerial roots.

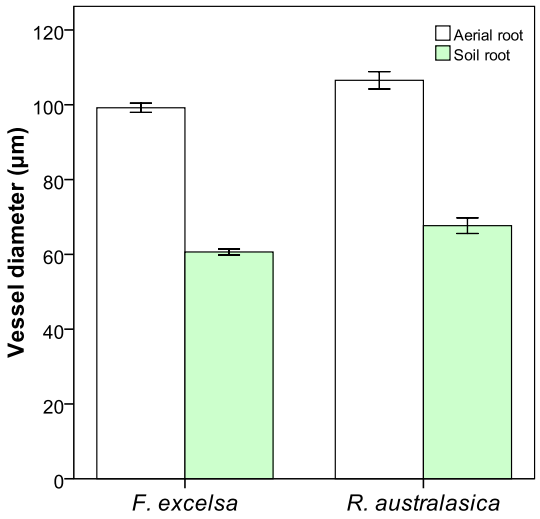


Fig 3.15 Mean vessel diameters of aerial and soil roots of *F. excelsa* and *R. australasica*. Error bars show 95% confidence intervals.

3.3.3. Hydraulic architecture parameters

Stem hydraulic conductivity (K_H) of *F. excelsa* was significantly different for all ontogenetic phases (F= 93.093; P<0.01) (Fig 3.16a), in which adult individuals had the highest values. Stem specific conductivity (K_S) (Fig 3.16b) and leaf specific conductivity (LSC) (Fig 3.16c) values of adult plants were also higher than for juvenile plants, although the values for LSC were not significantly different between adult and intermediate individuals (K_S, F= 73.251; P<0.01 and LSC, F= 8.759; P<0.01). On the other hand, Huber values of juvenile individuals were higher than adult plants (F= 29.078; P<0.01) (Fig 3.16d).

A similar pattern also occurs in *R. australasica* (Fig 3.16). Values of stem hydraulic conductivity, stem specific conductivity and leaf specific conductivity were higher for adult plants than for the other two growth stages (K_H, F= 96.497; P<0.01 and K_S, F= 49.532; P<0.01 and LSC, F= 5.915; P<0.01). However, values of K_S and LSC were not significantly different between intermediate and juvenile habits. Huber values of juvenile individuals were higher than adult and intermediate plants (F= 15.109; P<0.01).

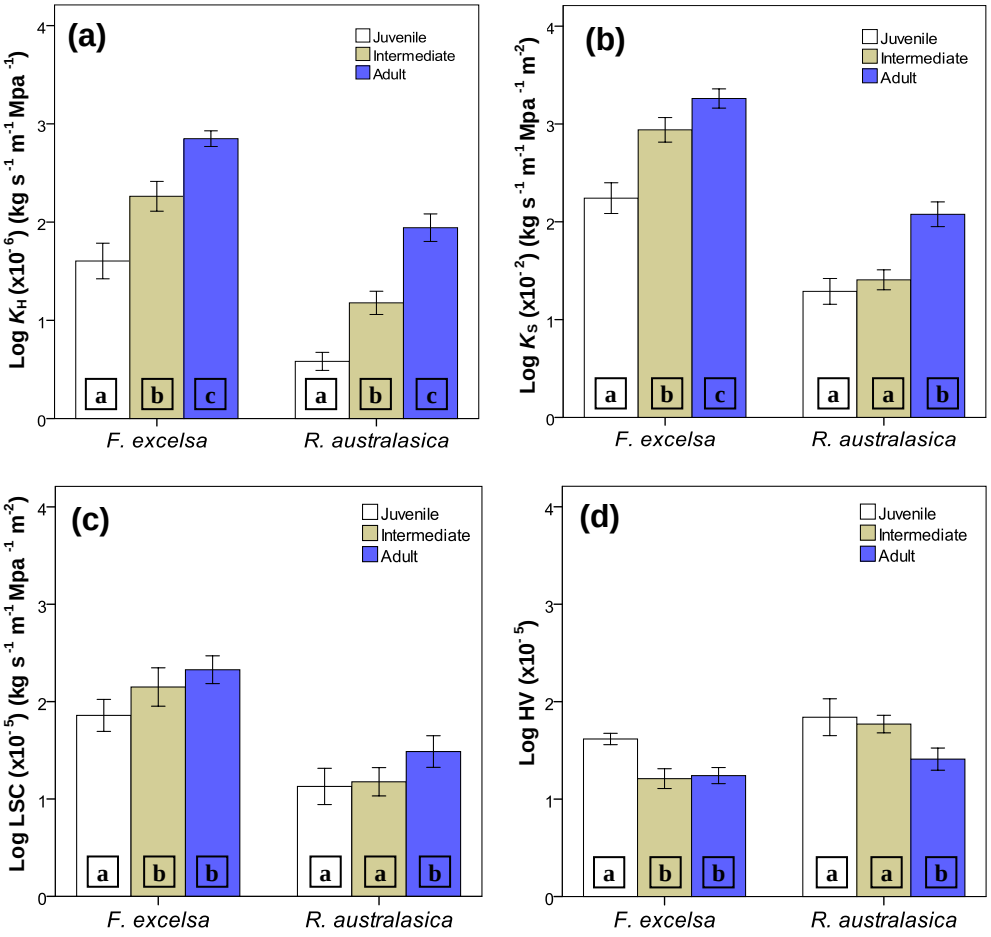


Fig. 3.16 Hydraulic architecture parameters, i.e. (a) stem hydraulic conductivity (K_H), (b) stem specific conductivity (K_S), (c) leaf specific conductivity (LSC) and (d) Huber value (HV), for different growth habits of *F. excelsa* and *R. australasica*. Data have been logarithmically transformed. Error bars show 95% confidence intervals. Different letters indicate that values are significantly different between habits of each species at α = 1 % and the same letter indicates that values are not significantly different at α = 5 %.

The patterns of hydraulic architecture parameters with height along the stem are shown in Figs. 3.17 and 3.18. The graphs show that lower values of stem hydraulic conductivity (Figs. 3.17a and 3.18a) and stem specific conductivity (Figs. 3.17b and 3.18b) of adult individuals were observed at the basal and distal parts of the stem. On the other hand, higher values of K_H and K_S were observed around the middle part of the stem length. This pattern is not really clear for juvenile stage plants, but there is an indication of this pattern for intermediate individuals. As plants become longer, it appears that the pattern of stem hydraulic conductivity and stem specific conductivity changes. Vessels are also narrower at the base of the stem and tend to increase in diameter toward the middle. The vessel diameter then decreases toward the plant shoots.

In contrast, the Huber value of both species tends to increase exponentially toward the distal end of the stem (Figs. 3.17d and 3.18d). Huber value is the measure of the investment of stem tissue per unit leaf area fed. Therefore, as fewer leaves are supported by a stem segment toward the distal end, the Huber value increases.

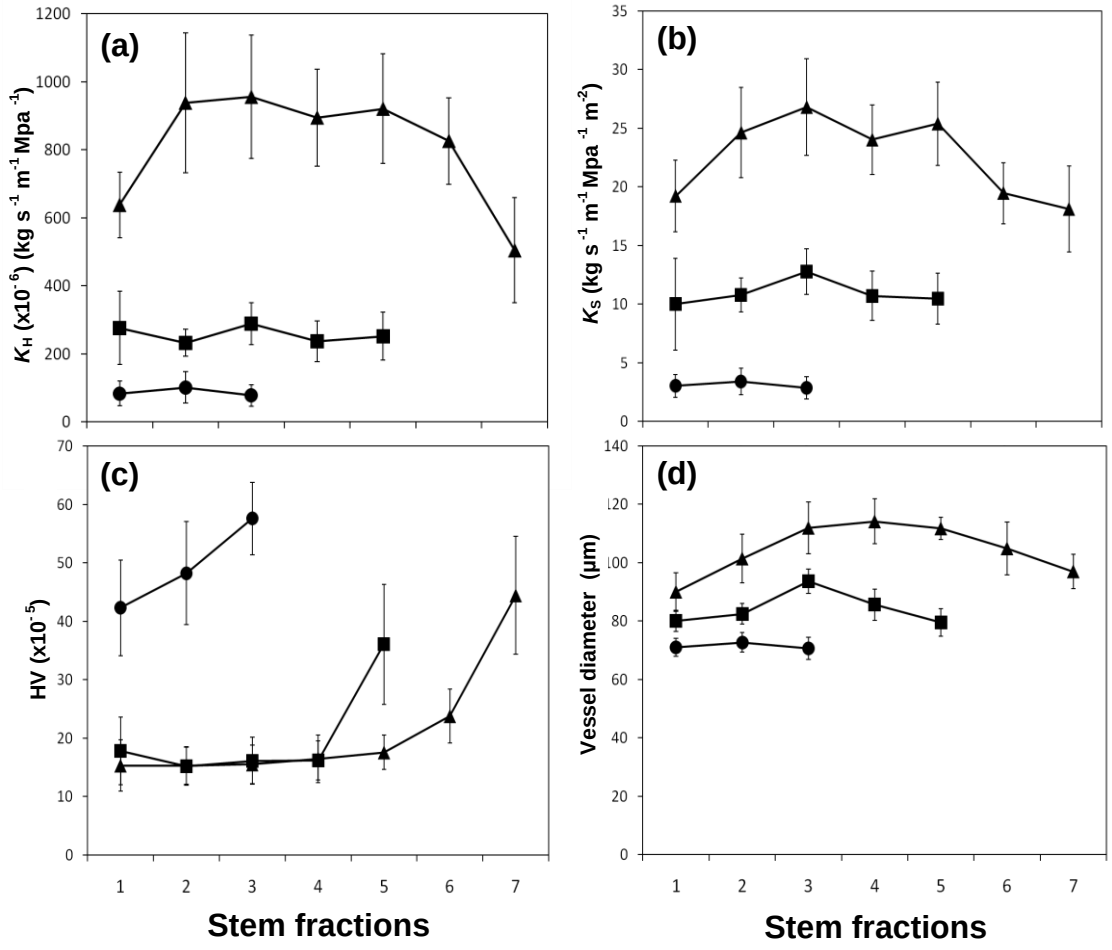


Fig. 3.17 (a) Stem hydraulic conductivity (K_H), (b) stem specific conductivity (K_S), (c) Huber value (HV) and (d) vessel diameter with distance along the stems of juvenile (●), intermediate (■) and adult (▲) *F. excelsa*. Stem fractions indicate the position on the stem. 1 is the basal part of the stem. The distal end of the stem is 3 for juvenile, 5 for intermediate and 7 for adult stages. Values are an average of six individuals of each growth stage and vertical bars represent standard errors.

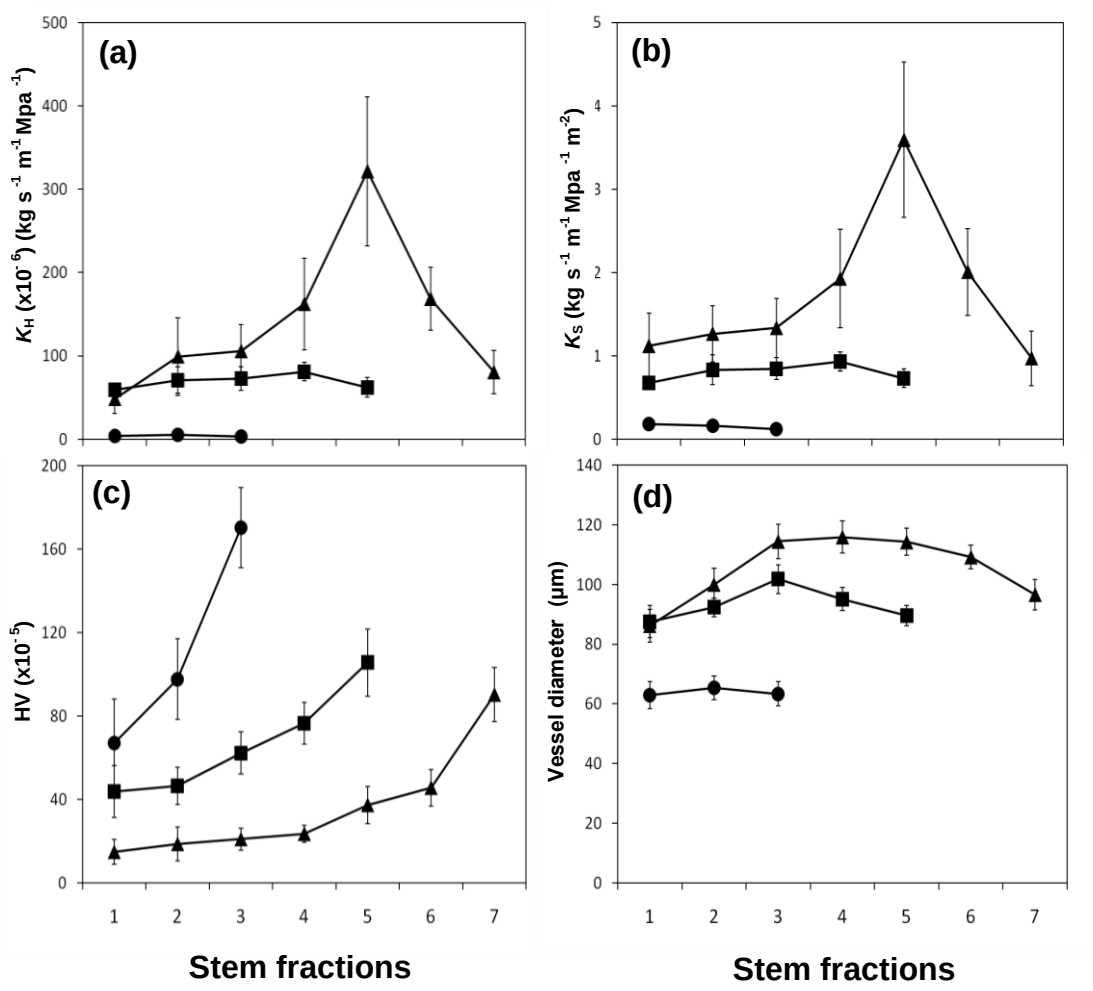


Fig. 3.18 (a) Stem hydraulic conductivity (K_H), (b) stem specific conductivity (K_S), (c) Huber value (HV) and (d) vessel diameter (d) with distance along the stems of juvenile (●), intermediate (■) and adult (▲) *R. australasica*. Stem fractions indicate the position on the stem. 1 is the basal part of the stem. The distal end of the stem is 3 for juvenile, 5 for intermediate and 7 for adult stages. Values are an average of six individuals of each growth stage and vertical bars represent standard errors.

3.4 DISCUSSION

The general anatomy of the xylem of the two study species is similar, in which each vascular bundle consists of one or two large metaxylem vessels and a few narrow protoxylem vessels. As the plant climbs the host, ages and develops into a semi-epiphytic adult individual, the parenchyma (soft tissues) also becomes more abundant (Figs. 3.6 and 3.7). According to Putz and Holbrook (1991), soft tissues in vines permit more mechanical flexibility and so they avoid physical damage while climbing. These soft tissues also increase the rate of recovery from damage.

There were clear differences in stem xylem anatomy in plants of both species with different habits. The adult plants have wider vessels than the intermediate and juvenile stage individuals (Fig. 3.8), which means that vessels of adult plants have a greater capacity to transport water (Tyree and Zimmerman 2002). Hypothetical hydraulic weighting of vessel diameter size classes based on the Hagen-Poiseuille law (Figs. 3.9 and 3.10) predicts that the wider vessels of adult individuals would result in much higher rates of water transport (Choat 2003).

Hydraulic properties of different ontogenetic stages of *F. excelsa* and *R. australasica* also differed. Values of stem hydraulic conductivity and stem specific conductivity (stem porosity) of the adult individuals were higher than for other growth stages (Figs. 3.16a and 3.16b). Differences in vessel sizes of differing growth stages, thus hydraulic parameters, may be related to the nature of their development, in which vascular stelar areas of adult individuals occupy more area of the stem than the other growth habits, which correlate to larger size of vessels (Fig. 3.4).

The increase in vessel size and hydraulic conductivity in adult plants of secondary hemi-epiphytes may also be a form of hydraulic plasticity in relation with increasing plant height. In their study on the hydraulic architecture of several phylogenetically different canopy tree species in tropical rainforests of Sulawesi, Indonesia, Zach *et al*. (2010) found that leaf specific conductivity, sapwood-area specific conductivity and vessel size increased with tree height. This adjustment in hydraulic conductivity is important for taller plants to compensate for the longer distance for water transport than in shorter plants.

The maximum vessel diameters of *F. excelsa* and *R. australasica* are smaller than vessels of *Monstera acuminata*, another secondary hemi-epiphytic vine (Lopez-Portillo *et al*. 2000). Maximum vessel diameters of *M. acuminata* can reach 200 µm, compared with maximum vessel diameters of 150 µm in *F. excelsa*, (Fig. 3.9c) and 170 µm in *R. australasica* (Fig. 3.10c). The vessel diameters of the study species are also generally lower than for several climbing palms studied by Fisher *et al*. (2002) (Table 3.1).

Table 3.1 Diameter of widest vessels of several climbing palms compared to current study species[1]. Values are the largest values from several sample collections studied (Fisher *et al*. 2002).

Species	Diameter of widest vessels (µm)
Calamus insignis	213
Daemonorops hystrix	243
Korthalsia echinometra	380
Korthalsia rigida	532
Korthalsia rostrata	205
Plectocomia elongate	458
Freycinetia excelsa[1]	150
Rhaphidophora australasica[1]	170

In most dicotyledons and conifers, tracheid or vessel diameters increase in a basipetal direction. This means that the widest vessels are in the lower part of the stem and decrease with increasing plant height (Tyree and Ewers 1996; Tyree and Zimmerman 2002). However, this pattern is reversed in secondary hemi-epiphytic vines. In *M. acuminata*, vessel diameter increases with height; therefore, the widest vessels are in the top part of the stem (Lopez-Portillo *et al*. 2000). A similar pattern was also found in *F. excelsa* (Fig. 3.17d) and *R. australasica* (Fig. 3.18d). However, in these two species the size then decreases again towards the stem apex, so the widest vessels occupy the middle part of the stem.

Values of stem hydraulic conductivity, stem specific conductivity and leaf specific conductivity of the semi-epiphytic adult stage of both study species were significantly higher than for other growth stages. This is consistent with the fact that adult individuals have wider vessels. As juvenile plants have less capacity to conduct

water, they would require a greater pressure gradient in order to sustain the same transpirational flux as the adult individuals (Choat 2003; Eamus 1999).

The research reported here for secondary hemi-epiphytes differs from the observations on primary hemi-epiphytes, i.e. *Ficus* species. Studies on seven *Ficus* species, Patiño *et al.* (1995), for example, found that epiphytic individuals have less conductive stems per unit leaf area, i.e. lower leaf specific conductivity than terrestrial individuals. This means that less water can be transported by the stem per unit leaf area for epiphytic *Ficus*. The hydraulic architecture of epiphytic *Ficus* is more affected by the stressful canopy conditions compared with rooted trees which may have an adequate water supply.

Values of leaf specific conductivity and stem specific conductivity of both species of secondary hemi-epiphytic vines are lower than for tropical lianas (woody vines) (Fig. 3.19). As the leaf specific conductivity is an appropriate tool for predicting hydraulic sufficiency, i.e. the relationship between hydraulic conductivity of the xylem in the stem and the amount of leaf area it must supply, it seems that herbaceous secondary hemi-epiphytic vines have a less effective hydraulic architecture to support water transport to the leaves than do woody vines. This fact may be associated with the ability of woody vines to reach higher into the canopy than herbaceous vines.

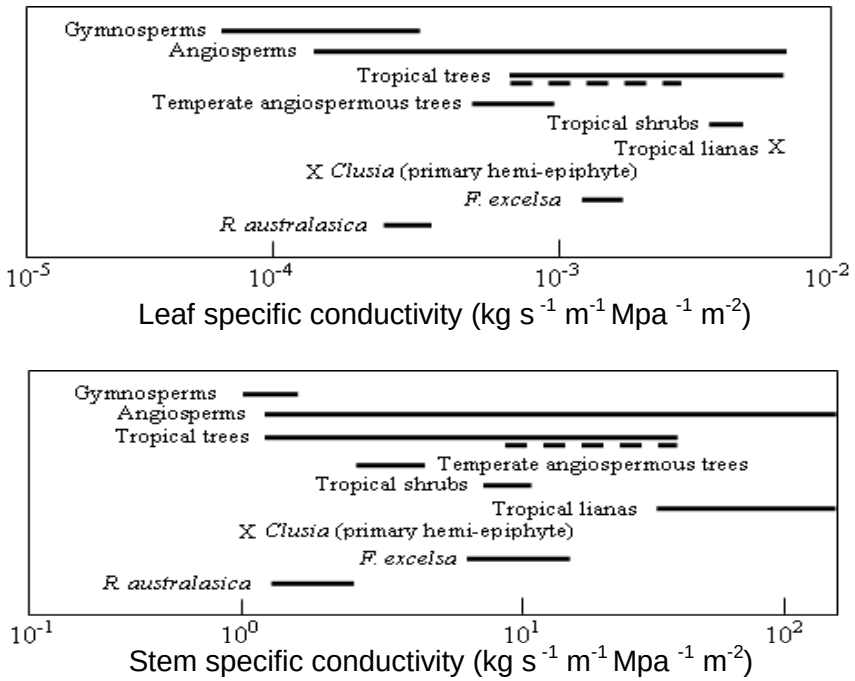


Fig. 3.19 Ranges of leaf specific conductivity and stem specific conductivity according to phylogeny or growth form, based on published literature. Dashed line below the tropical trees indicates *Ficus* spp. and "X" indicates that ranges are too short to be represented (from Tyree and Ewers 1996). The range of values for *F. excelsa* and *R. australasica* include juvenile, intermediate and adult individuals.

The patterns of stem hydraulic conductivity and stem specific conductivity along the plant stem (Figs. 3.17 and 3.18) are consistent with the pattern of vessel-diameter distribution. The stem base had lower hydraulic conductivity, which consequently may provide hydraulic restrictions. The lower hydraulic conductivity at the stem base of semi-epiphytic adult individuals can be attributed to two things. Firstly, as mentioned before, it is due to narrower vessels. Narrower vessels would result in lower flow rates of water transport (Choat 2003; Tyree and Zimmerman 2002).

Secondly, the lower hydraulic conductivity at the stem base may be related to the nature of development of monocotyledonous plants. As monocotyledonous vines, *F.*

excelsa and *R. australasica*, have stems of finite duration due to the lack of a vascular cambium and therefore of secondary growth (Carlquist 1991; Lopez-Portillo *et al*. 2000). The original primary xylem has to supply water for the leaves throughout the entire plant life cycle (Ewers *et al*. 1991). The basal part of the stem contains older tissues and, hence, they are less functional. These less functional tissues may slow the rate of water transport through the vessels.

As adult individuals have wider vessels and higher stem hydraulic conductivity than intermediate and juvenile individuals, it appears that there is less physical restriction in water transport for the adult growth stage. However, the hydraulic architecture parameter distribution with increasing plant height suggests that the basal parts of the stem may restrict water transport. The establishment of aerial roots has two advantages: through creating a more effective pathway to take up water from the soil and at the same time, providing a mechanism to escape from the hydraulic bottle-neck in the basal part of the stem.

Although aerial roots have smaller diameters than the plant stem, aerial roots have a similar vessel diameter (Fig. 3.15) compared to the stem vessel diameter of adult individuals (Fig. 3.8). This phenomenon partly explains the effectiveness of aerial roots in transporting water. However, further benefits of these aerial roots, such as improving the utilization of water from different parts of the soil profile, are yet unknown. No evidence has been found in the literature regarding whether aerial roots contribute to the partitioning of soil water resources.

3.5 CONCLUSIONS

Adult individuals of *F. excelsa* and *R. australasica* have wider vessels than intermediate and juvenile plants. Furthermore, this study found the reverse of the common pattern of vessel diameter distribution, whereby hydraulic conductivity, increases in the basipetal direction from the top of the plant to the base in most plants. The reverse pattern may be common in plants with a secondary hemi-epiphytic growth habit. A previous study also found this phenomenon in another species of secondary hemi-epiphyte, *M. acuminata*.

There are also differences in hydraulic architecture parameters between the three growth stages of the two study species. The change in plant-soil connectivity does not seem to physically restrict water transport, since the adult stage has a higher hydraulic conductivity than the juvenile stage. Lower hydraulic conductance in the basal parts of the stem provides a hydraulic resistance. However, this problem appears to be bypassed by the establishment of aerial roots.

The observations described in this chapter raise other questions. The first question is if there is no physical and architectural limitation to water uptake as the plant ages and becomes semi-epiphytic, are water relations and gas exchange affected by changes in seasonal availability? A second question is do aerial roots use water resources differently to the original soil roots, which may consequently contribute to hydraulic efficiency, during the ontogenetic development of secondary hemi-epiphytic vines? Answering these questions will give a better understanding of eco-physiology of secondary hemi-epiphytic vines.

CHAPTER 4

COMPARATIVE LEAF ANATOMY OF DIFFERENT GROWTH PHASES OF SECONDARY HEMI-EPIPHYTIC VINES

4.1. INTRODUCTION

The adaptations of plants to the environmental conditions in which they live are generally evident in their morphology, physiology and anatomy (Dickison 2000). The leaf is the most anatomically variable of plant organs, and leaf adaptations can be reliable indicators of environmental conditions. Many studies have investigated changes in leaf anatomy in relation to differences in light acclimatization (Ashton and Berlyn 1992; Oguchi *et al.* 2005; Oguchi *et al.* 2006; Sims and Pearcy 1992), salinity (Parida *et al.* 2004; Suárez and Medina 2005), water stress in xeromorphic and desert plants (Ehleringer and Mooney 1978; Gibson 1996) or water stress in epiphytic plants (Rada and Jaimez 1992; Loeschen *et al.* 1993). These adaptations may include differences in leaf and tissue thicknesses (Chartzoulakis *et al.* 2002; Chazdon and Kaufman 1993; Sims and Pearcy 1992), and the number and dimensions of stomata (Hetherington and Woodward 2003; Holbrook and Putz 1996a; Parlange and Waggoner 1970; Sam *et al.* 2000).

In the later stages of their development, secondary hemi-epiphytic vines lose their stem connection to the ground. The connection to the soil is then maintained by the development of aerial roots (Lopez-Portillo *et al*. 2000; Lüttge 1997; Moffet 2000; Patino *et al*. 1999). This shift in soil connectivity may be associated with changes in resource utilization by different growth habits of secondary hemi-epiphytic vines.

In the previous chapter, it was established that individuals of the adult phase of *F. excelsa* and *R. australasica* had wider vessels and higher rates of water conductivity than earlier developmental stages. Leaf anatomical responses to the shift in growth habits, nonetheless, are poorly understood. This study examined leaf anatomical characteristics of juvenile, intermediate and adult stages of *F. excelsa* and *R. australasica*. The hypothesis tested is that leaf anatomy differs between different developmental stages of the two study species. Variables examined were specific leaf area, stomatal distribution and dimension, stomatal density, leaf thickness, the thickness of the palisade parenchyma and spongy mesophyll layers, and the thickness of the upper and lower epidermal layers, including, if any, hypodermis, and the number of cell layers in the palisade parenchyma tissue.

4.2. MATERIAL AND METHODS

4.2.1 Plant materials and sampling strategy

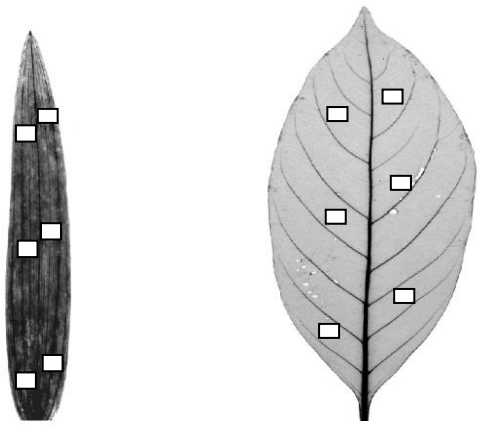
Freycinetia excelsa samples were collected from Paluma Range National Park and samples of *Rhaphidophora australasica* were collected from the Henrietta Creek Section of Wooroonooran National Park. A description of the study species is contained in Section 2.1 and a description of study sites is in Section 2.2.

Fifteen representative plants of each growth habit of each species were taken as samples. Fully expanded leaves were removed from each plant (Ferris *et al*. 2002). Two leaves of each plant sample were collected and stored in Formaldehyde-acetic acid-alcohol (FAA) for histological studies. Another leaf from each plant was kept in

a sealed plastic bag and was stored in a humidified container for specific leaf area measurement.

4.2.2 Stomatal distribution, density and length

The number of stomata per unit area (stomatal density) and stomatal distribution was observed at six different sites between the midrib and the leaf margin on each leaf for both abaxial (lower) and adaxial (upper) surfaces (Fig. 4.1) (Tay and Furukawa 2008), totalling 90 observations for each growth habit of each species. A portion of the leaf surface was hand-sectioned with a razor blade from each leaf observation site and was mounted onto a microscope slide. The sample was observed under a light microscope at a magnification of x100 (Olympus U-CMAD-2, Japan). Each sample was then photographed using an Olympus digital camera attached to the microscope. The resulting images were then viewed on a computer screen and the number of stomata per unit area (mm^2) was counted.



(a) (b)

Fig. 4.1 Positions of stomatal observations (□) on the leaves of (a) *F. excelsa* and (b) *R. australasica*.

From each leaf, 30 stomata were chosen randomly, resulting in measurement of 450 stomata per growth phase per species. Stomatal dimension is generally described in terms of the length and width between outer walls, away from the pore, of the guard cells (Holbrook and Putz 1996a). However, in this study only stomatal length was measured. Stomatal width was not measured as the level of openness of each stomata varied. The length of stomata was measured using ImageJ software (National Health Institute, USA).

4.2.3 Leaf tissue anatomy

Immediately prior to sectioning, FAA stored leaves were transferred to 70% alcohol and were cut into two segments of about 2x10 mm to fit into a histological cassette. These two segments were taken at about half way along the leaf blade between the midrib and the leaf margin. The processing of plant tissue samples followed the method described by Winsor (1994). Segments of leaves were processed in paraffin, embedded in wax, sectioned using a rotary microtome (8 µm) and mounted on a slide. To de-wax the sections, slides were immersed in xylene for two minutes, and then re-immersed in a second xylene solution for two minutes. The slides were rinsed through a series of alcohols, i.e. three absolute alcohols and one 70% alcohol (20 dips in each) and the sections were then hydrated in water. Sections were stained with Alcian blue. Finally, the sections on each slide were mounted in DPX mounting medium.

Sections on each slide were then photographed at four different random locations at a magnification of x100, which means that each leaf sample was represented by four tissue images. The resulting images were used to measure leaf blade thickness, the thickness of the palisade parenchyma and spongy mesophyll layers and the thickness of the upper and lower epidermal layers, including, if any, hypodermis. The thicknesses were measured using ImageJ software (National Health Institute, USA). For each tissue image, the measurements were conducted at three different random positions, resulting in 12 measurements for each leaf sample. In addition, the number of cell layers per unit width of section in the palisade parenchyma was also counted.

4.2.4 Specific leaf area

Specific leaf area (SLA) is the leaf area of a fresh leaf divided by its oven-dry mass, expressed in m^2 kg^{-1}. Another commonly used term leaf mass area (LMA) is simply 1/SLA (Cornelissen *et al*. 2003). The leaf area of samples was measured using a Paton Electronic Planimeter (Paton Industries Australia). Leaves were oven dried at 70^0C for two days and then weighed.

4.2.5 Statistical analysis

Differences between observed variables were analysed by applying ANOVA with a post hoc Tukey test (Zar 1999). Basic data management was carried out using Microsoft Excel spreadsheets. SPSS software was used for statistical analyses (Coakes *et al.* 2007).

4.3. RESULTS

4.3.1 Stomatal distribution and density

F. excelsa is amphistomatous, i.e. it has stomata on both the adaxial (upper) and abaxial (lower) surfaces of the leaf. The density of stomata is much higher on the lower surface than the upper surface. The number of stomata on the upper surface of *F. excelsa* leaves was less than 10% of the number of stomata on the lower surface for all developmental stages (Table 4.1). The adaxial stomata also tend to be located at the edge of the leaf. In contrast, *R. australasica* has stomata only on the lower leaf surface (hypostomatous).

Table 4.1 Mean ± standard deviation of the ratio of adaxial to abaxial stomata of different developmental stages of *F. excelsa*. As *R. australasica* has no adaxial stomata, the ratio of adaxial to abaxial stomata of this species does not exist. The same letter shows no significant statistical differences between developmental stages.

	Ratio of adaxial to abaxial stomata		
Species	**Juvenile**	**Intermediate**	**Adult**
F. excelsa	0.05 ± 0.05 a	0.06 ± 0.05 a	0.07 ± 0.07 a

The shift from terrestrially juvenile to semi-epiphytic adult life habit in the two species studied was associated with an increase in the stomatal density (Figs. 4.2, 4.3 and 4.4). The density of abaxial stomata of adult plants of *F. excelsa* and *R. australasica* was higher than for juvenile and intermediate congeners (*F. excelsa*: F= 13.480, P<0.01; *R. australasica*: F= 25.029, P<0.01) (Fig. 4.2), although the difference was not significant for adult and intermediate individuals of *F. excelsa*.

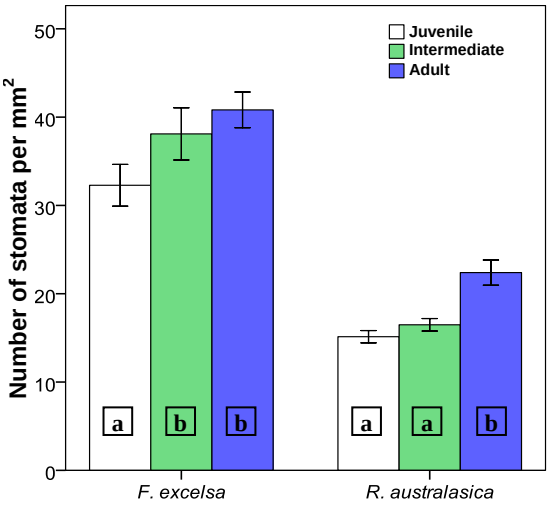


Fig. 4.2 The number of abaxial stomata of different growth habits of two secondary hemi-epiphytic vines. Error bars show 95% confidence intervals. The same letter indicates that values are not different at α = 5 % for each species. The number of leaf samples was 15 leaves of each ontogenetic phase of each species.

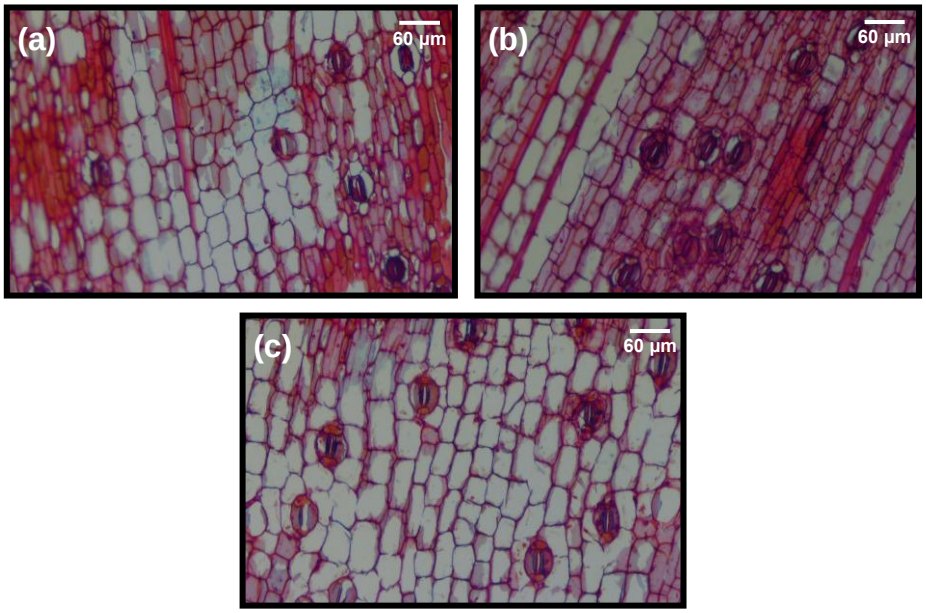


Fig. 4.3 Examples of the lower surface of leaves of (a) juvenile, (b) intermediate and (c) adult *F. excelsa* showing the density and size of stomata.

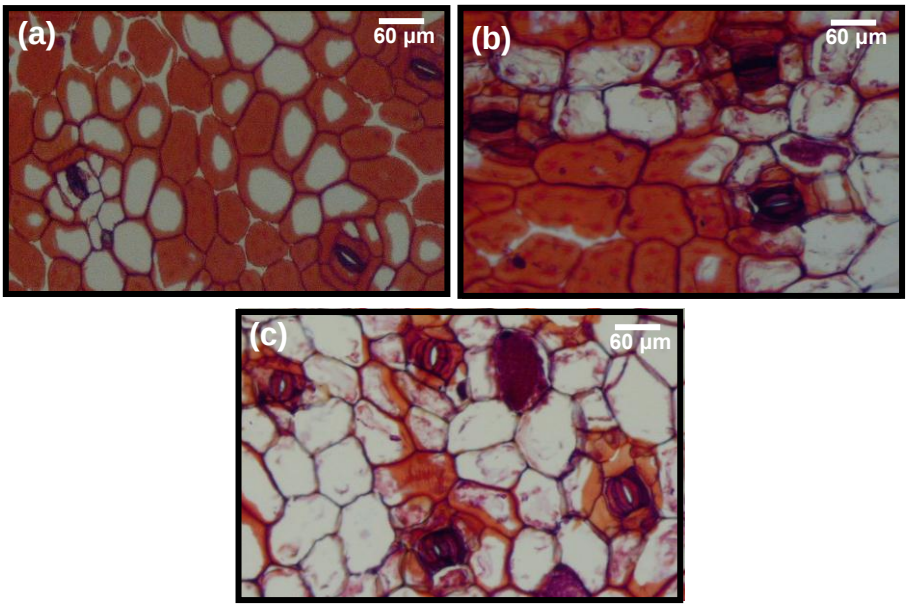


Fig. 4.4 Examples of the lower surface of leaves of (a) juvenile, (b) intermediate and (c) adult *R. australasica* showing the density and size of stomata.

4.3.2 Stomatal length

Plants of different ontogenetic stages of the secondary hemi-epiphytes studied had different lengths of stomata. Adult individuals of *F. excelsa* had significantly longer stomata than juveniles (F= 8.391, P <0.01) (Fig. 4.5). However, the average stomatal length of adult and intermediate individuals of this species did not differ significantly (Tukey test). A similar pattern was also found in *R. australasica*. Juveniles had significantly shorter stomata than did intermediate and adult congeners (F= 70.708, P<0.01) (Fig. 4.5).

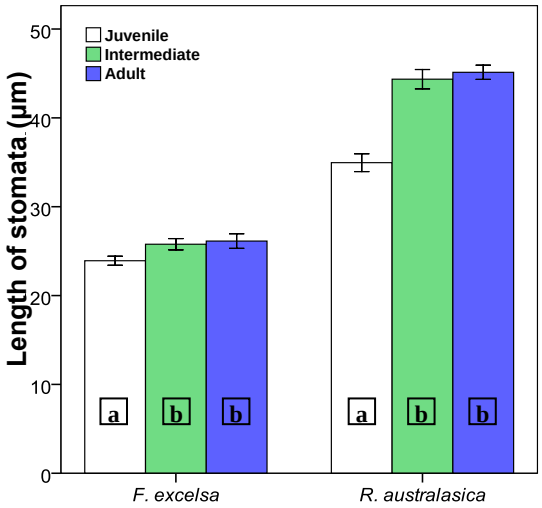


Fig. 4.5 The length of stomata of different ontogenetic phases of semi-epiphytic vines. Error bars are 95% confidence intervals. The same letter indicates that values are not different at α = 5 % for each species. 450 stomata from 15 leaves of each ontogenetic phase of each species were observed.

4.3.3 Quantitative leaf tissue anatomy

Observations on leaf and tissue layer thickness are shown in Figs. 4.6 and 4.7, and transverse sections of tissue of the two study species are shown in Figs. 4.8 and 4.9. For *F. excelsa*, there were no significant differences in leaf thickness and tissue layer thickness for juvenile, intermediate and adult individuals, except for the upper epidermal layer (Figs. 4.6 and 4.8). In contrast, leaves of adult individuals of *R. australasica* were thicker than intermediate and juvenile individuals (F= 25.155, P<0.01) (Figs. 4.7 and 4.9). Upper epidermal, palisade parenchyma and spongy mesophyll tissues of adult plants of *R. australasica* were also thicker than plants of other ontogenetic phases (F= 17.857, 33.135 and 24.438 respectively, with all P<0.01). The difference was not significant between intermediate and adult individuals for upper epidermis and palisade parenchyma (Fig. 4.7). Lower epidermal tissue, however, was thinner in adult plants than terrestrial congeners (F=

11.724, $P<0.01$). It appears that as leaves become thicker, the lower epidermis becomes thinner (Fig 4.9).

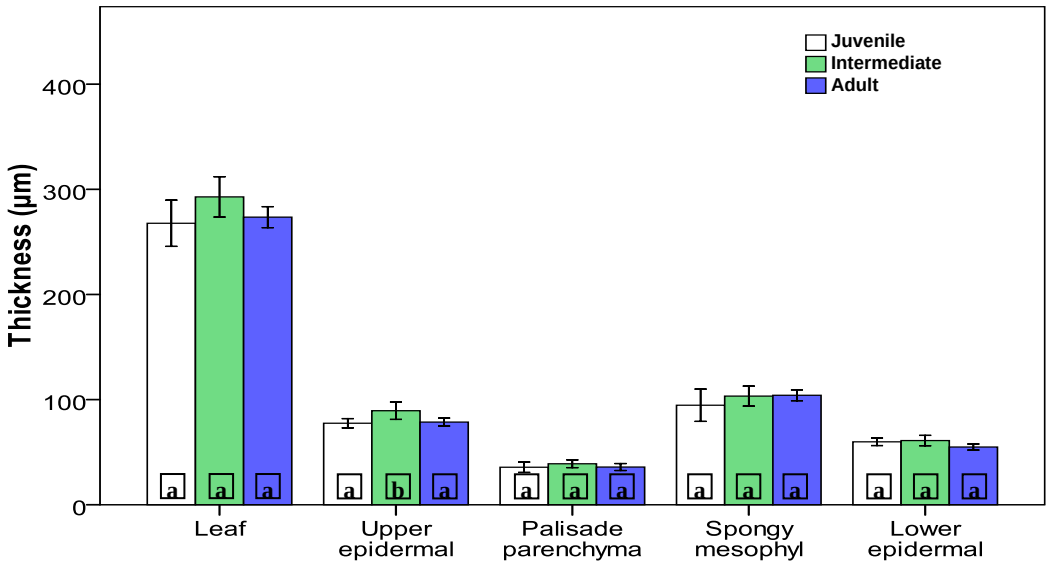


Fig. 4.6 Leaf and tissue layer thickness of *F. excelsa*. Error bars are 95% confidence intervals. Upper epidermal layer includes epidermis and hypodermis (see Fig. 4.8 for more detail). The same letter indicates that values of each variable are not different at α = 5 %.

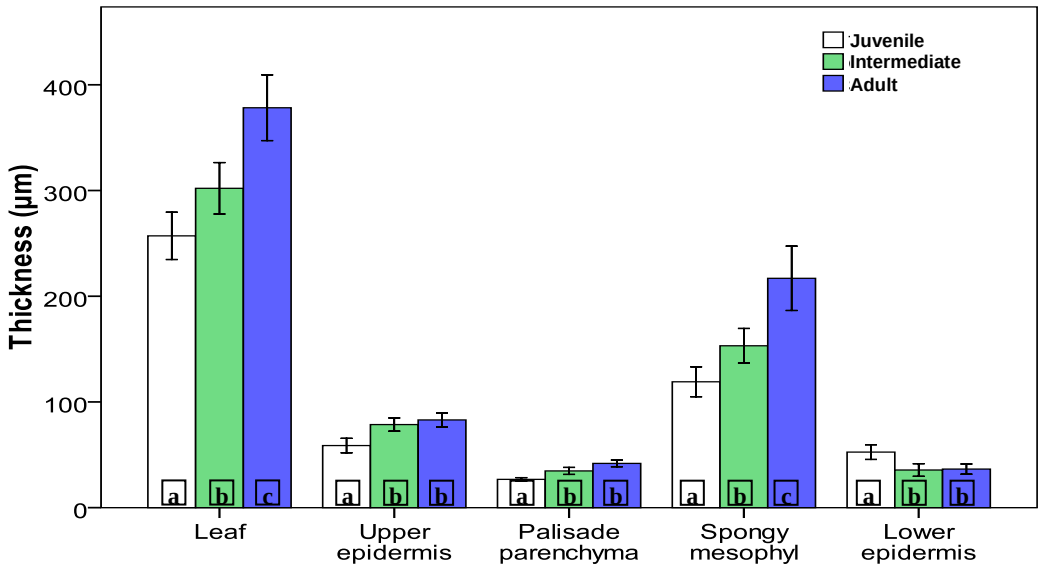


Fig. 4.7 Leaf and tissue layer thickness of *R. australasica*. Error bars are 95% confidence intervals. The same letter indicates that values of each variable are not different at α = 5 %.

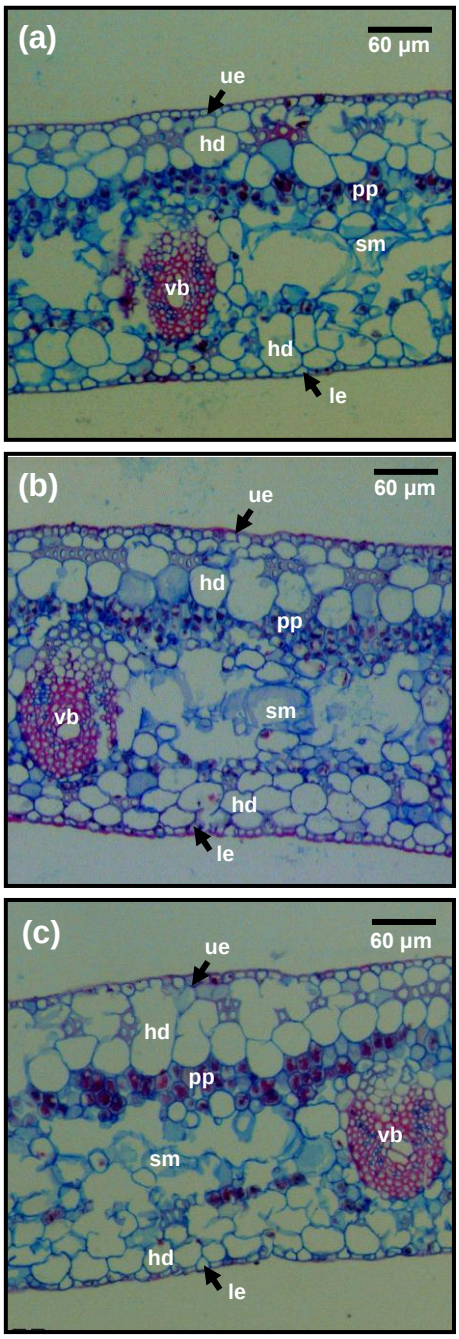


Fig. 4.8 Leaf tissue anatomy of (a) juvenile, (b) intermediate, and (c) adult *F. excelsa* showing upper epidermis (ue), hypodermis (hd), palisade parenchyma (pp), spongy mesophyll (sm), vascular bundle (vb) and lower epidermis (le). The epidermis and hypodermis collectively are referred to as epidermal tissue.

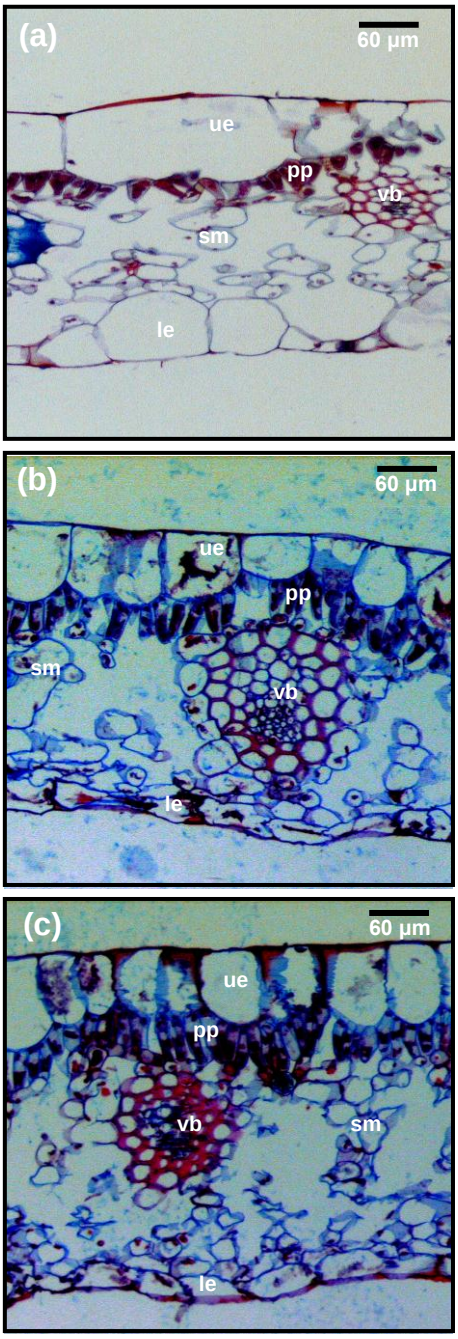


Fig. 4.9 Leaf tissue anatomy of (a) juvenile, (b) intermediate, and (c) adult *R. australasica* showing upper epidermis (ue), palisade parenchyma (pp), spongy mesophyll (sm), vascular bundle (vb) and lower epidermis (le).

F. excelsa has two to three cell layers in the palisade parenchyma. However, there were no significant differences in the number of palisade parenchyma cell layers between plants of differing ontogenetic phases (Fig. 4.10). *R. australasica* individuals of different developmental stages also had the same number of cell layers in the palisade parenchyma (Fig. 4.10).

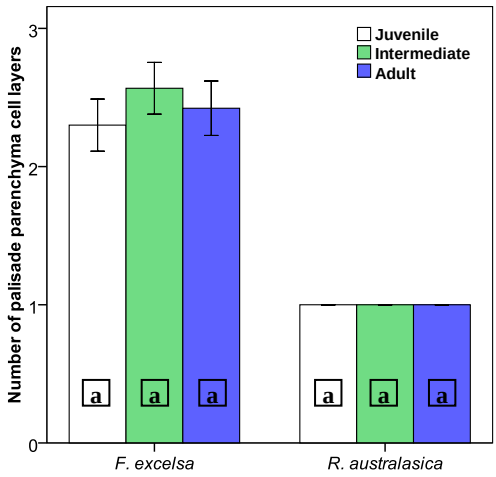


Fig. 4.10 The number of cell layers in the palisade parenchyma of *F. excelsa* and *R. australasica*. Error bars are 95% confidence intervals. The same letter indicates that values are not different at $\alpha = 5$ %.

4.3.4 Specific leaf area

The specific leaf area (SLA) of *F. excelsa* and *R. australasica* decreases with the shift in ontogenetic stages (Fig. 4.11). Juveniles of *F. excelsa* had significantly higher SLA than adult congeners (F= 5.303, P<0.01). For *R. australasica*, the SLA of juvenile plants was double that of adult individuals (F= 134.229, P<0.01).

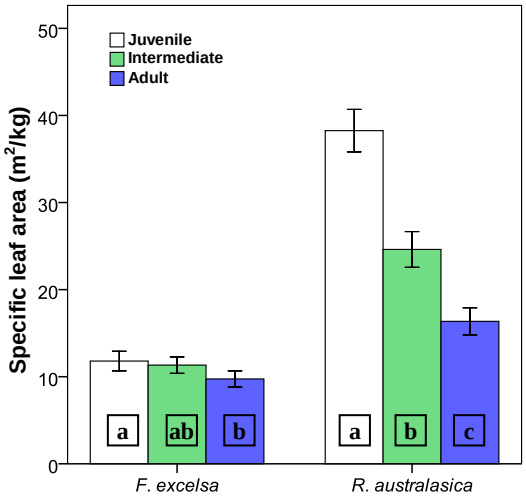


Fig. 4.11 Specific leaf areas of differing growth stages of *F. excelsa* and *R. australasica*. Error bars are 95% confidence intervals. The same letter indicates that values are not different at α = 5 %.

4.4. DISCUSSION

Microclimates at the canopy level differ from the forest floor (Whitmore 1998). Living in the canopy may provide plants with some advantages, including an increase in light availability and a reduced risk from terrestrial herbivores, fire and flooding (Holbrook and Putz 1996a). However, the canopy environment also may impose more stressful conditions for plants through the increase in possible physiological stresses due to a dry environment, higher light intensity in comparison to the forest floor, and a limited supply of nutrients (Benzing 1990; Holbrook and Putz 1996a; Lüttge 1989).

The ability of canopy-occupying plants, such as epiphytes and hemi-epiphytes, to survive in a stressful environment depends upon morphological and physiological adaptations (Lorenzo *et al*. 2010). Epiphytic plants have developed mechanisms to improve their water use efficiency by exhibiting CAM photosynthesis, xeromorphism, and water and organic debris impoundments (Benzing 1990; Lüttge 1997). In many species of epiphytic Araceae, which lack CAM (Winter *et al*. 1983; Zotz and Ziegler 1997), morphological and anatomical adaptations to water stress may include leaf deciduousness (Croat 1988), high leaf succulence, sclerophylly, and stomatal and epidermal resistance to water loss (Lorenzo *et al*. 2010).

Controlling water loss is the main strategy by which most plants counteract water stress. Rapid loss of water through transpiration is enhanced by many factors, including a large surface area, a thin cuticle and lack of hairs. Epidermal water loss can also be attributed to evaporation from around the stomatal complex (Muchow

and Sinclair 1989). Controlling stomatal geometry may reduce the water loss through the stomatal pore by resisting diffusion (Parlange and Waggoner 1970). Aasama *et al*. (2001) reported that there is a clear negative relationship between the length of the stomatal pore and sensitivity to increasing drought. Larger stomata were slower to close and more vulnerable to hydraulic dysfunction under drought.

There are contradicting arguments on the relative sensitivity of stomatal density to water stress. Holbrook and Putz (1996a), for example, argued that a lower stomatal density in leaves of epiphytic *Ficus*, compared with terrestrial tree congeners, is consistent with the frequent limitations to water availability in the epiphytic environment and the need for conservative water use in epiphytic stranglers. However, more stomata do not mean plants lose more water (Hetherington and Woodward 2003). A higher stomatal density may give leaves more control, to open and close stomata more rapidly in certain areas of leaves, consequently providing the capacity for leaves to rapidly increase stomatal conductance and maximize CO_2 diffusion into the leaf during favourable conditions for photosynthesis.

In general, plant physiologists and ecologists consider that stomata provide a balance between uptake of CO_2 for photosynthesis and preventing water loss (Jones 1997). Environmental conditions, such as light intensity, the concentration of atmospheric carbon dioxide and endogenous plant hormones, control stomatal aperture and development (Hetherington and Woodward, 2003). In vines, this physiological control is also important in order for the plants to be successful in co-existing with their hosts (Tay and Furukawa 2008).

Morphological and physiological adaptations of epiphytes may vary with plant size and/or developmental phases (Adam and Martins 1986; Lorenzo *et al*. 2010; Zotz and Tyree 1996). Lorenzo *et al*. (2010) found that, compared to young and adult individuals, seedlings of epiphytic *Anthurium scandens* (Araceae) had different leaf anatomical features, including lower leaf and tissue thickness and leaf succulence. On the other hand, epiphytes of the same age but different size may also express varied physiological responses to environmental conditions (Schmidt *et al*. 2001; Schmidt and Zotz 2001).

F. excelsa and *R. australasica* individuals showed no reduction in stomatal length as they become semi-epiphytic adults (Fig. 4.5). This is consistent with the work of Holbrook and Putz (1996a) who found no clear patterns of the effect of epiphytic growth habits on stomatal dimension in eight primary hemi-epiphytes. Epiphytic individuals of three *Ficus* species had shorter and narrower stomata than tree individuals, but this was not the case in the other five species studied, including *Ficus* and *Clusia*.

In *F. excelsa* and *R. australasica*, the ontogenetic shift from terrestrial juvenile to semi-epiphytic adult was also associated with a higher stomatal density (Fig. 4.2), and similar or thicker leaves and tissue layers, excluding the lower epidermis of *R. australasica* (Figs. 4.6 and 4.7). The opposite pattern of stomatal density and leaf anatomy occurs in primary hemi-epiphytes and casual epiphytes. Holbrook and Putz (1996a) found that the epiphytic juvenile stage of primary hemi-epiphytic *Ficus* had fewer stomata than their terrestrial adult tree counterparts. Plants of the epiphytic

stage of *Ficus* also had thinner leaves and tissue layers than did the terrestrial tree phase. Research on one species of casual epiphyte, i.e. species in which some individuals of a population are true epiphytes while others are terrestrial plants, showed a similar pattern to the primary hemi-epiphytic *Ficus*. Epiphytic *Anthurium bredemeyeri* (Araceae), in a tropical cloud forest of La Carbonera, Venezuela, had a significantly lower stomatal density than the terrestrial congener (Rada and Jaimez 1992).

Stomatal characteristics of *F. excelsa* and *R. australasica* indicate that the semi-epiphytic adult plants are well-adapted to a drier canopy environment. The semi-epiphytic adult plants had more stomata per unit area than terrestrial juvenile congeners, which may provide the larger plants with a greater control over CO_2 uptake and water loss through stomata. Stomatal control by reducing the stomatal length is one significant strategy in coping with water stress (Aasama *et al*. 2001), which was also not found in the semi-epiphytic adult phase of the two study species.

Epiphytes in the forest canopy have to adapt to the canopy environment. As limited water availability is the major characteristic of the canopy environment (Benzing 1990), canopy-dwelling plants must have several mechanisms to cope with drought, to acquire essential ions (Benzing 1990) and to have a high level of water use efficiency (Lüttge 1997). However, the results of the study on leaf anatomy of *F. excelsa* and *R. australasica* suggests that secondary hemi-epiphytes may not face as high a level of water stress as do true epiphytes or the epiphytic stage of primary hemi-epiphytes.

The specific leaf area (SLA) of *F. excelsa* and *R. australasica* was greater in juvenile plants than intermediate and adult congeners (Fig. 4.11). SLA is commonly found to have a significant negative correlation with leaf thickness (Vile *et al*. 2005). Leaves with high SLA (i.e. low leaf mass area (LMA)), are usually thinner and have low tissue density and low leaf construction cost (Osunkoya *et al*. 2010). In contrast, thicker leaves (i.e. lower SLA/higher LMA), may correlate to longevity of leaf life span (Chabot and Hicks 1982; Mediavilla *et al*. 2001; Putz *et al*. 1995), high construction cost (Westoby *et al*. 2002) and greater drought tolerance (Mediavilla *et al*. 2001; Salleo and Lo Gullo 1990). The fact that juvenile plants had higher SLA and thinner leaves, reflecting low investment in leaf structure, may indicate that leaves of juvenile individuals of secondary hemi-epiphytes are short-lived leaves (Chabot & Hicks 1982), compared with leaves of individuals of later stages of development. Juvenile plants also have to cope with low levels of light. As juvenile individuals of *F. excelsa* and *R. australasica* had higher SLA, they may also have low assimilation rates. Mediavilla *et al*. (2001) found that nitrogen per unit leaf area was positively correlated with LMA. Therefore, in plants with low LMA (high SLA), assimilation could be reduced by a lower allocation of nitrogen per unit area to the photosynthetic system than in plants with low SLA.

When secondary hemi-epiphytic vines reach the canopy by climbing a host and losing the lower part of their main stem, their connection to the ground is maintained by the development of aerial roots (Chapter 2 and 3). This gives secondary hemi-epiphytes two benefits: more access to light and a continued supply of water and nutrients from the soil. These connections act as a buffer in dealing with water

scarcity, which is the condition faced by true epiphytes (Williams-Linera and Lawton 1995) and the epiphytic phase of primary hemi-epiphytes (Holbrook and Putz 1996a).

The characteristics of different developmental stages of hemi-epiphytes may be determined by the different environments in which they live. Holbrook & Putz (1996c) found that well-watered epiphytic individuals of primary hemi-epiphytes of *Ficus* still had lower stomatal conductance than tree individuals. This suggests that the lower stomatal density of epiphytic stage individuals, compared to terrestrial congeners, may limit the maximal rate of stomatal conductance in *Ficus* (Holbrook & Putz 1996b), and is probably a result of long adaptation to the epiphytic living environment. By contrast, terrestrial juvenile plants of *F. excelsa* and *R. australasica* had lower stomatal densities than semi-epiphytic adult congeners. How these structural characteristics are expressed in terms of physiological function is still poorly understood. Therefore, more detailed physiological studies on secondary hemi-epiphytic vines are needed.

4.5. CONCLUSION

The ability of secondary hemi-epiphytic vines to vary stomatal characteristics, such as density and dimension, and leaf and tissue thicknesses in relation to the shift in their life phases may contribute to the physiological plasticity of these species. The semi-epiphytic adult plants had more stomata per unit area than terrestrial juvenile congeners, which may allow them to have better control in absorbing CO_2 and regulating transpirational loss. Larger stomata in adult individuals also do not reflect conservative use of water. These leaf anatomy attributes of the semi-epiphytic adult stage plants suggest that they are well-adapted to the canopy environment.

They may not face the high level of water stress found in a dry canopy environment, as they have no physical restriction in water transport during their developmental phases (Chapter 3). However, since the structural characteristics affect physiological function, more detailed physiological studies, including gas exchange and leaf water potential, are required. As they further develop aerial roots, the utilization of resources, such as water, during ontogenetic phases of these species needs to be investigated. The capacity of these plants to exploit resources will influence physiological performance, which consequently dictates the survival of the species in their environment.

CHAPTER 5

SEASONAL VARIATION IN LEAF WATER POTENTIAL AND GAS-EXCHANGE OF DIFFERENT DEVELOPMENTAL PHASES OF SECONDARY HEMI-EPIPHYTIC VINES

5.1 INTRODUCTION

As vines maintain leaves from the forest floor to the upper canopy, they may encounter more heterogeneous light environments compared with trees (Holbrook and Putz 1996b). In response, vines frequently exhibit plasticity of leaf characteristics, including changes in size and shape, which can reduce the physiological constraints of having large photosynthetic surface areas. Secondary hemi-epiphytic vines might not only experience heterogeneous light environments, but they also may need to overcome consequences of changes in the soil-plant connections that accompany the transformation from reliance on 'normal' roots to aerial roots.

In Chapter 3, it was established that semi-epiphytic climbing adults of *F. excelsa* and *R. australasica* had fewer physical limitations in stem water transport than terrestrial juveniles. The fact that the stem base may become a hydraulic constriction may also be overcome by the establishment aerial roots in adult individuals. As hydraulic properties dictate the capacity of the plant to transport water from the roots to the leaves; consequently, leaf water potential, gas-exchange and stomatal conductance can be different between plants of differing growth stages.

The leaf anatomy study (Chapter 4) showed that adult individuals of *F. excelsa* and *R. australasica* had relatively larger stomata and more stomata per unit area than conspecific juveniles. Differences in these structural characteristics may result in varied ability of the plants to absorb CO_2 from the atmosphere and to regulate water loss (Hetherington and Woodward 2003). Therefore, more detailed studies of physiological responses, including gas-exchange and leaf water potential, were undertaken.

The physiological responses of secondary hemi-epiphytes are not only expected to change due to changes in plant-soil connections, but also due to different environmental conditions across seasons. Patterns of water relations and gas-exchange might vary seasonally. Plants of a specific growth stage may be more susceptible to drought than those of other stages. How secondary hemi-epiphytes of different developmental stages respond to seasonal changes is poorly understood.

As most ecological studies on vines have investigated woody vines (lianas), research on the eco-physiology of herbaceous secondary hemi-epiphytes will also contribute to the deeper understanding of the ecophysiology of vines in general. Woody vines, for example, are among the most deep-rooted species in tropical forests (Holbrook and Putz 1996b), which equips them with the ability to absorb more water from the soil (Jackson *et al*. 1995), and perform well physiologically. However, lianas might not rely solely on deep soil water; Andrade *et al*. (2005) found that at the beginning of the dry season, lianas in seasonally dry tropical forests of Panama take up shallow sources of soil water. On the other hand, secondary hemi-epiphytes establish aerial

root connections to the ground in their later developmental phases. How these aerial roots contribute to the utilization of different soil water resources may also influence physiological performance of secondary hemi-epiphytes during ontogeny.

Seasonal and diurnal patterns of water relations and gas exchange were studied in two species of secondary hemi-epiphytic vines in north Queensland forests. This study aimed to characterize physiology and water relations during the life cycle of secondary hemi-epiphytic vines by comparing plants of different stages in different seasonal conditions. Questions to be answered include: (a) is there any physiological difference between the juvenile, intermediate and adult individuals? (b) Are physiological attributes influenced by seasonality? (c) How do physiological attributes correlate with each other and correlate to hydraulic architecture?

5.2 MATERIALS AND METHODS

5.2.1 Plant materials and study sites

Freycinetia excelsa was studied at Paluma Range National Park and *Rhaphidophora australasica* was studied in the Henrietta Creek Section of Wooroonooran National Park. Descriptions of the study species and growth habits are contained in Section 2.1, and descriptions of the study sites are in Section 2.2.

Four representative plants of each growth habit of each species were sampled. All plants were sampled from the sub-canopy environment (Figs. 5.1 and 5.2). Field studies were conducted during the dry season (which usually extends from May to November) and the wet season (usually December to April). Measurements for the dry season were undertaken in September and October 2009, and measurements for the wet season were conducted in April 2010.

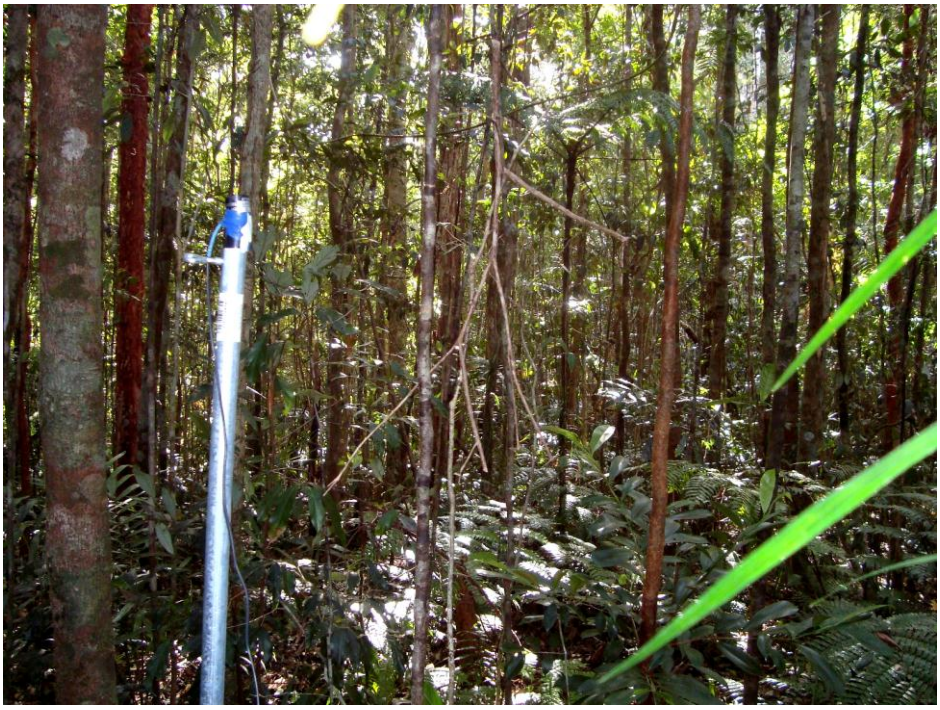


Fig. 5.1 The sub canopy environment of the study site in the Mt. Spec Section, Paluma Range National Park, where *F. excelsa* individuals were studied.

Fig. 5.2 The sub canopy environment of the study site in the Henrietta Creek Section, Wooroonooran National Park, where *R. australasica* individuals were studied.

5.2.2 Environmental variables

5.2.2.1 Rainfall data

Monthly rainfall from April 2009 to April 2010 was obtained from the Rainfall Bulletin published by the Bureau of Meteorology (BOM 2011) for two rainfall stations: Paluma Ivy Cottage Station for Paluma Range NP and Crawford Lookout Station for Wooroonooran NP. These stations were within 4 km of the study sites.

5.2.2.2 Soil moisture

Soil moisture was measured every 15 minutes for both study sites over several 24-hr periods of the field experiments for both the dry and wet seasons. The daily course of soil moisture was monitored at two different soil depths: 0-30 cm and 30-60 cm. Soil moisture probes were inserted in the soil and the data were recorded using MEA's Bug System (MEA, Australia).

5.2.2.3 Photosynthetically active radiation

The daily course of photosynthetically active radiation (PAR) of the sub-canopy environment was monitored at the study sites using a LI-190 quantum sensor attached to a LI-COR 6400 (LI-COR Inc., Nebraska) set up about 2 m above the ground.

5.2.3 Leaf water potential

Twelve fully mature leaves of four individuals of each growth stage were tagged for measuring water potential. Leaf water potential (ψ_L) was measured using a Scholander-type pressure chamber (PMS Instrument Co., Oregon). Patterns of water potential were monitored pre-dawn, mid-day and late-afternoon.

Leaves were harvested from the plant and were directly placed in the pressure chamber. The balance pressure required to force sap to the cut surface of the xylem was recorded as water potential.

5.2.4 Gas-exchange

Four individuals of each study species of each growth habit were selected to provide samples for gas-exchange measurements. Three fully expanded mature leaves were tagged for each individual. Measurements were carried out on sunny days at six different times (7.00, 9.00, 11.00, 13.00, 15.00 and 17.00 h) to document diurnal patterns of gas exchange.

Assimilation rate (A) (μmol CO_2 m^{-2} s^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1}) were measured using an infra red gas analysis (IRGA) system (LI-COR 6400, LI-COR Inc., Nebraska). Air flow was maintained at 300 μmol s^{-1}. This constant air flow rate was chosen for two reasons: to ensure adequate mixing of the gas in the chamber and to increase convective coupling between the chamber and the leaf (Field *et al*. 1989). CO_2 concentration

was maintained at 400 μmol m^{-1} s^{-1}. All measurements were made under ambient temperature.

Leaves of *F. excelsa* were smaller than the LI-COR sample chamber; therefore, the measurements for this species were adjusted to actual observed leaf area. The software program LI6400 Simulator version 5.3 was used for this adjustment.

As the plants were growing under the canopy, the light intensity received was not the same for each leaf studied. Based on observed photosynthetically active radiation (PAR) under the canopy at the study sites (Figs. 5.4 and 5.5), experimental light intensity was standardized for each time of observation, as is detailed in Table 5.1.

Table 5.1 Light intensity (PAR) applied to the leaf samples at each time of observation.

Time (hours)	**Light intensity (μmol $m^{-2}s^{-1}$)**
07.00	10
09.00	20
11.00	50
13.00	250
15.00	100
17.00	10

5.2.5 Statistical analysis

Estimated daily total carbon gain was calculated as the integrated area under the curve of diurnal assimilation measurements at the leaf level and used to provide mean values for each growth habit of each species. Differences between variables of the differing growth stages in differing seasons were calculated by employing linear models (ANOVA and *t*-test) using the SPSS statistical package. Linear regression was applied to characterize relationships between variables.

5.3 RESULTS

5.3.1 Seasonal variation in rainfall

Monthly rainfall varied between the dry and wet seasons (Fig. 5.3). There had been at least 4 months of very low rainfall (<20 mm) before the dry season field measurements in September/October 2009 were conducted. On the other hand, there was a high rainfall each month during the wet season before the measurements were made in April 2010. The greatest rainfall occurred in January, where the monthly rainfall was 835 mm and 590 mm at Paluma Range NP and Wooroonooran NP respectively.

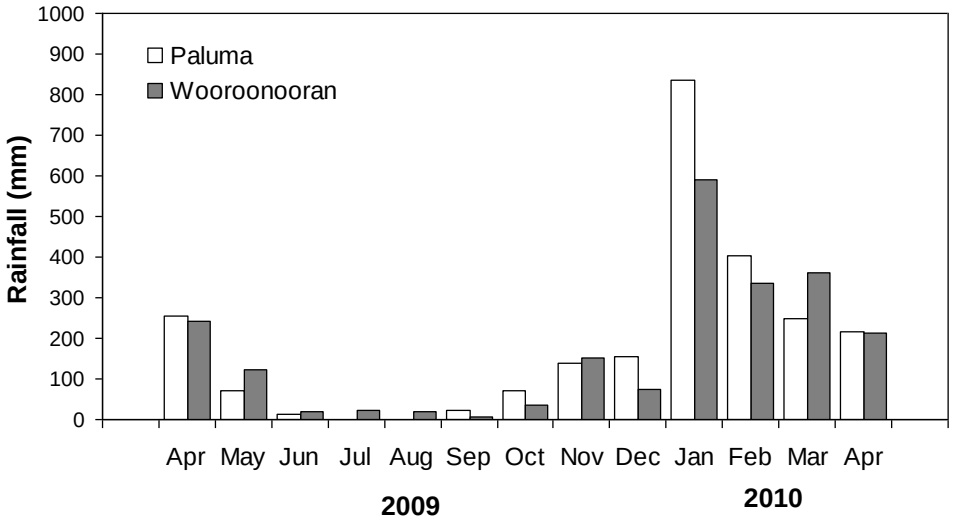


Fig. 5.3 Rainfall (mm) during the dry and wet seasons 2009-2010 at Mt. Spec Section, Paluma Range National Park and Henrietta Creek Section of Wooroonooran National Park.

5.3.2 Diurnal patterns of light intensity and seasonal variation of air and leaf temperature

Daily courses of photosynthetically active radiation (PAR) of the sub-canopy environment for both study sites are shown in Figs. 5.4 and 5.5. The study site at Mt. Spec was relatively more closed than at Henrietta Creek. The highest PAR recorded at Mt. Spec was about 250 µmol photons m^{-2} s^{-1}, compared with Henrietta Creek where it was about 600 µmol photons m^{-2} s^{-1}. However, in general daily PAR received by the sub canopy-environments of both sites was relatively low, i.e. between 10-20 µmol photons m^{-2} s^{-1}.

Although air temperature at both study sites was higher during the early morning in the wet season than in the dry season, there was a relatively constant air temperature during the day in the wet season (Figs. 5.4 and 5.5). There were variations in the daily air temperature for both sites during the dry season. Maximum air temperature at Mt. Spec did not differ greatly between the two seasons, about 25^0C. In contrast, maximum air temperature in the dry season at Henrietta Creek reached 35^0C, compared with 28^0C in the wet season. This reflects the more open canopy at the Henrietta Creek site.

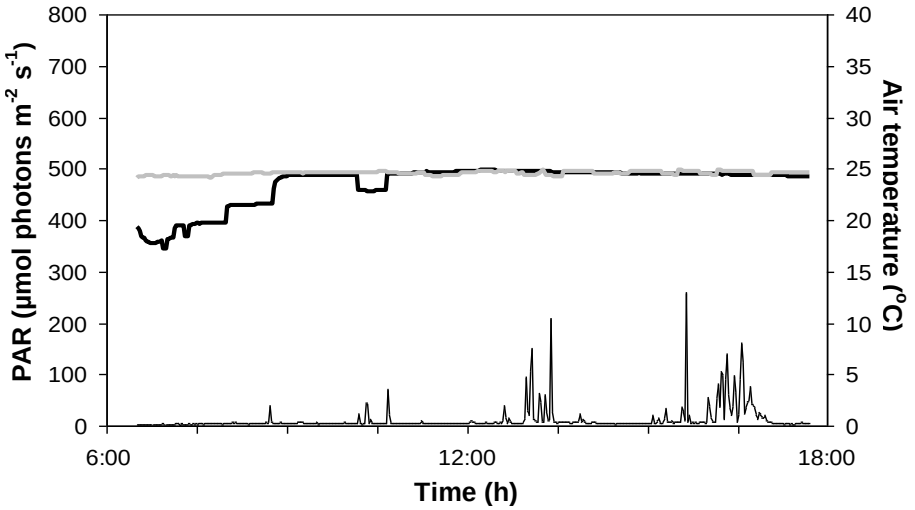


Fig. 5.4 Daily courses of photosynthetically active radiation (PAR) (µmol photons m^{-2} s^{-1}) (—) in the dry season and air temperature in the dry season (▬) (late September 2009) and wet season (▬) (early April 2010) under the canopy in the Mt. Spec Section, Paluma Range National Park, where samples of *F. excelsa* were studied.

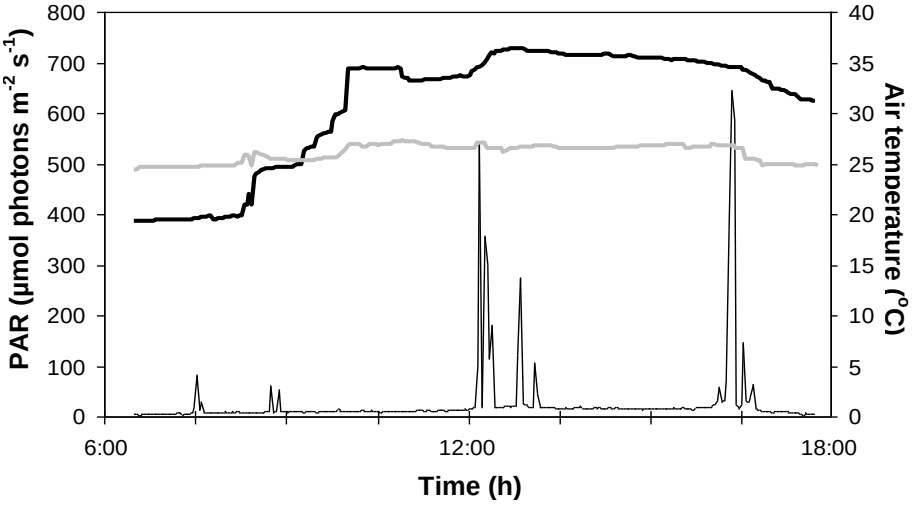


Fig. 5.5 Daily courses of photosynthetically active radiation (PAR) (µmol photons m^{-2} s^{-1}) (—) in the dry season and air temperature in the dry season (▬) (early October 2009) and wet season (▬) (mid April 2010) under the canopy in the Henrietta Creek Section of Wooroonooran National Park, where samples of *R. australasica* were studied.

Leaf temperatures were correlated with air temperatures (Figs. 5.6 and 5.7). This is indicated by high R^2 values for linear relationships between leaf and air temperatures for both species across seasonal periods (R^2 values for *F. excelsa* were 0.96 (dry season) and 0.94 (wet season), and R^2 values for *R. australasica* were 0.99 (dry season) and 0.93 (wet season)). The variation in leaf temperatures was greater in the dry season than in the wet season. Leaf minimum temperature for both species was lower in the dry season than in the wet season. Maximum leaf temperatures of *F. excelsa* were similar for both dry and wet seasons. On the other hand, the maximum leaf temperature of *R. australasica* was higher in the dry season than in the wet season.

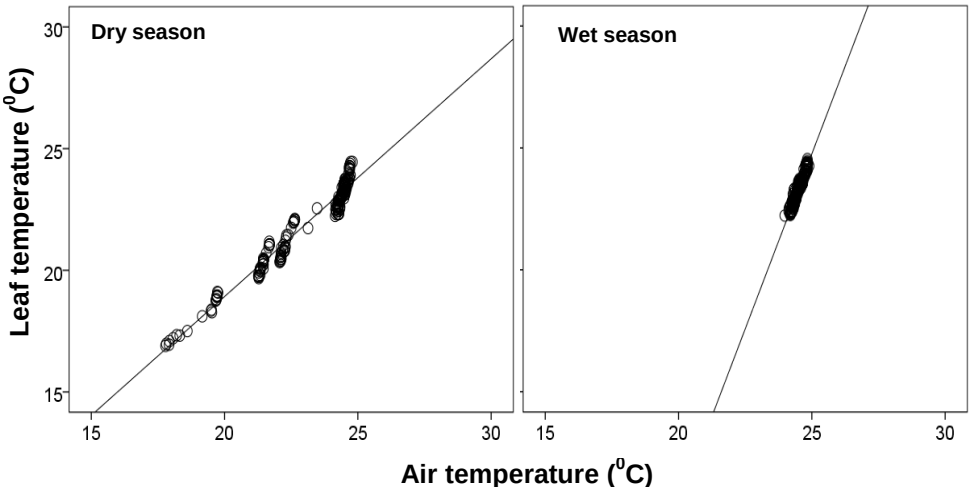


Fig. 5.6 Relationships between leaf temprature of *F. excelsa* and air temperatures (o) (^{0}C) in the dry and wet seasons. Data were pooled from the daily profiles of 12 leaf samples of all growth stages starting at 07:00 h and finishing at 17:00 h.

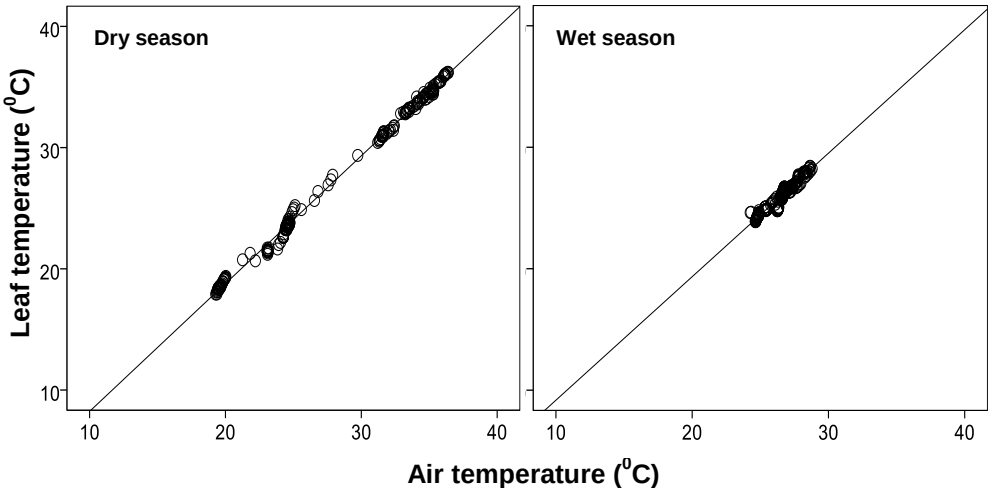


Fig. 5.7 Relationships between leaf temperature of *R. australasica* and air temperatures (o) (^{0}C) in the dry and wet seasons. Data were pooled from the daily profiles of 12 leaf samples of all growth stages starting at 07:00 h and finishing at 17:00 h.

5.3.3 Seasonal variation in soil moisture

Both study sites experienced low soil moisture (less than 20%) during the dry season (Figs. 5.8 and 5.9). This was a result of a long period of low rainfall (Fig 5.3). During the wet season, soil moisture increased considerably, reaching about 55% at Henrietta Creek (Fig. 5.9) and 35% at Mt. Spec (Fig. 5.8). Soil moisture in the top soil in the wet season was higher than at 30-60 cm depth, reflecting rainfall replenishment of water at the soil surface, in contrast to the dry season.

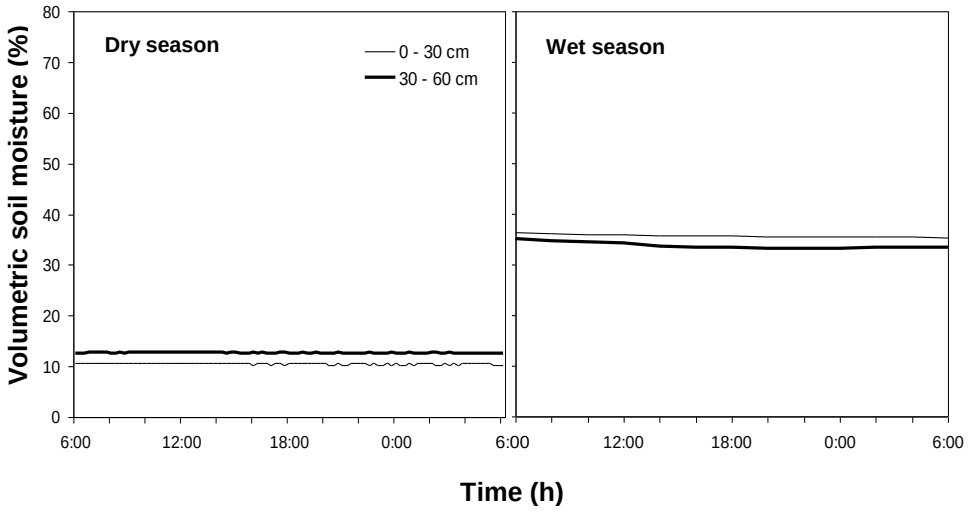


Fig. 5.8 Diurnal patterns of soil moisture at two different depths, i.e. 0-30 cm and 30-60 cm in the dry (late September 2009) and wet (early April 2010) seasons at Mt. Spec Section, Paluma Range National Park, where individuals of *F. excelsa* were studied.

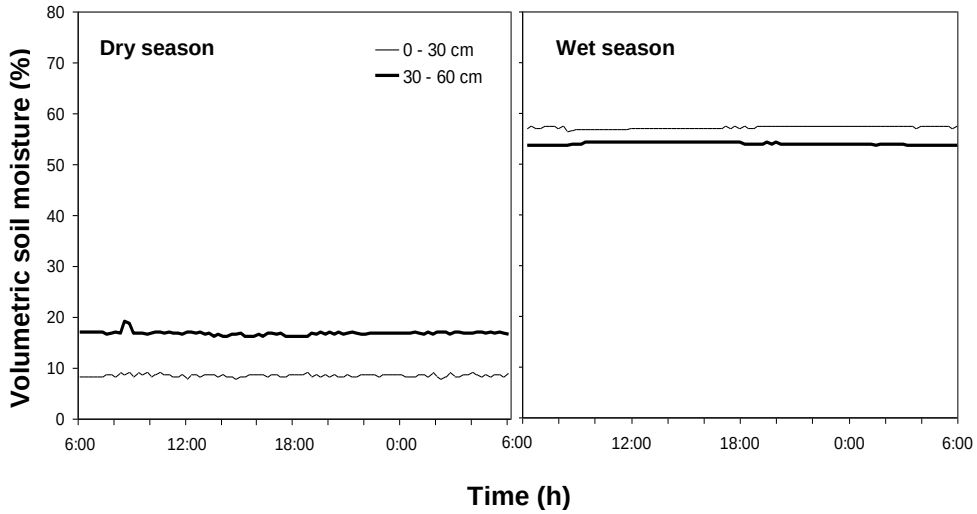


Fig. 5.9 Diurnal patterns of soil moisture at different depths, i.e. 0-30 cm and 30-60 cm in the dry (early October 2009) and wet (mid April 2010) seasons at Henrietta Creek Section, Wooroonooran National Park, where individuals of *R. australasica* were studied.

5.3.4 Seasonal and diurnal variation in leaf water potential

During the dry season, regardless of ontogenetic stage, leaf water potentials (ψ_L) of *F. excelsa* were less negative than -2.0 MPa (Fig. 5.10), with maximum values occurring pre-dawn (ψ_{pd}). Minimum values occurred at mid-day (ψ_{md}). Values of ψ_{pd} of individuals of the three habits were not significantly different (F= 2.303; P>0.05). Although juvenile individuals had lower values for mid-day (ψ_{md}) and late-afternoon (ψ_{la}) leaf water potentials, the differences were not significant (F= 2.923 for ψ_{md} and F= 3.131 for ψ_{la}; P>0.05).

Regardless of ontogenetic phase, ψ_L values of *R. australasica* in the dry season were less negative than -1.0 MPa (Fig 5.11). Maximum values of ψ_L occurred pre-dawn; however, unlike *F. excelsa*, minimum values of ψ_L of *R. australasica* occurred in the late afternoon (ψ_{la}). Adult individuals had significantly less negative values of ψ_L at all times compared with juvenile and climbing congeners (F= 9.434 for ψ_{pd}, F= 19.086 for ψ_{md} and F= 10.082 for ψ_{la}; P< 0.01).

In the wet season, ψ_L increased significantly for all habits of both species (Figs 5.10 and 5.11). Maximum values of ψ_{pd} of both species were above -0.5 MPa. Daytime values of ψ_L of *R. australasica* remained above -0.5 MPa for all juvenile, intermediate and adult individuals, whereas the minimum values of ψ_L of *F. excelsa* were less negative than -1 MPa. There were no significant differences between values of ψ_{pd} (F= 1.958; P>0.05) and ψ_{md} (F= 0.179; P>0.05) of *F. excelsa* individuals of all growth stages; however, values of ψ_{la} of the adult *F. excelsa* were more negative than for other growth habits (F= 11.097; P<0.01). There were no

significant differences between values of ψ_L of all habits of *R. australasica* at all times. Juveniles of *R. australasica* had more negative values of ψ_{la} (F= 19.505; P<0.05).

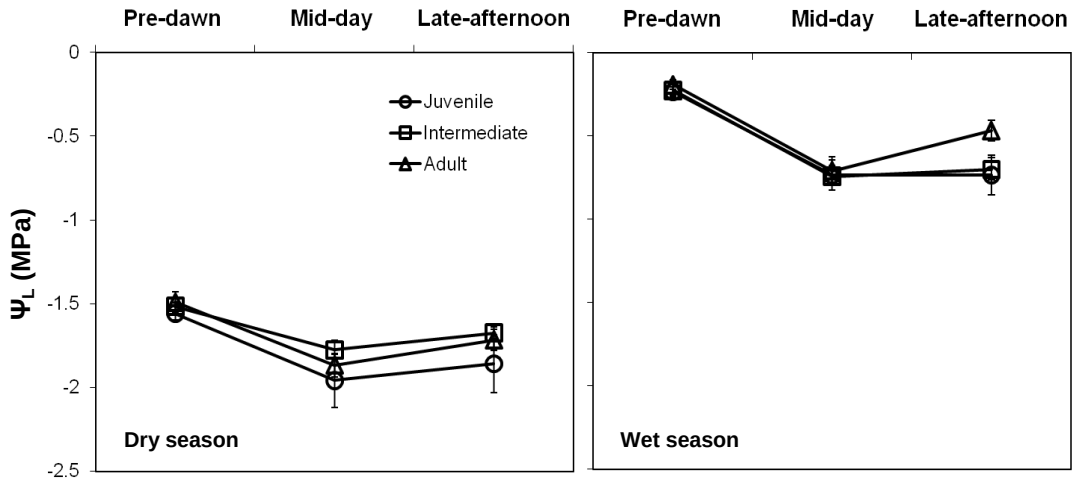


Fig. 5.10 Diurnal and seasonal variation in leaf water potential (Ψ_L) (MPa) of juvenile, intermediate and adult individuals of *F. excelsa* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

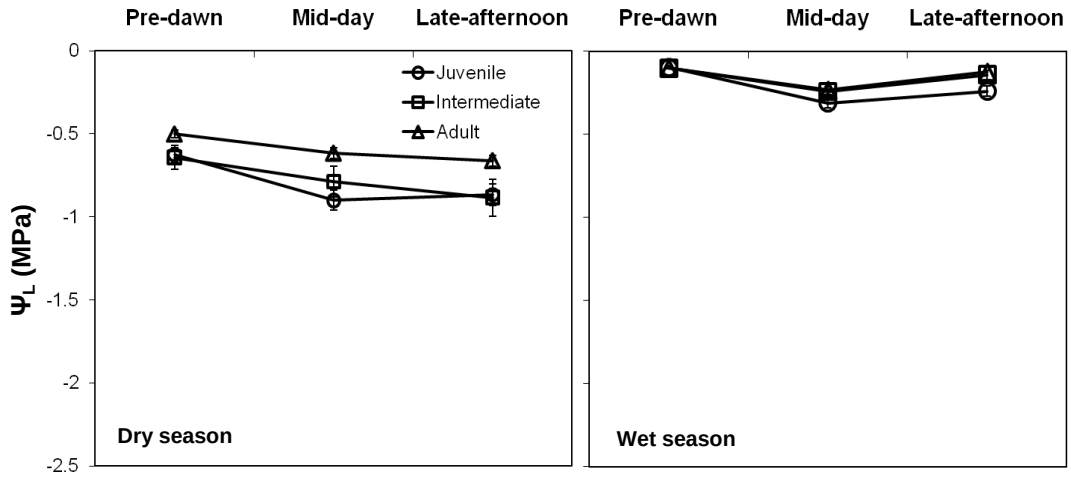


Fig. 5.11 Diurnal and seasonal variation in leaf water potential (Ψ_L) (MPa) of juvenile, intermediate and adult individuals of *R. australasica* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

5.3.5 Leaf gas exchange

5.3.5.1 Seasonal and diurnal patterns of assimilation

Seasonal and diurnal profiles of all habits of both study species show that, in general, assimilation rates were lower in the dry season than in the wet season (Figs. 5.12 and 5.13). The assimilation rates of juvenile individuals of both *F. excelsa* and *R. australasica* were most negatively affected by the environmental conditions during the dry season.

Total carbon gain shows more detailed differences in daily rates of assimilation (Figs. 5.14 and 5.15). The total carbon gains of *F. excelsa* were significantly higher in the wet season than in the dry season (Juvenile: t= -15.781, Intermediate: t=-8.268, Adult: t= -8.102; all Ps<0.01). Even though assimilation values of juvenile individuals of *F. excelsa* in the dry season were significantly lower than intermediate and adult congeners (F= 128.578; P<0.01), the values for the juvenile habit in the wet season were higher than assimilation rates of adult individuals (F=22.826; P<0.01).

A similar pattern occured for *R. australasica* in which total carbon gain was significantly higher in the wet season than in the dry season (Juvenile: t= -19.411, Intermediate: t= -7.904, Adult: t= -3.400; all Ps<0.01). Juvenile individuals of *R. australasica* also experienced the lowest carbon gain during the dry season (F= 74.922; P<0.01); however, there were no significant differences in carbon gain between plants of different growth stages during the wet season (F= 2.187; P>0.01).

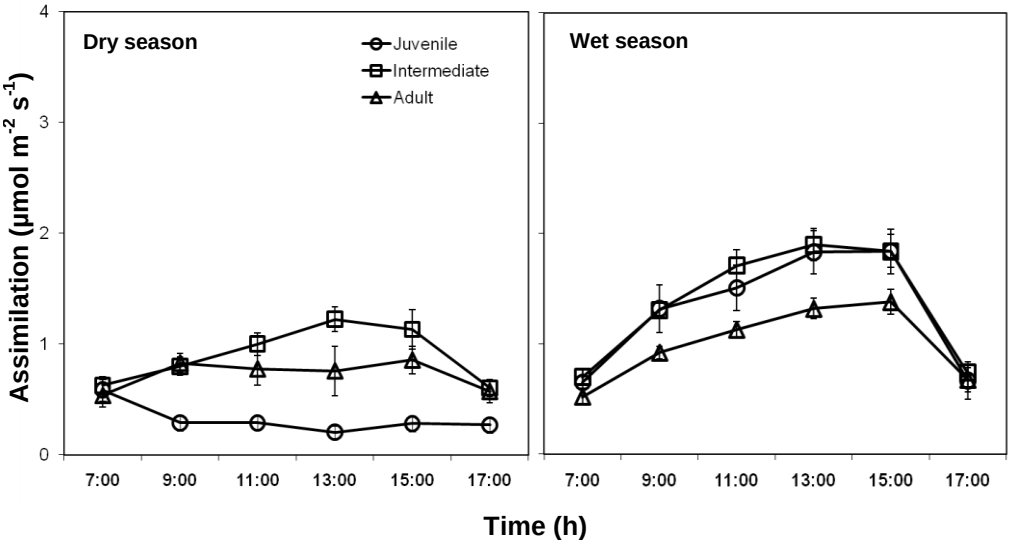


Fig. 5.12 Diurnal and seasonal variation in leaf assimilation (µmol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *F. excelsa* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

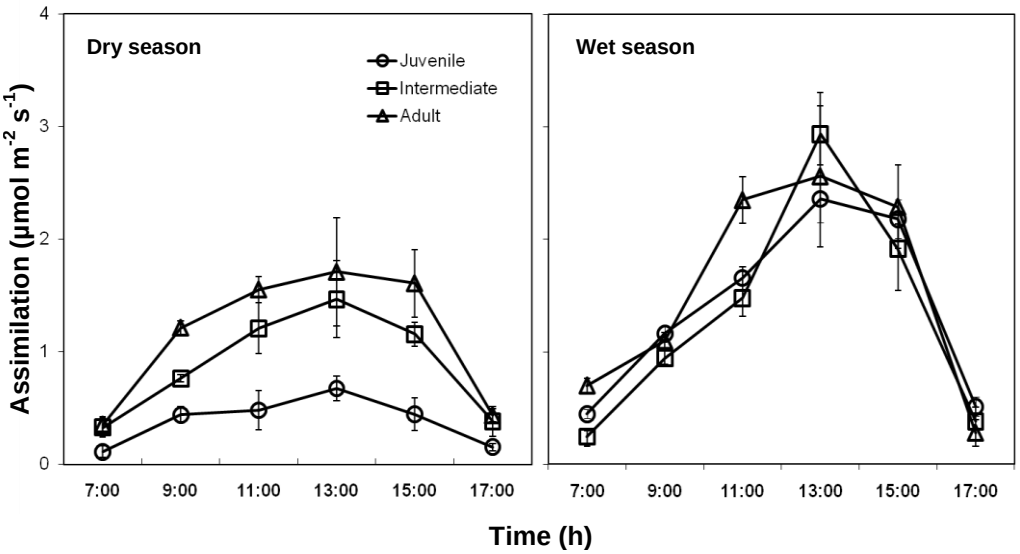


Fig. 5.13 Diurnal and seasonal variation in leaf assimilation (µmol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *R. australasica* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

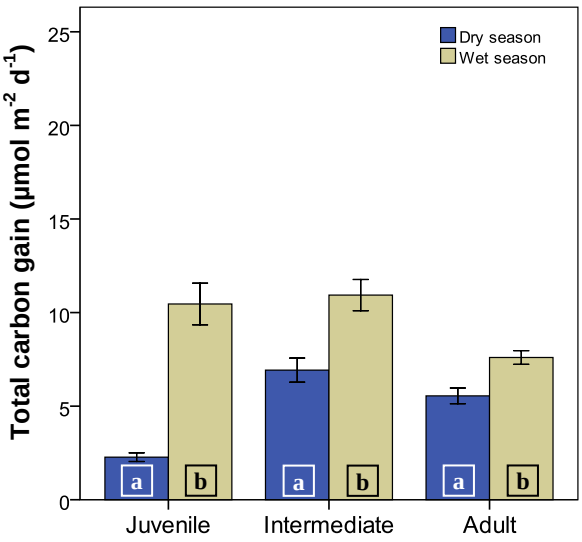


Fig. 5.14 Total carbon gain (µmol m^{-2} d^{-1}) of juvenile, intermediate and adult individuals of *F. excelsa* in the dry and wet seasons. Total carbon gain was calculated from the integrated areas of daily assimilation of each leaf sample of each growth stage (12 leaves for each growth stage). Error bars show 95% confidence intervals. Different letters indicate that values are significantly different between seasons of each habit at α = 1 %.

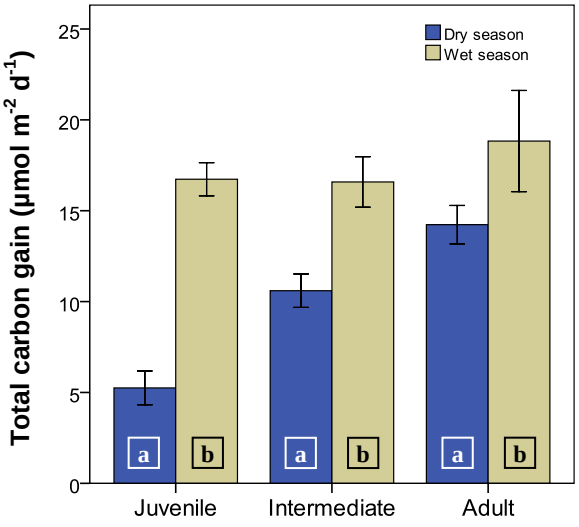


Fig. 5.15 Total carbon gain (µmol m^{-2} d^{-1}) of juvenile, intermediate and adult individuals of *R. australasica* in the dry and wet seasons. Total carbon gain was calculated from the integrated areas of daily assimilation of each leaf sample of each growth stage (12 leaves for each growth stage). Error bars show 95% confidence intervals. Different letters indicate that values are significantly different between seasons of each habit at α = 1 %.

5.3.5.2 Seasonal and diurnal patterns of stomatal conductance

Stomatal conductance (g_s) of both species was higher in the wet season than the dry season (Figs. 5.16 and 5.17). Patterns of maximum stomatal conductance (g_{smax}) varied between habits, species and seasons. In the dry season, g_{smax} of juvenile *F. excelsa* occurred in the morning, while individuals of intermediate and adult stages experienced g_{smax} in the afternoon (Fig. 5.16). g_{smax} of juvenile plants was significantly lower than intermediate and adult plants (F= 6.313; P<0.01). In the wet season, regardless of their ontogenetic stages, all plants experienced g_{smax} in the early afternoon, and there was no significant differences in the g_{smax} between individuals of differing developmental stages (F= 0.728; P>0.05).

On the other hand, g_{smax} of intermediate and adult individuals of *R. australasica* occurred in the late morning in the dry season, while juvenile congeners experienced g_{smax} in the early afternoon (Fig. 5.17). The value of g_{smax} of adult individuals was significantly higher than for juvenile congeners (F= 5.775; P<0.01). Similar to the pattern of stomatal conductance of *F. excelsa*, in the wet season *R. australasica* experienced maximum stomatal conductance in the early afternoon. During this season, there was no significant difference of g_{smax} values between individuals of juvenile, intermediate and adult phases (F= 0.571; P>0.05).

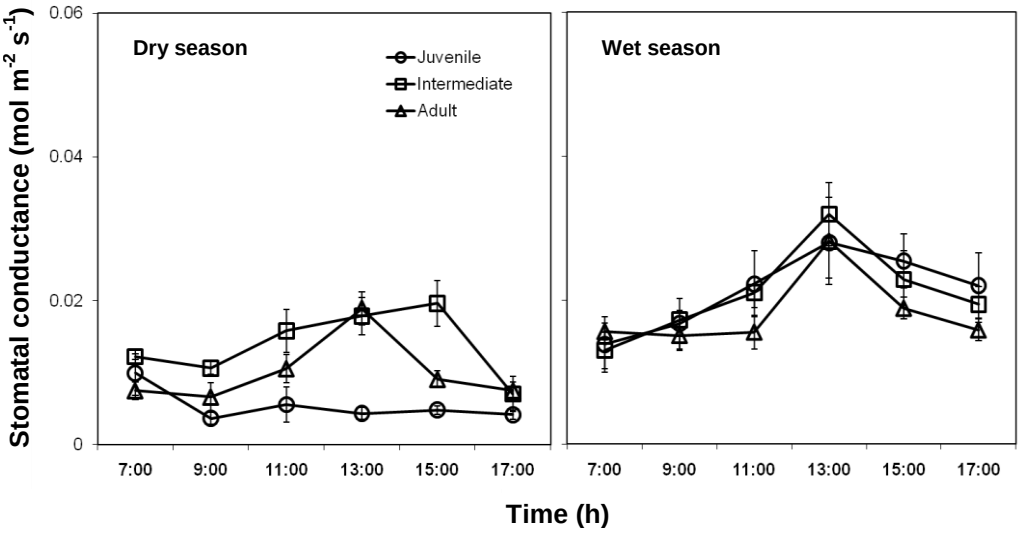


Fig. 5.16 Stomatal conductance (g_s) (mol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *F. excelsa* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

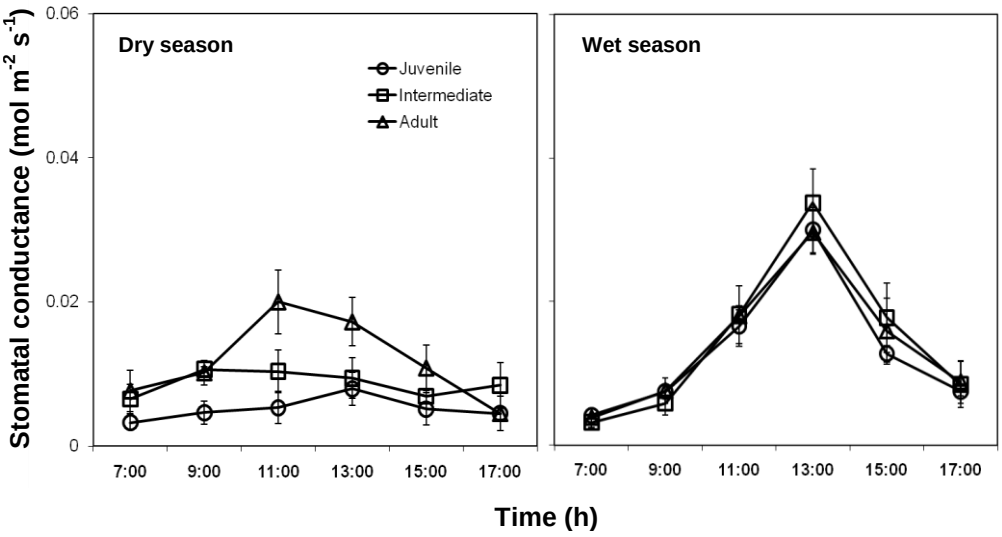


Fig. 5.17 Stomatal conductance (g_s) (mol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *R. australasica* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

5.3.5.3 Seasonal and diurnal patterns of transpiration

Consistent with the patterns of stomatal conductance, transpiration rate (E) of both species was higher in the wet season than the dry season for all growth stages (Figs. 5.18 and 5.19). In the dry season, the maximum transpiration rate (E_{max}) of intermediate stage individuals of *F. excelsa*, which occurred in the afternoon, was higher than for the adult and juvenile congeners (F= 5.314; P<0.05) (Fig. 5.18). Similar to the pattern of stomatal conductance, juvenile individuals of *F. excelsa* also experienced early morning E_{max}. In the wet season, E_{max} of plants of all habits occurred in the afternoon, with no significant differences in E_{max} between juvenile, intermediate and adult individuals (F= 0.418; P>0.05).

For *R. australasica*, during the dry season intermediate phase individuals experienced E_{max} in the morning and, on the other hand, E_{max} of juvenile and adult individuals occurred in the early afternoon (Fig. 5.19). There were significant differences between values of E_{max} for adult plants compared with intermediate and juvenile individuals (F= 27.129; P<0.01). Similarly to *F. excelsa*, E_{max} of *R. australasica* in the wet season occurred in the early afternoon and there were no significant differences between the values of E_{max} for all habits (F= 0.876; P>0.05).

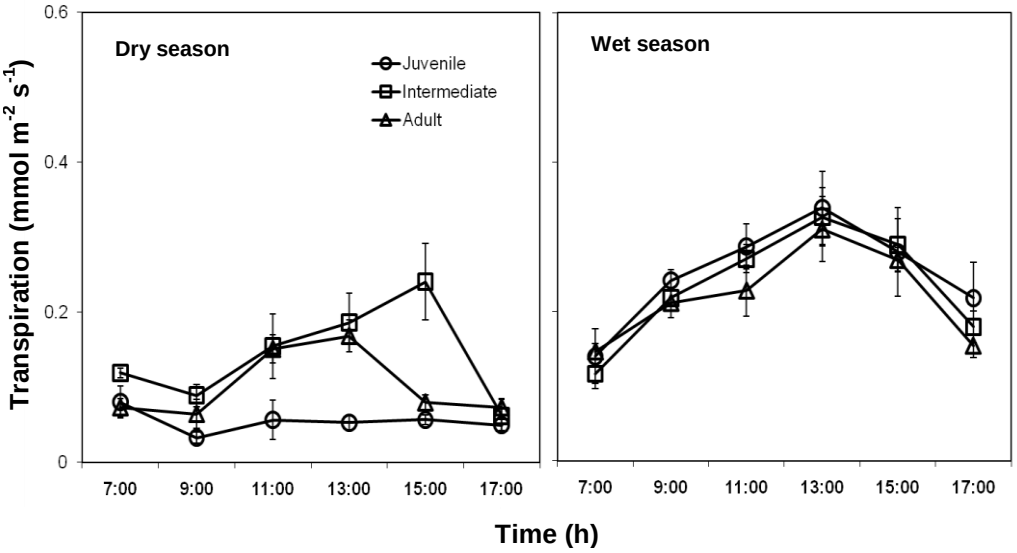


Fig. 5.18 Transpiration rates (E) (mmol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *F. excelsa* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

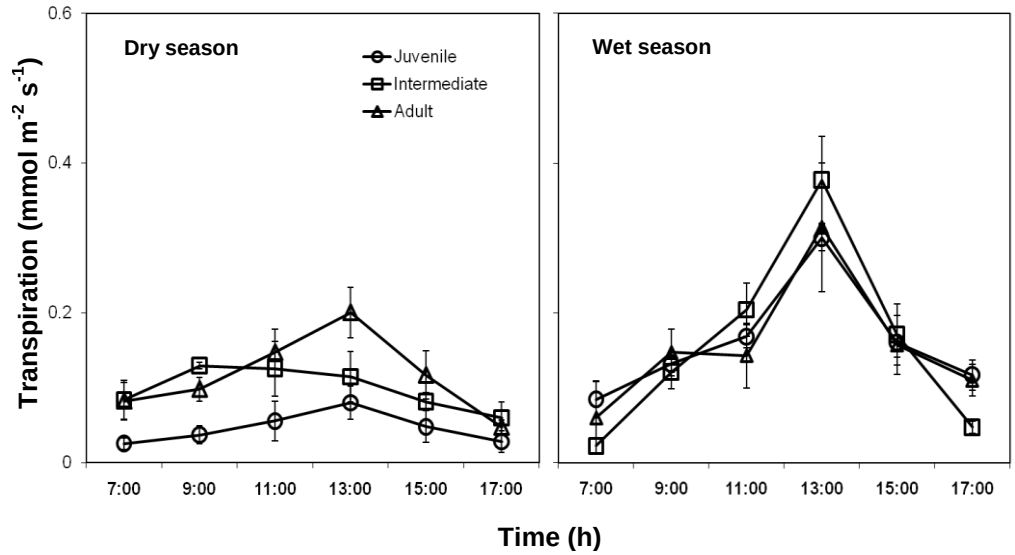


Fig. 5.19 Transpiration rates (E) (mmol m^{-2} s^{-1}) of juvenile, intermediate and adult individuals of *R. australasica* in the dry and wet seasons. Each point represents the mean of 12 measurements (three leaves on four plants). Error bars are 95% confidence intervals.

5.4 DISCUSSION

5.4.1 Water relations

The leaf water potentials of all habits of both species were much lower during the dry season than during the wet season. This low water potential correlated with lower soil moisture, when diurnal soil moisture was less than 20% (Figs. 5.8 and 5.9). Juvenile *F. excelsa* experienced slightly lower minimum water potential in the dry season, which was about -2.0 MPa, compared with the other two habits (Fig 5.10). Adult plants of *R. australasica* also had less negative values of leaf water potential than juvenile congeners (Fig. 5.11), which indicates better access to water for plants of this habit during the dry season (Choat *et al*. 2006). The water potential observations suggest that plants of different growth stages vary in their capacity to absorb water from the soil, including the possibility that plants utilize water from different parts of the soil profile. Juvenile individuals may only rely on surface water since their roots only occupy the shallow part of the soil. As the soil moisture of the top soil was lower than the sub soil in the dry season (Figs. 5.8 and 5.9), the shallow root systems of juvenile individuals may have restricted access to soil water pools. On the other hand, the aerial roots may provide deeper penetration into the ground for intermediate and adult stages of *F. excelsa*. Consequently, intermediate and adult plants are probably more able to utilize water from the sub-soil. This argument will be tested in Chapter 6.

In contrast, the greater water supply from the soil during the wet season lead to markedly increased leaf water potential of all habits of both species. All maximum

(pre-dawn) leaf water potentials were less negative than -0.5 MPa. There were no significant differences in leaf water potential between habits, except for the afternoon leaf water potential of *F. excelsa*.

The range of leaf water potential experienced by both species of secondary hemi-epiphytes was similar to that exhibited by two primary woody hemi-epiphytes, *Ficus trigonata* and *F. pertusa*, studied by Holbrook and Putz (1996c). Leaf water potentials of terrestrial-rooted and epiphytic plants of both *Ficus* species were also more negative during the dry season. Holbrook and Putz (1996c) also reported that terrestrial-rooted individuals of both *Ficus* species experienced lower leaf water potential than epiphytic plants during the dry season. Leaf water potential was similar for both habits in the wet season.

In a study of a casual epiphyte, i.e. a species in which some individuals of a population are true epiphytes while others are terrestrial plants, Rada and Jaimez (1992) showed that the leaf water potentials of terrestrial and epiphytic *Anthurium bredemeyeri* were more depressed during the dry season than the wet season. Even though, pre-dawn water potentials of both habits of *A. bredemeyeri* were less negative than -0.5 MPa for all seasons, leaf water potentials decreased in the dry season. However, unlike primary hemi-epiphytes (Holbrook and Putz 1996c) and both secondary hemi-epiphytes in this study, the epiphytic habit of *A. bredemeyeri* had more negative leaf water potentials than terrestrial congeners.

Unlike primary hemi-epiphytes, in which there are no connections to the ground during the epiphytic stage, semi-epiphytic adults of secondary hemi-epiphytes never lose connection to the soil. The soil connections in secondary hemi-epiphytic vines only change from normal roots to aerial roots. During this transition, plants of different growth stages exhibit differences in leaf water potential, especially during the dry season.

5.4.2 Gas-exchange

The first aim of the gas-exchange measurements of *F. excelsa* and *R. australasica* was to investigate the responses of the plants to conditions of the dry and wet seasons. The seasonal response of both study species is fairly typical, in which assimilation rates were higher during the wet season for all habits of *F. excelsa* and *R. australasica* (Figs. 5.12 and 5.13). High water availability on the one hand, and low evaporative demand on the other, provide excellent conditions for plants to absorb CO_2. This result agrees with other studies on the eco-physiology of the casual epiphytic *Anthurium bredemeyeri* (Rada Jaimez 1992), epiphytic *Anthurium scandens* (Lorenzo *et al*. 2010), several species of primary hemi-epiphytes, including *Ficus* (Ting *et al*. 1987; Holbrook and Putz 1996a; Holbrook and Putz 1996b) and *Clusia* (Ting *et al*. 1987), and several species of dry rain forest trees in north Queensland (Choat *et al*. 2006). In contrast, photosynthesis and stomatal conductance were reduced in the dry season which correlated with low water availability (Figs. 5.16 and 5.17) (Lüttge *et al*. 1986; Moricca and Ragazzi 2008).

The second aim was to compare physiological performances of *F. excelsa* and *R. australasica* of different ontogenetic stages. The behaviour of juvenile individuals of *F. excelsa* and *R. australasica* in this study is notable, as their assimilation rate increased significantly from the dry season to the wet season, compared with other habits (Figs. 5.14 and 5.15). This phenomenon may relate to the physical structure of the plants and different soil water availability during each season. As less water is available during the dry season than the wet season (Figs. 5.8 and 5.9), the lower hydraulic conductivity of the stems of juveniles (Chapter 3) may restrict water supply such that down-regulation of CO_2 uptake and stomatal opening are necessary to diminish water loss and maintain water potential. Water supplied to intermediate and adult plants by aerial roots variously inserted at a number of sites along a stem is evidently sufficient to sustain higher rates of CO_2 exchange and water loss.

Gas-exchange patterns demonstrate that, during the dry season, juveniles down-regulate more than intermediate and adult individuals, although they have "normal" roots and lower leaf area per xylem area (higher Huber value) (Chapter 3). This suggests that water limitation is greater for juveniles than for intermediate or adult plants. The relatively lower leaf water potentials of juvenile individuals in the dry season are consistent with this view. The logical explanation would be that the access of roots to soil water in juveniles is restricted by rooting depth. Hence, plants of this developmental phase might use water more conservatively than intermediate and adult individuals. This pattern contrasts with primary hemi-epiphytes, where epiphytic *Ficus* used water more conservatively than terrestrial congeners (Holbrook and Putz 1996b).

Intermediate and adult individuals of *F. excelsa* and *R. australasica* had higher carbon gain in the dry season compared with juveniles (Figs. 5.14 and 5.15). This fact emphasizes that more options to acquire soil water were available for intermediate and adult individuals. The connection to the soil through the development of aerial roots may allow these individuals to absorb water from all available sources down the soil profile (Chapter 6). The ability of adult plants to perform well physiologically across the season also supports the view that semi-epiphytic adult individuals are well-adapted to the canopy environment and they do not experience severe water stress.

The results from the hydraulic architecture measurements indicate that there was no physical limitation to water transport through the stem for the semi-epiphytic adult stage (Chapter 3). The vessel size and hydraulic architecture parameters along the plant stem path length also suggest that the basal part of the stem may be the site of hydraulic restrictions. This also explains the fact that terrestrial individuals of *F. excelsa* with original soil roots under-performed physiologically in the dry season and increased assimilation dramatically during the wet season. Hydraulic resistance in the basal part of the stem would reduce water transport, and thus assimilation rates. On the other hand, the establishment of aerial roots provide advantages for intermediate and adult individuals, such as creating a more effective way to take up water from deeper in the soil and also a mechanism to bypass the hydraulic bottle-neck in the basal part of the stem.

Although aerial roots of the semi-epiphytic habit have relatively smaller diameters than stems, they have similar sized vessels (Figs. 3.8 and 3.15). The size of vessels in the roots decreases in a basipetal direction, and wider vessels occupy the top part of aerial roots close to the intersection with the stem. This phenomenon partly explains the effectiveness of aerial roots in transporting water.

5.4 CONCLUSIONS

In the dry season, terrestrial plants used water more conservatively than intermediate and adult individuals. On the other hand, individuals of all habits performed relatively better in the wet season. This suggests that juveniles had greater water limitation during the dry season than did intermediate or adult plants, which is shown by the relatively lower leaf water potentials of juvenile individuals.

Stem of juveniles had less physical capacity to conduct water, which may consequently restrict water supply (Chapter 3). Therefore, down-regulation of CO_2 uptake and stomatal opening in the juvenile plants are necessary to diminish water loss and maintain water potential. The higher capacity to conduct water in the stem of intermediate and adult individuals and the establishment of aerial roots at a number of sites along a stem is evidently sufficient to sustain higher rates of CO_2 exchange and water loss.

Water use efficiency of carbon gain was not calculated in this chapter. The main objective of this chapter was to examine actual physiological responses of plants of different ontogeny in the field. I believe the data in this chapter, which include water

potential, carbon uptake/daily carbon gain, stomatal conductance and transpiration, were adequate to show the responses. However, the observation of $\delta^{13}C$ of leaves may provide more detailed assessments of water use efficiency and the extent of water stress experienced by the plants. Additionally, the measurement of leaf nutrient contents may also add some extra information of plant responses to the environment. Therefore, the investigation of $\delta^{13}C$ and leaf nutrients of different ontogeny of secondary hemi-epiphytes is considered for future research.

The role of different types of roots in water uptake from different sources and different soil depth levels is further examined in Chapter 6. In this chapter, the water sources of different habits of secondary hemi-epiphytic vines will be studied by measuring the stable hydrogen isotope composition of different water sources and comparing these hydrogen isotopes with the composition of the plant tissues.

CHAPTER 6

WATER RESOURCE UTILIZATION BY SECONDARY HEMI-EPIPHYTIC VINES

6.1 INTRODUCTION

The development of stable isotope techniques has provided an opportunity to better understand resource acquisition by plants, including water uptake (Dawson *et al*. 2002; Ehleringer and Dawson 1992; Flanagan and Ehleringer 1991). Hydrogen and/or oxygen stable isotopes have been used to study plant water acquisition in a range of species and ecosystems (Dawson 2002; Drake and Franks 2003). Root excavation may provide a snapshot of root distribution, but may not totally explain water resource uptake and utilization (Ehlinger and Dawson 1992; Meinzer *et al*. 1999). In tropical forests, high plant species density and diversity further complicate the study of plant root systems (Jackson *et al*. 1995; Meinzer *et al*. 1999). As there is no isotopic fractionation during water uptake by root tissue to the stem xylem (Dawson Ehleringer 1992; Thorburn and Ehleringer 1995), the source of a plant's water can be traced.

In an ecosystem, plants with different phenology tend to use different sources of soil water. Such spatial and temporal partitioning may result in reduced competition for water (Tilman 1982; Cody 1986; Meinzer *et al*. 1999). In a seasonally dry tropical forest, Meinzer *et al*. (1999) reported that smaller diameter trees appear to tap deeper sources of soil water than do trees with larger diameters. They also found that species with little seasonal variability of leaf fall tend to utilize deep sources of soil water,

especially during the dry season. The same patterns also occur in lowland tropical forests. Evergreen species accessed soil water at greater depth than did deciduous species (Jackson *et al*. 1995). However, based on studies of eight woody species in a Hawaiian dry forest, Stratton *et al*. (2000) found species that more actively tap water from deeper soil layers tended to exhibit greater seasonality of leaf production. Therefore, the basic instruments of soil water partitioning may include patterns and activities of rooting systems and leaf phenology (Meinzer *et al*. 1999).

Water sources other than soil water may also be important in many communities. In coastal ecosystems and seasonal rainforests, the importance of water derived from fog is significant during the dry season (Dawson 1998, Liu *et al*. 2004). Fog contributes significantly to the total hydrologic input of many ecosystems, including coastal ecosystems (Dawson 1998; Schemeauer and Cereceda 1991), mountainous cloud forests (Cavelier and Goldsten 1989; Cavelier *et al*. 1996; Holder 2004), Australian subtropical rainforest (Hutley *et al*. 1997) and tropical seasonal rainforest in China (Liu *et al*. 2004).

Utilization of water sources by various life forms of plants may also vary. Based on a study of the hydrogen stable isotope composition of xylem water, a liana (*Bauhina* sp.) was found to take up water from a deeper part of the soil profile compared with tree species (Jackson *et al*. 1995). However, it seems that lianas might not rely solely on deep soil water, as Andrade *et al*. (2005) found that at the beginning of the dry season, lianas in seasonally dry tropical forests of Panama take up shallow sources of

soil water. Epiphytes are well known to tap water from canopy substrates and cloud water (Benzing 1990).

However, few data exist on the variation in the use of different water resources by species that are unique in having several different life habits (Feild and Dawson 1998). A species exhibiting variation in habit over time may take up different sources of water during development. Feild and Dawson (1998) studied different life stages of the primary hemi-epiphyte *Didymopanax pittieri*, which is a plant that changes from an epiphytic to a terrestrial habit. Based on stable hydrogen isotopic composition, they found that epiphytic, hemi-epiphytic and terrestrial life stages of *Didymopanax pittieri* tapped different sources of water. Epiphytic stages tapped water exclusively from canopy substrate and cloud water. In contrast, terrestrial plants used only soil water. Hemi-epiphytic congeners, with their roots attached to canopy substrates as well as to the ground, seemed to use a mixture of different water sources.

The utilization of different sources of water may also occur in secondary hemi-epiphytes. As they change their connections to the soil from a stem with ordinary roots to aerial/adventitious roots, secondary hemi-epiphytic vines may undergo a shift in the nature of their resource use, including water uptake. However, the utilization of water resources in different developmental stages of secondary hemi-epiphytic vines is poorly understood. There are no data available on how much soil water partitioning occurs in different ontogenetic habits of secondary hemi-epiphytic

vines. The ability to utilize different sources of water may affect the performance of differing habits of these species in different seasons.

Based on leaf gas exchange and water potential studies (Chapter 5), differing ontogenetic phases of two secondary hemi-epiphytic species were observed to perform differently during the dry and wet seasons. In the dry season, for example, juvenile individuals of *F. excelsa* and *R. australasica* used water more conservatively than intermediate and adult individuals. On the other hand, intermediate and adult stage individuals performed relatively better, indicated by carbon gain, during both seasons. The leaf physiology of different growth phases of these secondary hemi-epiphytic vines may be related to water resource exploitation strategy.

This study aims to investigate utilization of water sources by individuals of differing growth phases of secondary hemi-epiphytic vines by studying stable hydrogen and oxygen isotopic compositions. It is hypothesized that there is a shift in water resource utilization with change in developmental stages of secondary hemi-epiphytic vines. This study, in conjunction with studies on leaf gas exchange and water potential (Chapter 5), will provide deeper understanding on how water resource utilization affects the performance of each growth habit of secondary hemi-epiphytic vines.

6.2 MATERIALS AND METHODS

6.2.1 Study sites and species

Freycinetia excelsa samples were collected from Paluma Range National Park and samples of *Rhaphidophora australasica* were collected from the Henrietta Creek Section of Wooroonooran National Park. A description of the study species is contained in Section 2.1 and a description of the study sites is in Section 2.2. Samples were collected in late April 2011, the end of the wet season and a transitional period to the dry season.

6.2.2 Rainfall data and soil moisture

The average annual rainfall data for the study sites and rainfall data for the period prior to the study were taken from the closest recording stations, i.e. Paluma Ivy Cottage Station for Paluma Range NP data and Crawford's Lookout for Wooroonooran NP data. The data were generated from climate data available from the Australian Bureau of Meteorology website (BOM 2011). Gravimetric soil water content was measured for several depths of soil. The fresh weight of the soil samples was determined before the samples were oven dried at 70^0C for three days and then re-weighed. The volumetric soil content is the ratio of water contained in the soil (soil fresh weight – soil dry weight) to the soil fresh weight. The samples for soil water content measurements were taken from the same soil samples for isotopic analysis (Section 6.2.3.2).

6.2.3 Stable hydrogen and oxygen isotope composition

6.2.3.1 Stem sample collection

Six to ten plants of each ontogenetic phase were chosen as samples. Stem samples were cut into segments that were sufficient to yield 6-10 segments of about 5 cm length each. All leaves and green stem tissues were removed from the stem segments. Green tissues and leaves were always avoided because they tend to be isotopically enriched relative to the source water (Ehleringer and Dawson 1992). Stem segments were immediately sealed in airtight plastic vials. These samples were placed in a humidified container while transported back to the laboratory. Before water was extracted, the samples were stored in a freezer.

6.2.3.2 Soil sample collection

In order to determine the isotopic composition of potential water sources from the soil profile, soil samples were collected using an auger at three different depths: 0-20 cm, 30-50 cm and 60-80 cm, at three locations at each study site, around the area where plant samples were collected. Soil samples were placed in capped plastic vials following the same procedure used for stem samples. In addition, water samples were also taken from creeks near the study areas.

6.2.3.3 Water extraction from soil and stem samples

Water was extracted from stem and soil samples by applying a cryogenic vacuum distillation process (Dawson 1993; Ehleringer *et al*. 2000, West *et al*. 2006). A schematic diagram of this process is presented in Fig. 6.1.

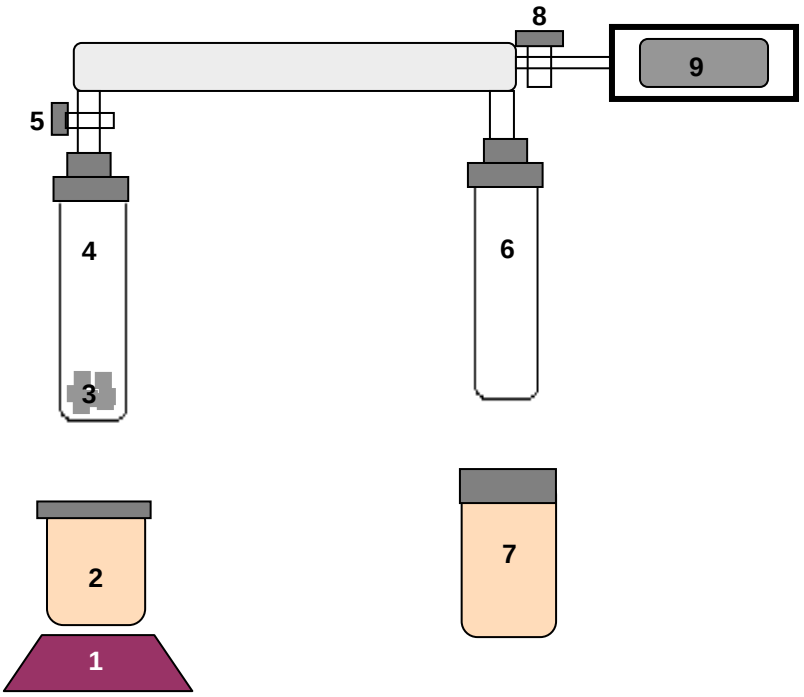


Fig. 6.1 A schematic diagram of cryogenic vacuum distillation line used in this study showing heater (1), beaker containing oil (Ondina) (2), soil/stem sample (3), extraction tube (25 mm in diameter) (4), first valve (5), collection tube (25 mm in diameter) (6), liquid nitrogen (LN_2) Dewar (7), second valve (8) and vacuum pump (Vacuumbrand, PC500 series) (9).

The cryogenic vacuum distillation method used in this study was adopted from Ehleringer *et al*. (2000). Soil and stem samples were kept frozen until extraction was conducted. Before the extraction began, the first valve (5) was closed and second valve (8) was open. The vacuum (9) pumped down the system to 5 hPa. After being taken from the freezer, soil or stem samples were cut into smaller segments and placed in the extraction tube (4). The extraction tube (4) was directly immersed into liquid nitrogen (7) for about five minutes to freeze the sample. Once the sample was frozen, the second valve (8) was closed and the first valve (5) was opened. After one minute, the first valve (5) was closed and the second valve (8) was opened. This

process was repeated until the pressure in the closed system reached 5 hPa. While the opening and closing of valves was conducted, the collection tube (6) was heated by a dryer (Aero 1600 plus) to ensure no moisturized air remained in the tube. Once the pressure in the system was 5 hPa and the second valve (8) was closed, the liquid nitrogen Dewar (7) was removed from extraction tube (4). The extraction tube (4) was immersed in the beaker (2) containing Ondina oil and then heated to 100^0C. The base of the collection tube (6) was immersed in liquid nitrogen (7). Ondina oil in the beaker (2) was maintained at 100^0C throughout the process. Once the extraction was finished, the collection tube (6) was removed, the water was thawed and then transferred into O-ring sealed storage vials. The samples were kept frozen until the isotopic analysis was conducted.

In a pre-extraction experiment, the cryogenic vacuum distillation apparatus was tested to determine the adequate extraction time required without water being fractionated during the extraction. The test showed that 45 minutes was the optimal extraction time for the system. Further tests established that in 45 minutes, 1 mL water could be totally extracted from stem samples and 1.5 mL water could be totally extracted from soil samples. Therefore, before extraction took place, some portions of each soil and stem sample were taken, oven dried and their water content was measured. Water content data were used to determine the weight of soil or stem samples that should be put in the extraction tube in order to yield 1 mL or 1.5 mL of water in 45 minutes.

6.2.3.4 Stable hydrogen and oxygen isotope analysis and mixing models

Stable hydrogen and oxygen isotope compositions were analysed using the mass spectroscopy facility at the Research School of Biological Sciences, Australian National University, Canberra. The stable hydrogen and oxygen isotope signatures of water samples were expressed in the internationally accepted delta notation (**$\delta^0/_{00}$**), where the ratio of the heavy to light isotopes in the sample (^{2}H/H or ^{18}O/^{16}O) is determined relative to an accepted standard (V-SMOW, Vienna standard mean ocean water) (Dawson 1998; Dawson *et al*. 2002; Gonfiantini 1978). The isotopic compositions were denoted as δD for hydrogen isotope ratio and δ^{18}O for oxygen isotope ratio. The formula is given below:

$$\boldsymbol{\delta}^0/_{00} = (R_{sample}/R_{standard}-1) \times 1000 \qquad \text{Formula 6.1}$$

As the potential sources of water for the plant samples hypothetically came from three different soil depths, a mixing model was applied to determine the contribution of each source to the total xylem water of each individual of differing growth stages (Dawson *et al*. 2002; Phillips and Greg 2003; Phillips *et al*. 2005). A standard linear mixing model using two isotopic signatures ($\boldsymbol{\delta}^1$ and $\boldsymbol{\delta}^2$) to the contributions (*f*) of three sources (a, b, c) to a mixture (m) is:

$$\boldsymbol{\delta}^1_m = f_a\,\delta^1_a + f_b\,\delta^1_b + f_c\,\delta^1_c$$

$$\boldsymbol{\delta}^2_m = f_a\,\delta^2_a + f_b\,\delta^2_b + f_c\,\delta^2_c$$

$$\mathbf{1} = f_a + f_b + f_c \qquad \text{Formula 6.2}$$

Before the above formula was applied, the relationship between hydrogen and oxygen stable isotopes was examined by a linear regression. A significant relationship means both isotope compositions are not independent values. Therefore, only one isotope signature was used to evaluate the relative contribution of each soil water source to the xylem water. The standard linear model in Formula 6.2 is not appropriate for an *n* isotope system with > *n*+1 sources (Phillips and Greg 2003). The software package, Iso-Source, which was created to analyse isotopic contributions of >*n*+1 sources, was employed. This software is freely available from http://www.epa.gov/wed/ pages/models.

6.2.4 Statistical analysis

Statistical analyses were conducted using the SPSS statistical package. Differences in stem water hydrogen stable isotope ratio for the three different growth habits were analysed by one-way ANOVA followed by a post-hoc Tukey test. The relationship between δD and $\delta^{18}O$ values was evaluated by employing linear model.

6.3 RESULTS

6.3.1 Rainfall and volumetric soil water content

The average monthly rainfall for 2008-2010 at the two study sites can be seen in Figs. 2.5 and 2.6. The total rainfall in the Mt. Spec Section, Paluma Range National Park for March 2011 was 1624.7 mm, which was higher than the average rainfall for March in the three preceding years. The total rainfall at this site for April 2011 (138.6 mm), however, was lower than the average rainfall for April 2008-2010. In the Henrietta Creek section, Wooroonooran National Park, the rainfall totals for March and April 2011, were 774 mm and 265 mm respectively, and were lower than the average rainfall for March and April 2008-2010. Volumetric soil water content varied at different soil depths (Fig. 6.2). At the soil surface, the volumetric soil water contents for both study sites were above 40%. The soil water content declined in the deeper parts of the soil profile.

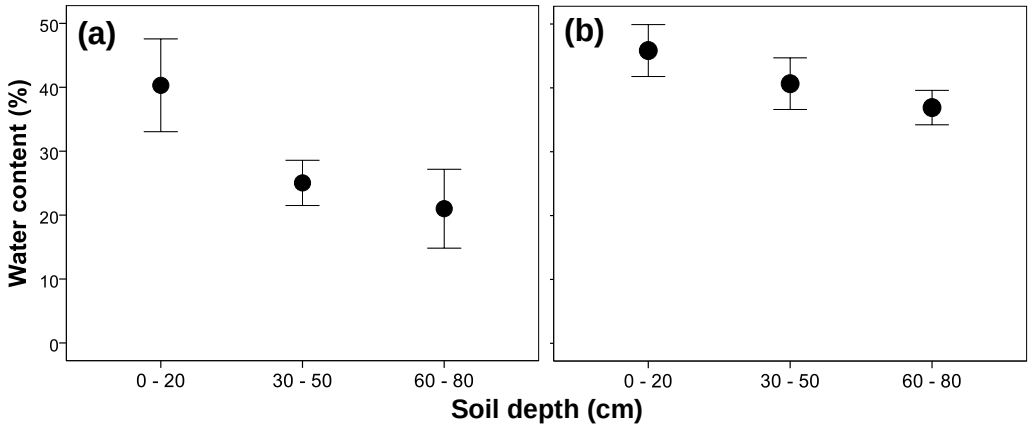


Fig. 6.2 Volumetric soil water content as a function of soil depths in the (a) Mt. Spec Section, Paluma Range National Park and (b) Henrietta Creek Section, Wooroonooran National Park when the study was conducted, late April 2011. Error bars show 95% confidence intervals.

6.3.2 Hydrogen stable isotope ratios of xylem and soil water

There was variation in the hydrogen stable isotope ratio (δD) of stem water of differing ontogenetic phases of *F. excelsa* and *R. australasica* (Fig. 6.3). In both species, juvenile individuals had the least negative δD values. Even though δD values of juvenile individuals of *F. excelsa* did not differ significantly from intermediate congeners, their differences to δD values of adult plants were significant (F= 8.538, P<0.01). For *R. australasica*, adult individuals had significantly lower δD values than for juveniles (F= 5.785, P<0.05). Differences in δD values of *F. excelsa* and *R. australasica* indicate that plants of different growth stages utilize water from dissimilar sources.

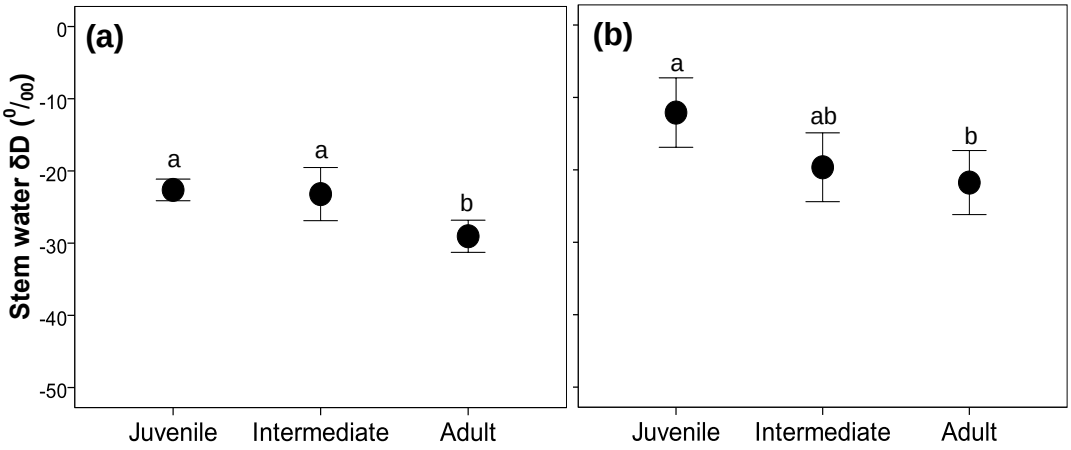


Fig. 6.3 Xylem water hydrogen isotope ratio (δD) for three different developmental stages of (a) *F. excelsa*, studied in the Mt. Spec Section, Paluma Range National Park and (b) *R. australasica*, studied in the Henrietta Creek Section, Wooroonooran National Park. The same letter indicates that values are not different at α = 5 % for each species. Error bars show 95% confidence intervals.

Soil water δD values were highest near the soil surface and decreased with increasing depth (Fig. 6.4). In the Mt. Spec section, Paluma Range National Park, where *F. excelsa* samples were collected, the average δD value near the surface (0 - 20 cm) was -24.18 $^{0}/_{00}$. The δD average value decreased to -34 $^{0}/_{00}$ at the 30-50 cm depth and -35.84 $^{0}/_{00}$ at 60-80 cm depth. The same patterns also occurred in the Henrietta Creek section, Wooroonooran National Park, where *R. australasica* individuals were studied. The average δD value near the surface (0-20 cm) was -15.21 $^{0}/_{00}$. The δD average value decreased to -25.87 $^{0}/_{00}$ at the 30-50 cm depth and -35.06 $^{0}/_{00}$ at 60-80 cm depth.

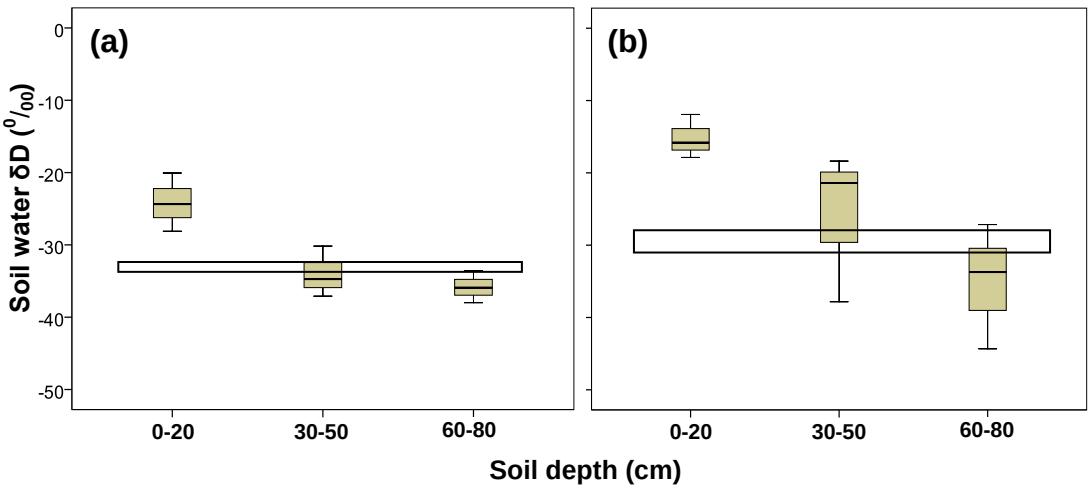


Fig. 6.4 The range of values of soil water hydrogen isotope ratio (δD) for three different depths in the (a) Mt. Spec Section, Paluma Range National Park and (b) Henrietta Creek Section, Wooroonooran National Park. The horizontal bands represent the range of δD values for water samples from nearby creeks of the study areas.

The δD values for stream water in both study areas overlapped with the δD values of the deeper part of the soil profiles (Fig. 6.4). Drake and Franks (2003), in their study on the Atherton Tableland, North Queensland, found that stable isotope ratios for stream water were always higher than for groundwater derived from between 2 m and 4 m below the soil surface. The differences, however, were much higher in the dry season than in the wet season. Therefore, it is hypothesized that δD values for ground water at both study sites were similar or lower than δD values for water derived from the deepest soil profile investigated in this research.

Differences in δD values in soil water indicate two points. Firstly, it indicates that there was an isotopic fractionation occurring in soil water of different depths. Secondly, it offers an opportunity to calculate the relative contribution of soil water from different depths of the soil profile to the xylem water in each growth stage of each species. The water uptake behaviour of plants can then be investigated.

6.3.3 Stable isotope mixing model

Based on linear regression models between the two isotopes, xylem water δD values of *F. excelsa* were significantly correlated with $\delta^{18}O$ values (R^2=0.77, F=82.201, P<0.01) (Fig. 6.5a). The same pattern occurs in *R. australasica*, in which a significant relationship was also observed between δD and $\delta^{18}O$ values (R^2= 0.85, F= 115.274, P<0.01) (Fig. 6.5b). As they were significantly correlated with each other, both isotope compositions are not independent values. Therefore, only one isotope signature, which is δD, was used and the Iso-Source package was applied.

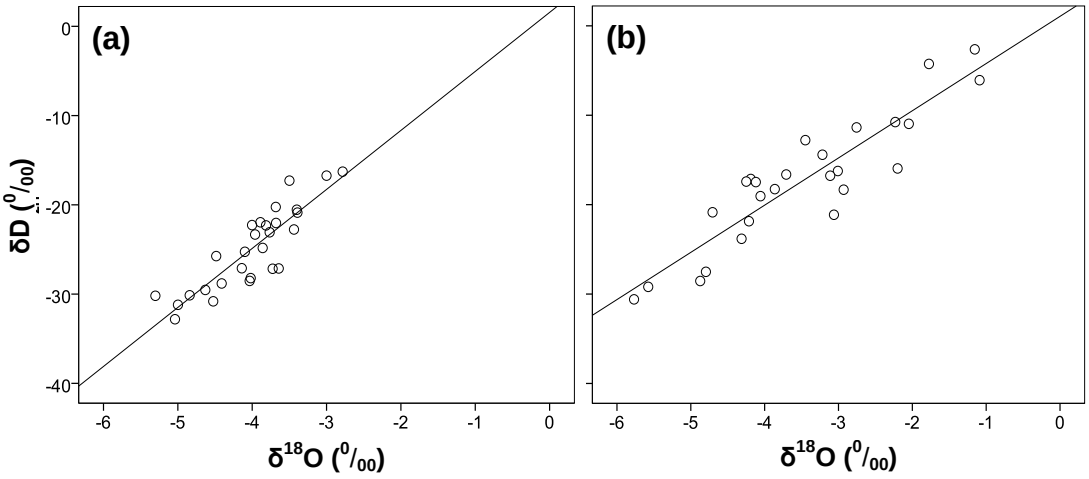


Fig. 6.5 Linear regressions between hydrogen (δD) and oxygen ($\delta^{18}O$) stable isotope ratios for xylem water of (a) *F. excelsa* and (b) *R. australasica*. The data were pooled from hydrogen and oxygen stable isotope ratios of all developmental stages of each species.

The results of the stable isotope mixing model indicate that plants of differing developmental phases of both study species utilized different sources of soil water (Tables 6.1 and 6.2). Water uptake by juvenile individuals appeared to be largely restricted to the upper part of the soil, contributing more than 90% of water in the juvenile stems. As the plants grow, the dependence on surface soil water decreased and the plants started utilizing water from deeper parts of the soil profile. Apparently adult individuals established connections, through their adventitious roots, to the deeper parts of the soil profile and utilized water from all available sources down the soil profile.

Table 6.1 Estimated proportion of potential water sources (%) at the end of the wet season (late April 2011), for three developmental stages of *F. excelsa*. Water source proportions were calculated using Iso-Source software (Philips and Gregg 2003). Soil water isotopic values represent average values of δD of each soil depth. The proportional water use of each developmental stage was calculated for each individual plant sample. The average and the range of minimum/maximum source proportions (in parentheses) are shown in the table.

	Proportion of potential water sources (%)		
Water sources	Juvenile	Intermediate	Adult
0-15 cm δD= -24.18	97 (86-100)	84 (44-100)	52 (19-97)
30-50 cm δD= -34	2 (0-8)	9 (3-32)	27 (2-47)
60-80 cm δD= -35.84	1 (0-8)	7 (3-25)	21 (2-35)

Table 6.2 Estimated proportion of potential water sources (%) at the end of wet season (late April 2011), for three growth/developmental stages of *R. australasica*. Water source proportions were calculated using Iso-Source software (Philips and Gregg 2003). Soil water isotopic values represent average values of δD of each soil depth. The proportional water use of each developmental stage was calculated for each individual plant sample. The average and the range of minimum/maximum source proportions (in parentheses) are shown in the table.

	Proportion of potential water sources (%)		
Water sources	Juvenile	Intermediate	Adult
0-20 cm δD= -15.21	91 (46-100)	69 (12-100)	55 (13-85)
30-50 cm δD= -25.87	6 (0-35)	14 (0-35)	25 (11-41)
60-80 cm δD= -35.06	3 (0-19)	17 (0-65)	20 (4-51)

6.4 DISCUSSION

Differences in stable hydrogen isotopic ratios between water derived from different layers of the soil profile were observed at the two sites in this study (Fig. 6.4). Water near the soil surface was found to have less negative hydrogen isotopic ratios, which means that the water is more isotopically enriched than water of deeper soil layers. During water uptake by root tissue to the stem xylem, isotopic fractionation can occur but it is rarely observed (Dawson Ehleringer 1992; Thorburn and Ehleringer 1995); therefore, the isotopic compositions of stem xylem water should reflect isotopic signatures of water sources.

Gradients of water hydrogen and/or oxygen isotopic compositions with soil depth have been observed in a wide range of ecosystems, including an alpine ecosystem on the Tibetan Plateau, China (Duan *et al*. 2008), tall-grass prairie in Kansas (Nippert and Knapp 2007), a short-grass steppe community of north-eastern Colorado (Dodd *et al*. 1998), savannah in West Africa (Le Roux *et al*. 1995) and Brazil (Jackson *et al*. 1999), Mediterranean-type ecosystems of south Western Australia (Dawson and Pate 1996), dry forest in Hawaii (Stratton *et al*. 2000) and Panama (Meinzer *et al*.1999), karst soils in seasonally dry tropical Mexico (Querejeta *et al*. 2007), tropical lowland forest in Panama (Jackson *et al*. 1995) and tropical riparian rainforest ecosystem on the Atherton Tableland, north Queensland, Australia (Drake and Franks 2003).

Hydrological processes influence the characteristics of soil water isotopic signatures. Rainwater adds meteoric water with a certain isotopic composition and evaporation

will result in isotopic enrichment (Barnes and Allison 1988; Drake and Franks 2003), due to the change in the proportion of heavy and light isotopes (Dawson *et al*. 2002). During evaporation, isotopic fractionation occurs as there are transitions between liquid water and water vapour (Fry 2006). The chemical bonds of lighter isotopes are usually weaker and easier to disintegrate. This fractionation which involves chemical reactions to break the bond consequently affects the variability of isotopic composition within the water cycle. In moderately wet soil, the water movement in the upper soil layers is by vapour diffusion and, on the other hand, liquid transport is significant in the deeper parts (Barnes and Allison 1988). Therefore, evaporation which drives the water into the atmosphere results in soil water near the surface becoming more isotopically enriched than water from deeper parts of soil profile (Allison and Hughes 1983). The isotopic composition of soil at depth is usually similar to the isotopic signature of groundwater (Drake and Franks 2003). The magnitude of isotopic composition differences with increasing soil depth, however, may be influenced by many factors, such as vegetation cover and soil types (Allison 1982; Allison and Hughes 1983), and seasonal atmospheric conditions (Drake and Franks 2003; Jackson *et al*. 1995).

Based on stable isotope mixing model calculations, variation was observed in the behaviour of *F. excelsa* and *R. australasica* individuals of different developmental stages in utilizing water from different layers of the soil. The results of this study show that water uptake by juvenile plants appears to be limited to near the soil surface, which means that juvenile individuals have limited rooting systems (personal observations). As they grow, the plants send more aerial roots down to the

ground, which penetrate deeper in the soil; thus, their ability to utilize water from deeper soil layers increases (Tables 6.1 and 6.2). Driven by available water from the surrounding soils, plants primarily use the water source that is most easily accessible for them (Thorburn and Ehleringer 1995).

Partitioning of soil water uptake by plants is a function of rooting systems and partly driven by leaf phenology (Meinzer *et al*. 1999). However, there are contradictory observations in regard to the correlation between soil water partitioning and leaf phenology. In their study in a lowland tropical forest of Panama, Jackson *et al*. (1995) found that drought-deciduous trees were using water from shallower soil layers compared to evergreen trees. Evergreen species had more access to deeper soil by establishing deep roots. This probably provides the capacity for evergreen species to maintain their leaves during the dry season. This is consistent with the greater fluctuations in water availability near the soil surface across the seasons. In a seasonally dry tropical forest in Panama, Meinzer *et al*. (1999) also found that species with little seasonal variability of leaf fall tend to utilize deep sources of soil water, especially during the dry season. However, based on studies of eight woody species in a Hawaiian dry forest, Stratton *et al*. (2000) found species that more actively tap water from deeper soil layers tended to exhibit larger seasonality of leaf production. They concluded that species tapping deeper sources of soil water appeared to behave as drought avoiders in that they maintained higher leaf water potentials during the dry season and tended to drop their foliage. These contradicting patterns indicate that relationships between leaf phenology and soil water acquisition

are complex, may be affected by phylogenetic background, and may also reflect site climatic and edaphic factors.

Rooting systems affect the ability of plants to effectively take up water. Plant rooting systems are strongly driven by water availability (Nippert and Knapp 2007), which consequently plays a very crucial role in water resource partitioning. Jackson *et al*. (1999) hypothesized that rapid development of deep roots to reach soil layers, where substantial water depletion may not occur, facilitates establishment and survival of small trees in seasonally dry tropical ecosystems. Duan *et al*. (2008) also found the utilization of water from deeper soil layers is more influenced by the development of deep roots rather than by plant size.

The utilization of water resources by plants is opportunistic. Meinzer *et al*. (1999) observed the ability of some trees to tap increasingly deeper soil water when the upper soil layers dried. Trees that were able to take up water from greater depths in the soil profile, as the dry season intensified, tended to show progressively increasing water use. By contrast, trees with less flexibility in exploitation of soil water sources tended to show declining rates of water use during the dry season. This pattern was also reported for woody species in other communities such as riparian vegetation (Mensforth *et al*. 1994; Thorburn and Walker 1994) and Australian heathland plants (Dawson and Pate 1996).

The stable isotope mixing model indicates that individuals of the adult stage of *F. excelsa* and *R. australasica* had more options to access water from different soil

layers than did individuals of the juvenile phase. However, it is apparent that water derived from near the soil surface still contributes significantly as the main source of water for individuals of the adult stage (Tables 6.1 and 6.2). Since the surface level contained more water than did deeper soil layers during the study period (Fig. 6.2), plants appear to utilize all available options for water sources. However, as the dynamics of soil moisture changes across seasons, plant behaviour in accessing water sources may also shift.

Patterns of water use of different species and growth forms may provide better understanding of patterns of plant community structure, diversity and productivity (Nippert and Knapp 2007). Information on the water use of different growth stages of certain species, on the other hand, may further our understanding of their ability to survive during their developmental phases. The capacity to acquire water from different parts of the soil affects the ability of plants to survive. In the case of secondary hemi-epiphytic vines, the development of aerial roots may provide deeper penetration into the soil profile. As aerial roots have wider vessels than primary roots (Chapter 3), they have a greater ability to conduct water. Deep soil at both study sites contained more water than water near the surface in the dry season (Chapter 5), which may facilitate the performance of individuals of the adult phase that had access to deeper parts of the soil profile.

In Chapter 5, it was observed that in the wet season juvenile plants performed relatively well compared with intermediate and adult individuals in terms of photosynthesis. However, juvenile plants had very low carbon assimilation rates in

the dry season; even though all forms operated at similar pre-dawn water potentials of -1.5 MPa and afternoon water potentials of not less than -2 MPa (see Chapter 5 for more detail). The lower hydraulic conductivity of the stems of juvenile plants (Chapter 3) may restrict water supply such that down-regulation of CO_2 uptake and stomatal opening are necessary to diminish water loss and maintain water potential. Hypothetically, as soil near the surface had lower water content than the deeper soil layers in the dry season (Chapter 5), juvenile plants, which could only access water in the upper layers, may face more water scarcity in the dry season. On the other hand, intermediate and adult individuals still had options to utilize water from deeper soil. The ability of plants with different developmental stages to utilize water from different parts of the soil will ultimately dictate the performance of the plants across the seasons.

6.5 CONCLUSION

Water derived from different layers of the soil profile at the two sites in this study had different stable isotopic signatures, with water near the soil surface more isotopically enriched than water at deeper soil layers. This difference was exploited to investigate the soil water source used by *F. excelsa* and *R. australasica* individuals of differing developmental stages. There was variation in the utilization of water with increasing soil depth by juvenile, intermediate and adult individuals of *F. excelsa* and *R. australasica*. Hydrogen isotope mixing model indicates that water uptake by juvenile plants was limited to the area near the soil surface; on the other hand, adult plants utilized water from all soil layers studied. The development of aerial roots in the later growth stages of secondary hemi-epiphytic *F. excelsa* and *R. australasica*

provides deeper penetration into the soil profile, which consequently dictates the capacity of plants to exploit all available soil water sources across seasonal periods. As the ability or inability to exploit water from different sources affects the performance of individuals of different growth stages, the information on ontogenetic water use furthers our understanding of the ability of secondary hemi-epiphytic vines to survive during their development.

CHAPTER 7

SYNOPSIS

7.1 ECOPHYSIOLOGICAL ATTRIBUTES OF SECONDARY HEMI-EPIPHYTES DURING ONTOGENY

Variation in the ecophysiological performance of plants in response to genotype, age, ontogeny and environmental heterogeneity is a widely accepted hypothesis (Donovan and Ehlinger 1992; Knapp and Fahnestock 1990; Zotz 2000; Zotzt *et al*. 2001). Major environmental factors influence the dynamics of individuals, as well as plant populations, including soil fertility and nutrient cycling, light, CO_2, temperature and humidity, which interact together to create a specific micro-climate. As environmental conditions may vary spatially and seasonally, plants have to continually respond and acclimate to conditions in which they live (Dickison 2000). The nature of any response is driven by both genetic inheritance and environmental factors.

However, the interactive effects of plant age, particularly size, and micro-environmental resources on the physiological performance of plants are not clear cut (Zotz 2000). De Soyza *et al*. (1996), for example, found that assimilation rate and stomatal conductance of small mesquite shrubs in the Chihuahuan Desert in southern New Mexico, varied compared with larger conspecific individuals. Smaller individuals performed better physiologically after high summer rainfall but worse after a dry period than did larger individuals. On the other hand, a consistent pattern of the leaf gas exchange was shown by the shrub *Chrysothamnus nauseosus* in the

semi-arid ecosystem in Utah, in which smaller plants had higher rates of photosynthesis, but lower long-term water use-efficiency (Donovan and Ehleringer 1992). For many tree species, larger individuals may show lower rates of photosynthesis than smaller individuals (Zotz 2000).

As the ability of plants to exploit soil resources is dictated by the development of root systems, variation in the ecophysiological performance of plants of differing age and size may be the consequence of differences in root depths and distribution (Knapp & Fahnestock 1990; Zotz 2000). Therefore, according to Zotz (2000), variation in the pattern of the effects of plant size in many plants is partly attributed to intra-individual variability in root system development in the soil and the condition of micro-environmental factors. Water availability at different soil depths and differences in the development of rooting system may benefit both small and large individuals. The behaviour of plants in utilizing water resources tends to be opportunistic (Meinzer *et al*. 1999), and the size-related performance of plants is driven by seasonal environmental conditions.

In some epiphytic plants, many morphological and physiological characters, including photosynthesis, stomatal conductance, leaf nitrogen and leaf anatomy, are a function of plant size, which may or may not be related to ontogenetic phases (Adams and Martin, 1986; Lorenzo *et al*. 2010; Schmidt *et al*. 2001; Zotz, 1997; Zotz *et al*. 2001). For some epiphytic plants, the size of plants is not necessarily an indicator of age/ontogenetic phases (Zotz 2000). Epiphytic plants of the same size may be of quite different ages. Therefore, the use of the terms “juvenile” and “adult”

for some epiphytes reflects more a temporal perspective rather than differences in plant size (Zotz 2000).

Size-related intra-specific ecophysiological variability may be found in most plants; however, in epiphytic plants, this is possibly more evident and predictable (Zotz 2000). A few centimetres differences in size between individuals may mask considerable variation in physiological properties such as photosynthetic capacity or *in situ* leaf carbon gain. Zotz (1997) found a consistent pattern of a size-related increase in photosynthetic capacity in the epiphytic orchid *Dimerandra emarginata*. In this experiment, all plants were growing under similar conditions and all plants were of similar age but were not of similar size. Therefore, changes in size were not due to differences in plant age. In their study on the epiphytic bromeliad *Vriesea sanguinolenta*, Schmidt and Zotz (2001) also observed size-related differences in leaf anatomy, morphology and physiology. These differences were attributed to a more reliable water supply for the rooting systems of larger individuals.

Further studies, which included more epiphytic species, showed that size-related ecophysiological attributes in epiphytes of similar ontogenetic phase are common. Schmidt *et al*. (2001) reported that a survey of ten vascular epiphyte species in Panama revealed an up to five-fold continuous increase in photosynthetic capacity from small to large plants. Moreover, when comparing only large individuals, the intra-specific variation in photosynthetic capacity was almost always higher than the inter-specific variation

On the other hand, Lorenzo *et al*. (2010) observed size-related differences in the epiphyte *Anthurium scandens*, which they attributed to plant ontogenetic phases. Seedlings of *A. scandens* had higher epidermal conductance to water loss ratios as well as lower values of leaf succulence and sclerophylly than did young and adult individuals. Adult individuals had higher retranslocation rates of leaf nitrogen during senescence than young individuals. Adult individuals also had thicker upper and lower epidermis, mesophyll, palisade and spongy parenchyma than seedling leaves. However, stomatal length and density did not differ between leaves of adults and seedlings. Stomatal conductance and leaf anatomical characteristics of young and adult individuals were similar. These results, including lower succulence and sclerophylly and less resistance to water loss in seedlings of *A. scandens*, indicate that a smaller sized seedling phase had a higher potential susceptibility to stressful abiotic conditions in comparison to young and adult individuals.

Secondary hemi-epiphytic vines start their life as ground-dwelling plants. Like other vines, the plant then climbs the host, but when the plant reaches maturity, the oldest portion of the stem dies. The plants then reconnect to the soil through aerial roots (Lopez-Portillo *et al*. 2000; Lüttge 1997; Moffet 2000). The change in soil connection from stem to aerial roots occurs in the two vine species in this research, i.e. *F. excelsa* and *R. australasica*.

In secondary hemi-epiphytic *F. excelsa* and *R. australasica*, differences in plant size are evident at different ontogenetic phases (Chapter 2). The results of this research show that there is variation in ecophysiological attributes of plants of different

ontogenetic phases. The differences in anatomical and physiological characters during ontogenetic development of secondary hemi-epiphytes may affect the ability of plants to respond to variation in environmental factors.

In terms of xylem anatomy, adult individuals of *F. excelsa* and *R. australasica* had wider vessels than intermediate and young plants. There were also differences in hydraulic architecture parameters, i.e. hydraulic conductivity, stem specific conductivity, leaf conductivity and Huber value, between adult, intermediate and juvenile individuals. Adult plants had a higher capacity to conduct water through the stem to the leaves than did individuals of early stage development.

The pattern of xylem vessel size with increasing height along the stem was also the opposite of that commonly found in trees. The base of the stem had narrower vessels and lower hydraulic conductivity than the middle part of the stem. This pattern was more pronounced as the plants became more mature and longer. The basipetal pattern of vessel size along the plant stem may be common in plants with a secondary hemi-epiphytic growth habit. Similar results for vessel diameter distribution and hydraulic conductivity were also observed in a previous study of the secondary hemi-epiphyte, *Monstera acuminata* (Lopez-Portillo *et al*. 2000). As adult individuals of *F. excelsa* and *R. australasica* had wider vessels and a higher capacity for hydraulic conductivity than intermediate and juvenile congeners, the change in plant-soil connectivity during ontogeny of these species does not physically restrict water transport. The well established aerial roots in adult individuals presumably provide a mechanism to bypass the lower hydraulic conductance in the basal parts of the stem.

The leaf anatomy study suggests that secondary hemi-epiphytes have not adapted especially to the hemi-epiphytic habit. The observations of anatomy are consistent with what is observed in canopy leaves. As the semi-epiphytic adult plants had more stomata per unit area than terrestrial juvenile congeners, it is likely that they have better control of the opening and closing stomata in certain areas of leaves. Consequently, they may have more control in absorbing CO_2 and regulating transpirational loss. Larger stomata in adult individuals also do not reflect conservative use of water. Therefore, the ontogenetic leaf anatomy attributes of secondary hemi-epiphytes suggest that the adult individuals appear to be well adapted to the canopy environment. This concept is consistent with the fact that secondary hemi-epiphytic vines have no physical restriction in water transport during their developmental phases.

In regard to the leaf physiology, leaf water potential was correlated with volumetric soil moisture, in that plants of all developmental phases were more affected during the dry season than the wet season. Consequently, assimilation rates were higher during the wet season for all growth stages of both species. High water availability in the wet season and relatively low evaporative demands provide excellent conditions for plants to absorb CO_2.

CO_2 exchange rates were similar for adult, intermediate and juvenile plants during the wet season; in contrast, CO_2 uptake by juvenile plants was most affected by dry season conditions. Juveniles exhibited the lowest CO_2 exchange in the dry season, but all forms operated at similar pre-dawn and afternoon water potentials, with the

exception of adult *R. australasica* which had less negative leaf water potentials. The lower hydraulic conductivity of the stems of juveniles may restrict water supply such that down-regulation of CO_2 uptake and stomatal opening are necessary to diminish water loss and maintain water potential. Water supplied to intermediate and adult plants by aerial roots variously inserted at a number of sites along a stem is evidently sufficient to sustain higher rates of CO_2 exchange and water loss.

There was also a variation in the utilization of water from different soil depths by juvenile, intermediate and adult individuals of *F. excelsa* and *R. australasica*. Based on the hydrogen stable isotope signatures of water derived from different layers of the soil profile, matched with isotope signatures of the stem water, plants of different ontogenetic phases differed in their use of soil water resources. Water uptake by juvenile individuals was limited to the upper part of the soil profile; on the other hand, adult plants utilized water from all soil layers studied. This suggests that the development of aerial roots in the later growth stages, of secondary hemi-epiphytic *F. excelsa* and *R. australasica*, not only bypasses the stem basal hydraulic resistance, but also provides deeper penetration into the soil profile, which consequently dictates the capacity of plants to exploit all available soil water sources across seasonal periods. The ability or inability to exploit water from different sources affects the performance of individuals of different ontogenetic stages.

The eco-physiological attributes of the secondary hemi-epiphytes *F. excelsa* and *R. australasica* indicate the ability of these plants to survive during their developmental phases. These attributes affect the performance of these plants and their capacity to

respond to environmental conditions. Based on their ecophysiological characters, this study shows that smaller size juveniles may have a higher potential susceptibility to stressful abiotic conditions (i.e. drought) in comparison to bigger adult congeners.

7.2 THE NATURE OF THE DEVELOPMENT OF SECONDARY HEMI-EPIPHYTES

Epiphytic environments are often characterized as stressful and the main challenges are associated with limited water and nutrients. Consequently epiphytic plants have evolved mechanisms to cope with drought and to acquire essential ions (Benzing 1990). True epiphytes, for example, are known to have a tendency to develop CAM photosynthesis, high leaf succulence and sclerophylly, a high level of water use efficiency and low rates of water loss, which relates to stomatal control and epidermal resistance (Benzing 1990; Holbrook and Putz 1996a; Lüttge 1997). This research indicates that secondary hemi-epiphytes exhibit hydraulic plasticity that assists in coping with life in the canopy and with the transition from depending upon 'normal' roots to aerial roots.

The ecophysiology of secondary hemi-epiphytes is probably more similar to the physiology of lianas (Holbrook and Putz 1996b), rather than epiphytes. Unlike primary hemi-epiphytes which undergo a clear shift between an epiphytic juvenile phase and a terrestrial adult phase (Holbrook and Putz 1996a; Moffet 2000; William-Linera and Lawton 1995), a shift in growth forms during ontogeny of secondary hemi-epiphytes may not involve a fully epiphytic stage (Lüttge 1997; Moffet 2000), as the plants never fully surrender their soil connection. The plants still maintain

their access to more abundant resources in the soil. Therefore, adaptations to stressful canopy environments are not essential for survival.

In secondary hemi-epiphytes, the development of aerial roots is not only an alternative means of taking up water and nutrients after the stem connection is severed, but also dictates the height that the plant can reach in the canopy. A study of two secondary hemi-epiphytic Araceae in Panama, by Meyer and Zotz (2004), showed that *Philodendron radiatum* was more likely to reach higher levels in the canopy since they had fast growing aerial roots. On the other hand, *Anthurium clavigerum* which had slower growing aerial roots was found much lower in the forest canopy. Aerial roots of *A. clavigerum* were never able to establish a connection with the soil from greater heights in the canopy before the roots die. Patino *et al*. (1999) also found that, in secondary hemi-epiphytic Araceae, aerial roots which are located close to the ground are more likely to connect with the soil.

Aerial roots may also be important for the longevity of monocotyledonous secondary hemi-epiphytes. As monocots, *F. excelsa* and *R. australasica*, have stems with finite duration due to the lack of a vascular cambium and therefore of secondary growth (Carlquist 1991; Lopez-Portillo *et al*. 2000). The basal part of the stem contains older tissues, which may be less functional. The establishment of aerial roots may not only be bypassing hydraulic resistance due to narrower vessels but also bypassing less functional tissues. As the growth rate of aerial roots affects the height that secondary hemi-epiphytes can reach in the canopy, I suggest that the growth rate of aerial roots

may also affect the longevity of the plants. This assertion, however, needs to be further examined.

7.3 CONCLUDING REMARKS

The association of changes in morphological characters of secondary hemi-epiphytes with ontogenetic development has been previously documented (Ray 1990; Lee and Richards 1991). My research has taken a more integrated approach to show that there are variations in vessel anatomy and hydraulic conductivity, leaf anatomy and physiology, and the utilization of soil water resources during the ontogenetic development of the secondary hemi-epiphytes *F. excelsa* and *R. australasica*. As they climb the host and live in the canopy, these two species do not appear to suffer from water stress as holo-epiphytes do.

The physiognomy of secondary hemi-epiphytes differs from epiphytes and vines. The plants' soil connections through aerial roots provide access to the soil, avoid a potential hydraulic bottleneck at the base of the stem and may contribute to the plants' longevity. This continued access to the soil can support a greater leaf area and biomass than most epiphytes. The modified xylem anatomy in adult plants compensates for the water demand of a plant with considerable leaf area. The multiple soil contact-points of the aerial roots also provide redundancy that is not available to most vines, which tend to be single stemmed.

However, some species of secondary hemi-epiphytic climbers may lose their stem connection to the soil and never reconnect to the ground through aerial roots in part of their life cycle. In that case, their ecophysiological characters may differ from observations reported in this research. Studies of their ontogenetic ecophysiological attributes will enrich our understanding of this type of life habit. As the behaviour of aerial roots may contribute to secondary hemi-epiphytic plant longevity, further studies may clarify the ecophysiology of secondary hemi-epiphytes and elucidate the functional ecology of aerial roots.

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