

**COMPARATIVE ECOLOGY AND PHYSIOLOGY OF AUSTRALIAN AND
BRAZILIAN MISTLETOE-HOST RELATIONSHIPS**

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Thesis submitted in fulfil
of the requirement for the degree of
Doctor of Philosophy

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May 2015

“Now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!”

Lewis Carroll – Alice Through the Looking Glass

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Summary

Mistletoes are parasitic angiosperms that attach to the host branch by a modified root (the haustorium) and acquire water and nutrients exclusively via the host xylem, at a very low carbon unit-cost. The association between mistletoes and their hosts is a fascinating co-evolutionary system and has long been used as a model to investigate plant resource use strategies. In this thesis I tackled resource use questions using a many-species, “comparative ecology” approach, focusing on mistletoe-host species pairs from both Australia and Brazil, with the overarching aim of identifying robust generalities among species. I investigated several key aspects of their ecology and physiology, including photosynthetic trait adaptations to aridity, nutrient resorption, leaf functional traits, investment in anti-herbivore defences, and herbivory itself. I also took the opportunity to revisit old hypotheses in the literature, such as the “N-parasitism hypothesis” and the “mimicry hypothesis”. My findings reveal that mistletoes show strong responses to environmental conditions, exhibiting similar trait-shifts as their hosts in relation to site aridity. Nevertheless, mistletoes are profligate water users compared to their hosts and showed substantially less efficient use of water in photosynthesis, both at wet and at dry sites. Mistletoes seemingly achieve reliably high rates of water flow from the host xylem via maintaining higher osmolarity in their leaf tissues than in hosts; this appears to have a substantial cost that is reflected in leaf respiration rates. Little support was found for the N-parasitism hypothesis in a global context, and in three low-P sites, where the lack of N resorption suggests that N is not a limiting nutrient for mistletoes. Moreover, natural selection may have favoured P resorption processes in mistletoes occurring in P-impooverished habitats, suggesting that an alternative ‘P-parasitism hypothesis’ could better explain these results, at least for these systems. I provided evidence that the evolution of mimicry in

Summary

Australian mistletoes could be associated with higher N availability in the hosts and with alkaloid-positive hosts, illustrating a case of Batesian mimicry. In a broader context, my results deliver significant new insights into the evolution of resource use in mistletoes, and address fundamental theories related to mistletoe ecology and physiology. They also provide general insights into adaptations of plants along environmental gradients and into the evolution of mimicry in Australian mistletoes.

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Certificate of candidate

I certify that the work in this thesis ‘Comparative Ecology and Physiology of Australian and Brazilian mistletoe-host relationships’ is an original piece of research and has been written by myself. The contributions of others are listed below (Statement of Contribution) and assistance by volunteers has been acknowledged either at the end of each chapter or in the Acknowledgement section. All sources of information and literature used are indicated in the thesis. This thesis has not previously been submitted in any form for a higher degree at any other university or institution.

Marina Corrêa Scalon

November 2014

Acknowledgements

This project was founded by Conselho Nacional do Desenvolvimento Científico e Tecnológico from the Brazilian Ministry of Sciences (CNPq) and by Macquarie University.

I am greatly thankful to my supervisor Ian Wright for the support and share of my enthusiasm for mistletoes, for amplifying my scientific view and for making this project possible.

To Augusto C. Franco, for the trust and the respect, and for all the support during my academic life and to be a source of inspiration for me. To all that helped during the time I spend in Franco's lab in Brazil: Cristiane, Sarah, Mendes, Wando, Marinho, William, Izabelly.

To the governmental agencies that provided the permits for plant collections and National Parks administration staff and rangers for being very helpful during field data collection. In particular, Whyalla Conservation Park, Royal NP, Ku-ring-gai NP, Howard Springs, Gundabooka NP, Innes NP, and Reserva Ecológica do IBGE (RECOR). To all administrative and academic staff at Macquarie University for the help and support.

To all the people who helped in the field, for the exchange of ideas and great fun during fieldwork in Australia and Brazil: Fabricius, Julieta, Julia, Allyson, Izabelly, Rose, Antônio Mendes.

Especially to Fabricius Domingos, my partner, not only for the help in the field and reviewing every chapter of my thesis, but also for all the love and care and the emotional support during my candidature. Thank you for sharing your life and your dreams with me. Te amo muito!

Acknowledgements

To all the beautiful people I had the honour to meet and spend some time together during this journey in Australia, my friends I will carry in my heart forever: Emma, James and Vashi, Anthony, Julieta, Yu Na, Denitsa, Saskia, Tina, Julia and Matt, Julietinha and Mark, Kasia, Anna, Wang Han, Allyson, Vincent, Francesca, Mayra, Felipe and Raquel. I will miss Friday drinks with Emma, Anthony and James: you are the best! Especially to my beautiful bokkie and Juli for the huge support during the final steps of this journey! Love you heaps!

To the other part of the beautiful people I met in Adelaide and from the MELFU lab: Luciana Moller and Luciano Beheregaray, Daniel and Mari, Ju and Pedro, Cat, Minami and Oliver, Astrid and Chris, Rafa, Edu and the beautiful kids Moana, Enzo, Pipe and Luiza who always have brought happiness to my life!

To the Wright's lab colleagues and friends, for all the scientific insights and lunches we shared together, Vincent, Allyson, Srikanta, Sas, Em, Rachel, Wu Kuang, Julia Cooke, and Raquel.

I am particularly grateful to my friends and family from Brazil, for the constant presence in my life and enormous encouragement. Primetas! To my mom Luciana, my sister Marcela and my dad Marcelo for making such a great part of me. Amo muito todos vocês!

Statement of contribution

I, Marina Corrêa Scalon, declare that the research contained in this thesis entitled ‘Comparative Ecology and Physiology of Australian and Brazilian mistletoe-host relationships’ is my own work. The contribution of co-authors and other sources are indicated below.

Chapter 2

‘A global analysis of water and nitrogen relationship between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses’

Ian J. Wright was involved in concept development and manuscript preparation:

Concept & development: MCS 60%

Data collection: MCS 100%

Data analyses: MCS 100%

Writing: MCS 80%

Chapter 3

‘Photosynthetic trait adaptations of parasitic mistletoes and their hosts in sites of contrasting aridity’

Ian J. Wright was involved in concept development and manuscript preparation, Augusto C.

Franco was involved in the manuscript preparation:

Concept & development: MCS 60%

Data collection: MCS 100%

Data analyses: MCS 100%

Writing: MCS 70%

Chapter 4

‘Nutrient Resorption in mistletoes from three P-deficient sites in Australia and Brazil’

Ian J. Wright was involved in concept development and manuscript preparation, Augusto C.

Franco was involved in the manuscript preparation:

Concept & development: MCS 70%

Data collection: MCS 100%

Data analyses: MCS 100%

Writing: MCS 90%

Chapter 5

‘Leaf lifespan, herbivory and leaf defence investment in mistletoes and their hosts’

Ian J. Wright and Augusto C. Franco were involved in concept development and manuscript preparation, Izabelly Sant’Ana was involved in the data collection.

Concept & development: MCS 80%

Data collection: MCS 95%

Data analyses: MCS 100%

Writing: MCS 90%

Chapter 1

General Introduction

General Introduction

A central challenge in plant ecology is to understand the adaptive significance of particular physiological traits or the combination of traits. As parasitic organisms, mistletoes have some unusual resource use strategies compared to non-parasitic plants. They have a completely different system to access nutrients and water (discussed below), and rely on the host capacity of acquiring nutrient and water from the soil. These differences are intriguing, especially because the evolutionary pressure to optimise the use of resources might not be the same for mistletoes as for non-parasitic plants. The general aim of my thesis was to explore the adaptive significance of so-called “functional” traits (Violle *et al.* 2007) in hemiparasitic plants. I looked at several aspects of mistletoe ecology and physiology – including photosynthetic adaptations to aridity, investment in chemical defences, mimicry and herbivory, and patterns of nutrient resorption – to answer how the parasitic lifestyle adaptation affects resource use strategies. By adopting a “comparative ecology approach” (i.e., comparison across many species), I was able to expand on previous studies, and test old and newly-generated hypotheses concerning mistletoe ecology and evolution, at an unprecedented scale across different environments in Australia and Brazil.

Background

The parasitic lifestyle evolved at least 11 times among angiosperms (Barkman *et al.* 2007). Parasites are defined as organisms that live in or on another organism taking resources partly or completely from their hosts and causing some negative effect to them (Anderson & May 1982; Vrijenhoek 1986). From an ecological perspective, a parasite does not only cause damage to the individual host by decreasing its fitness, but also have some impact on the entire host population (Anderson & May 1982). Indeed, parasitic angiosperms are known to have a major impact on growth, allometry and reproduction of hosts (Press 1998), which may

affect the balance between host and non-host species, leading to structural and compositional changes in the whole plant community and in the population dynamics of hosts (Smith & Reid 2000; Noetzli, Müller & Sieber 2003). Nonetheless, parasitic plants have also been associated with an increase in species diversity (Pennings & Callaway 1996; Press & Phoenix 2005), by suppressing dominance and helping to maintain subordinate species in some communities. These plants may be considered “keystone” species in some communities, since they mediate community interactions by host selection (Press & Phoenix 2005; Watson 2009), and alter the physical environment by potentially increasing nutrient cycling (Quested *et al.* 2003).

There are more than 4500 species of parasitic plants occurring worldwide (on every continent except Antarctica), and they occupy all distinct biomes on earth (Der & Nickrent 2008). Parasitic plants can be classified as either **holoparasites** (approximately 10% of the species), which are plants that lack chlorophyll and are exclusively dependant on both the xylem and the phloem of their hosts (e.g. *Cuscuta* species); or **hemiparasites**, which are plants that have functional photosynthetic leaves containing chlorophyll, and rely only on the xylem sap of their hosts (with intermediate cases also connecting to host’s phloem) (Stewart & Press 1990). Hemiparasites can be further divided into “root parasites”, when they are found attached to the roots of the hosts (e.g. *Santalum* species); or “mistletoes”, when they parasitise the branches or trunks of the hosts (e.g. *Amyema* species).

In this thesis, I focused on mistletoes, using their hosts to compare different sets of ecological and evolutionary strategies between the parasitic and non-parasitic lifestyles. Mistletoes are a polyphyletic group of aerial shoot hemiparasites from five clades in Santalales (Nickrent 2002), that together form the largest and most diverse functional group of parasitic plants (~50% of all parasitic plant species).

The haustorium

Mistletoes penetrate the bark of the hosts' branches connecting to their host xylem from which they access all the water and nutrients that they need to survive (Calder & Bernhardt 1983). The organ involved in the mistletoe-host connection is the 'haustorium', a modified root exclusive from parasitic angiosperms, which has functions of attaching to the host branch surface, invading the host vascular tissue, and establishing the continuum of mistletoe and host vascular system (Press & Graves 1995). Different connection types vary according to the species of the parasitic plant (Cameron & Seel 2007). For mistletoes specifically, direct transfer of host-derived solutes is very unlikely, since anatomical studies show there is not a direct connection between xylem cells of parasites and hosts (Calvin 1967; Dobbin & Kuijt 1974; Tennakoon, Pate & Arthur 1997). Moreover, there is strong evidence that the haustorium selectively absorb a range of host-derived solutes (Lamont 1983), such as various nitrogenous compounds (Pate, True & Rasins 1991; Pate 2001), organic acids, ions (Hibberd & Jeschke 2001) and carbohydrates (Richter & Popp 1987).

Differences between parasitic and non-parasitic angiosperms

Even though all plants need similar abiotic resources to grow, reproduce and survive – i.e., carbon, nutrients, water, and light – the functional traits of mistletoes are usually quite distinguishable from their hosts. The first remarkable difference can be found in the carbon economy. By having a modified root that taps into host tissues, mistletoes rely on the host's ability to acquire resources from the soil (i.e., water and nutrients) and thereby save on the carbon that would otherwise be allocated to build roots and trunks. However, this strategy has the intrinsic trade-off of lacking the control on root carbon allocation and storage (and possibility of symbiotic relationships with mycorrhizas, etc.), which can be particularly important in situations of water and nutrient limitations. Moreover, even though mistletoes

have functional leaves, they often show some level of heterotrophy, meaning that they obtain carbon directly from their hosts' xylem in the form of amino acids. In some cases more than 50%, but more commonly, around 20% of the carbon is known to be obtained heterotrophically, which also has consequences for their carbon balance (Marshall & Ehleringer 1990; Schulze *et al.* 1991; Marshall *et al.* 1994). The assimilation of amino acids would result in more enriched $\delta^{13}\text{C}$ signal in the mistletoe leaf because the carbon in the host xylem should be less negative than the $\delta^{13}\text{C}$ measured in the host leaves (Cernusak, Pate & Farquhar 2004).

The second remarkable difference between mistletoes and hosts relates to water use. In order to maintain the xylem flux gradient, mistletoes need to generate a more negative water potential in their shoots than their hosts. Indeed, mistletoes are known to exhibit faster transpiration and lower photosynthetic rates than hosts, leading to very low values of water use efficiency (WUE; the ratio of photosynthesis to transpiration) (Ehleringer, Cook & Tieszen 1986; Goldstein *et al.* 1989; Davidson & Pate 1992).

Finally, mistletoes also differ from hosts in terms of nutrient use. Because nutrients are transported in solution through the xylem, there is generally a close link between water and nutrient relations. Therefore, by achieving higher rates of transpiration, mistletoes also have a higher nutrient uptake *via* the haustorium and tend to show higher concentrations of macronutrients in their leaves, especially K, but also N, P, Ca and Mg (Lamont & Southall 1982; Glatzel 1983; Küppers 1992; Panvini & Eickmeier 1993). Furthermore, because mistletoes have limited physiological sinks, they can accumulate disproportional amounts of some nutrients in their leaves (Glatzel 1983; Glatzel & Geils 2009). In some situations, they might have to deal with unnecessary and undesirable nutrients, noticeably when growing on Al-accumulator hosts in tropical environments (Scalon, Haridasan & Franco 2013) or on halophytic species in mangroves (Goldstein *et al.* 1989; Orozco *et al.* 1990).

Most of these differences between parasitic and non-parasitic plants can be related to differences in the ecological strategies of use and acquisition of resources. The concept of ‘ecological strategy’ adopted here, as defined by Westoby (1998), expresses the opportunities and selective forces shaping the ecology of a plant species to sustain a population. Economic analogies are often used in plant ecology in order to help understanding the trade-offs that shape ecological strategies, and especially to describe the trade-offs inherent to physiological processes; i.e., to identify the costs, benefits and evolutionary constraints that underpin functional traits and traits relationships (Mooney & Gulmon 1982; Bloom, Chapin III & Mooney 1985; Wright *et al.* 2004; Prentice *et al.* 2014).

In this perspective, the costs to acquire a resource should be proportional to its availability at a given habitat. If the resource is limiting (i.e., the resource is expensive to acquire), the evolutionary pressure on autotrophic plants should cause a shift to use resources more efficiently. Water and nutrient limitations are common in most natural environments (Vitousek & Howarth 1991; Elser *et al.* 2007; LeBauer & Treseder 2008), so that the costs to obtain and maintain these resources in the plant can be considered high (Gutschick 1981). Strategies such as differential accumulation of nitrogen through the canopy (in parallel to vertical light gradients), nutrient resorption (control of leaf death time), longer leaf longevity, and investment in defence against herbivory are commonly found in nutrient limited environments (Aerts 1996; Eckstein, Karlsson & Weih 1999; Wright, Reich & Westoby 2001; Reich *et al.* 2003; Hikosaka 2004). Similarly, to optimize the water use in water limiting habitats, plants usually adopt some strategies such as strong stomatal control, leaf senescence during the dry season, higher investment in root structure, and leaf functional traits that permit more control over water lost.

For parasitic angiosperms, differences in resource acquisition are evident in relation to the hosts, and the unit-costs for water and nutrients are presumably very low compared to that

experienced by non-parasitic plants. However, little is known about trait adaptations of mistletoes facing shortage of different resources (e.g. in P-limited rather than N-limited environments). Moreover, it remains unexplored how the influence of lower resource costs affects the selective forces shaping the ecological strategies of mistletoes. Therefore, my research is aimed to fill knowledge gaps about the use and economy of water and nutrients in mistletoes.

Thesis outline

This thesis is presented as four different data chapters, each following the format of stand-alone manuscripts, book-ended with a relatively short general Introduction and general Discussion. Each chapter contains the relevant background and discussion, and they are described below in more detail, with major findings highlighted. It is important to emphasise that, for brevity, some chapters may refer to others throughout the text, especially in the Material and Methods section.

Chapter 2. A global analysis of water and nitrogen relationships between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses

In this chapter, I had two primary goals. Firstly, I aimed to test the generalities of the “mimicry hypothesis” and the “N-parasitism hypothesis”, which have both been proposed in published literature to explain mistletoe-host water and nutrient relations. Secondly, I investigated the influence of environmental conditions on leaf nitrogen concentration and leaf carbon isotopic signature in mistletoes and hosts. Understanding what drives mistletoe-host relations is fundamental to improving our knowledge of water and nitrogen use and economy in plants (mistletoes being an intriguing test-case). For this enterprise, I combined my own

field data with data from published literature on mistletoe leaf nitrogen concentration and leaf carbon isotopic signature. From my analysis across 168 mistletoes-host pairs distributed globally, we found no broad support for the N-parasitism hypothesis, but partial support for mimicry, with mimicry being associated with N-fixing hosts. Contrary to current belief, our findings suggest that nitrogen is not the limiting nutrient for mistletoes, at least not the main component driving the faster transpiration rates. Our results also give insight into the evolution of mimicry in mistletoes and show, for the first time, that mistletoes are also constrained by local water availability, exhibiting clear trait adaptations to environmental gradients.

Chapter 3. Photosynthetic trait adaptations of parasitic mistletoes and their hosts in sites of contrasting aridity

I sampled 42 mistletoe-host species pairs from five different sites with contrasting aridity located Brazil and Australia to investigate mistletoe and host photosynthetic trait responses to lower water availability. Based on previous work showing that mistletoes are profligate users of water (Ehleringer, Cook & Tieszen 1986; Marshall & Ehleringer 1990; Marshall, Dawson & Ehleringer 1994), but also could show close coordination with the stomatal behaviour of their hosts (Ullmann *et al.* 1985, Davidson & Pate 1992; Whittington & Sinclair 1988; Davidson, True & Pate 1989; Goldstein *et al.* 1989; Bowie & Ward 2004), we expected that mistletoes would exhibit some degree of adaptation to aridity, but noticeably dampened responses compared to that seen in hosts. Surprisingly, mistletoes showed tightly coupled responses with their hosts to environmental conditions, exhibiting trait response in parallel and to the same extent as host species to increasing aridity. Nevertheless, mistletoes were indeed profligate water users compared to their hosts as well as exhibited very high

respiration rates for a given photosynthetic capacity. In this chapter, I argue that P requirement, together with carbon, should be considered an alternative for the N-parasitism hypothesis, and I suggest that the low gross carbon gain could be the consequence of considerable costs associated with trade-off between mistletoes maintaining higher osmolarity to guarantee the continuous flow of the host xylem.

Chapter 4. Nutrient resorption in mistletoes from three low-P sites in Australia and Brazil

In this chapter, I investigated nutrient resorption patterns of macronutrients in parasitic mistletoes, across three different sites comprising 18 mistletoe-host species-pairs. I also looked at the relationship of resorption to leaf lifespan and herbivory rates. Previously it was reported (and become widely accepted) that mistletoes do not resorb nutrients from leaves before they are shed (Pate, True & Kuo 1991; Watson 2001; March & Watson 2010). Here, that was true for leaf N (averaging zero resorption), and also for Ca and Mg. However, mistletoes did generally resorb P and K, on average withdrawing ~30% and ~20% of nutrient while senescing, respectively. That said, mistletoes were still relatively inefficient in terms of nutrient resorption compared to non-mistletoes species, here and globally (which, on average, resorb 50% of N and 60% of P; Aerts 1996; Vergutz *et al.* 2012). Resorption efficiency was not strongly correlated with specific leaf area, but lower N and P concentration and lower N_{eff} and N_{sen} were related to longer leaf lifespan. Overall, the results suggest that P and K, rather than N, are the most notably limiting nutrients for mistletoes in these systems. Indeed, I suggest that the need to acquire sufficient P from host xylem may be a key driver of high transpiration rates in mistletoes in these systems (together with the need to acquire heterotrophic carbon), and that leaf K is used as an osmolyte to drive this process.

Chapter 5. Leaf lifespan, herbivory and leaf defence investment in mistletoes and their hosts

My first aim in Chapter 5 was to compare mistletoe and host investments in anti-herbivore defences and investigate chemical and physical defence trade-offs and syndromes. I used 35 mistletoes-host pairs across four different sites in Australia and Brazil and predicted that mistletoes would show lower investment in defence, by considering that general resources to construct a leaf would be less expensive for parasites compared to hosts. My second aim was to investigate the relationship between defence investment, leaf lifespan and their influence on herbivory rates, using 14 out of the initial 35 mistletoes-pairs. In this chapter, few of our initial expectations were supported and we found that mistletoes had generally higher carbon-based defence investment, and similar defence per unit of nitrogen. I also found evidence of tannins being transferred from hosts, while other classes of phenols and alkaloids were seemingly being excluded. There were differences in defence syndromes and trade-offs between mistletoes and hosts but, similarly, herbivory rates showed a negative relationship with leaf lifespan, in agreement with the hypothesis that long-lived leaves must be well defended. I also took the opportunity to investigate the evolutionary processes involved in the leaf morphological resemblance of Australian mistletoes and their hosts, with regards to anti-herbivory defence. Within Australian mistletoe-host pairs, I found a clear pattern of highly-mimic mistletoe species testing negative for alkaloids, while all associated hosts tested positive, which I interpreted as evidence of Batesian mimicry (i.e., the palatable mimic modelled the unpalatable host).

Chapter 6. General Discussion

Finally, in the last chapter I integrate the main findings of all data chapters and provide a general discussion of how my results relate to three key hypotheses in the literature: (1) the “leaf economics spectrum” (Wright *et al.* 2004); (2) the N-parasitism hypothesis (Schulze, Turner & Glatzel 1984); and (3) the mimicry hypothesis (Ehleringer *et al.* 1986). In addition, I identify a number of areas where mistletoe research still has the potential to generate key knowledge for functional ecology research.

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Chapter 2

A global analysis of water and nitrogen relationship between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses*

* Scalon, M. C., Wright, I. J. (2015) A global analysis of water and nitrogen relationships between mistletoes and their hosts: broad-scale tests of old and enduring hypotheses. *Functional Ecology*. doi: 10.1111/1365-2435.12418

Abstract

Mistletoes are known to use far more water per unit carbon fixed during photosynthesis than their hosts do (i.e., they have lower ‘water use efficiency’, WUE). The “nitrogen-parasitism hypothesis” posits that N is the most limiting resource for mistletoes, and that they use their faster transpiration rates to acquire sufficient N from the host xylem. In a rather different context, the “mimicry hypothesis” arose in the literature suggesting that some mistletoes mimic the morphology of host leaves in order to deploy higher-N leaves without suffering higher levels of herbivory, which would otherwise be expected. These two non-exclusive hypotheses share the common goal of trying to explain patterns of mistletoe leaf N concentration. We set out to test the generality of both hypotheses at broad geographic scale using data for 168 mistletoes-host pairs, from 39 sites, encompassing all continents except Antarctica. We drew together data from published literature and our own field data on two key plant functional traits that represent N content (leaf N concentration), and long-term WUE and degree of stomatal control (leaf carbon isotopic composition, $\delta^{13}\text{C}$). Key findings included (1) little support for the N-parasitism hypothesis: higher nitrogen was only marginally related to more similar $\delta^{13}\text{C}$ between hosts and mistletoes, and N-fixing hosts did not influence the difference in WUE; (2) partial support for the mimicry hypothesis: mimic mistletoes generally have greater N concentration when associated with N-fixing hosts. More broadly, our results suggest that mistletoes tend to show similar traits responses as their hosts to environmental drivers, such as water availability: they showed the same shifts in N_{mass} and $\delta^{13}\text{C}$ in relation to site precipitation as did their hosts, showing higher N_{mass} and WUE at drier sites. Contrary to current belief, our findings suggest that nitrogen is not the limiting nutrient for mistletoes, at least not the main component driving the faster transpiration rates. Our results also give insight into the evolution of mimicry in mistletoes and show, for the first time, that mistletoes are also constrained by local water availability, exhibiting clear trait adaptations to environmental gradients. By reconsidering

these issues at broad geographic scale and across a large number of species, our findings substantially modify current knowledge on the ecology and physiology of mistletoes and their hosts.

Key-words: carbon isotope, Loranthaceae, mimicry, N-parasitism, Santalaceae, Viscaceae, water use efficiency.

Introduction

Mistletoes are parasitic angiosperms that connect to the xylem of their host through a modified root system called a haustorium (Lamont & Southall 1982). Once this connection is established, the xylem solution flows from the host to the mistletoe, becoming its only source of water and nutrients (Calder & Bernhardt 1983; Press & Graves 1995). Being hemiparasites, mistletoes produce their own photosynthetically active leaves but, because there is no connection between the phloem of the two organisms, no photoassimilates are contributed back to the host (Glatzel & Geils 2009). Mistletoes are a widespread group, occurring on every continent in the world except Antarctica (Calder & Bernhardt 1983), and highest species diversity is found in the families Loranthaceae, with 73 genera and over 1500 species, and Santalaceae (formerly treated as the separate family Viscaceae), with 7 genera and over 450 species (Nickrent *et al.* 2010; Nickrent 2011).

Because mistletoes do not invest in a complex root system, the acquisition costs for water and nutrients are presumably far lower than those experienced by their hosts. Therefore, mistletoe-host interactions present a unique and intriguing study system to ecophysiologists interested in the water and nitrogen costs of photosynthesis (Schulze, Turner & Glatzel 1984; Ehleringer, Cook & Tieszen 1986; Orozco *et al.* 1990; Küppers 1992; Panvini & Eickmeier 1993; Marshall, Dawson & Ehleringer 1994; Bowie & Ward 2004). These costs are sometimes expressed as the ratios “water use efficiency” (WUE; ratio of photosynthetic rate to that of transpirational water loss) and “photosynthetic nitrogen use efficiency” (PNUE; ratio of ratio of photosynthesis to leaf N concentration) (Chapin *et al.* 1987; Evans 1989; Farquhar *et al.* 1989; Lambers, Chapin & Pons 1998).

Carbon isotope discrimination ($\delta^{13}\text{C}$) in leaf dry matter, reflecting discrimination against ^{13}C by Rubisco and PEP-carboxylase during photosynthesis, is used as a long-term estimator of $c_i:c_a$ ratio (ratio of leaf-internal to ambient CO_2 ; (Farquhar *et al.* 1989). Under a

given atmospheric humidity, lower ci:ca (higher $\delta^{13}\text{C}$), equates to higher WUE. Even under extreme drought condition, mistletoes generally show faster transpiration rates and far lower $\delta^{13}\text{C}$ than their hosts, and thus far lower WUE (Schulze, Turner & Glatzel 1984; Ullmann *et al.* 1985; Marshall *et al.* 1994; Escher *et al.* 2004; Escher *et al.* 2008; Glatzel & Geils 2009). Noting this profligate water use, teamed with lower leaf N concentrations than their hosts but very high accumulations of mobile cations such as K^+ and Ca^{2+} (Glatzel 1983; Schulze & Ehleringer 1984), Schulze *et al.* (1984) proposed the “**nitrogen-parasitism hypothesis**”, positing that nitrogen limitation is the key driver for rapid transpiration in mistletoes. Evidence in favour of this hypothesis includes enhanced mistletoe performance when growing on hosts with higher N concentration in the xylem, whether due to fertilizer application or to having N-fixing root symbionts; e.g. there have been reports of mistletoes showing less negative $\delta^{13}\text{C}$ signatures (Ehleringer, Cook & Tieszen 1986; Bannister & Strong 2001a), higher biomass, higher flower production (Schulze & Ehleringer 1984; Gibson & Watkinson 1989; Seel, Cooper & Press 1993) and lower herbivory rates (Adler 2002).

A contrasting – but not mutually-exclusive – hypothesis suggests that higher transpiration rates in mistletoes may be driven not only by the need of N, but also by the ability to acquire large amounts of carbon via the host xylem (“heterotrophic” carbon), in the form of amino acids (Marshall & Ehleringer 1990; Stewart & Press 1990; Schulze *et al.* 1991; Marshall *et al.* 1994). Estimates of how important this external source of carbon is to mistletoes vary widely. Early reports suggested that up to 60 % of C in the mistletoe *Phoradendron juniper* came via this pathway (Marshall & Ehleringer 1990), and around 50-70 % of C in five mistletoe species from Namib desert (Schulze *et al.* 1991). Subsequent reports – based on more and different species – suggested that heterotrophic carbon gain might be highly variable, ranging from 5-21 % (Marshall *et al.* 1994) in 11 mistletoes-host pairs from eastern Australia; 50-80 % (Richter *et al.* 1995) in 10 pairs from Namibia; and

from 35-78 % (Wang *et al.* 2008) in 3 pairs along the Kalahari Transect. In any case these estimates should be considered somewhat tentative, since they are based on differences in $\delta^{13}\text{C}$ between mistletoes and hosts, which presupposes that they are operating at a very different ci:ca. Certainly, this secondary source of carbon potentially has an impact on the mistletoe carbon isotope signature (Schulze *et al.* 1991).

A rather separate literature has focused on how similar or different mistletoes are from their hosts in terms of their leaf N concentration (N_{mass} hereafter). On the one hand, higher N_{mass} is generally associated with greater photosynthetic capacity; on the other, higher N_{mass} should, all else equal, make leaf tissue more attractive to herbivores (Mattson 1980; Mooney & Gulmon 1982; Marvier 1996). Considering these issues, and the remarkable resemblance between the leaves of many Australian mistletoes and their hosts (especially *Eucalyptus*, *Acacia* and *Casuarina* hosts), Barlow and Wiens (1977) described the “**mimicry hypothesis**”. Barlow & Wiens argued that mistletoes that mimic their hosts (“mimics”) can get away with having higher N_{mass} than their hosts without suffering serious herbivory, since – for larger herbivores, at least – they will not stand out as being different. By contrast, “non-mimic” species by definition do stand out visually; therefore the best strategy to avoid serious herbivory in that case would be to have similar or lower N_{mass} than their hosts. This hypothesis has broad-scale empirical support from mistletoe-hosts pairs measured in both Australia (Ehleringer *et al.* 1986b) and New Zealand (Bannister 1989). One concern with this hypothesis is what type of herbivores could be responsible. For example, in Australia various species of possums are known to eat leaves of both mistletoes and hosts; whereas in New Zealand there are no large vertebrate herbivores to explain this pattern (Bannister *et al.* 1989). Other concerns include whether mimicry really has any fitness benefit for the mistletoe (Canyon & Hill 1997; Schaefer & Ruxton 2009), or even whether mimicry truly exists (Blick, Burns & Moles 2012).

In this study, we revisited the nitrogen-parasitism and the mimicry hypotheses using a global dataset of leaf N and $\delta^{13}\text{C}$ discrimination data compiled from the literature and supplemented with new data from several sites in Australia. We investigated a range of issues related to N and water deployment/use, along the way testing several specific hypotheses (see below). We tested for generality (or otherwise) of trait relationships reported previously in regional analyses, considering the influence of site climate, and the extent to which observed trends (in N_{mass} and $\delta^{13}\text{C}$) were related to mistletoe taxonomy (family). Specific hypotheses and questions were as follows:

1. In relation to the N-parasitism hypothesis (Schulze *et al.* 1984; Ehleringer *et al.* 1986):
 - I. Is it globally true that mistletoes have more negative $\delta^{13}\text{C}$ than their hosts, implying that they operate at higher $c_i:c_a$ and have lower WUE?
 - II. Are host and mistletoe $\delta^{13}\text{C}$ positively correlated? This was previously reported by Bannister and Strong (2001a), presumably because heterotrophic carbon gain from the host influences $\delta^{13}\text{C}$ signature in the mistletoe (Ziegler 1995), but also because mistletoes and hosts might respond similarly to climatic variations (see also question 3).
 - III. Are host and mistletoe N_{mass} positively correlated? e.g., because the N concentration in the xylem sap is higher on hosts with higher N_{mass} (Schulze *et al.* 1991; Bannister & Strong 2001b; Wang *et al.* 2008).
 - IV. Is it generally the case that differences between mistletoes and their hosts in $\delta^{13}\text{C}$ are smaller on N-fixing hosts, or on hosts with higher N_{mass} (Ehleringer *et al.* 1985; Schulze *et al.* 1991; Marshall *et al.* 1994; Richter *et al.* 1995; Bannister & Strong 2001b)? These are situations where N concentration in the xylem sap of the host is presumed to be higher, with direct evidence that interspecific variation in leaf N is tightly correlated with variation in xylem sap N concentration (Stewart *et al.* 1992; Schmidt *et al.* 1998).

2. In relation to the mimicry hypothesis (Barlow & Wiens 1977; Ehleringer *et al.* 1986b; Bannister 1989):
 - I. Do host-mimic mistletoes have higher N_{mass} than their hosts, while non-mimic mistletoes show similar or lower (i.e. *not* higher) N_{mass} than their hosts?
3. Broader questions in relation to phylogeny and environmental influences:
 - I. Is there patterning in N_{mass} and $\delta^{13}\text{C}$ relationships of mistletoes and hosts in relation to mistletoe family (Loranthaceae vs Viscaceae)? Differences between families have been suggested by different authors, such as Shaw *et al.* (2004) and Aukema (2003), where Viscaceae mistletoes were suggested to have larger impacts on hosts compared to Loranthaceae mistletoes.
 - II. Does the difference in $\delta^{13}\text{C}$ between mistletoes and hosts vary according to site climate? In particular, is the difference greater at more arid sites? As suggested by Bannister & Strong (2001), in arid sites there should be stronger pressure on hosts to use water efficiently, but somewhat less pressure on mistletoes.
 - III. Do mistletoes show the same trend in leaf $\delta^{13}\text{C}$ and N_{mass} in relation to site aridity as do their hosts (and other species)? Or, do mistletoes show a dampened trend? e.g. because of weaker selective pressure to be efficient in their photosynthetic water use.

Material and Methods

Leaf N concentration and carbon isotope signature data from mistletoes and their hosts (“M-H pairs” hereafter) were compiled from the literature (135 different M-H pairs from 23 published papers), to which we added data from our own sites in Australia (33 M-H pairs), yielding a dataset comprising 168 M-H pairs from 39 sites (Table S1). When a given mistletoe species was reported growing on several different host species, each instance was considered a different M-H pair. The majority of the pairs were sampled in Australia (43.5

%), New Zealand (17.8 %) and United States (8.9 %). Eleven countries contributed the remaining 30 % of data (Fig. 1). Loranthaceae was the best represented mistletoe family (141 pairs), Viscaceae contributing the other 27. The best represented host family was Fabaceae, accounting for 45 pairs. We only had C isotope data for 93 of the 168 M-H pairs, of which 84 % included a Loranthaceous mistletoe and 16 % a Viscaceous mistletoe.

We also recorded site latitude and longitude, biome type, whether the host was a N-fixing species, and whether mistletoes were considered host-mimics by the authors or by Barlow and Wines (1977). An approximate latitude and longitude were derived from the written description of site location in cases when precise information on the geographical coordinates was not available in the original paper. Geographical coordinates were used to retrieve the mean annual temperature (MAT) and mean annual precipitation (MAP) from the CRU CL2.0 global climate dataset (New *et al.* 2002). Potential evapotranspiration (PET) was estimated following Wang, Prentice and Ni (2012) and moisture index was calculated as the ratio between MAP and PET (Table S2).

To the literature data we added information on M-H pairs that were sampled at four Australia locations between 2011-2013 (Table S1). Three fully-expanded sun leaves were collected from at least three different individuals per species, oven-dried at 60 °C for 72 hours, and finely ground in preparation for chemical analyses. For the $\delta^{13}\text{C}$ determination, leaves from the same species were bulked and analysed at the Mass Spectrometry Facility at the Australian National University, Canberra. For nitrogen analysis, individual samples analysed by LECO TruSpec CHN combustion technique at the Analytical Service Unit from the School of Agriculture and Food Science at The University of Queensland.

Data Analyses

Data for leaf N concentration (N_{mass} ; mg of N per g dry leaf mass) and climate variables were log-transformed to meet assumptions of normality (Shapiro-Wilk test of normality, $P > 0.1$). While paired t-tests are an appropriate statistic for quantifying mean differences between mistletoes and their hosts (which are intrinsically paired), this approach is limited to testing for differences in just one factor at a time. Therefore, we also used linear mixed effect analysis to compare the different aspects of our dataset simultaneously and to assess the relative importance of potential predictors of the *difference* in N concentration between mistletoes and hosts, and the *difference* in $\delta^{13}\text{C}$ isotope composition between mistletoes and hosts. Arithmetic differences in these properties were calculated in all cases as trait (mistletoe) – trait (host). As fixed-effects we considered the environmental factors (mean annual precipitation and temperature), the family of the mistletoe (Viscaceae or Loranthaceae), mimicry (yes or no), and nitrogen-fixing host (yes or no). Study location and the family of the host were treated as random effects.

Standardized major axis (SMA) slopes (Warton *et al.* 2006) were used to compare the best-fit proportional relationship of traits between mistletoes and hosts. Pearson correlation and ordinary least square (OLS) regression were used for quantifying relationships between N and $\delta^{13}\text{C}$ with climate (climate being the independent variables). All statistical analyses were performed using R software v. 2.13 (R Core Team). The package lme4 (Bates, Maechler & Bolker, 2012) was used for the linear mixed model analyses and SMATR v. 3 package (Warton *et al.* 2012) was used to test for differences between SMA slopes.

Results

Mistletoes typically showed lower $\delta^{13}\text{C}$ than their hosts (host mean \pm sd: -27.5 ± 2.7 ‰, mistletoe mean \pm sd: -29.5 ± 2.2 ‰; paired t-test: $P < 0.001$, $n = 93$), implying that mistletoes

typically operate at higher $c_i : c_a$ ratios (i.e., they are less water use efficient). Mistletoe and host carbon isotope signatures were positively correlated ($r^2 = 0.39$, $P < 0.001$), with a fitted slope not significantly different from 1 (SMA slope = 1.01, 95 % CIs = 0.86-1.19; Fig. 2a).

Overall there was no difference between mistletoe and host N_{mass} (host mean \pm sd: $16.1 \pm 6.6 \text{ mg.g}^{-1}$, mistletoe mean \pm sd: $16.3 \pm 8.8 \text{ mg.g}^{-1}$; paired t-test: $P = 0.298$, $n = 168$); and leaf N concentration of mistletoes strongly reflected that of their hosts ($r^2 = 0.35$, $P < 0.001$; Figure 2b). This relationship had a slope slightly steeper than 1 (SMA slope = 1.25, 95 % CIs = 1.10-1.41; $P < 0.001$, Fig. 2b).

According to the N-parasitism hypothesis, the lower the N concentration in the host xylem the more water mistletoes will need to transpire in order to fulfil their nitrogen requirements. By extension, a higher N_{mass} in the host leaves is expected to reflect smaller difference between mistletoe and host $\delta^{13}\text{C}$. We found only a marginally significant positive relationship between M-H differences in $\delta^{13}\text{C}$ and M-H differences in N_{mass} ($r^2 = 0.03$, $P = 0.08$, Fig. 3a). In addition, there was no patterning in M-H differences in carbon isotope signature in relation to whether the hosts were nitrogen fixers or not (Fig. 3b; $P = 0.49$).

There was a positive relationship between $\delta^{13}\text{C}$ and N_{mass} both in mistletoes ($r^2 = 0.24$, $P < 0.001$) and in hosts ($r^2 = 0.08$, $P < 0.01$; Fig. 4), meaning the lower the N_{mass} the lower the water use efficiency (more negative $\delta^{13}\text{C}$). The mistletoe-specific and host-specific relationships did not differ in slope ($P = 0.256$) but they were significantly offset ($P < 0.001$) such that, at a given N_{mass} , mistletoes had ca. 1.5 ‰ more negative $\delta^{13}\text{C}$ than their hosts.

Next, we tested predictions from the mimicry hypothesis. Overall there was no difference in N_{mass} between mimic and non-mimic mistletoes (mimics, mean \pm sd = $15.47 \pm 8.35 \text{ mg.g}^{-1}$, $n = 50$; non-mimics, mean \pm sd = $16.68 \pm 8.98 \text{ mg.g}^{-1}$, $n = 118$; $P = 0.40$). However, the family of the mistletoe together with the interaction between mimicry and N-fixing status of the host accounted for 28% of the variance found in M-H differences in leaf N

concentration ($r^2 = 0.28$, all $P < 0.001$, Table 1). The positive interaction between mimicry and N-fixing host showed that mistletoes considered mimics and parasitizing N-fixing hosts did indeed have higher N_{mass} than their hosts (ANOVA, $F_{1,163} = 7.86$, $P = 0.005$; in support of the mimicry hypothesis), whereas this was not the case for mimics growing on non-fixing hosts (Fig. 5). Consequently, neither mimicry (yes/no) nor host N-fixing status (yes/no) alone explained significant variation in M-H differences in N_{mass} . By contrast, mistletoe family did explain significant variation in M-H differences in N_{mass} : on average Viscaceae mistletoes had higher N_{mass} than their hosts (paired t-test, $P < 0.01$), while Loranthaceae mistletoes showed similar N_{mass} than their hosts (paired t-test, $P = 0.061$).

Table 1. Results of linear mixed effects models estimating effects of mimicry, nitrogen-fixing host, environmental aspects and family between mistletoe and host on (a) nitrogen leaf concentration difference; and (b) carbon isotopic composition difference. For each model, the sum of squares and *F*-values are shown. Significant non-zero slope estimates are highlighted ($P < 0.001$).

Variable	Predictor	Coefficient	<i>P</i>	<i>F</i>	d.f.	<i>r</i> ²
N _{mass} difference	General model		<0.001	8.72	6, 134	0.28
	Intercept	-3.92	0.03			
	Mimicry	1.35	0.34			
	N-fixing host	1.77	0.24			
	Mimicry* N-fixing host	6.58	<0.001			
	MAT	0.05	0.61			
	Precipitation	0.00	0.62			
	Family	10.10	<0.001			
δ ¹³ C difference	General model		<0.001	5.93	7, 84	0.33
	Intercept	0.20	0.73			
	N _{mass} difference	0.01	0.81			
	Mimicry	0.43	0.32			
	N-fixing host	0.64	0.22			
	Mimicry* N-fixing host	0.38	0.68			
	MAT	-0.18	<0.001			
	Precipitation	0.00	0.15			
	Family	0.59	0.28			

MAT: Mean annual temperature.

Environmental effects

As expected, host plants showed less negative $\delta^{13}\text{C}$ (higher WUE) at drier sites but, interestingly, the same was clearly true of mistletoes (trends in relation to precipitation shown in Fig. 6a, and in relation to site moisture index in Fig. 6b). Because of the similarity in mistletoe and host relationship slopes, site aridity did not explain significant variation in M-H differences in $\delta^{13}\text{C}$ (e.g. see mixed model results incorporating all effects in Table 1). Both species groups showed a weak but significant tendency for higher N_{mass} at drier sites (mistletoes: $r^2 = 0.07$; host: $r^2 = 0.13$; both $P < 0.01$, Fig. 6c).

Unexpectedly, we found that MAT explained 24 % of the M-H differences variation in $\delta^{13}\text{C}$ (Fig. 7a), and this effect was still highly significant when all other effects were accounted for (mixed model results; Table 1). Specifically, mistletoes and hosts did not on average differ in $\delta^{13}\text{C}$ (and thus WUE) at cold sites, while at warmer sites mistletoes were increasingly more profligate in water use than their hosts (they had lower WUE). Fig. 7b illustrates that this result was caused mainly by a response to MAT in mistletoes and not in hosts: mistletoes $\delta^{13}\text{C}$ decreased while $\delta^{13}\text{C}$ in hosts was relatively constant across the temperature gradient.

Discussion

To summarise the main results, we found support for lower $\delta^{13}\text{C}$ in mistletoes relative to their hosts, suggesting that they operate at lower $c_i:c_a$ (and are therefore less water use efficient), except at colder sites. We also showed that N_{mass} in mistletoe and host are positively correlated, and the same was found in relation to $\delta^{13}\text{C}$, suggesting coupled carbon and N metabolisms. Our results provided little support for the N parasitism hypothesis: more nitrogen in hosts (i.e., N-fixing hosts and higher N_{mass}) was not related to more similar WUE between hosts and mistletoes. However, we found clear support for the mimicry hypothesis

considering N-fixing hosts: mimic mistletoes had higher N_{mass} than their hosts, whereas non-mimic species did not differ. In contrast, no support for the mimicry hypothesis was found when considering non N-fixing hosts (or, indeed, when considering all species together). More broadly, we could see significant differences between the two mistletoe families (Viscaceae mistletoes showed higher N_{mass} , than their hosts, while Loranthaceae showed marginally lower N_{mass} than their hosts). We also found similar shifts of N_{mass} and $\delta^{13}\text{C}$ to site aridity in mistletoes and hosts, but differences in the patterning of $\delta^{13}\text{C}$ to site temperature.

Water use efficiency and the N-parasitism hypothesis

Ehleringer et al. (1985) showed that, across species sampled from three continents, M-H differences in $\delta^{13}\text{C}$ were smaller on hosts with higher leaf N concentration (N_{mass}). These authors argued that this constituted strong evidence in support of the N-parasitism hypothesis, reasoning that, given sufficient access to host N in the xylem stream, there would be less advantage to mistletoes having markedly lower WUE. Here we took a different approach to testing the hypothesis, considering individual pairs of mistletoe and host rather than using an average value for each continent, and we did not find the same strong pattern (Fig. 3a). Moreover, we showed that M-H differences in $\delta^{13}\text{C}$ are no lower on N-fixing hosts than on non-fixing hosts, suggesting that higher N in the host xylem does not seemingly influence WUE in mistletoes (Fig. 3b). At best, we found very weak support for this contention, with host N_{mass} explaining just 8% of variation in M-H differences in $\delta^{13}\text{C}$ (Fig. 3a).

One could interpret the positive relationship between N concentration and $\delta^{13}\text{C}$ in mistletoes (Fig. 4) as the outcome of a strategy to extract more nitrogen from hosts by maintaining a steep differential in xylem water pressure (via keeping the stomata open), which would support the nitrogen-parasitism hypothesis. However, a similarly positive slope was found for the relationship in hosts, suggesting that there is little difference between

mistletoe and host water and N use behaviour in this regard. For instance, N_{mass} is known to be positively correlated with $\delta^{13}\text{C}$ (Högberg, Johannisson & Hällgren 1993; Guehl, Fort & Ferhi 1995; Sparks & Ehleringer 1997) because of the strong influence of nitrogen on photosynthetic capacity (Evans 1989), and the negative correlation (all else being equal) between photosynthesis with intercellular CO_2 concentration (Farquhar, Ehleringer & Hubick 1989; Sparks & Ehleringer 1997). In summary, we found little support for the N-parasitism hypothesis, although it was indeed true that mistletoes operated with lower WUE.

One explanation for this lower intercept value (lower WUE) in the regression between $\delta^{13}\text{C}$ and N_{mass} in mistletoes (Fig. 4) could be that mistletoes usually develop inside the canopy of the hosts, so that the average light availability and the microclimate they experience are different (Watson 2001; Cooney, Watson & Young 2006), in turn influencing intercellular CO_2 concentration ($c_i : c_a$). Indeed, several studies indicate that leaf $\delta^{13}\text{C}$ can vary with canopy position, becoming more negative as leaves become more shaded (Medina & Minchin 1980; Francey *et al.* 1985; Ehleringer *et al.* 1986a). Da Silveira *et al.* (1989) found that the difference in $\delta^{13}\text{C}$ values between plants grown in the sun treatment and in forest shade was over 6‰, while Ehleringer *et al.* (1986a) found values from 2.8 to 4.2‰ lower in understory leaf samples compared to canopy leaves. If leaves collected from mistletoes for analyses were formed in the shade, and leaves from the host in the sun, there is the possibility that the pattern we found may be a result of different environmental conditions during leaf development.

The carbon parasitism hypothesis (Marshall & Ehleringer 1990; Schulze *et al.* 1991; Marshall *et al.* 1994) also does not help to explain why mistletoes exhibit such a low $\delta^{13}\text{C}$ signal compared to their hosts. The carbon retrieved from the host xylem is expected to be less negative than the $\delta^{13}\text{C}$ measured in the host leaves, because structural carbon from dry matter in leaves shows higher discrimination compared to the xylem sap (Evans *et al.* 1986;

Cernusak, Pate & Farquhar 2002; Keitel *et al.* 2003), and heterotrophic tissues are ^{13}C -enriched compared to leaves (Cernusak *et al.* 2009). Therefore, the higher assimilation of amino acids from the host xylem should result in more enriched $\delta^{13}\text{C}$ signal (*less negative*) in the mistletoe leaf (Cernusak, Pate & Farquhar 2004). For example, holoparasitic plants, which derive all their carbon from the host, exhibit a $\delta^{13}\text{C}$ signal 1.0 to 1.5‰ less negative than their hosts (Cernusak, Pate & Farquhar 2004). Therefore, for mistletoes, if it were possible to measure the $\delta^{13}\text{C}$ of photosynthetic carbon only (i.e., not including any carbon from the host) then these values should be even more negative than the $\delta^{13}\text{C}$ signatures of the observed (combined heterotrophic and autotrophic) carbon. In addition, the current models used to calculate heterotrophy in mistletoes are still rather untrustworthy, yielding unrealistic values when mistletoes have similar or higher $\delta^{13}\text{C}$ compared to their hosts (Bannister & Strong 2001a; Tennakoon, Chak & Bolin 2011). In order to fully understand all the mechanisms underlying mistletoe carbon balance and water use we need better models or approaches to verifying the extent to which heterotrophic carbon gain helps to explain mistletoe carbon isotopic signature.

Mimicry hypothesis

Overall, host-mimic mistletoes in this study did not show higher N_{mass} in relation to their hosts, compared to differences seen for non-mimic mistletoes. However, when mimicry was considered together with nitrogen-fixing ability of the host, we found a significant effect on the host-mistletoe N_{mass} difference. N-fixing host alone was not a significant factor, nor the mimicry, but only the interaction between the two factors (Table 1, Fig. 5), suggesting that the positive interaction between mimicry and N-fixing hosts might be a result of a combined additive effect. Host-mimic mistletoes parasitizing non-N-fixing hosts do not show the same trend, perhaps due to limiting N concentration in the host xylem (Fig. 5). Considering optimal

defence theory (McKey 1974; Rosenthal, Janzen & Applebaum 1979), it is reasonable to assume that higher N concentration in mistletoe leaves relative to the surrounding vegetation will increase their attractiveness to herbivores, leading to a greater selective advantage for investing on herbivore avoidance strategies. Coincidentally, N-fixing plants usually do have higher amounts of N-based toxic defences, such as alkaloids, cyanogenic glycosides, metal-binding factors and protease inhibitors (McKey 1974; Mattson 1980; Johnson, Liu & Bentley 1987; Møller 2010). There is the possibility that mistletoes on N-fixing hosts could also accumulate N-based defences from the hosts, and the evolution of mimicry could be favoured in these situations where the presence of an N-fixing host affords the luxury of have higher N concentration compared to the hosts.

It is important to recognise that there is some confusion in the literature regarding the application of the terms “mimicry” and “crypsis” in cases of mistletoe and host leaf resemblance (Vane-Wright 1980). If herbivores are searching exclusively for mistletoe leaves but are deceived because they are indistinguishable from host leaves, it is a case of protective crypsis (Endler 1981). Protective crypsis implies that the mistletoe should have traits that otherwise would make their leaves more attractive to herbivores, such as higher N_{mass} (as a proxy for higher leaf palatability). However, if herbivores already actively avoid leaves from a specific host, mistletoes would benefit from being morphologically similar to the host leaves, and it would consist an example of Batesian mimicry (Vane-Wright 1980). In this case, mistletoes would not necessarily have higher N_{mass} , but their hosts should have lower palatability traits (or higher chemical and physical defences) compared to the surrounding vegetation. Further investigation into herbivory rates and investment in chemical and physical defences are needed to determine if there is support for this hypothesis, which would help to explain the evolution of leaf morphological resemblance in mistletoes and hosts.

Family differences between mistletoe traits

Differences in N concentration between mistletoes and host leaves could also be explained by inherent differences between the two distinct families (Viscaceae and Loranthaceae, Table 1). For instance, Viscaceae species in this study tended to show higher N_{mass} compared to Loranthaceae species. Loranthaceae family originated in the South Hemisphere, between fragments of Gondwana (Raven & Axelrod 1974), and is mainly distributed in the tropical region (Geils et al. 2002) whereas Viscaceae is thought to have originated in East Asia and radiated through Laurasia, occurring mostly in tropical and temperate zones of the Northern Hemisphere (Geils, Cibrián Tovar & Moody 2002). Although Viscaceae and Loranthaceae were considered closely related families and even classified as a single family in the past (Engler & Krause 1935), there are significant differences between them (Kuijt 1969; Polhill & Wiens 1998). More recently, the two families are considered to have evolved parasitism independently and are classified as non-sister taxa (Nickrent *et al.* 2010; Nickrent 2011).

All Viscaceae mistletoes have small flowers, are connected by a single haustorium and have pale-green leaves, while Loranthaceae mistletoes almost exclusively have large, colourful flowers, can develop multiple connections by epicortical roots and are highly varied in leaf colour (Nickrent 2011). Implicit differences in the evolutionary history between the two families might be reflected not only in these morphological traits, but also in their physiology and the resource exploitation strategy of their hosts. The higher N_{mass} in Viscaceae suggest that this family might indeed have a greater impact on the host, as suggested in previous studies (Aukema 2003; Shaw, Watson & Mathiasen 2004), though the specific mechanism remains to be clarified.

Environmental factors affecting host-mistletoe leaf traits

Increasing aridity was correlated with higher $\delta^{13}\text{C}$ signature (Figs 6a, 6b) and higher leaf N_{mass} (Fig. 6c) for both mistletoes and hosts. The tendency for higher aridity to be associated with less negative $\delta^{13}\text{C}$ in non-parasitic, C_3 plants has been demonstrated in many studies, both regionally and globally (Stewart *et al.* 1995; Weiguo *et al.* 2005; Diefendorf *et al.* 2010; Hartman & Danin 2010; Prentice *et al.* 2011; Ma *et al.* 2012). Plants from more arid climates also tend to have higher leaf N per unit area (Wright, Reich & Westoby 2003; Wright *et al.* 2005; Prentice *et al.* 2011). Mistletoes also become more conservative in their water use as aridity increases (Fig. 6b), suggesting that they are not only capable of adjusting some of the physiological traits to couple with their hosts' characteristics (Figs 1a, 1b), but also respond similarly to environment differences in water availability (Fig. 6).

Unexpectedly, M-H differences in leaf $\delta^{13}\text{C}$ were negatively correlated with mean annual temperature (Table 1; Fig. 7a) with a mean difference of *ca.* 3 ‰ at sites with MAT of 25 °C but no mean difference at sites with MAT of *ca.* 5 °C. Neither of the underlying trends (i.e. in mistletoes or hosts) was as consistent as the combined trend; still, it was clear that the trend in M-H differences was largely driven by that in mistletoes, there being no relationship between leaf $\delta^{13}\text{C}$ and MAT in host plants (Fig. 7b). The trend in mistletoes, indicating lower average ci:ca at colder sites, is consistent with the predicted and then observed trend seen in non-mistletoe species along a temperature gradient in eastern Australia (Prentice *et al.* 2014; but see Diefendorf *et al.* 2010), where the prediction of lower ci:ca at colder sites was mainly due to the effect of temperature on Rubisco kinetics. Why this was seen here in mistletoes but not hosts is unknown, as is the overall significance of this trend in M-H differences in $\delta^{13}\text{C}$ with respect to site temperature. What we can say is that this result was still clearly observed when variation in a wide variety of other factors (of both hosts and mistletoes) was simultaneously accounted for (Table 1).

Conclusion

We found little support for the N-parasitism hypothesis and partial support for the mimicry hypothesis in a global context. Mistletoes considered to be mimics and occurring on N-fixing host had higher N concentrations compared to the host, suggesting that the evolution of mimicry in mistletoes could be associated with higher N availability in the hosts. We also found that N_{mass} is patterned with respect to different taxonomic groups, with Viscaceae showing higher N_{mass} than hosts compared to the tropical Loranthaceae mistletoes. Our study shows, for the first time, that mistletoes and hosts have similar responses to precipitation and moisture index gradients considering water and nitrogen use in a global context, but also respond differently in terms of a temperature gradient.

Acknowledgements

We acknowledge CNPq for financial support. We thank Allyson Eller, Julieta Garcia-Russell, Julia Cooke and Fabricius Domingos for the valuable help in the field; and Vincent Maire and Emma F. Gray for help with the analyses. We thank Wright Lab members and Lucas Cernusak for comments on various earlier versions of the manuscript.

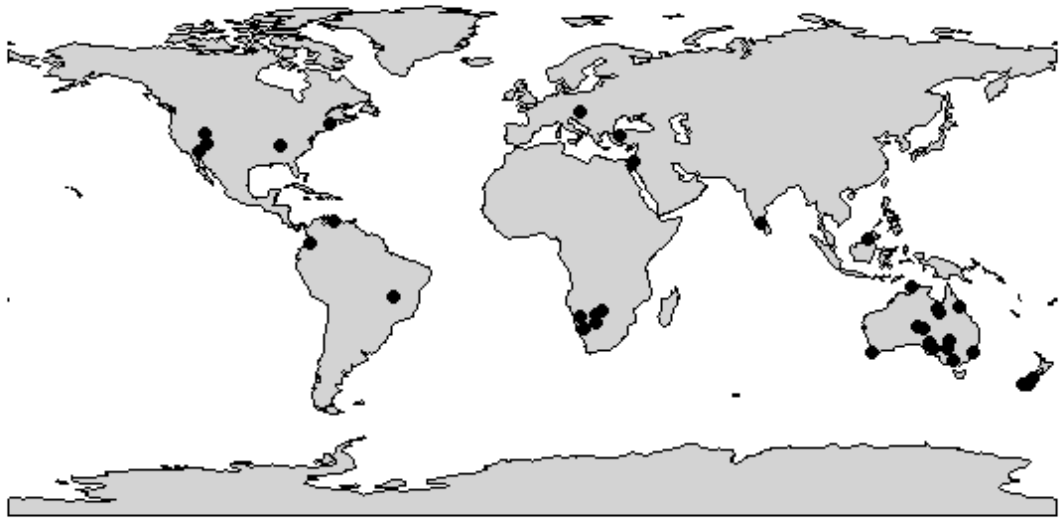


Figure 1. The distribution of the mistletoe-host pairs data globally.

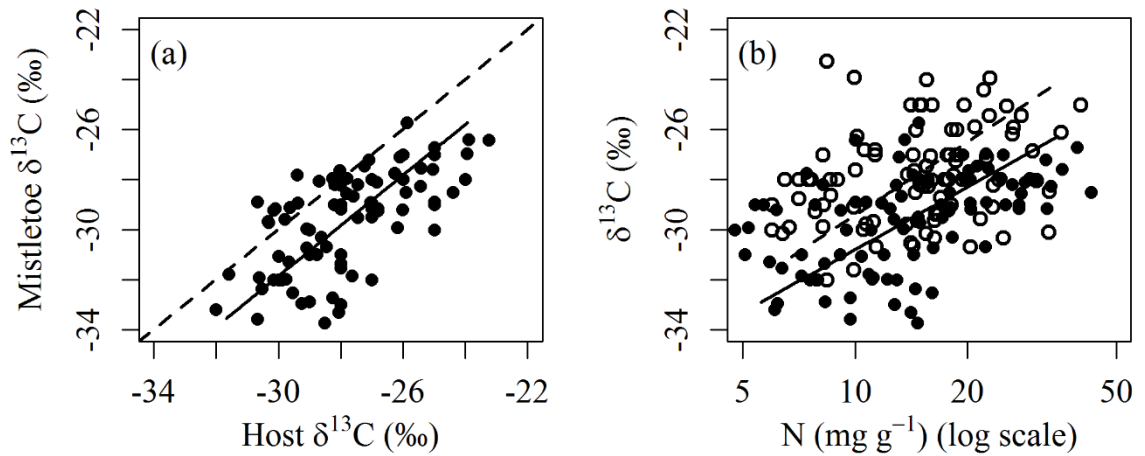


Figure 2. Positive relationship between mistletoes and hosts across different mistletoes-pair species reported on the literature and our own data for (a) carbon isotope discrimination (slope (95% confidence intervals) = 1.01 (0.86, 1.19); $r^2 = 0.39$, $P < 0.0001$); and (b) leaf N concentration (slope (95% confidence intervals) = 1.25 (1.10, 1.41); $r^2 = 0.35$, $P < 0.0001$). The dashed line corresponds to the 1:1 relationship and the solid line represents the fitted line based on the SMA values.

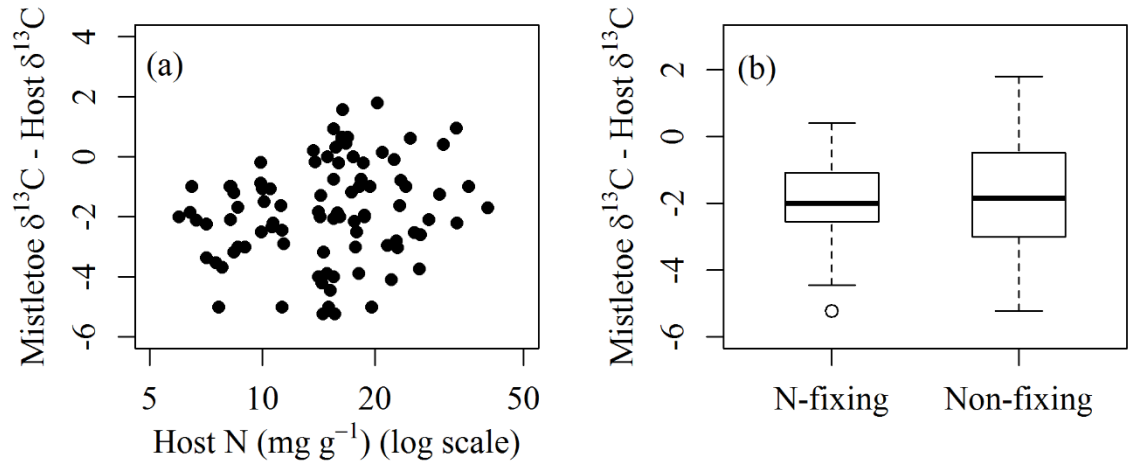


Figure 3. (a) Mistletoe to host difference in $\delta^{13}\text{C}$ in relation to host leaf N concentration ($r^2 = 0.03$, $P = 0.08$); and (b) pair-wise comparison between mistletoe to host difference in $\delta^{13}\text{C}$ in N-fixing ($n = 19$) and non-fixing hosts ($n = 71$; $t = -0.69$, $P = 0.49$).

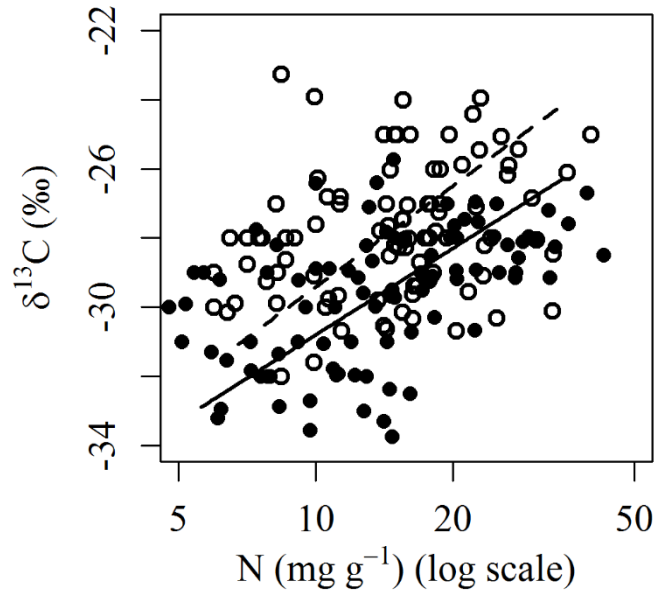


Figure 4. Standardized major axis (SMA) relationship between $\delta^{13}\text{C}$ and leaf N concentration for mistletoes (filled symbols, solid line; slope (95% confidence intervals) = 0.83 (0.79, 1.00); $r^2 = 0.24$, $P < 0.001$) and hosts (empty symbols, dashed line; slope (95% confidence intervals) = 0.97 (0.80, 1.19); $r^2 = 0.08$, $P = 0.004$). Common slope (95% confidence intervals) $\beta = 0.89$ (0.71, 1.02).

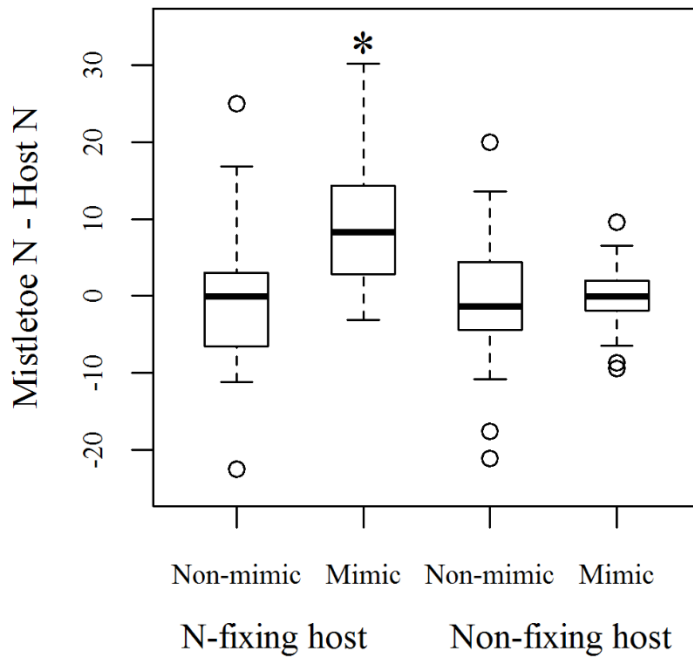


Figure 5. Pair-wise comparison between mistletoe to host difference in N concentration in mimic ($n = 50$) and non-mimic ($n = 118$) mistletoes growing on N-fixing ($n = 48$) and non-fixing hosts ($n = 119$). The continuous line within the box shows the median, error bars show 10 and 90 percentiles and open circles represent outliers. Mimic mistletoes growing on N-fixing hosts shows higher difference values than the other groups (ANOVA, $F_{1,163} = 7.86$, $P = 0.005$).

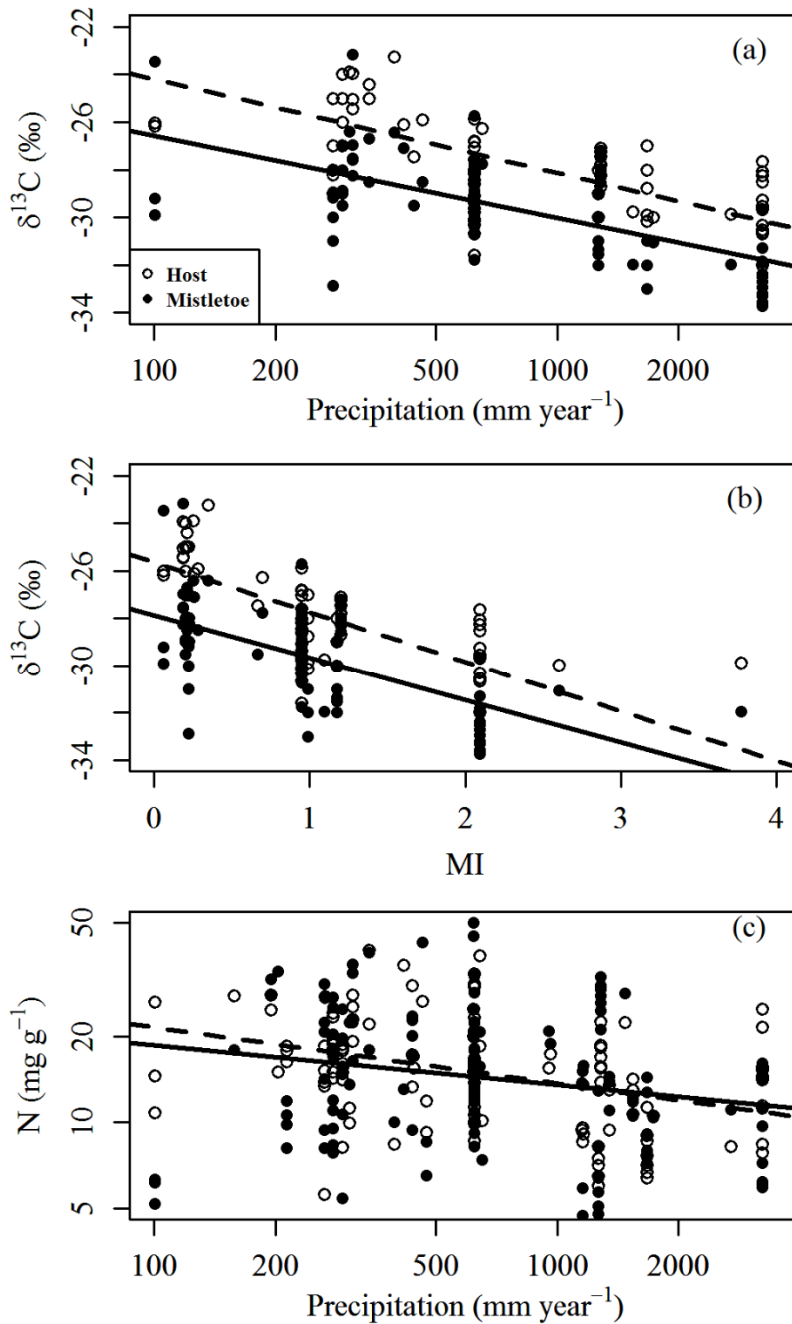


Figure 6. Relationship between $\delta^{13}\text{C}$ and (a) precipitation (mistletoes: $r^2 = 0.41$; host: $r^2 = 0.35$; $P < 0.001$); and (b) moisture index for mistletoes ($r^2 = 0.35$, $P < 0.001$) and hosts ($r^2 = 0.12$, $P < 0.001$). (c) Relationship between N concentration and precipitation (mistletoes: $r^2 = 0.07$; host: $r^2 = 0.13$; $P < 0.01$) for mistletoes (empty symbols) and hosts (filled symbols) across a precipitation gradient. Moisture index was calculated as the ratio between mean annual precipitation and potential evapotranspiration.

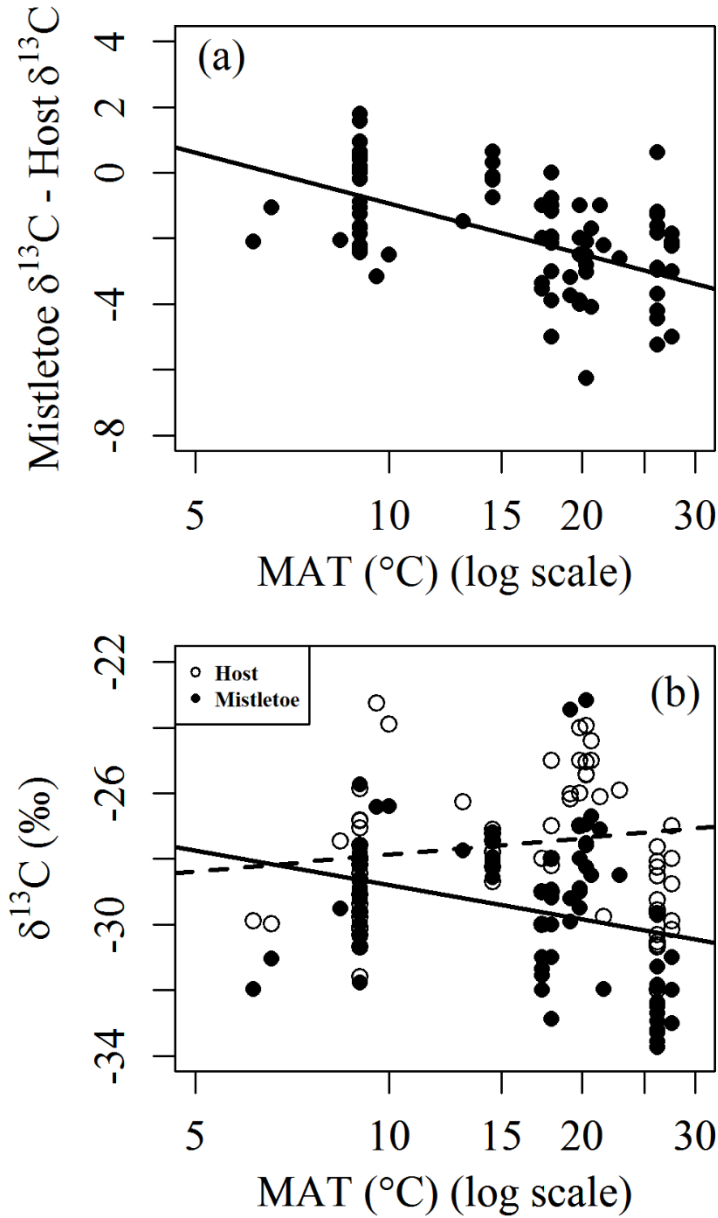


Figure 7. (a) Mistletoe to host $\delta^{13}\text{C}$ difference become higher in warmer sites. The more negative the difference, the greater the difference between mistletoe and host $\delta^{13}\text{C}$ ($r^2 = 0.24$, $P < 0.0001$, $n = 93$). (b) Relationship between $\delta^{13}\text{C}$ and mean annual temperature for mistletoes (empty symbols, dashed line; $r^2 = 0.08$, $P = 0.003$) and hosts (filled symbols, solid line; $P = 0.26$).

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Supplemental Data

Table S1. Mistletoe-hosts pairs considered in this study.

Ref	site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N _{mass}	Host N _{mass}	Mimicry	N-fixing	Host δ ¹³ C	Mistletoe δ ¹³ C
1	1	<i>Korthalsella lindsayii</i>	Viscaceae	<i>Melicope simplex</i>	Rutaceae	32.6	33	Yes	No	-30.1	-29.15
1	1	<i>Korthalsella lindsayii</i>	Viscaceae	<i>Coprosma crassifolia</i>	Rubiaceae	18	16.3	Yes	No	-29.63	-29.1
1	1	<i>Korthalsella salicornioides</i>	Viscaceae	<i>Erica lusitanica</i>	Ericaceae	18.2	8.6	Yes	No	-28.62	-30.3
1	1	<i>Korthalsella salicornioides</i>	Viscaceae	<i>Erica vagans</i>	Ericaceae	12.7	13.7	Yes	No	-29.79	-29.59
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Melicope simplex</i>	Rutaceae	22.3	33.1	No	No	-28.46	-30.67
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Coprosma rotundifolia</i>	Rubiaceae	10	20.3	No	No	-30.68	-28.88
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Coprosma propinqua</i>	Rubiaceae	9.15	15.5	No	No	-30.15	-29.22
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Coprosma crassifolia</i>	Rubiaceae	11.8	16.7	No	No	-29.38	-28.94
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Hoheria angustifolia</i>	Malvaceae	16.2	23.3	No	No	-29.09	-30.72
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Melicytus ramiflorus</i>	Violaceae	14.8	20.9	Yes	No	-25.87	-25.73
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Kunzea ericoides</i>	Myrtaceae	22.5	15.9	No	No	-27.05	-28.91
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Schefflera digitata</i>	Araliaceae	14.2	15.4	Yes	No		
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Rubus schmidelioides</i>	Rosaceae	15	14.3	No	No		
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Rubus cissoides</i>	Rosaceae	12.4	10.6	No	No	-26.81	-29.15
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Pittosporum tenuifolium</i>	Pittosporaceae	13.3	10	Yes	No	-27.6	-28.66
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Olearia ilicifolia</i>	Asteraceae	12	10	Yes	No		
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Chamaecytisus palmensis</i>	Fabaceae	20.1	30.5	No	Yes	-28.04	-27.63
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Teline monspessulana</i>	Fabaceae	28.5	29.8	No	Yes	-26.84	-28.1
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Malus domestica</i>	Rosaceae	14.3	16.4	No	No	-29.39	-27.82
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Crataegus monogyna</i>	Rosaceae	14.6	16.3	No	No	-30.33	-29.68
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Cotoneaster simmondsii</i>	Rosaceae	12.9	16	No	No	-28.01	-28.21
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Ribes sanguineum</i>	Grossulariaceae	17.8	14.7	No	No		
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Prunus laurocerasus</i>	Rosaceae	17.8	11.3	Yes	No	-26.81	-29.25

Ref	site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N_{mass}	Host N_{mass}	Mimicry	N-fixing	Host $\delta^{13}C$	Mistletoe $\delta^{13}C$
1	1	<i>Loranthus micranthus</i>	Loranthaceae	<i>Erica lusitanica</i>	Ericaceae	13.5	9.9	No	No	-29.09	-29.97
1	1	<i>Tupeia antarctica</i>	Loranthaceae	<i>Coprosma propinqua</i>	Rubiaceae	8.2	16.3	No	No		
1	1	<i>Tupeia antarctica</i>	Loranthaceae	<i>Carpodetus serratus</i>	Rousseaceae	8.2	14.9	No	No	-28.19	-28.19
1	1	<i>Tupeia antarctica</i>	Loranthaceae	<i>Loranthus micranthus</i>	Loranthaceae	10.9	9.9	Yes	No	-31.59	-31.77
1	2	<i>Korthalsella salicornioides</i>	Viscaceae	<i>Kunzea ericoides</i>	Myrtaceae	17.1	15.5	Yes	No	-27.46	-29.51
1	3	<i>Elytranthe flavida</i>	Loranthaceae	<i>Nothofagus solandri var. cl.</i>	Nothofagaceae	10.4	10.5	No	No	-29.99	-31.05
1	4	<i>Elytranthe tetrapetala</i>	Loranthaceae	<i>Nothofagus solandri var. cl.</i>	Nothofagaceae	11.1	8.2	No	No	-29.88	-31.97
2	5	<i>Lysiana murrayi</i>	Loranthaceae	<i>Acacia brachystachya</i>	Fabaceae	46.8	16.6	Yes	Yes		
2	5	<i>Anyema gibberulum</i>	Loranthaceae	<i>Gravillea wickhamii</i>	Proteaceae	11.8	8.5	No	No		
2	5	<i>Anyema linophyllum</i>	Loranthaceae	<i>Casuarina cristata</i>	Casuarinaceae	13.1	8.7	Yes	No		
2	5	<i>Lysiana murrayi</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	31.4	22.1	Yes	Yes		
2	5	<i>Anyema quandang</i>	Loranthaceae	<i>Acacia papyrocarpa</i>	Fabaceae	20	15.7	Yes	Yes		
2	5	<i>Anyema quandang</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	9.7	20.9	No	Yes		
2	5	<i>Anyema quandang</i>	Loranthaceae	<i>Acacia brachystachya</i>	Fabaceae	13.1	17.8	No	Yes		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Heterodendrum oleifolium</i>	Sapindaceae	22.3	17.5	Yes	No		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Templetonia egena</i>	Fabaceae	16.1	14.9	No	Yes		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	15.6	15.8	No	Yes		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Myoporum platycarpum</i>	Scrophulariaceae	10.1	13.8	No	No		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Acacia brachystachya</i>	Fabaceae	10.9	20.9	No	Yes		
2	5	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Pittosporum phylliraeoides</i>	Pittosporaceae	12.5	12.3	Yes	No		
2	5	<i>Anyema sanguinea</i>	Loranthaceae	<i>Eucalyptus sp.</i>	Myrtaceae	8.6	8.7	Yes	No		
2	5	<i>Anyema miquelii</i>	Loranthaceae	<i>Eucalyptus largiflorens</i>	Myrtaceae	9.8	10.2	Yes	No		
2	5	<i>Anyema miquelii</i>	Loranthaceae	<i>Eucalyptus crebra</i>	Myrtaceae	10.1	11.2	Yes	No		
2	5	<i>Anyema miquelii</i>	Loranthaceae	<i>Eucalyptus sideroxylon</i>	Myrtaceae	10.8	13	Yes	No		
2	5	<i>Anyema miquelii</i>	Loranthaceae	<i>Angophora costata</i>	Myrtaceae	10.3	15.3	No	No		
2	5	<i>Anyema cambaguei</i>	Loranthaceae	<i>Casuarina cunninghamiana</i>	Casuarinaceae	17.9	20.5	Yes	No		
2	5	<i>Anyema mackayense</i>	Loranthaceae	<i>Avicennia marina</i>	Acanthaceae	8.3	17.7	Yes	No		

Ref site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N _{mass}	Host N _{mass}	Mimicry	N-fixing	Host $\delta^{13}\text{C}$	Mistletoe $\delta^{13}\text{C}$
2	5	<i>Dendrophthoe vitellina</i>	Loranthaceae	<i>Casuarina glauca</i>	Casuarinaceae	16.5	No	No	12.5	
2	5	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia monticola</i>	Fabaceae	19.8	No	Yes	16	
2	5	<i>Lysiana spathulata</i>	Loranthaceae	<i>Callitris columellaris</i>	Cupressaceae	7.1	No	No	6.9	
2	5	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	14.6	No	Yes	17.3	
2	5	<i>Lysiana casurinae</i>	Loranthaceae	<i>Gossypium robinsonii</i>	Malvaceae	14.3	No	No	18.6	
2	5	<i>Lysiana casurinae</i>	Loranthaceae	<i>Acacia acradenia</i>	Fabaceae	13.7	No	Yes	11.5	
2	6	<i>Amyema preissii</i>	Loranthaceae	<i>Acacia brachystachya</i>	Fabaceae	33.8	Yes	Yes	15	
2	7	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Acacia victoriae</i>	Fabaceae	27.6	No	Yes	27.5	
8	7	<i>Lysiana exocarpi</i>	Loranthaceae	<i>Acacia victoriae</i>	Fabaceae	27.58	No	Yes	27.58	
2	8	<i>Lysiana murrayi</i>	Loranthaceae	<i>Acacia kempeana</i>	Fabaceae	30.6	No	Yes	13.8	
2	8	<i>Amyema preissii</i>	Loranthaceae	<i>Acacia kempeana</i>	Fabaceae	20.8	No	Yes	13.8	
2	8	<i>Amyema gibberulum</i>	Loranthaceae	<i>Hakea eyreana</i>	Proteaceae	9.4	Yes	No	5.6	
2	8	<i>Amyema maidenii</i>	Loranthaceae	<i>Acacia kempeana</i>	Fabaceae	22.4	Yes	Yes	15.2	
2	8	<i>Lysiana murrayi</i>	Loranthaceae	<i>Acacia tetragonophylla</i>	Fabaceae	14.2	No	Yes	13.4	
8	8	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia cowleana</i>	Fabaceae	8.12	No	Yes	18.48	
2	9	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia monticola</i>	Fabaceae	10.6	No	Yes	17.8	
2	9	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia cowleana</i>	Fabaceae	8.1	No	Yes	18.5	
2	9	<i>Amyema maidenii</i>	Loranthaceae	<i>Acacia cowleana</i>	Fabaceae	11.9	No	Yes	18.5	
2	9	<i>Amyema maidenii</i>	Loranthaceae	<i>Acacia coriacea</i>	Fabaceae	9.8	No	Yes	16.3	
2	10	<i>Diplatia grandibractea</i>	Loranthaceae	<i>Eucalyptus leucophloia</i>	Myrtaceae	6.5	Yes	No	9.2	
8	10	<i>Diplatia grandibractea</i>	Loranthaceae	<i>Eucalyptus leucophloia</i>	Myrtaceae	8.54	Yes	No	11.9	
2	11	<i>Lysiana subfalcata</i>	Loranthaceae	<i>Atalaya hemiglauc</i>	Sapindaceae	23.6	Yes	No	17.2	
2	11	<i>Lysiana spathulata</i>	Loranthaceae	<i>Acacia farnesiana</i>	Fabaceae	20.1	No	Yes	30.2	
2	11	<i>Lysiana subfalcata</i>	Loranthaceae	<i>Cassia oligophylla</i>	Fabaceae	9.4	No	Yes	13.3	
8	11	<i>Lysiana subfalcata</i>	Loranthaceae	<i>Atalaya hemiglauc</i>	Sapindaceae	22.82	Yes	No	17.22	
2	12	<i>Lysiana subfalcata maritima</i>	Loranthaceae	<i>Cerriops tagal</i>	Rhizophoraceae	13.9	Yes	No	9.4	
8	12	<i>Lysiana subfalcata</i>	Loranthaceae	<i>Cerriops tagal</i>	Rhizophoraceae	13.86	Yes	No	9.38	

Ref	site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N_{mass}	Host N_{mass}	Mimicry	N-fixing	Host $\delta^{13}C$	Mistletoe $\delta^{13}C$
3	13	<i>Phoradendron juniperinum</i>	Viscaceae	<i>Juniperus osteosperma</i>	Cupressaceae	10	8.4	No	No	-23.25	-26.42
4	14	<i>Phthirusa pyrifolia</i>	Loranthaceae	<i>Citrus reticulata</i>	Rutaceae	28.3	22.4	No	No		
5	15	<i>Loranthus europaeus</i>	Loranthaceae	<i>Quercus petraea</i>	Fagaceae	20.8	38.4	No	No		
11	15	<i>Loranthus europaeus</i>	Loranthaceae	<i>Quercus robur</i>	Fagaceae	15.68	18.48	No	No		
6	16	<i>Anyema preissii</i>	Loranthaceae	<i>Acacia acuminata</i>	Fabaceae	18.9	17.4	Yes	Yes		
7	17	<i>Phoradendron juniperinum</i>	Viscaceae	<i>Juniperus osteosperma</i>	Cupressaceae	13.58	9.94	No	No	-23.9	-26.4
7	17	<i>Phoradendron juniperinum</i>	Viscaceae	<i>Juniperus osteosperma</i>	Cupressaceae	22.4	11.2	No	No		
9	18	<i>Phthirusa maritima</i>	Loranthaceae	<i>Conocarpus erectus</i>	Combretaceae	5.87	8.55	No	No		
9	18	<i>Phthirusa maritima</i>	Loranthaceae	<i>Coccoloba uvifera</i>	Polygonaceae	4.7	9.6	No	No		
10	19	<i>Odontella welwitschii</i>	Loranthaceae	<i>Acacia recifens</i>	Fabaceae	33.46	22.82	No	Yes	-25.44	-28.25
10	19	<i>Plicosepalus undulatus</i>	Loranthaceae	<i>Acacia (5sp)</i>	Fabaceae	22.68	27.86	No	Yes	-25.43	-27.53
10	19	<i>Septulina glauca</i>	Loranthaceae	<i>Euphorbia (2sp.)</i>	Euphorbiaceae	16.38	19.18	No	No	-16.9	-23.16
10	19	<i>Tapinanthus oleifolius</i>	Loranthaceae	30 sp.		22.4	22.96	No	No	-23.94	-26.96
10	19	<i>Viscum rotundifolium</i>	Viscaceae	<i>Ziziphus mucronata</i>	Bignoniaceae	35.84	25.48	No	No	-25.05	-27.58
11	20	<i>Plicosepalus acaciae</i>	Loranthaceae	<i>Acacia raddiana</i>	Fabaceae	17.9	27.7	No	Yes		
12	21	<i>Dendrophthoe glabescens</i>	Loranthaceae	<i>Eucalyptus platyphylla</i>	Myrtaceae	11	13	No	No		
12	21	<i>Anyema biniflora</i>	Loranthaceae	<i>Eucalyptus tessellaris</i>	Myrtaceae	14.5	13.5	Yes	No		
12	21	<i>Dendrophthoe glabescens</i>	Loranthaceae	<i>Eucalyptus tessellaris</i>	Myrtaceae	11	13	Yes	No		
13	22	<i>Anyema miquelii</i>	Loranthaceae	<i>Eucalyptus behrriana</i>	Myrtaceae	7.406	10.108	No	No	-26.26	-27.75
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Ulmus americana</i>	Ulmaceae	27.8	18.33	No	No	-27.81	-28.56
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Carya ovata</i>	Fagaceae	26.3	15.52	No	No	-27.46	-28.2
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Fraxinus sp.</i>	Oleaceae	21.21	18.61	No	No	-27.24	-27.45
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Ulmus serotina</i>	Ulmaceae	24.57	13.82	No	No	-27.79	-27.95
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Maclura pomifera</i>	Moraceae	32.35	22.48	No	No	-27.1	-27.2
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Nyssa sylvatica</i>	Nyssaceae	29.27	15.71	No	No	-28.26	-27.95
14	23	<i>Phoradendron leucarpum</i>	Viscaceae	<i>Juglans nigra</i>	Juglandaceae	30.21	16.9	No	No	-28.7	-28.05
15	24	<i>Arceuthobium pusillum</i>	Viscaceae	<i>Picea rubens</i>	Pinaceae	15.8	9.1	No	No		

Ref site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N_{mass}	Host N_{mass}	Mimicry	N-fixing	Host $\delta^{13}C$	Mistletoe $\delta^{13}C$
15	25	<i>Arceuthobium pusillum</i>	Viscaceae	<i>Picea glauca</i>	Pinaceae	13.7	9.4	No	No	
16	26	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Euphorbia virosa</i>	Euphorbiaceae	6.3	10.78	No	No	-14
16	26	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Salvadora persica</i>	Salvadoraceae	5.18	26.32	No	No	-26.17
16	26	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Tamarix usneoides</i>	Tamaricaceae	6.16	14.56	No	No	-26.02
17	27	<i>Scurrula ferruginea</i>	Loranthaceae	<i>Tabebuia pallida</i>	Bignoniaceae	7.2	14.4	No	No	-27.64
17	27	<i>Scurrula ferruginea</i>	Loranthaceae	<i>Tabebuia rosea</i>	Bignoniaceae	16.1	21.6	No	No	-29.55
17	27	<i>Scurrula ferruginea</i>	Loranthaceae	<i>Vitex pinnata</i>	Lamiaceae	11.2	14.3	No	No	-30.63
17	27	<i>Macrosolen cochinchinensis</i>	Loranthaceae	<i>Artocarpus heterophyllus</i>	Moraceae	5.9	11.2	No	No	-29.67
17	27	<i>Macrosolen cochinchinensis</i>	Loranthaceae	<i>Calophyllum inophyllum</i>	Calophyllaceae	6.1	8.4	No	No	-32
17	27	<i>Macrosolen cochinchinensis</i>	Loranthaceae	<i>Mangifera indica</i>	Anacardiaceae	14.1	15.6	No	No	-28.07
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Andira inermis</i>	Fabaceae	14.7	14.5	No	Yes	-28.51
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Averrhoa bilimbi</i>	Oxalidaceae	14.9	24.9	No	No	-30.32
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Baphia nitida</i>	Fabaceae	9.7	15.2	No	Yes	-28.26
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Eugenia aquea</i>	Myrtaceae	9.7	11.4	No	No	-30.68
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Ixora coccinea</i>	Rubiaceae	14.5	14.1	No	No	-30.53
17	27	<i>Dendrophthoe curvata</i>	Loranthaceae	<i>Melastoma malabathricum</i>	Melastomataceae	6.2	7.8	No	No	-29.26
18	28	<i>Viscum album subsp. album</i>	Viscaceae	<i>Crataegus monogyna</i>	Rosaceae	25	20	No	No	
18	28	<i>Viscum album subsp. album</i>	Viscaceae	<i>Robinia pseudoacacia</i>	Fabaceae	50	25	No	Yes	
18	28	<i>Viscum album subsp. album</i>	Viscaceae	<i>Salix alba</i>	Salicaceae	45	25	No	No	
18	28	<i>Viscum album subsp. album</i>	Viscaceae	<i>Populus alba</i>	Salicaceae	25	15	No	No	
19	29	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Acacia leuderitzii</i>	Fabaceae	17.9	22.1	No	Yes	-24.4
19	29	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Acacia mellifera</i>	Fabaceae	39.3	40.1	No	Yes	-25
19	30	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Acacia mellifera</i>	Fabaceae	13.1	35.6	No	Yes	-26.1
19	31	<i>Tapinanthus oleifolius</i>	Loranthaceae	<i>Colophospermum mopane</i>	Fabaceae	42.8	26.5	No	Yes	-25.9
20	32	<i>Arceuthobium pusillum</i>	Viscaceae	<i>Picea glauca</i>	Pinaceae	15.1	13.6	No	No	
21	33	<i>Phoradendron californicum</i>	Viscaceae	<i>Phoradendron californicum</i>	Loranthaceae	31.7	28	No	No	
21	33	<i>Phoradendron californicum</i>	Viscaceae	<i>Cercidium floridum</i>	Fabaceae	28	24.8	No	Yes	

Ref site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N_{mass}	Host N_{mass}	Mimicry	N-fixing	Host $\delta^{13}C$	Mistletoe $\delta^{13}C$
22	34	<i>Dendrophthoe falcata</i>	Loranthaceae	<i>Azadirachta indica</i>	Meliaceae	20.9	15.5	No	No	
23	35	<i>Psittacanthus robustus</i>	Loranthaceae	<i>Miconia albicans</i>	Melastomataceae	11.82	13	No	No	
23	35	<i>Phthirusa ovata</i>	Loranthaceae	<i>Miconia albicans</i>	Melastomataceae	10.52	14.16	No	No	
23	35	<i>Phthirusa ovata</i>	Loranthaceae	<i>Byrsonima verbascifolia</i>	Malpighiaceae	12.2	10.68	No	No	-29.76
0	36	<i>Anyema miquelli</i>	Loranthaceae	<i>Eucalyptus miniata</i>	Myrtaceae	14.337	8.577	Yes	No	-28
0	36	<i>Anyema sanguinea</i>	Loranthaceae	<i>Corymbia blesseri</i>	Myrtaceae	7.945	6.66	Yes	No	-29.89
0	36	<i>Anyema sanguinea</i>	Loranthaceae	<i>Corymbia porrecta</i>	Myrtaceae	7.8326	6.402	Yes	No	-30.15
0	36	<i>Anyema sanguinea</i>	Loranthaceae	<i>Eucalyptus miniata</i>	Myrtaceae	7.1895	8.9768	Yes	No	-28
0	36	<i>Anyema sanguinea</i>	Loranthaceae	<i>Eucalyptus tetrodonta</i>	Myrtaceae	9.1252	7.0706	Yes	No	-28.76
0	36	<i>Dendrophthoe odontocalyx</i>	Loranthaceae	<i>Grevillea pteridifolia</i>	Proteaceae	12.747	7.628	No	No	-28
0	36	<i>Decaisnina signata</i>	Loranthaceae	<i>Xanthostemon paradoxus</i>	Myrtaceae	7.5612	11.287	No	No	-27
0	37	<i>Dendrophthoe vitelina</i>	Loranthaceae	<i>Angophora costata</i>	Myrtaceae	5.68	6.48	No	No	-28
0	37	<i>Dendrophthoe vitelina</i>	Loranthaceae	<i>Eucalyptus spp.</i>	Myrtaceae	8.275	7.075	No	No	-28
0	37	<i>Muellerina eucalyptoides</i>	Loranthaceae	<i>Eucalyptus hemastoma</i>	Myrtaceae	5.0833	5.98	Yes	No	-29
0	37	<i>Muellerina eucalyptoides</i>	Loranthaceae	<i>Eucalyptus spp.</i>	Myrtaceae	6.375	7.5	Yes	No	-28
0	37	<i>Muellerina eucalyptoides</i>	Loranthaceae	<i>Eucalyptus spp.</i>	Myrtaceae	4.7667	8.2333	Yes	No	-29
0	37	<i>Notothixos subaureus</i>	Viscaceae	<i>Eucalyptus hemastoma</i>	Myrtaceae	12.92	5.98	No	No	-30
0	38	<i>Anyema miraculosa</i>	Loranthaceae	<i>Myoporum platycarpum</i>	Myoporaceae	5.4056	14.106	No	No	-25
0	38	<i>Anyema preissii</i>	Loranthaceae	<i>Acacia victoriae</i>	Fabaceae	14.721	17.862	Yes	Yes	-27
0	38	<i>Anyema quandang</i>	Loranthaceae	<i>Acacia papyrocarpa</i>	Fabaceae	19.728	18.732	No	Yes	-26
0	38	<i>Lysiana exocarpii</i>	Loranthaceae	<i>Acacia sp.</i>	Fabaceae	24.914	16.082	No	Yes	-25
0	38	<i>Lysiana exocarpii</i>	Loranthaceae	<i>Myoporum platycarpum</i>	Myoporaceae	10.696	14.896	No	No	-25
0	38	<i>Lysiana exocarpii</i>	Loranthaceae	<i>Eremophila longifolia</i>	Scrophulariaceae	15.678	15.526	No	No	-24
0	38	<i>Lysiana exocarpii</i>	Loranthaceae	<i>Santalum acuminatum</i>	Santalaceae	19.425	18.156	Yes	No	-26
0	38	<i>Lysiana exocarpii</i>	Loranthaceae	<i>Pittosporum angustifolium</i>	Pittosporaceae	14.896	8.1874	No	No	-27
0	39	<i>Anyema miraculosa</i>	Loranthaceae	<i>Myoporum platycarpum</i>	Myoporaceae	11.94	17.75	No	No	-28
0	39	<i>Anyema lucasii</i>	Loranthaceae	<i>Flindersia maculosa</i>	Rutaceae	8.3089	18.088	Yes	No	-29

Ref	site	Mistletoe species	Mistletoe Family	Host species	Host Family	Mistletoe N _{mass}	Host N _{mass}	Mimicry	N-fixing	Host $\delta^{13}\text{C}$	Mistletoe $\delta^{13}\text{C}$
0	39	<i>Amyema preissii</i>	Loranthaceae	<i>Acacia spp.</i>	Fabaceae	20.354	18.716	Yes	Yes	-27	-28.94
0	39	<i>Lysiana linearifolia</i>	Loranthaceae	<i>Eremophila mitchellii</i>	Scrophulariaceae	7.8141	14.272	Yes	No	-27	-29
0	39	<i>Amyema miraculosa</i>	Loranthaceae	<i>Eremophila longifolia</i>	Scrophulariaceae	11.034	15.03	No	No	-25	-30
0	39	<i>Amyema maidenii</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	27.41	17.614	Yes	Yes	-27	-29.15
0	39	<i>Amyema maidenii</i>	Loranthaceae	<i>Acacia harpophylla</i>	Fabaceae	25.252	19.366	No	Yes	-28	-29
0	39	<i>Amyema miraculosa</i>	Loranthaceae	<i>Eremophila mitchellii</i>	Scrophulariaceae	9.4905	19.595	No	No	-25	-30
0	39	<i>Lysiana exocarpia</i>	Loranthaceae	<i>Alectryon oleifolius</i>	Sapindaceae	27.432	23.438	No	No	-28.22	-29
0	39	<i>Amyema preissii</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	20.358	17.487	Yes	Yes	-28	-28
0	39	<i>Amyema preissii</i>	Loranthaceae	<i>Flindersia maculosa</i>	Rutaceae	17.013	24.217	No	No	-28	-29
0	39	<i>Lysiana exocarpia</i>	Loranthaceae	<i>Acacia aneura</i>	Fabaceae	20.406	17.324	No	Yes	-28	-29.18

*References: 0. Own field data; 1. Bannister 1989; Bannister & Strong 2001; 2. Ullmann *et al.* 1985; Ehleringer *et al.* 1986; 3. Ehleringer, Cook & Tieszen 1986; 4. El-Sharkawy, Cock & Hernandez 1986; 5. Glatzel 1983; 6. Lamont & Southall 1982; 7. Marshall, Dawson & Ehleringer 1993; 8. Marshall *et al.* 1994; 9. Orozco *et al.* 1990; 10. Schulze *et al.* 1991; 11. Bowie & Ward 2004; 12. Canyon & Hill 1997; 13. Küppers 1992; 14. Panvini & Eickmeier 1993; 15. Reblin, Logan & Tissue 2006; 16. Richter *et al.* 1995; 17. Tennakoon, Chak & Bolin 2011; 18. Türe, Bökük & Aşan 2010; 19. Wang *et al.* 2008; 20. Logan, Huhn & Tissue 2008; 21. Ehleringer & Schulze 1985; 22. Karunaichamy, Paliwal & Arp 1999; 23. Lüttge *et al.* 1998; Scalón, Haridasan & Franco 2013.

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Table S2. Climatic variables of sites from which mistletoes-hosts pair data was collected: elevation, moisture index (MI), annual evapotranspiration (PET), mean annual temperature (MAT), mean annual precipitation (MAP). References for sites as in Table S1.

Site	Country	Latitude	Longitude	Elevation	MI	PET	MAT	MAP
1	New Zealand	45°50'S	170°33'E	455	0.95	656	9.0	621.5
2	New Zealand	45°18'S	170°30'E	454	0.67	663	8.4	441.5
3	New Zealand	45°18'S	168°10'E	1410	2.60	665	6.6	1732
4	New Zealand	43°10'S	171°41'E	1719	3.77	716	6.2	2702.2
6	Australia	31°24'S	136°46'E	101	0.15	1314	18.0	203.3
7	Australia	32°80'S	142°50'E	61	0.21	1288	18.4	266.1
8	Australia	25°40'S	131°90'E	500	0.17	1516	20.0	264.6
9	Australia	26°46'S	134°20'E	305	0.14	1527	20.8	213.4
10	Australia	19°60'S	139°00'E	281	0.28	1691	25.4	473.9
11	Australia	20°73'S	139°50'E	366	0.26	1660	23.9	436.6
12	Australia	19°15'S	146°45'E	47	0.90	1488	23.3	1346.8
13	USA	40°21'N	112°36'W	1576	0.35	1137	9.6	394
14	Colombia	03°25'N	76°35'W	989	1.08	1358	24.5	1472.8
15	Austria	48°12'N	16°22'E	343	0.90	708	9.5	641.3
16	Australia	31°41'S	116°30'E	197	0.91	105	15.6	960.9
17	USA	37°00'N	112°00'W	1538	0.25	1223	10.0	305.5
18	Venezuela	10°48'N	68°19'W	17	0.74	1570	25.1	1157.3
19	Namibia	22°50'S	16°45'E	1525	0.18	1700	20.3	311.4
20	Israel	31°15'N	34°48'E	156	0.11	1458	19.8	158.2
21	Australia	19°00'S	146°46'E	5	0.91	1486	23.3	1346.8
22	Australia	37°50'S	144°50'E	516	0.69	932	13.0	649.8
23	USA	36°10'00"N	86°47'00"W	174	1.20	1065	14.5	1279.2
24	USA	43.766° N	69.312°W	5	1.52	763	7.7	1161.2
25	USA	43.855° N	69.559° W	5	1.49	768	7.7	1148.5
26	Namibia	26°50'S	17°45'E	1099	0.06	1623	19.2	100.3
27	Borneo	04°52'N	115°00'E	532	2.09	1542	26.2	3230.6
28	Turkey	39°50'N	30°30'E	1280	0.59	1043	9.1	619
29	Botswana	24°17'S	21°89'E	1117	0.21	1619	20.7	340.8
30	Botswana	21°65'S	21°81'E	1129	0.25	1628	21.3	415.4
31	Botswana	19°93'S	23°59'E	945	0.28	1658	22.9	463.9
32	USA	43°47'N	69°40'W	5	1.50	768	7.7	1155.2
33	USA	34°57'N	114°25'W	867	0.12	1557	19.8	195
34	India	09°55'N	78°10'E	94	0.62	1532	27.8	954.5
35	Brazil	15°47'S	47°55'W	999	1.09	1409	21.6	1539.2
36	Australia	12°30'S	130°45'E	5	0.99	1688	27.6	1668.6
37	Australia	33.63'S	151.26'E	148	1.17	1079	17.3	1266.6
38	Australia	30°35'S	143°17'E	180	0.20	1454	19.8	293.6
39	Australia	32.78'S	137.66'E	135	0.22	1256	17.9	277.9

Chapter 3

Photosynthetic trait adaptations of parasitic mistletoes and their hosts in sites of contrasting aridity

Abstract

It has been argued that xylem-tapping mistletoes have little ability to regulate their use of water, even when parasitising host plants under water stress. We investigated key photosynthetic traits, including leaf nutrient concentration, gas exchange measurements, leaf dark respiration, and specific leaf area (SLA), in 42 mistletoe-host species-pairs sampled from five sites of contrasting aridity located in Australia and Brazil. We proposed two different extreme scenarios as expectations: one in which mistletoes would show very relaxed selective pressure to optimise water use, exhibiting no adaptive trait in response to increasing aridity; and other, where we would find exactly the similar trait responses as seen in their hosts. Our results generally agreed with our second scenario. Both hosts and mistletoes displayed expected key trends in relation to site aridity, i.e., strong control over water loss, high leaf nitrogen (N) and phosphorus (P) concentrations per unit leaf area, and low mean SLA. Nevertheless, mistletoes are profligate water users compared to their hosts and showed substantially less efficient use of water in photosynthesis (and higher $c_i : c_a$) across all different sites. Mistletoes also had far higher dark respiration rates for a given photosynthetic capacity, presumably related to the considerable costs associated with maintaining higher osmolarity in leaf tissues than in hosts, to guarantee reliable water flow from the host xylem. We also suggest an alternative for the N-parasitism hypothesis, considering that P might be required in large concentrations and possibly driving, at least partly, the high transpiration rates of mistletoes. Despite fundamental differences in photosynthetic traits, mistletoes regulate the use of resources, exhibiting trait responses in parallel and to the same extent as host species to increasing aridity.

Key words: Carbon gain, nitrogen, phosphorus, dark respiration, hemiparasites

Introduction

Mistletoes are photosynthetic hemi-parasitic plants that attach *via* a modified root (haustorium) to the xylem stream of their host, from where they access all the water and nutrients they need to survive (Glatzel & Geils 2009). By not needing to invest in roots, the energetic per-unit costs for *acquiring* water and nitrogen should be presumably very low in mistletoes; at least, far lower than that experienced by their hosts. By contrast, presumably mistletoes and hosts suffer similar per-unit costs for nitrogen *maintenance*, since there is no reason to believe that they differ in the need to continually break down and resynthesise nitrogen-rich compounds, such as Rubisco. This “protein turnover” process amounts to quite a considerable respiration cost to plants (De Vries 1975). Thus, considering both the *acquisition* and *maintenance* costs of water and nitrogen, one might argue that nitrogen is relatively cheaper for mistletoes than for their hosts, while water is especially cheap, or perhaps almost negligible. The difference between relative costs for water and nitrogen should have implications for the manner in which they use these resources during photosynthesis (Bloom, Chapin III & Mooney 1985; Wright, Reich & Westoby 2003), and reasonably then, on how this manner varies with site properties, such as increasing aridity.

For non-parasitic species there is an extensive literature demonstrating that plants show more conservative water-use strategies at more arid sites. For example, in the short term (e.g., minutes to hours to days), photosynthetic carbon gain can be maximised in relation to transpirational water loss *via* modifying stomatal behaviour in relation to atmospheric humidity (Farquhar & Sharkey 1982; Schulze & Hall 1982; Farquhar, Ehleringer & Hubick 1989). On longer (ecological) time-scales, we might instead focus on functional traits differences among species and the manner in which these traits vary with site aridity.

From this view point, two of the most striking leaf-level adaptations to higher aridity in woody plants are: (1) species from arid habitats tend to have thick leaves with low specific

leaf area (SLA, the ratio of leaf area per dry mass) (Niinemets 2001; Wright *et al.* 2004); and (2) they tend to deploy leaves with high nitrogen concentration per area (N_{area}) (Field, Merino & Mooney 1983; Cunningham, Summerhayes & Westoby 1999; Wright *et al.* 2004). The increase in N_{area} is generally associated with greater CO_2 drawdown during photosynthesis (i.e., lower intercellular CO_2 concentration - c_i), meaning that plants can achieve a given carbon assimilation rate (A_{area}) with a lower stomatal conductance to both CO_2 and water (g_s), thus economising on water use (Wright, Reich & Westoby 2001; Prentice *et al.* 2011).

Mistletoes are well known to be profligate users of water (Ehleringer, Cook & Tieszen 1986; Marshall & Ehleringer 1990; Marshall, Dawson & Ehleringer 1994). They usually exhibit higher transpiration rates and g_s than their hosts, and achieve lower A_{area} , resulting in a very low water use efficiency (the ratio of A_{area} to transpiration; Ullmann *et al.* 1985; Escher *et al.* 2004; Glatzel & Geils 2009). Various hypotheses have been proposed to explain this pattern. The most recognized one is the ‘N-parasitism hypothesis’ (Schulze, Turner & Glatzel 1984), which posits that mistletoes are most strongly limited by access to nitrogen, hence they operate at very high transpiration rates in order to acquire sufficient N from the host xylem stream. Following a similar principle is the C-parasitism hypothesis (Marshall & Ehleringer 1990), based on reports of partial heterotrophy (i.e., part of the carbon in the mistletoes is actually transferred from the host xylem, in the form of amino acids; Marshall & Ehleringer 1990; Schulze *et al.* 1991; Marshall *et al.* 1994). The C-parasitism hypothesis predicts that higher transpiration rates would be necessary, not only to acquire N, but also to obtain heterotrophic carbon.

A complementary question to this scenario is the degree to which mistletoes do or do not show stomatal responses to varying atmospheric humidity. Early work suggested that mistletoes simply lacked stomatal control (Vareschi & Pannier 1953), or perhaps showed only limited control, even under extreme drought condition (Schulze, Turner & Glatzel 1984;

Marshall *et al.* 1994; Escher *et al.* 2008). By contrast, other studies showed close coordination of host and mistletoe stomatal behaviour during the day, suggesting that the unrestricted water use by the parasite could represent a disadvantage, especially if the host is severely affected (Ullmann *et al.* 1985, Davidson, 1992 #7971; Whittington & Sinclair 1988; Davidson, True & Pate 1989; Goldstein *et al.* 1989; Bowie & Ward 2004).

These contrasting responses raise questions of what sort of trait adaptations mistletoes show in relation to site aridity, and how similar or different these responses are to that of their hosts. Broadly speaking, there were two extreme scenarios to consider: (1) Mistletoes would show little or no apparent adaptation to aridity; or (2) Mistletoes would show similar adaptations to aridity as their hosts. The first scenario might be possible if water is indeed essentially free for mistletoes, meaning that they experience little or no evolutionary selective pressure to optimize the water use; or, alternatively, if rampant water use is so fundamentally important to the mistletoe lifestyle (i.e., N- or C- parasitism hypotheses) that this dominates over other selective pressures. The second scenario might be possible if, evolutionarily speaking, the most successful parasite strategy is to be frugal with water and thus, minimally detrimental to hosts. Indeed, universal parasitic theories suggest that, for obligatory parasites, there should be a balance of the virulence (i.e., the decrease of host's fitness) to avoid host death and maintain the parasite alive for longer periods of time (Levin & Pimentel 1981; Anderson & May 1982). Finally, a third scenario is also possible in which mistletoes would exhibit *some* degree of adaptation to aridity, but noticeably dampened responses compared to that seen in hosts because of possible relaxed selective pressure to use water more efficiently (Stewart & Press 1990).

We explored these issues using a comparative, multi-species framework. We sampled 42 mistletoe-host pairs across four sites in Australia and one in Brazil that varied widely in moisture index (ratio of annual precipitation to potential evapotranspiration). We also used

this opportunity to test for general trait differences between mistletoes and hosts across all pairs and among sites. Based especially on previous results for Australian sclerophyllous trees and shrubs in New South Wales (Wright et al 2001), but also on references given above, our expectations for host plants were that species at more arid sites would have:

- (1) Higher N_{area} (achieved either via lower SLA or via higher N_{mass}), and therefore:
- (2) Lower ratio of leaf internal to external CO_2 ($c_i : c_a$); and therefore, lower stomatal conductance g_s at a given photosynthetic capacity A_{area} .
- (3) Higher average dark respiration rate R_{area} , but especially higher R_{area} at a given A_{area} (i.e., higher respiratory costs of operating at drier sites).
- (4) Higher leaf phosphorous per area (P_{area}).

In relation to mistletoes, under the first scenario described above, in which water should be almost free for mistletoes, we would expect no systematic responses in these traits in relation to site aridity. Under the second scenario, where the balance of virulence is important, mistletoes would show these same trait differences in relation to site aridity, in parallel – and to the same extent – to what was seen in host species. Finally, in general, whether responding strongly or not at all to aridity - or somewhere in between -, we also expected that mistletoes, being profligate water users, would operate at higher $c_i : c_a$ compared to their hosts and thus, at higher g_s for a given A_{area} (using more water per unit of photosynthetically-fixed carbon); and, possibly, at higher g_s , absolutely.

Material and Methods

Our dataset is composed of 42 mistletoes found in different hosts from a variety of families, spanning a range of ecological and phylogenetic levels. The sites were chosen based on the abundance of mistletoe-host (M-H) pairs and the contrasting vegetation type, annual

precipitation and moisture index (MI). Geographical coordinates were used to retrieve the mean annual temperature (MAT) and mean annual precipitation (MAP) from the CRU CL2.0 global climate dataset (New *et al.* 2002). Potential evapotranspiration (PET) was estimated following Wang, Prentice and Ni (2012) and moisture index was calculated as the ratio between MAP and PET (Wang, Prentice & Ni 2012). For analyses, the sites were divided into dry ($MI < 0.3$) and wet ($MI > 0.7$) sites. Table 1 shows the study sites representing different environmental conditions, with the distinct climate properties. The number of species sampled varied between sites according to the availability of mistletoe-host pairs in the area. In each site, four to six individuals of 6-12 mistletoe-host pairs were sampled (Table 2).

Sites descriptions

Australian Savanna: The site was located in Howard Springs National Park, approximately 35 km southeast of Darwin in Northern Territory, Australia ($130^{\circ}45'E$, $12^{\circ}30'S$). It consists of a typical tropical savanna with markedly seasonality, whereas 95% of the 1750 mm mean annual rainfall is restricted to the wet season (December to March; Williams *et al.* 1997). The soil in the area is well-drained, highly weathered, laterised and low in nutrient status (Hutley, O'grady & Eamus 2000). The vegetation in the study site consists of open-forest dominated by *Eucalyptus tetrodonta* (F. Muell.) and *Eucalyptus miniata* (Cunn. Ex Schauer) dominant open-forest, and an understory of small trees, shrubs and C4 grasses. Mistletoes in the site tend to occur in the most abundant species (listed in Table 2).

Cerrado (Brazilian Savanna): The site is located in the Natural Reserve of the Roncador belonging to the Brazilian Institute of Geography and Statistics (RECOR/IBGE), 35km south of Brasilia-DF, Brazil ($47^{\circ}53'W$, $15^{\circ}56'S$) and part of the Environmental Protection Area Gama-Cabeça de Veado, with a total of 10,000 hectares of continuous protected area. The

savanna of central Brazil is the most diverse savanna in the world in terms of floristic composition (Solbrig 1996), and considered a biodiversity hotspot for conservation (Myers *et al.* 2000). Soil nutrient availability usually limits tree growth and density, mainly because of the low availability of P and Ca (Silva *et al.* 2013) associated with high soil acidity (pH around 5.5), high Al availability (Haridasan 2001) and fire disturbance (Eiten 1972). The average annual precipitation in this area is approximately 1500 mm with a pronounced dry season from May through September and a mean annual temperature that ranges from 20 to 26°C (Oliveira-Filho *et al.* 1989). The predominant soils are deep and well-drained Oxisols, but hydromorphic soils also occur associated with watercourses (Dantas & Batalha, 2011).

Australian closed woodland: The two different sites are located in distinct parks around Sydney area (New South Wales): Ku-ring-gai National Park (33°63'S, 151°26'E) and Royal National Park (34°17'S, 151°05'E). Although our samples are spread into the two different sites, the climatic variables are similar, both sites vegetation are dominated by evergreen trees, and the sandstone soils are characterized by extreme low nutrient content. In Royal NP, the site is located on a sheltered sandstone slope and the vegetation is dominated by red gum (*Angophora costata*) together with a eucalypt woodland community (NPWS 2000; Tozer *et al.* 2010). The site in Ku-ring-gai NP is also located in a sheltered sandstone and the vegetation is dominated by eucalypts species, specially forest grey box (*Eucalyptus moluccana*), spotted gum (*Eucalyptus maculata*), and scribbly gum (*Eucalyptus haemastoma*), classified as a low eucalypt woodland (NPWS 2002).

Australian chenopod shrubland: We sampled M-H pairs located in Whyalla National park and around the area of the park, ~ 10km north of Whyalla, South Australia (32°94'S, 137°53'E). The vegetation consists of a chenopod shrubland dominated by bladder saltbush

(*Atriplex vesicaria*) and pearl bluebush (*Maireana sedifolia*) with scattered trees, such as western myall trees (*Acacia papyrocarpa*) and sugarwood (*Myoporum platycarpum*), which are potential hosts for the mistletoes species. The climate is semi-arid, averaging 300 clear and sunny days per year (NPWS 1998), and the soils are deep and stratified (Jessup & Wright 1971). The soils of the areas we sampled are classified as clay sand soils.

Australian semi-arid woodland: The site was located at Gundabooka National Park, north-western New South Wales (30°42'S, 145°56'E), and approximately 50 km south of Bourke. The climate of the region is semi-arid, with very low annual rainfall and high temperatures in summer (NPWS 2005). The vegetation consists of an open woodland community dominated by mulga shrubs (*Acacia aneura*), ironwood (*Acacia excelsa*) and leopardwood (*Flindersia maculosa*). The region has been severely impacted by feral goats grazing (Russell, Letnic & Fleming 2011), and now the park has a high abundance of less palatable shrubs, such as *Eremophila*, *Senna* and *Dodonea* species (NPWS 2005). Mistletoes in the area occur at a very high density and parasitizing most of the dominant species.

Table 1. Site locations and climates. Mean annual precipitation (MAP) and mean annual temperatures (MAT) were obtained from the CRU CL2.0 global gridded dataset (New *et al.* 2002). Mean annual evapotranspiration (PET) was estimated following Wang, Prentice and Ni (2012) and moisture index was calculated as the ratio between MAP and PET.

Site vegetation	Location	Coordinates	MAT (°C) (Min-Max)	MI*	MAP (mm)	PET (mm)
Close woodland	Ku-ring-gai NP Royal NP (Sydney, NSW, Australia)	33°63'S 151°26'E 34°17'S 151°05'E	17.2 (13.7-21.7)	0.95	1267	1331
Chenopod shrubland	Whyalla Park (Whyalla, SA, Australia)	32°94'S 137°53'E	17.9 (11.4-23.7)	0.19	278	1465
Savanna 1	Howard Springs (Darwin, NT, Australia)	12°30'S 130°45'E	27.6 (23.2-32.0)	0.78	1669	2147
Semi-arid woodland	Gundabooka NP (Bourke, NSW, Australia)	30°42'S 145°56'E	19.8 (13.1-28.0)	0.15	294	1888
Savanna 2	IBGE Ecological Reserve (Brasília, DF, Brazil)	15°55'S 47°51'E	25.8 (14.4-31.2)	1.03	1478	1434

*MI = MAP/PET

Table 2. List of the studied mistletoe-host pairs species at each site location.

Site	Mistletoe	Host	Host Family
Closed woodland (Sydney) n=6	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus hemastoma</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus moluccana</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus</i> spp.	Myrtaceae
	<i>Amyema congener</i>	<i>Allocasuarina littoralis</i>	Casuarinaceae
	<i>Dendrophthoe vitellina</i>	<i>Eucalyptus</i> sp.	Myrtaceae
	<i>Dendrophthoe vitellina</i>	<i>Angophora costata</i>	Myrtaceae
Chenopod shrubland (Whyalla) n=8	<i>Amyema quandang</i>	<i>Acacia papyrocarpa</i>	Fabaceae
	<i>Lysiana exocarpii</i>	<i>Eremophila longifolia</i>	Scrophuliaceae
	<i>Lysiana exocarpii</i>	<i>Acacia</i> sp.	Fabaceae
	<i>Lysiana exocarpii</i>	<i>Pittosporum angustifolium</i>	Pittosporaceae
	<i>Lysiana exocarpii</i>	<i>Myoporum platycarpum</i>	Scrophuliaceae
	<i>Amyema miraculosa</i>	<i>Myoporum platycarpum</i>	Scrophuliaceae
	<i>Amyema presii</i>	<i>Acacia victoriae</i>	Fabaceae
	<i>Lysiana exocarpii</i>	<i>Santalum acuminatum</i>	Santalaceae
Savanna 1 (Darwin) n=7	<i>Amyema sanguinea</i>	<i>Corymbia porrecta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Eucalyptus miniata</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Eucalyptus tetradonta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Corymbia blesseri</i>	Myrtaceae
	<i>Amyema miquelli</i>	<i>Eucalyptus miniata</i>	Myrtaceae
	<i>Dendrophthoe odontocalyx</i>	<i>Grevillea pteridifolia</i>	Proteaceae
	<i>Decaisnina signata</i>	<i>Xanthostemon paradoxus</i>	Myrtaceae
Semi-arid woodland (Bourke) n=12	<i>Amyema miraculosa</i>	<i>Eremophila longifolia</i>	Scrophuliaceae
	<i>Amyema miraculosa</i>	<i>Myoporum platycarpum</i>	Scrophuliaceae
	<i>Amyema miraculosa</i>	<i>Eremophila mitchellii</i>	Scrophuliaceae
	<i>Lysiana exocarpi</i>	<i>Alectryon oleifolius</i>	Sapindaceae
	<i>Amyema lucasii</i>	<i>Flindersia maculosa</i>	Rutaceae
	<i>Amyema preissii</i>	<i>Acacia aneura</i>	Fabaceae
	<i>Amyema preissii</i>	<i>Senna eremophila</i>	Fabaceae
	<i>Amyema preissii</i>	<i>Flindersia maculosa</i>	Rutaceae
	<i>Amyema mandeirii</i>	<i>Acacia harpophylla</i>	Fabaceae
	<i>Amyema mandeirii</i>	<i>Acacia aneura</i>	Fabaceae
	<i>Lysiana linearifolia</i>	<i>Eremophila mitchellii</i>	Scrophuliaceae
	<i>Lysiana exocarpi</i>	<i>Acacia aneura</i>	Fabaceae
Savanna 2 (Brasília) n=9	<i>Phoradendron</i> sp.	<i>Miconia albicans</i>	Melastomataceae
	<i>Struthanthus polyanthus</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Psittacanthus robustus</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Phthirusa ovata</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Psittacanthus robustus</i>	<i>Qualea grandiflora</i>	Vochysiaceae
	<i>Psittacanthus robustus</i>	<i>Qualea parviflora</i>	Vochysiaceae
	<i>Psittacanthus robustus</i>	<i>Qualea multiflora</i>	Vochysiaceae
	<i>Phthirusa ovata</i>	<i>Dalbergia miscolobium</i>	Fabaceae
	<i>Phthirusa ovata</i>	<i>Styrax ferrugineus</i>	Styracaceae

Traits measurement

In the field, we measured photosynthetic rate (A_{\max}), stomata conductance (g_s), and transpiration rates (E) simultaneously at saturating light intensity, using portable infra-red gas analysis systems, either a Licor 6400 (Australian sites) or a LCpro (Brazilian site). After a maximum period of 6 hours, dark respiration was measured in the laboratory in detached branches that were maintained watered in a cooler at 25 °C when possible, or measured at between 26.8 and 35.1 °C, with these measurements transformed to 25 °C using the formula for temperature dependence of R described by Atkin, Bruhn and Tjoelker (2005):

$$R_2 = R_1 \{ 3.09 - 0.0435 [(T_2 + T_1) / 2] e^{[(T_2 - T_1) / 10]} \} \quad \text{Eqn 1}$$

where R_1 and T_1 are the respiration rate and temperature measured, and R_2 and T_2 are the respiration rate and temperature of interest (25 °C), respectively.

In addition, fully expanded mature leaves were collected, scanned and the area was measured using the software Image J (Abràmoff, Magalhães & Ram 2004). After drying for 72h, the leaves were weighed and the specific leaf area (SLA) was calculated as the ratio between fresh area and dry mass. The dried leaves were ground and Australian samples were sent to the Analytical Service Unit from the School of Agriculture and Food Science at The University of Queensland for N and P determination by LECO TruSpec CHN combustion analyser and ICP-OES technique, respectively. Brazilian samples were sent to Laboratorio de Agroquímica e Meio Ambiente at Universidade Estadual de Maringa (PR/Brazil) for N and P determination by Kjeldahl digestion and UV-Vis spectroscopy, respectively. All trait measurements were performed in five to seven individuals of each M-H pair.

Data analyses

All measurements were converted to mass and/or to an area basis for further analysis. Individual measurements were averaged for each species-pair-site combination, and all data were log-transformed for the assumption of normality (Kolmogorov-Smirnov test, $P < 0.05$ for all variables). Because of the intrinsic paired nature of the data, we used paired t-tests to test for systematic differences between individual traits in mistletoes and their hosts. To test for individual trait trends in relation to habit (parasite or host) and site aridity (wet or dry) we used two-way analysis of variance (ANOVA). We also fitted standardized major axis (SMA) slopes to describe bivariate relationships between key traits ($g_s - A_{area}$, $R_{d-area} - A_{area}$) and to compare the homogeneity and shifts of these relationships between dry and wet sites and between mistletoes and hosts (Warton *et al.* 2006). Software R v. 2.13 (R Development Core Team 2008) was used to perform all statistical analysis, and the package SMATR version 3 (Warton *et al.* 2012) to test for SMA slopes differences, considering all tests to be significant when $P > 0.05$.

Results

Trait patterning with site aridity

Overall, our results generally agreed with our second prediction, that mistletoes exhibited clear trait adaptations to aridity, in parallel and to the same extent as host species (Figs 1 and S1, Table 4). In both mistletoes and hosts, species at dry sites had higher N_{area} and P_{area} . Inspection of boxplots in Figure 1 shows that the higher N_{area} at dry sites (Fig. 1e) was achieved both via lower SLA (Fig. 1i) and higher N_{mass} (Fig. 1d), but in mistletoes more so via lower SLA (i.e, there was no significant difference in N_{mass} between dry- and wet-site mistletoe species, whereas there was in hosts). In both mistletoes and hosts, the higher P_{area}

seen in dry-site species (Fig. 1h) was clearly due both to their lower SLA and to their higher P_{mass} (Fig. 1g).

In both mistletoes and hosts, dry-site species showed greater CO_2 drawdown during photosynthesis (lower $c_i : c_a$; Fig. 1c), in support of the contention that higher N_{area} is implicated in decreasing photosynthetic water use. As a result, we expected that species from dry sites would show lower g_s at a given A_{area} , but in fact, we found stronger-than-expected patterning with aridity such that, in both mistletoes and hosts, dry-site species were operating *both* at lower g_s and at lower A_{area} (Figs 1a,f; 2a).

We found no support for the prediction of higher $R_{\text{d-area}}$ at drier sites (Fig. 1b). Instead, shifts in $R_{\text{d-area}}$ mirrored those in A_{area} , $R_{\text{d-area}}$ being significantly lower at dry sites in mistletoes while, in hosts, the apparent decrease was not supported statistically. Based on patterns reported by Wright *et al* (2001), at dry sites, we also predicted higher $R_{\text{d-area}}$ at a given A_{area} ; this pattern was also not observed (Fig. 2b).

Trait differences between mistletoes and their hosts

Considering all species, there were several remarkable trait differences between mistletoes and hosts (Table 3). Mistletoes averaged 1.4-fold lower SLA (paired t-test, $P < 0.01$), 1.4 times higher N_{area} ($P < 0.01$), 2.5-fold higher P_{area} , 2-fold higher P_{mass} , 1.5 times higher $c_i : c_a$ ($P < 0.01$) and 2.4 times higher stomatal conductance ($P < 0.001$).

Divided by rainfall zone, mistletoes on average showed 1.6-fold (wet sites) and 1.2-fold higher (dry sites) stomatal conductance at a given A_{area} , compared to hosts (differences in slope elevation both $P < 0.001$; Fig. 2a). Mistletoes also showed higher respiration relative to photosynthetic rates, $R_{\text{d-area}}$ being on average 2.7-fold higher (wet sites) and 1.3-fold higher (dry sites), for a given A_{area} (Fig. 2b). Considered as a ratio, overall, $R_{\text{d-area}} : A_{\text{area}}$ was twice as

high in mistletoes than in hosts (mean \pm SD: 0.39 ± 0.21 versus 0.20 ± 0.09 ; paired t-test, $P < 0.001$).

These mean trait differences between hosts and mistletoes traits were generally consistent across sites (Table 3). In each of the studied sites, compared to their hosts, mistletoes showed higher N_{area} , P_{area} , P_{mass} and g_s ; lower SLA and A_{area} , and similar $R_{\text{d-area}}$ and N_{mass} . The few exceptions were at the Cerrado site, where mistletoes showed higher $R_{\text{d-area}}$; at Sydney closed woodland, where mistletoes and hosts exhibited similar N_{area} and g_s ; and at Gundabooka semi-arid woodland, with similar A_{area} and g_s (Table 3).

Table 3. Trait mean (SD) for mistletoes (M) and host plants (H) and the mistletoe:host ratio (M:H) for the different studied sites and the total average value. Pair-wise differences between mistletoes and hosts are indicated by the symbol * within each site considered (paired t-tests, $P < 0.05$).

Trait	Semi-arid woodland			Chenopod shrubland			Australian Savanna			Closed woodland			Cerrado			Total		
	M	H	M:H	M	H	M:H	M	H	M:H	M	H	M:H	M	H	M:H	M	H	M:H
N _{area}	6.4 (3.0)	4.7 (1.8)	1.4*	5.8 (2.2)	4.4 (1.7)	1.3*	2.4 (0.4)	1.5 (0.3)	1.7*	1.8 (0.3)	1.7 (0.5)	1.1	4.6 (1.0)	3.1 (0.4)	1.5*	4.7 (2.6)	3.3 (1.8)	1.4*
P _{area}	0.36 (0.35)	0.21 (0.05)	1.7*	0.83 (0.4)	0.25 (0.04)	3.3*	0.23 (0.07)	0.09 (0.02)	2.6*	0.15 (0.02)	0.09 (0.02)	1.7*	0.3 (0.06)	0.12 (0.04)	2.3*	0.5 (0.4)	0.2 (0.1)	2.8*
SLA	28.7 (4.5)	44.6 (15.2)	0.6*	27.5 (2.9)	38.9 (11.8)	0.7*	40.6 (12.8)	56.1 (8.5)	0.7*	33.9 (7.3)	46.5 (14.3)	0.7*	46.2 (5.7)	64.9 (15.3)	0.7*	35.0 (10.0)	50.1 (16.0)	0.7*
A _{area}	3.9 (1.5)	4.6 (2.6)	0.8	6.7 (2.3)	9.5 (2.0)	0.7*	10.6 (1.5)	17.6 (2.7)	0.6*	6.6 (1.5)	17.1 (2.5)	0.4*	10.1 (2.4)	13.4 (2.1)	0.8*	7.3 (3.3)	11.2 (5.6)	0.6*
R _{area}	1.19 (0.29)	1.11 (0.44)	1.1	2.67 (0.91)	2.42 (0.49)	1.1	4.61 (1.34)	3.56 (1.12)	1.3	2.82 (0.73)	2.18 (1.23)	1.3	2.39 (0.89)	1.49 (0.34)	1.7*	2.52 (1.4)	1.85 (1.2)	1.4
g _s	0.05 (0.03)	0.03 (0.02)	1.7	0.15 (0.04)	0.09 (0.02)	1.7*	1.80 (0.52)	0.52 (0.15)	3.5*	0.41 (0.12)	0.30 (0.12)	1.4	0.24 (0.04)	0.17 (0.05)	1.4*	0.46 (0.66)	0.19 (0.18)	2.4*
c _i :c _a	0.59 (0.09)	0.39 (0.08)	1.5*	0.75 (0.09)	0.53 (0.08)	1.4*	0.93 (0.02)	0.76 (0.06)	1.2*	0.86 (0.05)	0.53 (0.12)	1.6*	0.77 (0.05)	0.56 (0.05)	1.4*	0.75 (0.14)	0.54 (0.14)	1.4*
N _{mass}	1.76 (0.74)	1.84 (0.27)	1	1.57 (0.59)	1.54 (0.37)	1	0.95 (0.29)	0.81 (0.17)	1.2	0.60 (0.14)	0.71 (0.09)	0.8	2.08 (0.48)	1.95 (0.58)	1.1	1.49 (0.72)	1.48 (0.59)	1
P _{mass}	0.17 (0.09)	0.09 (0.02)	1.9*	0.23 (0.12)	0.09 (0.02)	2.6*	0.09 (0.03)	0.05 (0.02)	1.8*	0.05 (0.01)	0.04 (0.01)	1.3*	0.12 (0.01)	0.08 (0.02)	1.5*	0.14 (0.09)	0.07 (0.03)	2.0*

Two-way ANOVA shows an individual significant effect of site (wet or dry) for every trait studied, and of habit (host or mistletoes) for most of the traits, except N_{mass} and $R_{\text{d-area}}$ (Table 4). The effect of the two-way interaction between site and habit was restricted to A_{area} , g_s , and leaf P concentration (P_{area} and P_{mass}), suggesting that increasing aridity affected differently mistletoes and hosts for these traits. However, the non-significant interaction between site and habit for SLA, leaf N concentration (N_{area} and N_{mass}) and $R_{\text{d-area}}$ suggest the opposite; i.e., plants were affected in similar ways for these traits, irrespectively of being mistletoe or host.

Table 4. *F*-values and *P*-values for two-way ANOVA on the influence of site moisture zone (wet or dry) and habit (mistletoe or host) on leaf functional traits.

Trait	Source of variation	df	<i>F</i>	<i>P</i>
A _{area}	Site	1	117.96	< 0.001
	Habit	1	39.73	< 0.001
	Site*Habit	1	13.88	< 0.001
	Residuals	80		
N _{area}	Site	1	45.82	< 0.001
	Habit	1	10.688	< 0.001
	Site*Habit	1	0.644	0.425
	Residuals	80		
g _s	Site	1	28.05	< 0.001
	Habit	1	8.909	0.004
	Site*Habit	1	6.610	0.012
	Residuals	80		
R _{area}	Site	1	18.56	< 0.001
	Habit	1	4.44	0.038
	Site*Habit	1	2.175	0.144
	Residuals	80		
P _{area}	Site	1	45.53	< 0.001
	Habit	1	46.38	< 0.001
	Site*Habit	1	16.63	< 0.001
	Residuals	80		
SLA	Site	1	32.131	< 0.001
	Habit	1	36.943	< 0.001
	Site*Habit	1	0.101	0.752
	Residuals	80		
N _{mass}	Site	1	8.698	0.04
	Habit	1	0.004	0.953
	Site*Habit	1	0.225	0.676
	Residuals	80		
P _{mass}	Site	1	24.783	< 0.001
	Habit	1	26.911	< 0.001
	Site*Habit	1	6.855	0.011
	Residuals	80		

Discussion

Our overarching question in this research was whether mistletoes exhibited clear photosynthetic trait adaptations to aridity; and, if so, whether these were similar or dampened in comparison to those seen in hosts. In order to ask this question we first needed to ascertain that host species exhibited the key, expected adaptations to site aridity (higher N_{area} and lower $c_i : c_a$), which in fact, they did. As expected, dry-site host species also showed higher P_{area} and lower SLA; but, counter to predictions, there was no evidence of higher leaf respiration at dry sites (absolutely, or relative to photosynthetic rates).

Our overall answer to the overarching question was that mistletoes clearly showed the same aridity-related shifts in leaf traits as their hosts, and that these shifts were of the same general magnitude. This trend was demonstrated at a considerable level of generality, considering 42 species-pairs from five sites on two continents. This finding was unexpected and divergent from the idea that water must be relatively “free” for mistletoes; in which case, there should be little selective pressure for them to use it efficiently. Based on previous studies, we broadly expected to see at least *some* degree of aridity-adaptations in mistletoes, but for this to be noticeably dampened compared to what was seen in hosts (as suggested by Stewart & Press (1990) to be generally true of leafy parasites). Below, we discuss this issue, the general matter of leaf nutrient adaptations to aridity, and then move on to discuss the finding that respiration in relation to photosynthesis was twice higher in mistletoes than in their hosts.

Similar trait adaptation to aridity in mistletoes and hosts

In line with the idea that water is very cheap for mistletoes, here as elsewhere (e.g. Chapter 2) we found clear evidence that mistletoes are profligate water users compared to their hosts, overall, at dry and wet sites. This was shown from instantaneous measurements of

photosynthesis, with mistletoes maintaining generally higher stomatal conductance to water (g_s) and higher $c_i : c_a$ at all five study sites (Table 3), and thus presumably also operating with higher transpiration rates, and lower water use efficiency (ratio of A_{area} to transpiration).

It is well understood that mistletoes must generate and maintain lower leaf water potentials (LWPs) than their hosts (Glatzel 1983; Hollinger 1983; Schulze, Turner & Glatzel 1984; Ehleringer, Cook & Tieszen 1986) to maintain high transpiration rates. The reported mechanism of how this is achieved is by accumulating great quantities of osmolytes (Ehleringer, Cook & Tieszen 1986) and having succulent fleshy leaves with higher water storage capacity (Ehleringer, Cook & Tieszen 1986; Whittington & Sinclair 1988; Richter & Popp 1992; Popp *et al.* 1995). LWPs in dry-site hosts are presumably lower than in wet-site hosts, because soil moisture is in shorter supply. The question that then arises is whether LWPs in dry-site mistletoes are similarly lower, or whether the difference between mistletoes and hosts in LWP is smaller at dry sites.

Here, we did not investigate LWP, so this question cannot be answered directly, but we are confident that there was no evidence of dampened response to aridity. Our results accord with those of Ullmann *et al.* (1985) who, considering a wide range of mistletoes and hosts across a transect in central Australia, observed that, while daily average leaf conductance were uniformly higher in mistletoes, diurnal time courses of stomatal regulation were seemingly tightly coordinated in mistletoe-host pairs. From our results and from some complimentary findings elsewhere (e.g., Ullmann *et al.* (1985); Davidson and Pate (1992); Whittington and Sinclair (1988); and Bowie and Ward (2004)), we could confirm mistletoes stomata behaviour is responding to environmental influence. However, we might need to consider whether these responses can also be mediated by host-induced responses, with stomatal closure in mistletoes being partially controlled by chemical signals (e.g. ABA) produced by the host roots, that are then transferred to mistletoes *via* host xylem. We are

unable to explore this possibility based on our own work. Nevertheless, we note that there is evidence against the ABA-mechanism for the mistletoe *Viscum album*, where Escher *et al.* (2008) corroborate the strong control by the mistletoe over the influx of ABA from the host.

Higher N_{area} and P_{area} at arid sites

We found that species from drier sites, whether mistletoes or hosts, had generally 2 times higher N_{area} and 2.5 times higher P_{area} than wet-site species, due both to lower SLA at drier sites, and generally higher nutrient concentrations per leaf mass. It is reasonably well known that woody species in arid and high irradiance regions tend to have higher N_{area} (Mooney, Ferrar & Slatyer 1978; Cunningham, Summerhayes & Westoby 1999; Reich *et al.* 1999; Wright, Reich & Westoby 2001; Farquhar, Buckley & Miller 2002; Wright *et al.* 2005). As outlined in the Introduction, this is likely associated with an enhanced ability to drawdown CO_2 during photosynthesis (lower $c_i : c_a$), and thus, to being more economical in photosynthetic water use (Wright, Reich & Westoby 2003; Prentice *et al.* 2014). Note however, this is not to say that dry-site species necessarily use *less* water per unit photosynthetic C fixed than wet-site species; whether or not this is the case depends on the relative magnitude of the shift in g_s versus that in atmospheric VPD, transpiration being a function of both. What is far less understood, however, is the significance is of higher P_{area} at lower rainfall sites – as seen here and previously also in NSW (Wright, Reich & Westoby 2001) and globally for woody species (Wright *et al.* 2004). In all cases, the higher P_{area} was driven both by lower SLA and by higher P_{mass} .

Leaf phosphorus is found in molecules such as ATP and NADPH. Generally speaking, higher leaf P enables a higher carboxylation capacity for a given leaf N (Niinemets *et al.* 1999; Reich, Oleksyn & Wright 2009) and perhaps, simply for this reason, combining higher leaf P with higher leaf N could consist an advantageous ecological strategy, especially at more

arid sites, where this would mean losing less water for a given rate of carbon gain. Indeed, there are reports of a role for leaf P in enhancing water use efficiency but, to our knowledge, no convincing mechanisms have been suggested, let alone established. For example, in *Quercus ilex* forest subjected experimentally to lower soil moisture, leaf P_{mass} increased, while P concentration in roots and stems decreased, suggesting that plants were mobilising P towards leaves, presumably to improve water use efficiency (Sardans & Peñuelas 2007). Similarly, in experiments with *Eucalyptus grandis* it has been shown that P fertilization enhances growth and water use efficiency for droughted seedlings (Graciano, Guiamét & Goya 2005), though no mechanism was proposed for this effect. This topic remains little understood but potentially of very significant interest, especially in semi-arid Australia and Brazil where P is generally considered the most limiting soil nutrient, and rainfall is low or seasonally restricted. Our results here serve to illustrate that high P_{area} in arid regions may be an even more general trend than previously suspected, found even in hemi-parasitic mistletoes.

Moreover, following the N-parasitism hypothesis principles, if there is indeed some specific limiting nutrient driving the high transpiration rates in mistletoes leaves, it might be more sensible to consider P as this limiting resource, at least for the systems we investigated. This inference is not only based on the results we found here, but also our results showed in Chapter 2, where no general support was found for the N-parasitism globally, and from Chapter 4, where there was strong evidence that N is not a limiting nutrient, not being resorbed by mistletoes; while P was at least partially resorbed.

Mistletoes higher respiration costs

In a previous study concerning several dozen woody perennials in NSW, Wright, Reich & Westoby (2001) showed that dry-site species were incurring higher leaf respiration costs, both

absolutely and at a given photosynthetic rate. This trend was later generalised to global scale (Wright *et al.* 2006), and interpreted as reflecting the higher cost of “doing business” at arid, high irradiance sites. Mechanisms invoked included higher respiratory costs of repairing UV-related damage to photosystem II; production of protective pigments, such as xanthophyll; various costs associated with dealing with photoinhibition; and respiratory costs associated with maintaining solute gradients, which should be especially important at arid sites (Wright *et al.* 2006).

Actually, here we did not find such pattern, with mistletoes and hosts showing lower $R_{d\text{-area}}$ and lower A_{area} at drier sites. Presumably, lower A_{area} at drier sites flows both from the lower g_s together with other factors thought common in arid and semi-arid environments, e.g. higher leaf-internal CO_2 diffusion limitations due to thicker and denser leaves, and down-regulation of photosynthesis, by metabolic impairment (Chaves *et al.* 2002; Flexas *et al.* 2004). In general, one would expect lower respiration rates to be paired with lower photosynthetic rates because major contributors to photosynthesis (and related processes) have significant respiration costs: e.g. continual turnover of photosynthesis-related proteins (most notably, but not only Rubisco), and phloem loading of photosynthates. Therefore, on this basis, our results (lower $R_{d\text{-area}}$ at drier sites) seem to make sense; it is just the discrepancy with the results from Wright, Reich & Westoby (2001) and (2006) that we are unable to reconcile.

Putting site aridity aside, the other key result here was that mistletoes showed considerably higher $R_{d\text{-area}} : A_{\text{area}}$ ratio (averaging 0.39), suggesting 2-fold higher maintenance costs at a given carbon gain in comparison with their hosts. In fact, mistletoes showed generally lower A_{area} but similar $R_{d\text{-area}}$ to their hosts (Table 3). Various studies have shown that mistletoes usually show lower photosynthetic rates compared to their hosts (Hollinger 1983; El-Sharkawy, Cock & Hernandez 1986; Orozco *et al.* 1990; Küppers 1992; Marshall *et*

al. 1994; Flanagan, Marshall & Ehleringer 2006). This lower A_{area} may be caused by differences in mesophyll structure (e.g. undifferentiated mesophyll decreasing mesophyll conductance; Stewart & Press (1990); Khan *et al.* (2009); Shahryar, Robabeh & Narges (2012)), low chlorophyll concentration (Johnson & Choinski 1993; Tuquet & Sallé 1996; Strong, Bannister & Burritt 2000) and low Rubisco and photosystem II activity (Strong, Bannister & Burritt 2000; Chen *et al.* 2013).

However, less well understood is why mistletoes show such high respiration rates for a given photosynthetic capacity and, indeed, how such this strategy is even feasible; i.e., how it results in sufficient net carbon gain to support their growth. In relation to how feasible it is, probably the main possibility is that mistletoes may receive considerable carbon from their host via the xylem stream, transported as amino acids (Raven 1983; Schulze, Turner & Glatzel 1984; Marshall & Ehleringer 1990; Marshall *et al.* 1994), helping them to achieve an overall positive carbon balance (Stewart & Press 1990).

Possible factors leading to higher $R_{\text{d-area}}$ at a given A_{area} include: (1) heterotrophy itself, there being energetic costs to using host-derived carbon, as for any carbon C substrates (Amthor 2000; Thornley & Cannell 2000); and (2) maintenance of large ion gradients between cellular compartments. Mistletoes have limited sinks and higher nutrient uptake via haustorium by higher rates of transpiration, and can accumulate disproportional amounts of some nutrients in their leaves, which can lead to metabolism unbalance. There are reports of disproportional hyperaccumulation of ions and heavy metals, such as potassium (Lamont & Southall 1982; Schulze, Turner & Glatzel 1984; Scalon, Haridasan & Franco 2013), aluminium (Scalon, Haridasan & Franco 2013), and sodium (Goldstein *et al.* 1989). Indeed, dealing with nutrient imbalance might be the one of the most substantial metabolic challenges for mistletoes. In addition, as outlined above, mistletoes need to maintain lower LWP than found in host leaves in order to maintain high transpiration rates. Popp *et al.* (1995) showed

evidence that increasing leaf succulence of two African mistletoes species was an adaptation to keep ion concentration at a tolerable level. Moreover, it has been proposed that selective intake of nitrogen-containing compounds, such as polyols, proline, and glycinebetaine may be an important advantage for the parasite (Tennakoon & Pate 1996; Frost, Lopez-Gutierrez & Purrington 2003), because these compounds act as osmoprotectants (i.e., protect the cell from the consequences of osmotic stress; Neales & Sharkey 1981; Sakamoto & Murata 2002). Therefore, mistletoes may need to balance between maintaining higher osmolarity, to guarantee lower water potential and the continuous flow of the host xylem, with the considerable costs associated with it.

Conclusions

In this study, we focused on adaptations to aridity in mistletoes and their host. Our results indicate that, even though mistletoes are profligate water users compared to their hosts, they showed same aridity-related shifts, and of the same general magnitude, in leaf traits as their hosts. Perhaps in these systems, the high transpiration rates of mistletoes can best be understood as driven by the need to obtain both carbon and phosphorous from their hosts, rather than nitrogen. Higher leaf P concentration in mistletoes from dry sites illustrate that high P_{area} in arid regions may be an even more general trend than previously suspected, involved possibly in water saving strategies. Moreover, we discussed the probable trade-off between mistletoes maintaining higher osmolarity, to guarantee lower water potential and the continuous flow of the host xylem, with the considerable costs associated with it, resulting in very high respiration rates for a given photosynthetic capacity.

Acknowledgements

We acknowledge CNPq for financial support and Australian National Parks and RECOR/IBGE offices for permits and logistic support. We also thank Izabelly Sant’Ana, Allyson Eller, Julieta Garcia-Russell, Julia Cooke and Fabricius Domingos for the valuable help in the field.

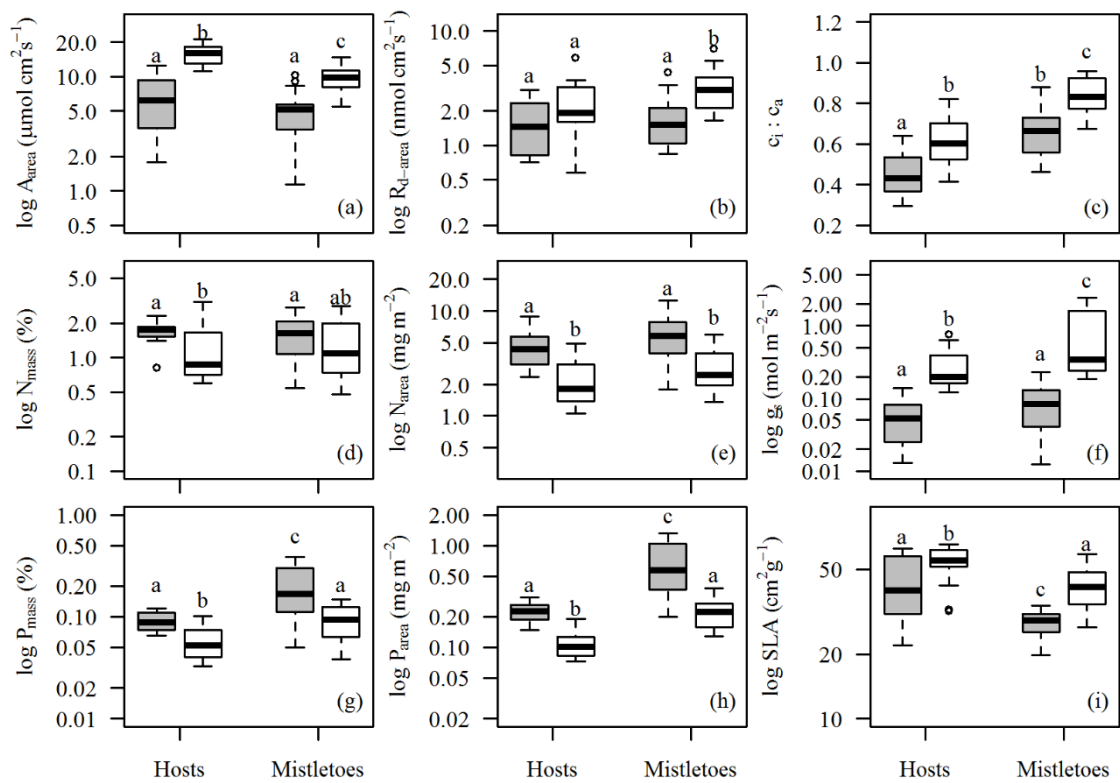


Figure 1. Boxplots of traits for mistletoes and hosts in dry (grey boxes) and wet (white boxes) in area and mass basis: leaf carbon assimilation ratio (A_{area}); leaf dark respiration rate ($R_{\text{d-area}}$); internal to ambient CO_2 ratio ($c_i : c_a$); nitrogen leaf concentration (N_{area} and N_{mass}); stomatal conductance (g_s); phosphorus leaf concentration (P_{area} and P_{mass}); and specific leaf area (SLA). Distinct letters denote significant differences (ANOVA, $P < 0.05$; Table S1).

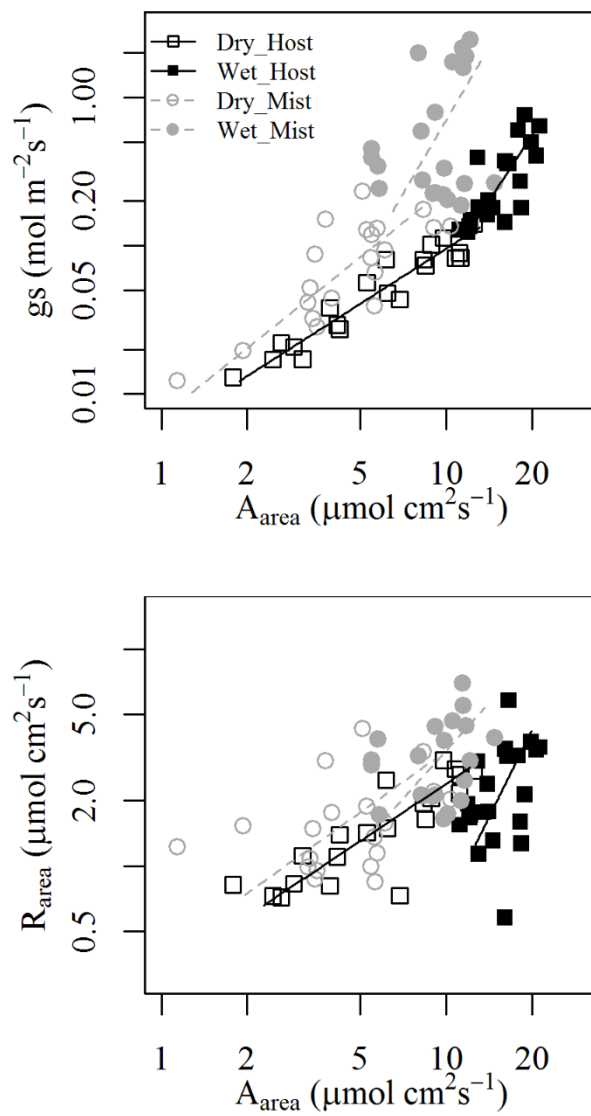


Figure 2. Standardized major axis (SMA) relationships between g_s and $R_{d\text{-area}}$ on A_{area} for hosts (squares, continuous line) and mistletoes (circles, dashed lines). (a) Common slopes fitted within wet sites (filled symbols, $MI > 0.7$): 0.32 (95% CI 0.25, 0.42), and dry sites (empty symbols, $MI < 0.3$): 0.78 (0.67, 0.88); differed in elevation across mistletoes and hosts (wet/ dry sites: Wald statistic: 68.3/ 30.97; $P < 0.001$). (b) Common slopes fitted within wet sites (circles, $MI > 0.7$): 1.99 (95% CI 1.44, 2.76), and dry sites (squares, $MI < 0.3$): 0.89 (0.71, 1.11); differed in elevation across mistletoes and hosts (wet/ dry sites: Wald statistic: 33.07/ 4.52; $P < 0.05$).

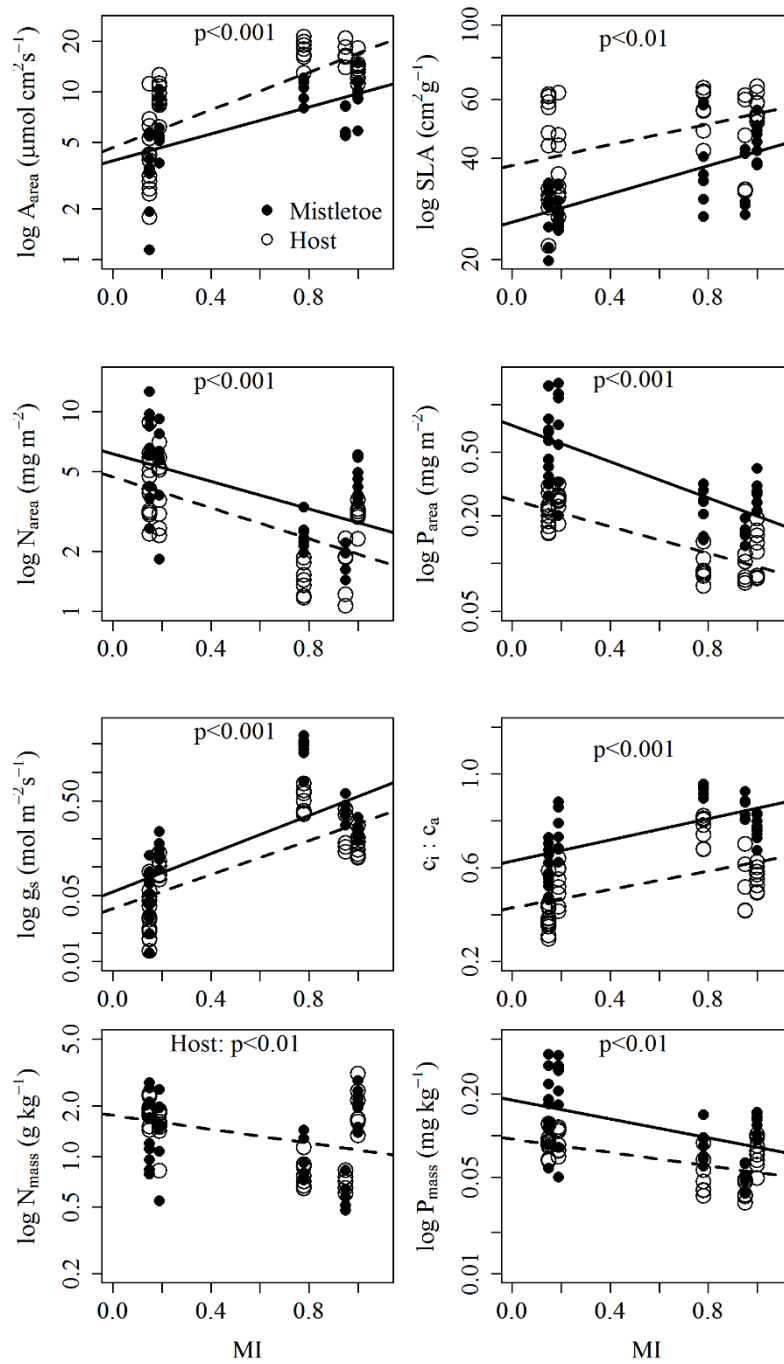


Figure S1. Linear regression between traits and climatic moisture index (MI) for mistletoes (black symbols, continuous line) and hosts (white symbols, dashed lines).

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Chapter 4.

Nutrient resorption in mistletoes from three low-P sites in Australia and Brazil

Abstract

Leaf nutrient resorption – the process of withdrawing leaf nutrients prior to leaf fall – is an important process that influences nutrient availability and cycling in ecosystems. Mistletoes have been suggested to be particularly inefficient in nutrient resorption because there is little selective advantage to be efficient in this respect, acquiring all their nutrients from the host xylem, thus at relatively low cost. Actually, very few studies have quantified nutrient resorption in mistletoes. Here we investigated nutrient resorption efficiency and proficiency patterns in 18 parasitic mistletoes species distributed across three different sites, each one with notably low P levels in the soil, focusing on nitrogen (N), phosphorus (P) and other essential macronutrients (potassium –K, calcium –Ca, and magnesium –Mg). We also investigated the relationship between nutrient resorption and specific leaf area (SLA) and leaf lifespan (LL). We did not find evidence for N, Ca or Mg resorption. Only two species showed N efficiency higher than 25%, while most of the studied species showed no resorption (values close to 0%). In some species, there was even “negative” resorption, meaning that senesced leaves were N-enriched compared to recently-mature green leaves (i.e., N accumulated over leaf life). However, P and K were generally resorbed, with P and K efficiency averaging ~30% and ~20%, respectively. Longer LL was associated with lower N and P concentrations in senesced leaves, and with lower N_{eff} . Our findings suggest that, even though mistletoes are relatively inefficient in terms of nutrient resorption, on low-P soils their ecological and evolutionary strategies for conserving phosphorous involve modulation of both leaf lifespan and P concentration in senesced leaves (just as seen in non-mistletoe species). Overall, our data show that mistletoes (at least in these systems) can reabsorb host-xylem limiting nutrients, such as P and K, but seemingly do not need to resorb N, providing evidence against the previous assumption that N would be a limiting nutrient and an important factor driving mistletoes water use.

Keywords: nutrient resorption proficiency, nutrient resorption efficiency, specific leaf area, leaf lifespan, parasitic plants, potassium, calcium, magnesium.

Introduction

Nutrient resorption is defined as the process of mobilizing nutrients from old leaves to new organs in plants (Aerts 1996) and is an important aspect of nutrient conservation, directly influencing nutrient cycles in ecosystems (Killingbeck 1996). Reducing nutrient losses potentially reduces the need of acquiring new nutrients, so having higher nutrient resorption might be especially advantageous in habitats where soil nutrients are especially scarce (Aerts 1999; Aerts & Chapin 1999). In fact, low nutrient concentration in the litter, together with long leaf lifespan (LL; achieved via low specific leaf area), are considered the main plant adaptations for enhancing nutrient conservation in infertile habitats (Escudero *et al.* 1992; Wright & Westoby 2003; Güsewell 2004). On average, ~62% of leaf N and ~65% of leaf P are resorbed from leaves before they are shed, although these values can vary substantially among species (Vergutz *et al.* 2012; but earlier estimates averaged ~50%, see Aerts 1996; Yuan & Chen (2009)). Vergutz *et al.* (2012) also showed that plants resorb very significant proportions of leaf potassium (K), averaging ~70%, but far less of elements, such as Ca and Mg, averaging ~11% and ~28%, respectively.

Following Killingbeck (1996), nutrient resorption “efficiency” (Nut_{eff}) is defined here as the proportion of nutrients withdrawn during senescence considered in relation to their initial concentration in green leaves, while resorption “proficiency” (Nut_{sen}) is defined as the final nutrient concentration in senesced leaves (Killingbeck 1996; i.e., species with high resorption proficiency reduce nutrient concentrations in senesced leaves down to very low levels). Resorption efficiency reflects the amount of nutrient invested in foliage that was conserved, summarizing nutrient demand and withdrawal. Nutrient proficiency is known to significantly vary between species within a single site, but to be maintained within species, even between different experimental plots (Wright & Westoby 2003; Hättenschwiler *et al.* 2008). Presumably, Nut_{sen} reflects plants biochemical limitations, as well as adaptive

strategies to minimise nutrient losses (Killingbeck 1996). Therefore, plants from more fertile soils would probably show lower Nut_{sen} in comparison with species from low nutrient sites, even though Nut_{eff} does not seem to vary widely across habitat types or fertility gradients (Aerts 1996; Wright & Westoby 2003).

Nitrogen and phosphorus are commonly assumed to be the most limiting (crucial and expensive) nutrients of primary production in terrestrial environments (Vitousek & Howarth 1991; Elser *et al.* 2007; LeBauer & Treseder 2008). Similarly, other key nutrients for plant growth, such as Ca, K, and Mg may also be expensive to obtain and could influence ecosystems processes, such as vegetation structure, primary production and nutrient cycling (Vitousek & Sanford 1986; Vitousek & Howarth 1991; Eviner & Chapin III 2003). Because nutrients are limiting resources and because trade-offs exist between the energy expended on acquiring and maintaining nutrients and the energy used for other metabolic purposes, it is expected that natural selection will drive evolutionary shifts favouring more efficient nutrient use in plants. Different strategies may be involved in this process, such as optimal differential accumulation through the canopy (Sands 1995; Niinemets 2007); investment in defence against herbivory (Mattson 1980; Ohnmeiss & Baldwin 1994); control over the timing of leaf death (meaning that nutrients can be resorbed prior to losing leaves; Reich *et al.* 1991); and evolutionary “choices” about which compounds in leaves are broken down and withdrawn during the resorption process, and which are left behind (Wright & Westoby 2003). In resorption, as in many other key processes, there may be broad differences between evergreen and deciduous species (i.e., evergreen species show lower N resorption efficiency, and deciduous species usually have higher leaf N and P concentration), N-fixing and non-fixing plants (i.e., higher leaf N concentration and lower N resorption efficiency in N-fixing plants), and phylogenetic trends (Aerts 1996; Killingbeck 1996; Vergutz *et al.* 2012).

Parasitic plants, such as xylem-tapping mistletoes, can be found over all different biomes in the world (Kuijt 1969). Mistletoes do not have a conventional root system; instead, they develop a connection to their hosts xylem - the haustorium-, through which they can acquire all required mineral nutrients and water (Calder & Bernhardt 1983). Therefore, the energetic costs required for nutrient acquisition in mistletoes is supposedly much lower compared to non-parasitic plants that need to surpass different challenges to access nutrient in the soils, such as investing largely in carbon allocation to roots, and establishing symbiotic relationships with N-fixing bacteria. From a mistletoe perspective, the energetic costs of translocating nutrients from old leaves and making them available for younger leaves could possibly be higher than simply acquiring more nutrients from the xylem of their hosts. Indeed, some previous mistletoe resorption studies reported that they did not show evidence of pre-senescence retrieval of nutrients (Pate, True & Rasins 1991; March & Watson 2007; March & Watson 2010).

In this chapter, we examined nutrient resorption in mistletoes, using 18 mistletoes (from 10 different species) growing on 18 different host species and sampled in sites located in Australia and Brazil. We aimed to (1) quantify nutrient resorption efficiency and proficiency, focusing on N, P, Ca, Mg and K; (2) investigate the relationship between nutrient resorption and two key functional traits, leaf lifespan (LL) and specific leaf area (SLA); and (3) assess differences in resorption between vegetation types. Based on the arguments given above, we expected that mistletoes would have low levels of nutrient resorption efficiency and proficiency. We expected to find differences between Australian and Brazilian mistletoes because of divergent evolutionary histories and distinct nutrient limitations in the soils. In addition, we also predicted that species with longer leaf lifespans would withdraw nutrients down to lower levels during resorption (i.e., show high proficiency), as found for Australian non-parasitic plants (Wright & Westoby 2003).

Material and Methods

We sampled mistletoes at three different sites across Central Brazil and Australia (Table 1). All sites were located in National Parks but differed in vegetation type, seasonality and nutrient availability. A soil sample was collected from the surface layer (0–10 cm) in 4 different locations at each site. At each site, at least five individuals of six different mistletoe-host species-pairs were sampled (Table 2). Therefore, every mistletoe species on a different host was considered a different observation unit.

Table 1. Site locations, climates and soil properties. Mean annual precipitation (MAP) and mean annual temperatures (MAT) were obtained from the CRU CL2.0 global gridded dataset (New *et al.* 2002).

Site vegetation	Location	Coordinates	MAT (°C) (Min-Max)	MAP (mm)	Total P (ppm)	Total N (%)
Closed woodland	Ku-ring-gai NP	33°63'S 151°26'E	17.2 (13.7-21.7)	1266.6	191.4 (25.7)	0.079 (0.012)
	Royal NP (Sydney, NSW, Australia)	34°17'S 151°05'E				
Savanna 1	Howard Springs (Darwin, NT, Australia)	12°30'S 130°45'E	27.6 (23.2-32.0)	1668.6	62.6 (3.1)	0.067 (0.02)
Savanna 2	IBGE Ecological Reserve (Brasília, DF, Brazil)	15°55'S 47°51'E	25.8 (14.4-31.2)	1478.0	207.1 (12.3)	0.140 (0.028)

Table 2. Species list of mistletoes used in this study and the host it was parasitising. Leaf lifespan (LL) data were collected for species in bold.

Site	Mistletoe	Host	Host Family
Closed Woodland (Sydney) n=6	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus hemastoma</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus moluccana</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus</i> spp.	Myrtaceae
	<i>Amyema congener</i>	<i>Allocasuarina littoralis</i>	Casuarinaceae
	<i>Dendrophthoe vitellina</i>	<i>Eucalyptus</i> sp.	Myrtaceae
	<i>Dendrophthoe vitellina</i>	<i>Angophora costata</i>	Myrtaceae
Australian Savanna (Darwin) n=6	<i>Amyema sanguinea</i>	<i>Corymbia porrecta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Eucalyptus tetradonta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Corymbia blesseri</i>	Myrtaceae
	<i>Amyema miquelli</i>	<i>Eucalyptus miniata</i>	Myrtaceae
	<i>Dendrophthoe odontocalyx</i>	<i>Grevillea pteridifolia</i>	Proteaceae
	<i>Dacrydium signata</i>	<i>Xanthostemon paradoxus</i>	Myrtaceae
Brazilian Savanna (Brasília) n=6	<i>Phthirusa ovata</i>	<i>Stryphnodendron adstringens</i>	Fabaceae
	<i>Phthirusa ovata</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Psittacanthus robustus</i>	<i>Qualea grandiflora</i>	Vochysiaceae
	<i>Phoradendron crassifolium</i>	<i>Tapirira guianensis</i>	Anacardiaceae
	<i>Phthirusa ovata</i>	<i>Dalbergia miscolobium</i>	Fabaceae
	<i>Phthirusa ovata</i>	<i>Piptocarpha rotundifolia</i>	Compositae

Sites descriptions

Australian Savanna: The site is located in Howard Springs National Park, approximately 35 km southeast of Darwin in Northern Territory, Australia (130°45'E, 12°30'S). It consists of tropical savanna with marked seasonality, where 95% of the 1750 mm mean annual rainfall is restricted to the wet season (December to March) (Williams *et al.* 1997). The soil in the area is well drained, highly weathered, laterised and low in nutrient (Hutley, O'Grady & Eamus 2000). The vegetation in the study site consists of savanna with a variety of canopy species but most notably *Eucalyptus tetrodonta* (F. Muell.) and *E. miniata* (Cunn. Ex Schauer), and an understory of small-medium trees and shrubs, and C4 grasses. Mistletoes in the site tend to occur on the most abundant species (listed in Table 2).

Cerrado (Brazilian Savanna): The site is located in the Natural Reserve of the Roncador belonging to the Brazilian Institute of Geography and Statistics (RECOR/IBGE), 35km south of Brasilia-DF, Brazil (47°53'W, 15°56'S) and is part of the Environmental Protection Area Gama-Cabeça de Veado, with a total of 10,000 hectares of continuous vegetation. The savanna of central Brazil is the most diverse savanna in the world in terms of floristic composition (Solbrig 1996), and considered a biodiversity hotspot for conservation (Myers *et al.* 2000). Soil nutrient availability usually limits tree growth and density, mainly because of the low availability of P and Ca (Silva *et al.* 2013) associated with high soil acidity (pH around 5.5), high Al availability (Haridasan 2001) and fire disturbance (Eiten 1972). The average annual precipitation in this area is approximately 1500 mm with a pronounced dry season from May through September and a mean annual temperature that ranges from 20 to 26°C (Oliveira-Filho *et al.* 1989). The predominant soils are deep and well drained Oxisols, but hydromorphic soils also occur associated with watercourses (Dantas & Batalha 2011).

Australian closed woodland: The two different sites are located in distinct parks around Sydney area: Ku-ring-gai National Park (33°63'S, 151°26'E) and Royal National Park (34°17'S, 151°05'E). Both sites are characterized by sandstone soils with extremely low nutrient content. The Royal NP site is located on a sheltered sandstone slope and the vegetation is dominated by *Angophora costata* (smooth-barked apple) together with a eucalypt woodland community (NPWS 2000; Tozer *et al.* 2010). The site in Ku-ring-gai Chase NP is also located on sheltered sandstone and the vegetation is dominated by eucalypt species, especially forest grey box (*Eucalyptus moluccana*), spotted gum (*Eucalyptus maculata*), and scribbly gum (*Eucalyptus haemastoma*; NPWS 2002).

Leaf trait measurements

Mature, fully expanded leaves and senesced leaves were collected from 5-7 different individuals over 1-2 years period at each site, on at least two different occasions. We classified as senesced the leaves in which an abscission layer was formed so that a gentle flicking of the branch would remove them.

Leaf samples were scanned and the area was measured using the software Image J (Abràmoff, Magalhães & Ram 2004). After oven-drying for 72 hours, leaves were weighed and the specific leaf area (SLA, mm g⁻¹) was calculated as the ratio between fresh area and dry mass. The dried leaves were ground and Australian samples were sent to the Analytical Service Unit from the School of Agriculture and Food Science at The University of Queensland for macro and micronutrients determination by combustion and ICP-OES technique. Total N was measured using a LECO TruSpec CHN combustion analyser. Total P, K, Ca and Mg were measured using an ICP-OES analyser, following Nitric perchloric acid digestion. Brazilian samples were sent to Laboratório de Agroquímica e Meio Ambiente at Universidade Estadual de Maringá (PR/Brazil) for N and P determination by Kjeldahl

digestion and UV-Vis spectroscopy, respectively. The other nutrients (Ca, Mg, and K) were determined by atomic absorption spectrometry.

Mass-based concentrations of leaf nutrients do not account for structural changes in soluble carbon and may underestimate resorption efficiency. Therefore, resorption efficiency measured on an area basis is preferred because it controls for these structural changes (Chapin III, Schulze & Mooney 1990). We calculated resorption efficiency (N_{eff} and P_{eff}) as the proportion of nutrients in senesced leaves relative to the green leaves, on an area basis. Average N and P concentration in senesced leaves was interpreted as resorption proficiency (N_{sen} and P_{sen}) according to Killingbeck (1996).

We also collected data on leaf lifespan (LL) for six of the mistletoes sampled in Australian closed woodland and three sampled in the Brazilian savannas sites (Table 2). Four branches of each individual were used to measure LL, based on leaf turnover rates (Wright & Cannon 2001). All leaves from each branch were sequentially numbered and revisited every three to four months for at least one complete year (12-18 months). LL was calculated as the inverse of the mortality rate (number of dead leaves per number of leaves at beginning of census/ period of time).

Data Analyses

All data were analysed using R software version 3.1.0 (R Development Core Team 2008). Leaf traits data were log10-transformed for normality assumptions, with the exception of nutrient proportional efficiency (%) that was deemed normal. All final datasets showed normal distribution as tested by a Shapiro-Wilk normality test ($P > 0.05$). We used paired t-tests to compare between green and senesced leaves and two-way analysis of variance (ANOVA) to test for effects of leaf age ($n = 2$ groups: green and senesced; 18 mistletoes per group) and differences between sites ($n = 3$ groups: Australian savanna, Australian closed

woodland, Brazilian savanna; six mistletoes per group). We fitted standardized major axis (SMA) slopes and used Pearson correlation analysis to explore trait-trait relationships, using package SMATR version 3.0 (Warton *et al.* 2012). All statistical tests assumed significance at $P < 0.05$. Marginally significant results are also reported when $0.10 > P > 0.05$.

Results

N concentration (N_{mass}) varied six-fold, while P_{mass} varied around four-fold in both green and senescent leaves among species. On average, leaf N_{mass} concentration increased by around 7% from green to senesced leaves, while P_{mass} concentration decreased by 33%. Mistletoes parasitizing nitrogen-fixing hosts (*Dalbergia miscolobium* and *Stryphnodendron adstringens*) in the Cerrado showed the highest N_{green} (2.8 and 2.5 mg g⁻¹) and N_{sen} values (2.8 and 2.7 mg g⁻¹).

Brazilian Cerrado mistletoes showed significantly higher green-leaf and senesced-leaf N_{mass} and P_{mass} compared to mistletoes from other sites, while Australian closed woodland mistletoes showed higher K_{mass} (Table 3, Fig. 1). There were no differences between sites in mean SLA, Ca_{mass} and Mg_{mass} across all species (Table 3). Between leaf samples of differing ages, the only difference detected was in P_{mass} (Table 3) where, in all sites, P_{green} was significantly higher than P_{sen} (Fig. 1 and Fig. S1). There was no interaction between site and leaf-age for any of the macronutrients or for site and SLA (Table 3).

Table 3. *F*-values and *P*-values for two-way ANOVA on the influence of leaf age and site on nutrient concentrations and specific leaf area (SLA).

Trait	Source of variation	df	<i>F</i>	<i>P</i>
N _{mass}	Leaf age	1	1.240	0.274
	Site	2	27.081	< 0.001
	Leaf Age*Site	2	0.271	0.764
	Residuals	30		
P _{mass}	Leaf age	1	13.804	< 0.001
	Site	2	5.815	0.007
	Leaf Age*Site	2	0.590	0.560
	Residuals	30		
K _{mass}	Leaf age	1	0.948	0.339
	Site	2	6.384	0.005
	Leaf Age*Site	2	1.175	0.323
	Residuals	30		
Mg _{mass}	Leaf age	1	0.120	0.731
	Site	2	2.446	0.105
	Leaf Age*Site	2	1.012	0.377
	Residuals	30		
Ca _{mass}	Leaf age	1	1.295	0.265
	Site	2	0.193	0.826
	Leaf Age*Site	2	0.007	0.993
	Residuals	30		
SLA	Leaf age	1	1.143	0.293
	Site	2	1.425	0.256
	Leaf Age*Site	2	0.201	0.819
	Residuals	30		

By considering green-leaf and senesced-leaf nutrient concentrations on a per-area basis, we incorporated any age-related changes in SLA when calculating resorption efficiencies. On average, SLA was 10% (± 9.29) lower in senesced leaves than in mature green leaves (paired t-test, $P < 0.001$). Mean N_{eff} resorption ranged from -36.6% in *Phthirusa ovata* parasitising *Stryphnodendron adstringens* to 38.7% in *Amyema sanguinea* parasitising *Eucalyptus tetrodonta* among the 18 mistletoe pairs (mean 2.3%). Site means were not significantly different from zero (ranging from -0.7% to 5.5%), and did not differ from one another (Fig. 2). P_{eff} resorption varied from 6.5% to 63.7% among species (mean 28.5%). P_{eff} site means were significantly higher than zero, ranging from 17.9% to 34.5% but, again, these were not significantly different, since there was substantial variation among species within each site. Generally, Ca accumulated in senesced leaves while K was reabsorbed, averaging -29.3%, and 19.3% resorption efficiency, respectively (Fig. 2).

SLA was positively correlated with P_{green} for all species together, but not for savanna sites individually (Table 4). No correlation was found between N or P resorption efficiency or proficiency and SLA. N_{green} and P_{green} were positively correlated for all sites combined (Fig. 3), while N_{sen} and P_{sen} , N_{eff} and P_{eff} were only marginally significantly correlated (Table 4). From green leaves to senesced leaves, the N-P relationship shifted: N was generally maintained constant, while P was lower in senesced leaves, with senesced leaves showing ~1.5 times lower P for a given N (Fig. 3).

In the nine pairs for which LL data were collected, there was a negative relationship between LL and N_{green} , P_{green} , N_{sen} and P_{sen} ; and a strong negative relationship ($r^2 > 0.60$) between LL and N_{eff} , but not between LL and P_{eff} (Table 5, Fig. 4). Leaf lifespan averaged 2.2 years, and it was shorter for the three Brazilian pairs (ranging from 1.3 to 1.7 years) compared to the six Australian pairs (ranging from 2.0 to 3.3 years).

Table 4. Pearson correlation between leaf traits (r^2 and P -values) at different locations and for all species considered together. Significant correlations ($P < 0.05$) are highlighted and the symbol * represents marginally significant correlations ($0.05 < P < 0.1$).

Trait	Sydney (n=6)	Darwin (n=6)	Brazil (n=6)	All species (n=18)
N _{green} , P _{green}	0.83, 0.011	0.22, 0.352	0.06, 0.639	0.52, <0.001
N _{sen} , P _{sen}	0.69, 0.040	0.44, 0.148	0.05, 0.677	0.17, 0.093*
N _{green} , SLA	0.68, 0.04	0.37, 0.196	0.07, 0.572	0.11, 0.178
N _{sen} , SLA	0.43, 0.154	0.72, 0.031	0.04, 0.894	0.10, 0.219
P _{green} , SLA	0.78, 0.019	0.44, 0.148	0.21, 0.290	0.29, 0.023
P _{sen} , SLA	0.08, 0.584	0.18, 0.401	0.08, 0.577	0.04, 0.423
N _{eff} , SLA	0.47, 0.131	0.001, 0.934	0.27, 0.340	0.05, 0.385
P _{eff} , SLA	0.27, 0.289	0.22, 0.354	0.15, 0.444	0.12, 0.171
N _{eff} , P _{eff}	0.40, 0.176	0.21, 0.355	0.12, 0.506	0.18, 0.081*

Table 5. Pearson correlation (r^2 and P -values) between leaf lifespan and nutrient concentrations and resorption efficiencies ($n = 9$).

Trait	All species
N_{green} , LL	-0.71, 0.004
N_{sen} , LL	-0.61, 0.012
P_{gre} , LL	-0.58, 0.018
P_{sen} , LL	-0.38, 0.049
N_{eff} , LL	-0.69, 0.005
P_{eff} , LL	-0.05, 0.571

Discussion

Nutrient resorption and implications for the N-parasitism hypothesis

Contradicting ideas that N resorption might be important for all plants independent of life-form (Eckstein, Karlsson & Weih 1999), but in line with previous work on Australian mistletoes from *Amyema* genus (Pate, True & Kuo 1991; March & Watson 2010), our study shows that in fact very little N is resorbed from senesced leaves in mistletoes from Australian and Brazilian systems. The data presented here is more comprehensive than seen in previous studies, containing nine genera of mistletoes from three vegetation types on two continents, thereby providing stronger support for the lack of N resorption in mistletoes. We found very little evidence of N resorption, restricted only to two species that showed N_{eff} higher than 25% (*Amyema sanguinea* in *Eucalyptus tetradonta* with 26.8% and *Phthirusa ovata* in *Miconia albicans* with 26.1% N_{eff}). Most of the studied species showed none (values close to 0%) or even negative resorption, indicating that senesced leaves of some mistletoes species accumulate more N before shedding, and are actually N-enriched compared to recently-matured green leaves (Fig. 1 and Fig. 2).

Despite negligible resorption of N, we found clear evidence of P resorption in mistletoes (P_{eff} ranging from 6.5% to 63.7% among species, Fig. 2). Many non-parasitic species are reported to resorb on average 50-60% of the P, coupled with ~50% of N resorption (Wright & Westoby 2003; Kobe, Lepczyk & Iyer 2005; Vergutz *et al.* 2012). For mistletoes in this study, on average, P was more resorbed compared to N. As a result, there was a weaker relationship between N and P concentration in senesced leaves compared to N and P concentration in green leaves (Table 4, Fig. 3). Moreover, only a marginally significant relationship between N and P efficiencies was found (Table 4), suggesting that the resorption process of those two nutrients is largely decoupled in mistletoes.

Considering that N was not resorbed, it seems reasonable to imply that nitrogen is not a limiting nutrient for mistletoes, otherwise selection would have favoured the optimization of N use, and diminished N loss by efficient N resorption. As demonstrated by Chapin III and Moilanen (1991) in a manipulative experiment with Alaskan birch, plants growing in abnormally high nutrient concentration showed very low nutrient resorption efficiency. Therefore, a lack of N resorption is strong evidence against the N-parasitism hypothesis suggested by Schulze, Turner and Glatzel (1984) to explain the evolution of the mistletoe habit. This hypothesis is based on the principle that N is the most limiting nutrient for mistletoes and, as such, should be the main driver of faster transpiration rates and lower water use efficiency (i.e., the ratio of carbon gain per unit of water loss) that mistletoes usually show. Therefore, lower water use efficiency drives xylem flow from host to mistletoes leaves so that sufficient nitrogen would be acquired. This hypothesis was not supported in a global scale analysis (Chapter 2), where higher N concentration in the host leaves was not related to more similar water use efficiency between mistletoes and hosts. Our results here also support the hypothesis that N is not the most limiting nutrient for mistletoes, or, at least, is not the nutrient that should be driving transpiration rates.

In relation to the other nutrients, Mg was not significantly resorbed, and mistletoes showed a high accumulation of Ca from green to senesced leaves (Fig. 2). Ca is generally conserved in senesced leaves of terrestrial plants because it is a structural element in cell walls (Van Heerwaarden, Toet & Aerts 2003; but see Vergutz *et al.* 2012). In contrast, K was generally resorbed in mistletoes, with K_{eff} averaging 20% and (ranging from -39.8% to 56.4%, Fig. 2). Nonetheless, 20% K resorption is much lower than the previously reported average of 70% from a global dataset of resorption rates (Vergutz *et al.* 2012). In mistletoes, K is usually found in disproportionally higher concentrations compared to non-parasitic plants (Lamont & Southall 1982; Schulze, Turner & Glatzel 1984; Scalon, Haridasan & Franco

2013). It was once suggested that K accumulation could be an active process in mistletoes (Lamont & Southall 1982) because K^+ plays an important role as neutralizing anions, stabilizing pH and osmotic potential, and maintaining the cell turgor and membrane integrity (Mengel & Arneke 1982; Marschnert, Kirkby & Engels 1997; Amtmann & Rubio 2012). However, perhaps a more plausible hypothesis is that K accumulates passively due to its high phloem mobility and the lack of appropriate sinks (Glatzel 1983; Glatzel & Geils 2009). If passive accumulation is the case, the resorption process of highly mobile ions should be unnecessary. However, our results show that K is generally resorbed, suggesting that the higher concentrations of K in mistletoes leaves may be a physiological requirement. Another possibility to consider, taking into account the relatively low values of K_{eff} found in this study is that K is not being actively resorbed, but leached from old leaves through rainfall before shedding. Concerns over the overestimation of K_{eff} due to ignorance of the effect of leaching was previously pointed out by Wang, Wang and Lin (2003) while studying nutrient resorption of a mangrove species in China, because K is suggested to be one of the inorganic nutrient leached in greatest quantities (Tukey Jr 1970). On the contrary, organic bounded nutrients, such as N and P are not readily leached from leaves (Tukey Jr 1970; Chapin III & Moilanen 1991; Aerts & Chapin 1999) and their resorption values are more reliable.

Differences in nutrient economy between mistletoes vs. non-parasitic plants

Non-parasitic plants have two pathways for acquiring nutrients used in producing new tissue: root uptake and retranslocation from old organs. The unit-cost of acquiring nutrients from the soil may vary according to site nutrient availability (Bloom, Chapin III & Mooney 1985), so that if the soil is nutrient deficient, plants are reported to accelerate the senescing process (Ono, Terashima & Watanabe 1996). As discussed by Wright and Westoby (2003), the resorption process also has a cost, and the balance between use of soil-derived and resorption-

derived nutrients should be set by their relative costs. The relative costs not only vary according to the amount of nutrient, but also depend on the compounds from which they are derived. For example, N can occur in multiple forms in the soil (nitrate, ammonium and organic N) and different species can have different preference over one of these forms, depending on the relative costs to absorb and assimilate it (Atkin 1996; Aerts & Chapin 1999). Similarly, the cost of resorbed nutrients depends on which compounds are broken down and mobilised during the senescence process (Lambers, Chapin & Pons 1998).

Expanding on this concept, mistletoes also have two nutrient sources: resorption of nutrients from old organs, or host-derived nutrients (i.e., acquiring nutrients directly from the host xylem). If nutrients in the host xylem are abundant, the costs of acquiring them should be very inexpensive compared to resorption-derived nutrients. By contrast, if any nutrient is found in low concentration in the host xylem and limits mistletoes nutritional requirement, resorbing it from old leaves might be cheaper than acquiring it from the host. Moreover, mistletoes face physiological constraints and lack the ability to develop natural strategies seen in non-parasitic plants to deal with low nutrient concentration, such as allocating more carbon to expand the root system, associating with symbiotic bacteria or fungi from the soil, or even modifying the soil environment to enhance nutrient availability (Aerts & Chapin 1999). Therefore, they are subject to whatever the host plant has to offer, and may have to tolerate differences in xylem chemistry and deal with nutrient deficiency (Glatzel & Geils 2009). All sites in this study are commonly considered low-P environments, with low soil nutrient availability (Table 1). The host plants are adapted to survive in these conditions with low P requirement, reflecting in very low P leaf concentration in green leaves compared to the average 1.4 mg g^{-1} from 496 plant species distributed globally and reported by Vergutz *et al.* (2012) (P concentration of host plants mature leaves (mean \pm sd): Australian savanna: $0.5 \pm 0.18 \text{ mg g}^{-1}$; Australian closed woodland: $0.4 \pm 0.07 \text{ mg g}^{-1}$; Cerrado: $0.7 \pm 0.15 \text{ mg g}^{-1}$). The

fact that mistletoes resorb a fair amount of P indicates possible P limitation in the host xylem in all three different sites. It also shows that mistletoes have the capacity to obtain nutrients from resorption and would adapt to balance between the two different nutrient sources once the costs for host-derived nutrients exceeds the resorption nutrient costs. Therefore, our results suggest that mistletoes growing in low-P environments and parasitising hosts adapted to deal with very low P availability, might also suffer similar P limitation and the resorption process may have been favoured by natural selection in these species.

Even though soil fertility or climatic variables were not markedly different among sites (Table 1), cerrado mistletoes showed higher N_{mass} and P_{mass} , and lower leaf lifespan compared to mistletoes from the other sites. However, there was not a strong pattern of nutrient resorption related to individual sites, which suggests that environmental differences did not affect nutrient use and resorption patterns of mistletoes (Table 3, Fig. 2).

Resorption and functional trait relationships

Nutrients can be conserved in the plant biomass by resorption or by extending the time the plant organs lives (Eckstein, Karlsson & Weih 1999). Therefore, in species with short leaf longevity, the retranslocation process is very important for nutrient conservation (Jonasson 1989; Aerts & Chapin 1999). However, because of the small variation of leaf nutrient resorption across species from nutrient-rich and nutrient-poor habitats, leaf longevity is suggested to be a more important adaptation to lower fertility than the resorption process itself, as concluded by many studies with different functional types of plants (Escudero *et al.* 1992; Reich, Walters & Ellsworth 1992; Aerts & Chapin 1999). Moreover, the negative effects of increasing LL, such as investing heavily on leaves that are more robust with a longer time to pay off their construction costs, were suggested to be surpassed by its positive effect on time of nutrient retention in the biomass in a study of woody species in Central

Spain (Escudero *et al.* 1992). Indeed, our results show that species with longer LL had lower nutrient concentrations in senesced leaves (Table 5), suggesting that selection has minimized nutrient losses in mistletoes both *via* extending LL and *via* high nutrient proficiency. The same relationship between N and P concentration and LL was found in non-parasitic Australian plants (Wright & Westoby 2003), although these authors did not find a significant relationship between N_{eff} and LL. In the present study, not only N_{green} and P_{green} , but also P_{sen} , N_{sen} and N_{eff} were negatively correlated with LL for mistletoes, showing that species with shorter-lived leaves may compensate nutrient losses with higher proportional N_{eff} . Nonetheless, they show higher concentration of N and P in senesced leaves compared to species with longer-lived leaves (Fig. 4).

While LL was negatively associated with N and P concentrations (Table 5), SLA was only related to P_{green} (Table 4) and decreased ~10% from green to senesced leaves (Fig. 1). Although leaves tend to get thicker with age (Hikosaka 2005), mass loss during senescence is common (Van Heerwaarden, Toet & Aerts 2003; Vergutz *et al.* 2012) and is mainly caused by resorption of carbon together with other nutrients, especially nitrogenous compounds (Chapin III, Schulze & Mooney 1990). Because N was not significantly resorbed in mistletoes, carbon may also have accumulated, resulting in lower SLA in senesced leaves.

Resorption and implications for litter

It is suggested that parasitic plants, including mistletoes, should be important for nutrient cycling, because nutrients that are not resorbed will circulate through litterfall and become available to the plant community (Watson 2001; March & Watson 2007; March & Watson 2010). We found support for the fact that mistletoes have high N concentration in senesced leaves, thus suggesting a potential effect in nutrient cycling in the ecosystem. However, because we do not have data on the contribution of mistletoes to litter biomass or their leaf

area index at the studied sites, it is not possible to estimate the influence they might have in the studied systems.

Nevertheless, the process of remineralisation might take several years (Berg 2000; Berg & McClaugherty 2008) and the nutrients in the litterfall may be considered nutrient losses to the plant population, at least in a short term. For example, the decomposition rates in the cerrado are considered very slow (around 15% per year), and it is suggested that the majority of nutrients remain in the vegetation itself, having high rates of resorption (up to 80% of P_{eff} ; Nardoto *et al.* 2006; Kozovits *et al.* 2007). If mistletoe litter is decomposed faster, because of possible lower C:N ratio (Berg & Staaf 1980; Taylor *et al.* 1989), then it might be the case that mistletoes contribute to a faster nutrient cycling in the system. A fast decomposition was observed for root hemiparasites at a sub-arctic environment (Quested *et al.* 2003; Quested, Press & Callaghan 2003; Quested *et al.* 2005) however, to the best of our knowledge, decomposition patterns have never been investigated for mistletoes. Further studies on mistletoe decomposition rates might help to elucidate their influence in ecosystem functioning, especially in habitats where nutrients are typically locked up in living biomass.

Conclusion

Our results showed that N, Mg and Ca are not resorbed in mistletoe species sampled across two continents, but P and K were generally resorbed. The lack of N resorption together with the fact that P is on average 30% resorbed suggest that N is not a limiting nutrient for mistletoes, contradicting the N-parasitism hypothesis. Moreover, mistletoes parasitising hosts with very low P availability may also suffer similar P limitation and the resorption process may have been favoured by natural selection in these species occurring in these low-P sites. Our findings also show that, even though mistletoes are relatively inefficient in terms of

nutrient resorption, species with higher nutrient resorption proficiency expand their time of nutrient retention by having longer-living leaves.

Acknowledgements

We acknowledge CNPq for financial support and Australian National Parks and RECOR/IBGE offices for permits and logistic support. We also thank Izabelly Sant’Ana, Allyson Eller, Julieta Garcia-Russell, Julia Cooke and Fabricius Domingos for the valuable help in the field; and Rachel Gallagher, Fabricius Domingos and Raquel Miatto for providing useful feedback on earlier versions of this chapter.

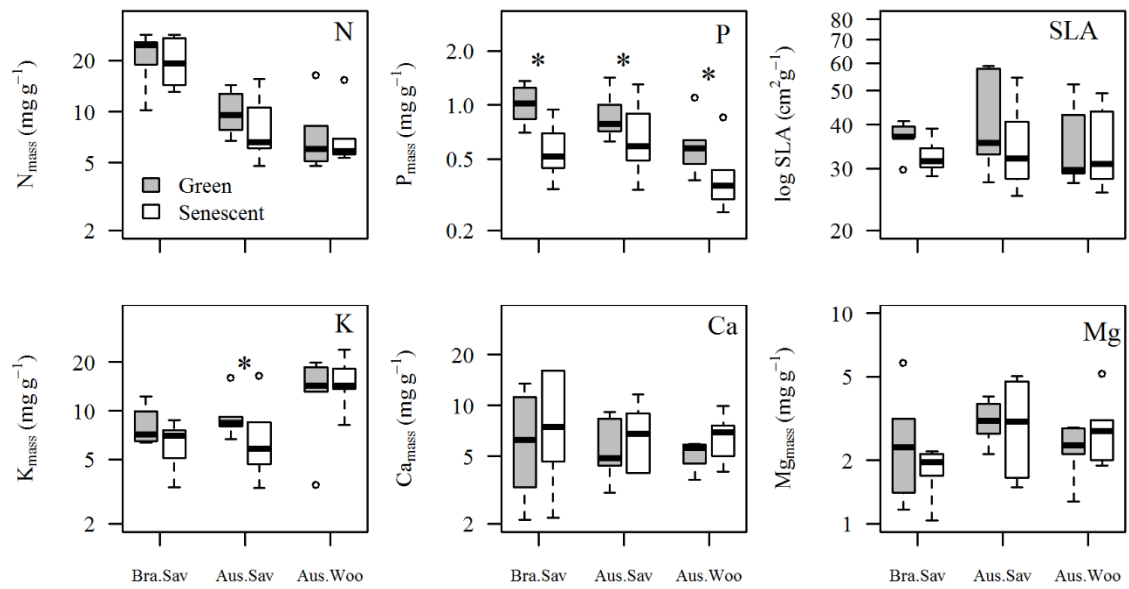


Figure 1. Boxplot of N, P, K, Ca and Mg concentration per mass, and specific leaf area (SLA) for mistletoes at the three studied sites, for green leaves (grey boxes) and senescent leaves (white box). The continuous line within the box shows the median and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. The symbol * denotes significant differences between the green and senescent leaves (paired t-tests, $P < 0.05$).

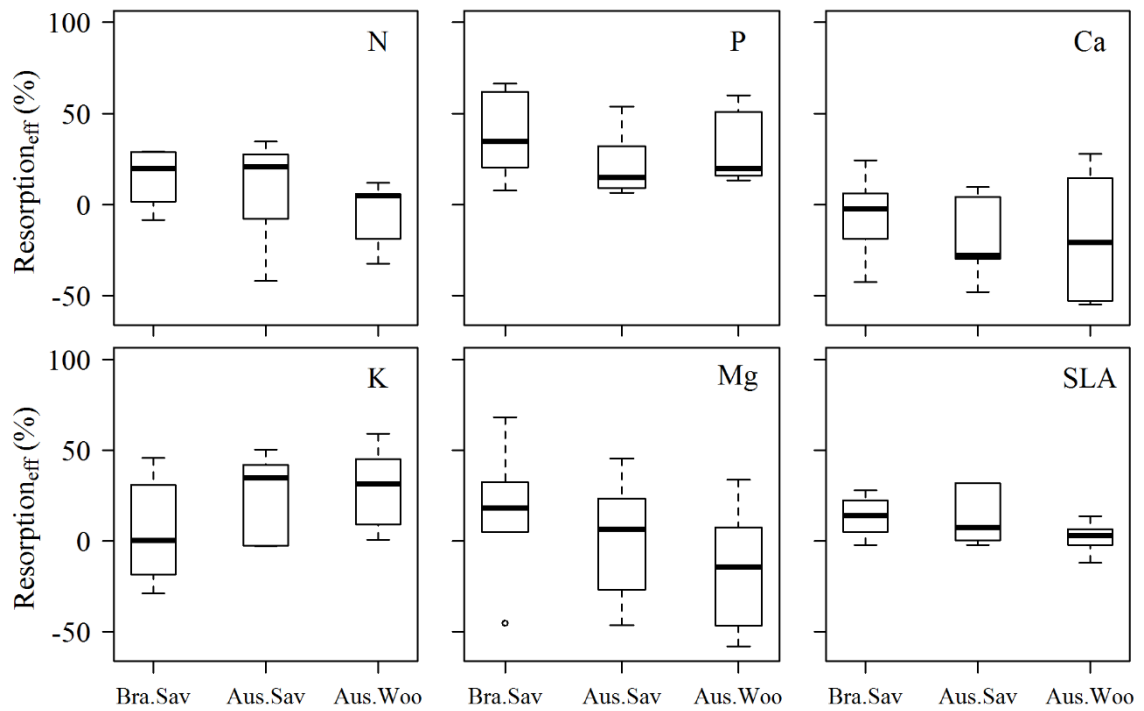


Figure 2. Boxplot of N, P, Ca, K, Mg and SLA proportional resorption efficiency of mistletoes at the three studied sites. The continuous line within the box shows the median, and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. There was no significant difference between sites (ANOVA, all $P > 0.05$).

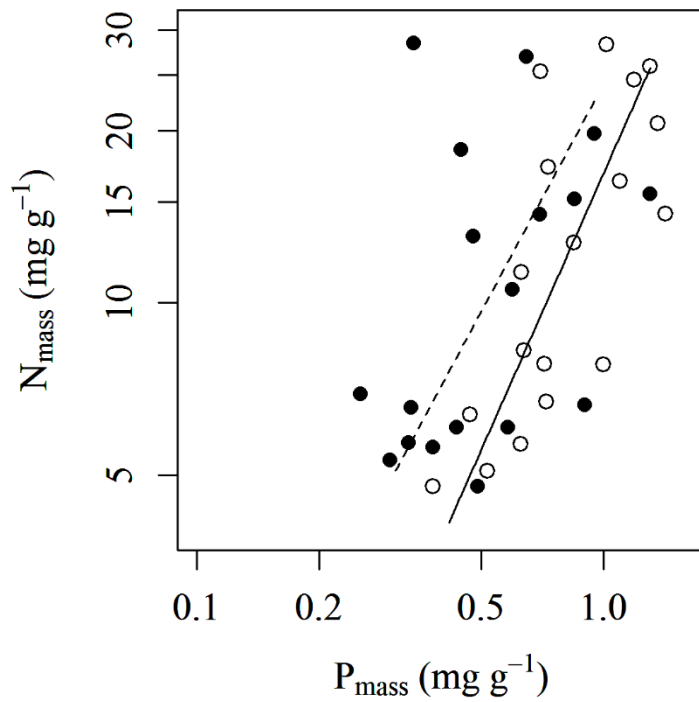


Figure 3. Standardized major axis (SMA) relationship between P and N concentration in green (white symbols, continuous line) and senesced (black symbols, dashed line) leaves. Correlations statistics are given in Table 3. Common slope (CI) of 1.49 (1.11, 1.99) for old and green leaves with an elevation shift (Wald statistic: 4.04, $P = 0.044$).

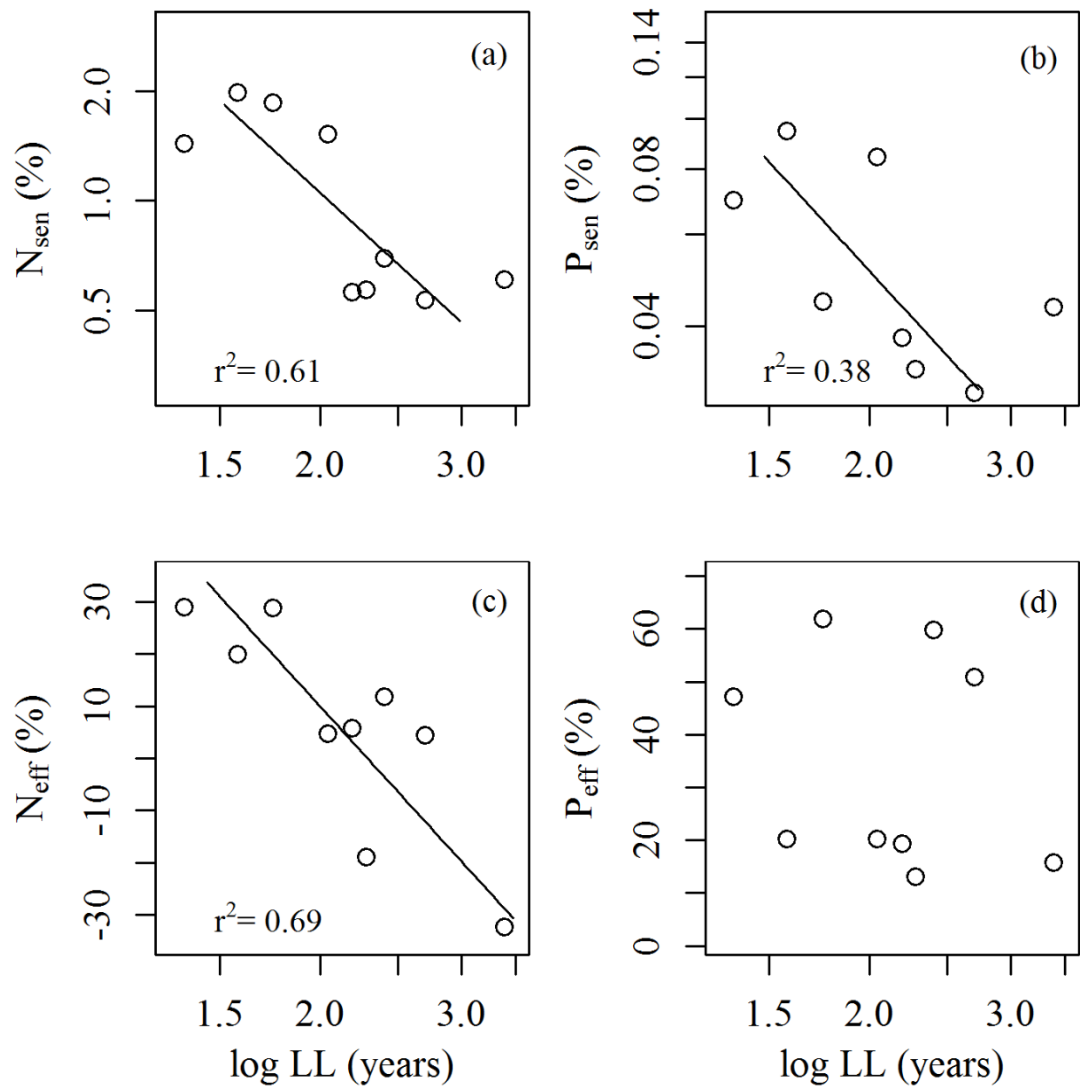


Figure 4. Relationship between leaf lifespan and N and P senesced leaf concentration (a, b), and resorption efficiency (c, d). Correlations statistics are given in Table 4.

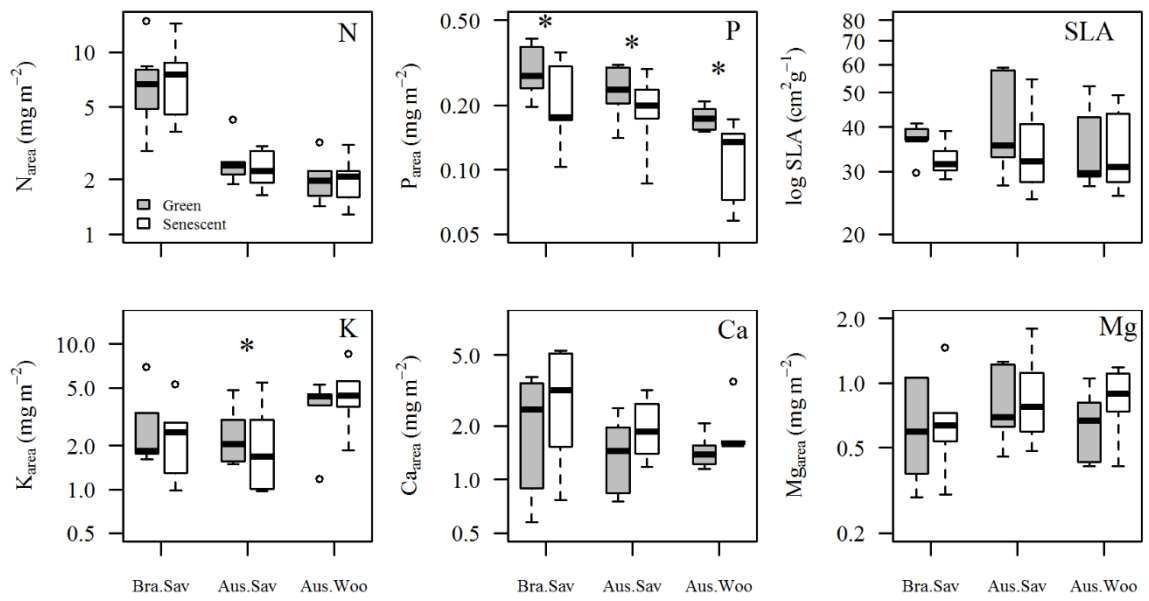


Figure S1. Boxplot of N, P, K, Ca and Mg concentration per area, and specific leaf area (SLA) for mistletoes at the three studied sites, for green leaves (grey boxes) and senescent leaves (white box). The continuous line within the box shows the median and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. The symbol * denotes significant differences between green and senescent leaves (paired t-tests, $P < 0.05$).

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Chapter 5.

Leaf lifespan, herbivory and leaf defence investment in mistletoes and their hosts

Abstract

Investment in defence against herbivory is costly for plants in the sense that the resources invested could have been used for other productive purposes. Parasitic plants, such as mistletoes, have been used as a model system to investigate resource balance and use in plants because they acquire water and nutrients exclusively through the xylem of the host plant. However, investment in defence traits and their influence on herbivore damage has remained unevaluated in mistletoes. We used 35 mistletoe-host species pairs sampled across four sites in Australia and Brazil to compare differences between mistletoes and hosts in chemical defence investment, and to investigate possible trade-offs and syndromes between different defence types. We also took the opportunity to investigate the evolutionary processes involved in the leaf morphological resemblance of Australian mistletoes and their hosts. Mistletoes had similar leaf nitrogen and generally higher carbon-based defence investment compared to their hosts (higher tannin concentration and lower SLA). Considering N-based defences, only 29% of the mistletoes tested positive for alkaloids, while alkaloids were found in 57% of the hosts. Among Australian species pairs, we found clear patterning for highly-mimic mistletoe species testing negative for alkaloids, while all associated hosts tested positive. This result illustrates an evidence of Batesian mimicry and suggests that alkaloids are not being transferred from hosts. Tannin concentrations in mistletoe leaves were highly correlated to that of their hosts, while phenols were weakly correlated, implying that tannins are being absorbed and other classes of phenols are being excluded. We found evidence for differences in defence syndromes and trade-offs between mistletoes and hosts, where lower SLA was related to higher tannin concentration in mistletoes, and higher SLA was related to higher phenol concentration in hosts. We also used 14 out of those 35 pairs to investigate the relationship between defence, herbivory rates and leaf lifespan. Mistletoes showed marginally

higher herbivory rates, but similar leaf lifespan. We found that lower SLA and lower N_{mass} were related to longer leaf lifespan for hosts, with mistletoes showing a similar tendency. Lower herbivory rates were related to longer leaf lifespan for mistletoes and hosts in agreement with the hypothesis that long-lived leaves must be well defended because leaf damage increases with time. Our results, therefore, provide an advance in the study of plant-herbivore and parasite-host theories, giving insights into the fundamental role secondary compounds may have on host selection, especially in the case of mimic mistletoes in Australia.

Keywords: phenol, tannin, alkaloids, nitrogen, Loranthaceae, specific leaf area, mimicry

Introduction

For all plants, nitrogen (N) limitation together with herbivory damage are generally considered the major constraints to growth, with N being a critical element for plants and also for herbivores. It is known that a great part of leaf N is found in the carboxylating enzyme Rubisco, in proteins of the Calvin cycle, and in thylakoids, thus N is closely related to photosynthetic capacity (Evans 1989; Hikosaka 2004). Therefore, all else equal, possessing higher leaf N concentration per unit of leaf mass (N_{mass}) is normally associated with a greater carbon (C) gain, which can be invested in growth, defence, and/or vegetative reproduction (Chapin III, Schulze & Mooney 1990). In contrast, higher leaf N_{mass} might also make leaves more attractive to the herbivores (Kytö, Niemelä & Larsson 1996; Marvier 1996), in turn leading to a decrease in plant fitness.

Mistletoes are parasitic angiosperms that attach to a host plant branch and connect to their xylem, this connection becoming their only source of water and nutrients (Calder & Bernhardt 1983). Anatomical studies have shown that there is no connection with the phloem (Lamont 1983; Press & Graves 1995), and no direct lumen-to-lumen connection between xylem cells of parasites and hosts (Calvin 1967; Dobbin & Kuijt 1974; Tennakoon, Pate & Arthur 1997). The transfer of minerals from host to parasites occur not only *via* the apoplastic continuum, but also *via* active loading after selective uptake and metabolism of solutes from parasite parenchyma cell walls (Lamont & Southall 1982; Lamont 1983). However, there is a broad variation between plant parasite species in terms of the types of solutes acquired and the manner in which these solutes are obtained (Tennakoon, Pate & Stewart 1997).

As parasitic organisms receiving their resources from the host trees, their unit-costs for acquiring both water and nitrogen are potentially far lower than that experienced by their hosts. If mistletoes maintain higher leaf N concentration (especially higher N:C ratio) than

their hosts, because of the lower unit-cost involved in acquiring N through the host xylem, they could potentially suffer higher herbivory pressures. Interestingly, the fact that some parasitic plants can afford to exhibit higher N concentrations in their leaves compared to the surrounding vegetation was suggested as the selective force behind the cryptic mimicry observed in the Australian and New Zealand mistletoes, which allows them to avoid vertebrate herbivory (Barlow & Wiens 1977; Ehleringer *et al.* 1986; Bannister 1989).

Regarding insect herbivory damage, higher leaf N concentration is not the only influence on its amount and rate, as leaf structural traits together with chemical defences mutually act to defend leaves (Peeters 2002). Plant investment in leaf structural reinforcement (e.g. thicker cuticle and/or epidermis; more lignified vasculature) is generally associated with lower specific leaf area (SLA, the ratio of leaf area per leaf dry mass). SLA is an important morphological trait, highly correlated with herbivory rates (Coley 1983), that reflects a basic trade-off in leaf construction: how much is invested in leaf biomass in relation to the light-capturing surface area. Having lower SLA may lead to longer leaf lifespan (LL) by ensuring resistance not only against herbivores, but also against physical damages (Wright & Cannon 2001). In addition, higher investment in chemical defences (i.e. tannins, phenols and other defensive compounds) are also correlated with longer LL (Chabot & Hicks 1982; Coley, Bryant & Chapin III 1985), because long-lived leaves should be exposed to the risk of herbivory damage for a longer period (Bryant *et al.* 1985; Williams, Field & Mooney 1989).

Chemical defences are secondary compounds stored in leaf tissues that have toxic effects on herbivores. Very commonly, they are classified according to whether they predominantly contain N (N-based defences; e.g., alkaloids, cyanogenic glycosides) or whether they are predominantly composed of C (C-based defences; e.g. phenols, tannins). C-based defences may be either constitutive or induced (in response to herbivory), but in both cases they act by lowering the digestibility of proteins and reducing bioavailability of amino

acids (Barbeau & Kinsella 1983; Coley 1986; Hättenschwiler & Vitousek 2000). By contrast, N-based defences are directly toxic to herbivores and thus are an important feeding deterrent (Waller, Nowacki & Edmund 1978; Roberts & Wink 1998).

According to the **resource availability hypothesis** (Coley, Bryant & Chapin III 1985), nutrient availability in the environment is a major control of plant allocation to secondary defence compounds. In environments with higher N availability, the cost of acquiring C relatively to N is greater, and N-based defences are less expensive to construct, so that N-based defence syndromes tend to be favoured by natural selection (Gartlan *et al.* 1980; Craine *et al.* 2003). In contrast, C-based defences should generally be favoured in N-deficient environments (Coley, Bryant & Chapin III 1985). This trade-off occurs because investing in defence is costly considering that these resources could be used, in theory, for other productive purposes, such as reproduction and growth (Mooney & Gulmon 1982; Coley, Bryant & Chapin III 1985; Berenbaum 1995). The current understanding of many aspects in plant ecology with respect to nutrient use is almost exclusively focused on N. However, in phosphorus (P) deficient soils, such as the well-weathered soils in Australia and South America, it is expected that P should also have a fundamental role in the plants economy, possibly affecting allocation to defence (Adams, Attiwill & Wang 1995; Sampedro, Moreira & Zas 2011; but see Koricheva *et al.* 1998).

Evolutionarily speaking, the amount of resources invested in defence should be determined by the intensity of herbivory in a given vegetation type (Mooney & Gulmon 1982). A trade-off between investment in physical and chemical defence has also been suggested, due to the limited pool of resources available for direct investment in defences (Read *et al.* 2009), although empirical evidence for this trade-off has not always been found (Moles *et al.* 2013; Cárdenas *et al.* 2014). Many different studies have suggested that species

may be both chemically and physically well defended, exhibiting a synergic syndrome of defence traits combinations (Kursar & Coley 2003; Agrawal & Fishbein 2006; Futuyma & Agrawal 2009; Agrawal 2011; Moles *et al.* 2013).

Parasitic angiosperms, such as holoparasites, root parasites and mistletoes, have been reported to have high phenolic concentrations (Khanna *et al.* 1968; Luczkiewicz *et al.* 2001) and high amounts of condensed tannins (Salatino, Kraus & Salatino 1993; Leitão *et al.* 2013). Phytochemical studies of mistletoes have been gaining recent attention because of the anti-tumour properties found in extracts of the European genus *Viscum* (Viscaceae) (Kienle & Kienle 2010). Mistletoes are also largely used in traditional and alternative medicine (Fernández *et al.* 1998; Deeni & Sadiq 2002; Bussing 2003), and pharmacological research has expanded to various members of the family Loranthaceae, with reports of antimicrobial activity in mistletoes of different genera; e.g. *Tapinanthus dodoneifolius* (Deeni & Sadiq 2002), *Loranthus micranthus* (Cemaluk & Nwankwo 2012), *Struthanthus vulgaris* (Vieira *et al.* 2005).

Because past research has focused mostly on the pharmacological aspects of mistletoe biochemistry, there are few comparative ecological studies on mistletoes leaf defences, or on herbivory. Canyon and Hill (1997), studying two different Australian mistletoes species, showed that even with tougher leaves, mistletoes suffered higher rates of herbivory compared to their hosts, irrespectively of having higher or lower N concentration. They attributed this result to the higher water content of the mistletoe leaves. Very similar results were found in another study investigating a species of an Australian mistletoe (*Amyema miquelli*) (Burns, Cunningham & Watson 2011). Urness (1969) also described higher leaf water content in six North American mistletoe species, and verified the forage value of mistletoes, with high digestibility and carbohydrate content, but low protein and mineral content.

Nonetheless, fundamental ecological questions related to the investment in chemical defences by mistletoes remain unevaluated. It is generally unknown whether mistletoes suffer similar or different levels of herbivory compared to their hosts, or whether they are more or less defended. Our aim was to investigate the relationship between anti-herbivory defence investment, herbivory rates and leaf lifespan in mistletoes compared to their non-parasitic hosts.

Firstly, we compared mistletoes and hosts in terms of defence investment, leaf functional traits, and traits correlations. Because N should be relatively cheap for mistletoes to acquire, we expected that they would show lower investment in chemical and physical defence per unit of leaf N and, as a consequence, exhibit higher herbivory rates. Moreover, considering that the relative cost of losing leaf tissue by herbivory should be cheaper for mistletoes, we expect them to show lower defence investment in general, compared to their hosts. We also predict some correlation between host and mistletoes defence investment, which could indicate that mistletoes might get some chemical compounds transferred from the host xylem.

Secondly, we tested for trade-offs between a very general index of physical defence (SLA) and chemical defences (tannins and phenols), and between N-based and C-based defences. Based on the lower unit-cost of N-acquisition for mistletoes, we expected them to show higher N-based defence (i.e., the proportion of alkaloid-positive species) investment compared to their hosts.

Thirdly, we analysed the effect of defence traits on herbivory and on leaf lifespan. Regardless of being host or mistletoe, we predicted positive relationships between investment in defence and leaf lifespan and negative relationships between investment in defence and herbivory rates.

We also took the opportunity to investigate the evolutionary processes involved in the leaf morphological resemblance of Australian mistletoes and their hosts. If mimic mistletoes prevent herbivory damage by herbivores that actively avoid leaves from a specific host because of its lower palatability traits (as suggested in Chapter 2), we expect mimic-mistletoes' hosts to show higher investment in defence compared to non-mimic mistletoes' hosts.

Material and Methods

All sites were located within National Park reserves in Australia and Brazil and consisted of different vegetation types. A detailed description of the sites can be found in Chapter 3. Briefly, we sampled four to seven individuals of 35 mistletoe-host pairs (M-H). We sampled seventeen M-H pairs across two savanna-type vegetation communities, ten of them in Brazil (the cerrados), and seven in Australian savanna. In Australia, we also sampled twelve M-H pairs in semi-arid woodland in Central-eastern Australia, and six, located in closed woodland vegetation in the Sydney area (Table 1). Australian mistletoes were classified as highly-mimic, mimic or non-mimic following Barlow and Wiens (1977).

Table 1. List of the studied mistletoe-host pair species at each site location (n = 35). Species marked in bold correspond to those from which leaf lifespan and herbivory data were collected (n = 14).

Site	Mistletoe	Host	Host Family
Closed woodland (Sydney) n = 6	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus hemastoma</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus moluccana</i>	Myrtaceae
	<i>Muellerina eucalyptoides</i>	<i>Eucalyptus spp.</i>	Myrtaceae
	<i>Amyema congener</i>	<i>Allocasuarina littoralis</i>	Casuarinaceae
	<i>Dendrophthoe vitellina</i>	<i>Eucalyptus sp.</i>	Myrtaceae
	<i>Dendrophthoe vitellina</i>	<i>Angophora costata</i>	Myrtaceae
Savanna 1 (Darwin) n = 7	<i>Amyema sanguinea</i>	<i>Corymbia porrecta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Eucalyptus miniata</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Eucalyptus tetradonta</i>	Myrtaceae
	<i>Amyema sanguinea</i>	<i>Corymbia blesseri</i>	Myrtaceae
	<i>Amyema miquelli</i>	<i>Eucalyptus miniata</i>	Myrtaceae
	<i>Dendrophthoe odontocalyx</i>	<i>Grevillea pteridifolia</i>	Proteaceae
Semi-arid woodland (Bourke) n = 12	<i>Decaisnina signata</i>	<i>Xanthostemon paradoxus</i>	Myrtaceae
	<i>Amyema miraculosa</i>	<i>Eremophila longifolia</i>	Scrophuliaceae
	<i>Amyema miraculosa</i>	<i>Myoporum platycarpum</i>	Scrophuliaceae
	<i>Amyema miraculosa</i>	<i>Eremophila mitchellii</i>	Scrophuliaceae
	<i>Lysiana exocarpi</i>	<i>Alectryon oleifolius</i>	Sapindaceae
	<i>Amyema lucasii</i>	<i>Flindersia maculosa</i>	Rutaceae
	<i>Amyema preissii</i>	<i>Acacia aneura</i>	Fabaceae
	<i>Amyema preissii</i>	<i>Senna eremophila</i>	Fabaceae
	<i>Amyema preissii</i>	<i>Flindersia maculosa</i>	Rutaceae
	<i>Amyema maidenii</i>	<i>Acacia harpophylla</i>	Fabaceae
	<i>Amyema maidenii</i>	<i>Acacia aneura</i>	Fabaceae
Cerrado (Brasília) n = 10	<i>Lysiana linearifolia</i>	<i>Eremophila mitchellii</i>	Scrophuliaceae
	<i>Lysiana exocarpi</i>	<i>Acacia aneura</i>	Fabaceae
	<i>Phoradendron sp.</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Struthanthus polyanthus</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Psittacanthus robustus</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Phthirusa ovata</i>	<i>Miconia albicans</i>	Melastomataceae
	<i>Psittacanthus robustus</i>	<i>Qualea grandiflora</i>	Vochysiaceae
	<i>Psittacanthus robustus</i>	<i>Qualea parviflora</i>	Vochysiaceae
	<i>Phthirusa ovata</i>	<i>Styrax ferrugineus</i>	Styracaceae
	<i>Phthirusa ovata</i>	<i>Dalbergia miscolobium</i>	Fabaceae
	<i>Phoradendron tunaeforme</i>	<i>Guapira areolata</i>	Nyctaginaceae
	<i>Phoradendron crassifolium</i>	<i>Tapirira guianensis</i>	Anacardiaceae

Leaf traits

Mature, fully-expanded leaves were collected from five to seven individual of each M-H pair, immediately scanned and the area was measured using Image J software (Abràmoff, Magalhães & Ram, 2004). The leaves were dried in the oven for 72 hours at 65°C and weighed. Specific leaf area (SLA) was calculated as the ratio of fresh leaf area to leaf dry mass.

The same leaves were finely ground for chemical defence analyses. Australian samples were sent to the Analytical Service Unit from the School of Agriculture and Food Science at The University of Queensland for N determination using a LECO TruSpec CHN combustion analyser, and for total P determination using an ICP-OES analyser, following Nitric perchloric acid digestion. Brazilian samples were sent to “Laboratório de Agroquímica e Meio Ambiente da Universidade Estadual de Maringá” (PR-Brazil) for N and P determination by Kjeldahl digestion and UV-Vis spectroscopy, respectively.

Total phenolic content (Ph) was determined by Folin-Ciocalteu assay, as described by Singleton & Rossi (1965) and Singleton, Orthofer & Lamuela-Raventos (1999). Briefly, phenolics were extracted from 20 mg of plant tissue with 12 ml acetone (70%) in an ultrasonic bath for 20 min followed by centrifugation. Total phenolics were quantified colorimetrically at 760 nm, with a standard curve of gallic acid and expressed as mg gallic acid equivalents g⁻¹ dry mass of plant tissue. The non-tannin phenolics were calculated by adding polyvinylpyrrolidone (PVPP) as a tannin binder to 1 ml of the extract followed by 15 min at 4 °C and centrifugation. The supernatant was collected and phenolics were again measured as described above (Folin-Ciocalteu assay). Tannin content (Ta) was calculated as the difference between the Ph and the non-tannin phenolics (Makkar *et al.* 1993; Makkar 2003). Ph and Ta were conducted in triplicate.

We used 10 mg leaf material in 1 ml methanol (80%) extract to test for the presence of alkaloids. To the filtered extract, we added 1-2 drops of the Dragendoff's reagent. This reagent forms an orange precipitate in the presence of alkaloids and has been demonstrated to be useful in predicting the presence or absence of alkaloids in plant tissue (Popl, Fahnrich & Tatar 1990; Raffaaf 1996).

Leaf lifespan and herbivory rates

We monitored 14 out of the 35 mistletoes-host pairs in Australian closed woodland and Brazilian cerrado (Table 1) for 12-18 months to investigate herbivory rates and LL. Four branches of five individual were used to measure LL, based on leaf turnover rates (Wright & Cannon 2001). All leaves from each branch were sequentially numbered and revisited every 3-4 months for 12 months. LL was calculated as the inverse of the mortality rate (number of dead leaves/ number of leaves at the beginning of census/ period of time). For 32 leaves marked on each individual, the average leaf area lost to herbivory was estimated (always by the same person (MCS)), by visually surveying the percentage of area removed or damaged in each census, totalling a number 160 leaves for each mistletoe and each host species considered. All damage to the surface of the leaf (including the action of chewers, mines, galls, and fungus) was considered broadly as "herbivory". We excluded from our estimation the leaves that were completely removed, because it was not possible to infer if it was caused exclusively by herbivores or by any other factor (physical damage, senescence, etc.). Initial analyses included the deciduous host species *Qualea grandiflora*. As it turned out, this species was a clear outlier in any analyses including traits, herbivory rates and leaf lifespan, presumably because of its very distinct defence strategy against herbivores, attracting mutualistic ants with pairs of extra floral nectaries along the stems (Costa, Oliveira-Filho &

Oliveira 1992). Because of this strategy, and as it was the only deciduous host species, we chose not to include it in further analyses.

Physical defences

For the 6 pairs located in the Australian closed woodland, we used a universal testing machine (Instron 5542; Instron, Canton, MA, USA) with custom-made penetrometer (Onoda, Schieving & Anten 2008) for conducting punch tests, measuring the force to punch (N), and a dial gauge micrometre to measure leaf thickness. The force to punch and leaf thickness were measured in three different points of the leaf lamina, avoiding the primary and secondary veins. Leaf toughness (N m^{-1}) was calculated as the force to punch divided by leaf thickness. Extra leaves were used to measure dry matter content (DMC). Squares of 1 cm^2 area were removed from the extra leaves and placed in water for 24 hours to saturate. The squares were weighed and placed in the oven until completely dry for 72 hours at 65°C , and then weighed again. DMC was calculated as the ratio of leaf dry mass to leaf saturated mass.

Data Analyses

All data were analysed using R software version 3.1.0 (R Development Core Team 2008). Leaf and defence trait data were log-transformed to satisfy standard assumptions for normality. We used paired t-tests to test for trait differences between mistletoes and their hosts, and Fisher's exact test for the categorical variable (alkaloid positive or negative). To maintain the paired nature of the data, we also used the mistletoe to host trait difference (always calculated as mistletoe trait – host trait) to test for differences between sites with one-way analysis of variance (ANOVA). Ordinary least squares (OLS) regression and Pearson correlation were used for quantifying the relationship between mistletoe and host defence traits, and for verifying trade-offs between defence traits. We fitted standardized major axis

(SMA) slopes to explore the relationship between defence traits and LL, and to compare slopes between mistletoes and hosts, using package SMATR version 3.0 (Warton *et al.* 2012). All statistical tests assumed significance at $P < 0.05$, but marginally significant results ($0.05 < P < 0.1$) are also reported, and noted as such.

Results

Patterning of Mistletoe-Host differences among sites

There were clear tendencies for mistletoes and their hosts to differ in SLA ($P < 0.001$), with mistletoes showing lower SLA at all sites, and overall (Table 2, Fig.1). For the 6 pairs on which we measured other physical properties, we found that mistletoe leaves were thicker (mean \pm sd: 1.13 ± 0.18 versus 0.45 ± 0.11 mm), tougher (mean \pm sd: 1869 ± 216 versus 3651 ± 776 N m⁻¹) and had lower DMC (mean \pm sd: 0.29 ± 0.02 versus 0.48 ± 0.01 mg mg⁻¹). Conversely, mistletoes and hosts showed similar N_{mass} ($P = 0.964$), whether within individual sites or considered overall (Fig. 1). Consequently, the ratio SLA : N_{mass} was lower in mistletoe compared to host across all mistletoe species, and lower in mistletoes in the two savanna sites (cerrado and Australian savanna, Table 2).

Trends for defence chemicals were less clear, and generally, there was greater variation between species than it was seen for SLA or N_{mass} . Total Ta averaged 2.78 % in hosts (ranging from 0.06 to 15.84 %) and 3.89 % in mistletoes (ranging 0.11 to 11.19 %). Combining all species, mistletoes showed higher Ta compared to their hosts (paired t-tests, $P = 0.027$, Figure S1); but this difference was observed only within one of four individual sites (semi-arid woodland, Table 2, Fig. 1).

Ph did not differ between hosts and mistletoes in general ($P = 0.404$), but mistletoes showed higher Ph at the semi-arid woodland site and lower Ph at the closed woodland site,

compared to their hosts (Fig. 1; Table 2). The patterning of $Ph : N_{mass}$ and $Ta : N_{mass}$ broadly mirrored Ph and Ta respectively, with mistletoes in semi-arid woodland showing higher values compared to their hosts.

Australian closed woodland and Brazilian cerrado mistletoes and hosts showed lower Ta than species at other sites (Table 2). However, because both mistletoe and host species from Australian savannas and Australian closed woodland species showed lower N_{mass} , the proportion of defence investment per unit of N ($Ph : N_{mass}$ and $Ta : N_{mass}$) was higher in those sites (Table 2). Surprisingly, a higher proportion of alkaloid-positive hosts was found in Australian savanna (86%) and Australian closed woodland (83%), despite showing the lowest N_{mass} values (Table 2). Australian semi-arid woodland mistletoes species were generally better defended than their hosts, showing higher Ph , higher Ta , and the highest proportion of alkaloid-positive mistletoes species (58%, Table 2).

Relationships between Mistletoe and Host defence traits

Among all sites, there was a tight positive relationship between mistletoe and host N_{mass} ($r^2 = 0.61$, $P < 0.001$) and Ta ($r^2 = 0.71$, $P < 0.001$, Fig. 2), while a weaker relationship for Ph ($r^2 = 0.14$, $P = 0.026$) and no correlation between mistletoe and host SLA ($P > 0.1$, Fig. 2).

On average, 60% of host species (20 out of the 35) and 30% of mistletoes (9 out of 35) tested positive for alkaloids. In only four pairs were both mistletoes and hosts alkaloid-positive, suggesting that most mistletoes do not acquire alkaloids from the host xylem (or perhaps none do).

Table 2. Trait mean (SD) for mistletoe (M) and host plants (H) for each studied site and the total average value. Significant differences between mistletoes and hosts in each site and total are indicated by the symbol * (paired t-tests or chi-squared tests, $P < 0.05$).

Trait	Brazilian Cerrado n = 10		Australian savanna n = 7		Semi-arid woodland n = 12		Closed woodland n = 6		Total n = 35	
	M	H	M	H	M	H	M	H	M	H
SLA	43.32 (8.70)	68.36* (15.58)	39.60 (12.41)	57.08* (8.15)	27.88 (3.88)	44.51* (14.45)	33.28 (5.39)	48.31* (12.23)	35.56 (10.35)	50.49* (16.88)
SLA : N _{mass}	21.45 (7.27)	36.52* (10.16)	41.25 (6.51)	68.88* (10.38)	19.33 (9.04)	24.26 (8.66)	49.10 (14.77)	67.61 (27.97)	29.42 (15.22)	44.12* (22.78)
N _{mass} (%)	2.04 (0.53)	1.97 (0.61)	0.96 (0.26)	0.84 (0.15)	1.74 (0.73)	1.85 (0.28)	0.78 (0.39)	0.77 (0.16)	1.54 (0.75)	1.50 (0.66)
Ta (%)	1.25 (1.07)	1.23 (1.08)	7.22 (2.53)	7.02 (4.00)	5.14 (2.66)	2.23* (1.26)	1.38 (0.52)	1.17 (0.81)	3.80 (3.19)	2.76* (3.04)
Ta : N _{mass}	0.72 (0.81)	0.71 (0.79)	7.94 (2.63)	8.93 (6.01)	3.38 (1.89)	1.23* (0.69)	2.05 (0.98)	1.58 (1.30)	3.30 (3.11)	2.68 (4.27)
Ph (%)	8.69 (3.94)	9.99 (3.59)	9.95 (3.02)	10.39 (5.21)	9.65 (4.43)	4.94* (2.06)	6.78 (0.84)	9.41* (2.34)	8.94 (3.83)	8.24 (4.22)
Ph : N _{mass}	5.13 (4.16)	5.56 (2.97)	11.01 (3.45)	13.09 (7.80)	6.43 (3.46)	2.74* (1.20)	12.38 (5.01)	13.38 (4.31)	7.99 (4.97)	7.47 (6.36)
Alkaloid-positive (% number species)	10	50	29	86*	58	33	0	83*	29	57*

N_{mass}: nitrogen concentration; SLA: specific leaf area; Ph: total phenolics concentration; Ta: total tannins concentration

Generalised defence syndromes vs. trade-offs between defence types

Trade-offs between chemical and physical defence were not very clear: relationships between SLA and other chemical defences were not consistent across mistletoes and hosts. Figure 3 shows a negative relationship between SLA and Ta for mistletoes ($r^2 = 0.16$, $P = 0.018$; Fig. 3a), although a wide range of Ta could be seen at any SLA, and especially among high SLA species. No relationship was seen between Ta and SLA in host species. By contrast, SLA was weakly and positively correlated with Ph in hosts ($r^2 = 0.14$, $P = 0.044$; Fig. 3b), but there was no relationship among mistletoe species.

A trade-off between N-based and C-based defence was not supported by our results. Alkaloid-positive mistletoes showed higher Ta concentration ($P = 0.019$, Fig. 4a) compared to alkaloid-negative mistletoes. There was no general difference between alkaloid-positive and alkaloid-negative mistletoes and host species for total Ph or for SLA (all $P > 0.1$, Fig. 4b and 4c), while hosts that tested positive for alkaloids also showed significantly lower N_{mass} concentration ($P = 0.006$, Fig. 4d).

Herbivory and leaf lifespan relationship with defence

Annual herbivory rate ranged from 11.4% to 29.1% for hosts (mean \pm SD: $18.9 \pm 6.6\%$) and from 15.0% to 54.5% for mistletoes (mean \pm SD: $26.1 \pm 11.1\%$), being on average marginally higher in mistletoes compared to their hosts (paired t-test, $P = 0.067$). However, mistletoes and hosts showed no differences in mean LL (paired t-test, $P = 0.572$). Herbivory was negatively related to LL for mistletoes ($r^2 = 0.48$, $P = 0.006$) and (marginally) for hosts ($r^2 = 0.20$, $P = 0.088$), indicating that species suffering less herbivory achieved longer LL (Fig. 5a, Table 3). We did not find any relationship between herbivory and chemical defences (Ta and Ph, Table 3) or between herbivory and physical traits (SLA, Fig. 5b), but herbivory was positively correlated to leaf N_{mass} in host species ($r^2 = 0.46$, $P = 0.010$; Figure 5c).

Longer leaf lifespan was associated with lower SLA and lower N_{mass} for hosts, and mistletoes tended to follow the same pattern with similar slopes (not significantly different), but a weaker correlation (Table 3, Fig. 4a and 4b). On average, mistletoes achieved 2/3 the LL for a given SLA (i.e., the elevation of LL-SLA slopes differed; Wald-test: 25.41, $P < 0.0001$, Fig. 4a). Host LL was positively correlated with phenolic concentration per unit of N (Fig. 4c, Table 3), presumably reflecting the negative LL-N relationship; while tannin investment (Ta and Ta : N_{mass} and Ph) were not related to longer LL, either for mistletoes or hosts (Fig. 4d, Table 3). Neither LL nor herbivory rate differed between alkaloid-positive and alkaloid-negative hosts ($P > 0.1$). It was not possible to verify whether this relationship occurred in mistletoes because only one out of the 14 species tested positive for alkaloids (*Phoradendron tunaeforme*).

Table 3. Pearson correlation between leaf defence traits, leaf lifespan (LL) and herbivory rates (r^2 and P -values, sample size in parentheses).

Traits	Mistletoes	Hosts
LL, N_{mass}	0.19, 0.115 (14)	0.64, 0.001 (13)
LL, P_{mass}	0.18, 0.147 (14)	0.74, <0.001 (13)
LL, SLA	0.09, 0.297 (14)	0.41, 0.022 (13)
LL, Ph	0.06, 0.387 (14)	0.144, 0.223 (13)
LL, Ta	0.00, 0.909 (14)	0.00, 0.786 (13)
LL, Ph: N_{mass}	0.008, 0.752 (14)	0.47, 0.001 (13)
LL, Ta: N_{mass}	0.03, 0.571 (14)	0.11, 0.286 (13)
LL, Herbivory	0.30, 0.042 (14)	0.20, 0.088 (13)
Herbivory, SLA	0.00, 0.959 (14)	0.11, 0.276 (13)
Herbivory, N_{mass}	0.06, 0.407 (14)	0.46, 0.010 (13)
Herbivory, P_{mass}	0.06, 0.439 (14)	0.34, 0.047 (13)
Herbivory, Ph	0.04, 0.494 (14)	0.00, 0.941 (13)
Herbivory, Ta	0.09, 0.312 (14)	0.185, 0.143 (13)
Herbivory, Ph: N_{mass}	0.07, 0.364 (14)	0.23, 0.100 (13)
Herbivory, Ta: N_{mass}	0.01, 0.672 (14)	0.03, 0.576 (13)
SLA, Ta	0.16, 0.018 (35)	0.00, 0.768 (35)
SLA, Ph	0.00, 0.964 (35)	0.14, 0.044 (35)
SLA, N_{mass}	0.03, 0.335 (35)	0.01, 0.617 (35)
SLA, P_{mass}	0.00, 0.917 (35)	0.02, 0.393 (35)

N_{mass} : nitrogen concentration; SLA: specific leaf area; Ph: total phenolics concentration; Ta: total tannins concentration; LL: leaf lifespan.

All traits were log-transformed prior to analysis.

Batesian mimicry in Australian mistletoes

Within the 25 Australian pairs, we found a strong pattern of alkaloid-negative mimic mistletoes parasitising alkaloid-positive hosts, while all alkaloid-positive mistletoes occurred when the mistletoe was considered “not mimic” or “not highly mimic” (Table S1). Hosts with mimic-mistletoes usually exhibited higher Ph and lower N_{mass} , and consequently higher Ta : N_{mass} and higher Ph : N_{mass} (paired t-tests, all $P < 0.05$), mirroring the associated mistletoe (Fig. S2).

Discussion

Table 4 summarises our results in relation to the expectations we outlined in the introduction, few of which were supported. Specifically, key expectations that mistletoes would have higher defence investment (either absolute or per unit of nitrogen), higher prevalence of N-based defences, and that defence investment in both mistletoes and hosts would decrease herbivory rates, were not supported. However, there were still a number of very clear and interesting results (specifically, a strong relationship between mistletoes and hosts in terms of N_{mass} and tannins, evidence of generalised defence syndromes in mistletoes, a negative relationship between herbivory and leaf lifespan), which we discuss below.

Table 4. Summary of expectations, tests and results

General expectations	Specific expectations and tests	Support
	In comparison to their hosts (paired t-tests) mistletoes will have:	
1. Mistletoes will have lower defence investment than hosts	<ul style="list-style-type: none"> Lower Ta Lower Ph Higher SLA 	<ul style="list-style-type: none"> None (<i>Higher</i> Ta across all species, and at 1 of 4 sites) None (<i>Similar</i> across all species <i>lower</i> Ph at the closed woodland, but <i>higher</i> Ph at the closed woodland) None (<i>Lower</i> SLA across all species and at each site)
2. Mistletoes will have lower defence investment per unit leaf N	In comparison to their hosts (paired t-tests) mistletoes will have: <ul style="list-style-type: none"> Lower Ta : N_{mass} Lower Ph : N_{mass} Higher SLA : N_{mass} 	<ul style="list-style-type: none"> None (no difference) None (no difference) None (<i>Lower</i> SLA : N_{mass} across all species and at two sites)
3. Mistletoes derive chemical defence compounds from host xylem	<ul style="list-style-type: none"> Positive correlation between host and mistletoe Ta Positive correlation between host and mistletoe Ph 	<ul style="list-style-type: none"> Yes, very strong ($r^2 = 0.71$) Yes ($r^2 = 0.14$, $P = 0.026$)
4. Mistletoes will show higher prevalence of N-based defences (alkaloids)	<ul style="list-style-type: none"> Higher proportion of alkaloid-positive mistletoe species (Fisher's exact test) 	<ul style="list-style-type: none"> None (only 9 mistletoes species tested positive for alkaloids against 20 hosts)

	<ul style="list-style-type: none"> Negative correlation between SLA and Ta Negative correlation between SLA and Ph Lower SLA in species with alkaloids (t-test) Higher Ta in species with alkaloids (t-test) Higher Ph in species with alkaloids (t-test) 	<ul style="list-style-type: none"> Mistletoes: yes ($r^2 = 0.16$, $P = 0.018$); Hosts: none. Mistletoes: none; Hosts: opposite (<i>positive</i> $r^2 = 0.14$) None in either mistletoes or hosts Mistletoes: yes ($P = 0.019$); Hosts: none. None in either mistletoes or hosts
5. In either or both mistletoes and hosts we may see evidence of generalised defence syndromes. (** any opposite findings are instead support for <i>trade-offs</i> between defence types)		
6. Mistletoes will suffer higher herbivory than their hosts	<ul style="list-style-type: none"> Paired t-test of annual accrued herbivory (loss of leaf area) 	<ul style="list-style-type: none"> Weak support: marginally higher herbivory ($P = 0.067$)
7. In both mistletoes and hosts higher investment in defence serves to lengthen average leaf lifespan (LL)	<ul style="list-style-type: none"> Positive correlation between LL and Ta Positive correlation between LL and Ph Negative correlation between LL and SLA LL will be longer in species with alkaloids (t-test) Negative correlation between LL and Herbivory 	<ul style="list-style-type: none"> None, in either mistletoes or hosts None, in either mistletoes or hosts Mistletoes: none; Hosts: yes ($r^2 = 0.41$) None – only tested for hosts: (t-test: $t = 0.01$, $P = 0.994$) Mistletoes: yes ($r^2 = -0.48$, $P = 0.006$), Hosts: weak ($r^2 = -0.20$, $P = 0.088$)
8. In both mistletoes and hosts higher investment in defence serves to decrease herbivory	<ul style="list-style-type: none"> Positive correlation between Herbivory and Ta Positive correlation between Herbivory and Ph Negative correlation between Herbivory and SLA Lower herbivory in species with alkaloids (t-test) 	<ul style="list-style-type: none"> None, in either mistletoes or hosts None, in either mistletoes or hosts None, in either mistletoes or hosts None – only tested for hosts: (t-test: $t = -0.16$, $P = 0.884$)
9. Australian mimic-mistletoes' hosts will be better defended than hosts with non-mimic-mistletoes	<p>Hosts with mimic mistletoes will have:</p> <ul style="list-style-type: none"> Higher $Ta:N_{mass}$ Higher $Ph:N_{mass}$ Lower SLA Lower N_{mass} Higher proportion of alkaloid-positive species 	<ul style="list-style-type: none"> Yes Yes None Yes Yes

Relationships between Mistletoe and Host defence traits

A close relationship between mistletoe and host N_{mass} ($r^2 = 0.61$, $P < 0.001$) is a well-known and expected pattern found in the global analysis from Chapter 2, and also in previous studies (Schulze *et al.* 1991; Bannister & Strong 2001; Wang *et al.* 2008). Presumably, this relationship occurs because the host xylem is the only source of N for mistletoes. Moreover, secondary compounds transferred from hosts could potentially benefit mistletoes, decreasing herbivory rates. Many reports from root parasites of the *Castilleja*, *Orobanch*e and *Orthocarpus* genera (Scrophulariaceae) and for mistletoes of the *Viscum* genus (Loranthaceae) show that they obtain alkaloids from their hosts (Wink, Witte & Hartmann 1981; Boros *et al.* 1991; Cordero, Serrano & Gonzalez 1993; Adler 2000; Adler & Wink 2001; Adler 2002; Ilesanmi 2011). When parasitizing a host with leaf alkaloids, the root parasite *Castilleja miniata* showed lower herbivory compared to the same species in a non-alkaloid host (Adler 2000). Therefore, acquiring alkaloids directly from the xylem of the hosts could be an advantage for the mistletoe. However, both mistletoes and hosts tested positive for alkaloids in only four out of the 35 pairs studied here. In the remaining 16 alkaloid-positive hosts, mistletoes did not show presence of alkaloids; while six alkaloid-positive mistletoes were parasitizing hosts without alkaloids, suggesting the majority of the mistletoes species are self-sufficient in constructing alkaloids. This result also suggests that alkaloids are not being transferred from hosts. As discussed by Adler and Wink (2001), the mechanism of storing and transporting alkaloids can differ between parasitic plants and hosts, which may possess different enzymes for alkaloid transport. Moreover, mistletoes may have the advantage of not needing to invest in alkaloids when growing on alkaloid-positive hosts, especially if they can “hide” in the host canopy (this aspect is further discussed below).

In contrast, we found a strong correlation between mistletoes and hosts in tannin concentration ($r^2 = 0.71$, Fig. 3). There are two interpretations for this pattern: mistletoes can

access tannins from the host xylem and deploy them in their leaves; or mistletoes suffer similar herbivory pressure to their hosts, and gain some fitness advantage by regulating tannin concentration to match that of their hosts. Tannins are found dissolved in parenchymatic cells, such as the cells from the secondary xylem, and there are reports of tannins being transferred to xylem-feeding insects (Raven 1983; Crews *et al.* 1998; Wallis & Chen 2012). Therefore, the most likely scenario is that tannins are being transferred to the mistletoes. In addition, even though tannins constitute a specific class of phenols, the relationship between mistletoe and host concentrations of total phenolics was not as strong as the relationship between tannins ($r^2 = 0.14$, $P = 0.026$). The only other study we are aware of that investigated transference of phenolics in mistletoes reported that phenolic concentrations of the mistletoe *Dendrophthoe falcata* were not related to phenolic concentrations in five different hosts (Khanna *et al.* 1968). There is evidence that some solutes are transferred directly from the host xylem to the mistletoe and others are metabolized before transfer, or excluded and not absorbed (Pate, True & Rasins 1991). Here, the case may be that tannins are being absorbed and other classes of phenols are being excluded.

The active exclusion of secondary compounds may also explain the absence of a strong concurrence in alkaloids, especially if alkaloids from the hosts are capable to affect mistletoes negatively. Alkaloids introduced to other plants can have allelopathic effects by inhibiting chlorophyll synthesis, internode elongation, and general growth (Waller, Nowacki & Edmund 1978; Roberts & Wink 1998). However, as argued by Pennings and Callaway (2002) the physiological link between hosts and mistletoes might be an obstacle, and preventing self-poisoning may be a challenge. Little is known about the effects of host alkaloids on mistletoes, but there is evidence for exclusion of nicotine-based alkaloids by *Cuscuta gronovii* and *Orobanche muteli*, both parasitic angiosperms, when growing on tobacco hosts (Wazel 1952). Clearly, there is still need for further research in this area.

Generalised defence syndromes vs. trade-offs between defence types

Many current hypotheses regarding plant defence investment can be divided into two categories: resource allocation trade-offs, or synergic syndromes of trait combinations (Ballhorn *et al.* 2014). The first is based on the prediction that trade-offs between different anti-herbivore traits occur because a limited pool of resources can be allocated between different types of defence for different types of herbivores (Eck *et al.* 2001; Read *et al.* 2009); or, alternatively, because the defences may be redundant (i.e., efficient against the same type of herbivore; Herms & Mattson (1992)). The latter (synergic syndromes) suggests additive interactions between defensive traits, with multiple trait combinations evolving together (Kursar & Coley 2003; Agrawal & Fishbein 2006; Futuyma & Agrawal 2009; Agrawal 2011). Our results suggest that mistletoes and hosts have rather different strategies in relation to physical and C-based chemical defence investment. Robust, low SLA leaves of mistletoes showed higher tannin concentration (in support of a generalised defence syndrome), while low SLA leaves of hosts showed less investment in phenolics (in support of a trade-off between structural and C-based defence; Fig. 3). Mistletoes apparently may not be subjected to the same trade-off, especially because of the transference of tannins from the hosts discussed above, resulting in a positive syndrome correlation of lower SLA and higher tannin concentration.

We did not find evidence of a trade-off between N-based and C-based defence investment, in either mistletoes or hosts. There was no difference between alkaloid-positive and alkaloid-negative hosts in relation to the investment in tannins and total phenols or SLA (Fig. 6). Interestingly, alkaloid-positive mistletoes showed higher leaf tannin concentration compared to alkaloid-negative species (Fig. 4). The absence of a trade-off between N-based and C-based defences and the evidence of a trade-off between SLA and C-based defences

gives support to the idea that defences using the same resource are more likely to display stronger trade-offs, as suggested by Moles *et al.* (2013).

Herbivory and leaf lifespan relationships with defence

Mistletoes showed marginally significantly higher herbivory rates compared to their hosts in the two sites that herbivory was monitored, despite apparently investing more in defence (lower SLA, higher Ta, and higher Ph at 2 of 4 sites, Table 3). Herbivory was not correlated with chemical defences, but it was positively related to N_{mass} and P_{mass} for host plants, meaning that host leaves with higher nutritional values suffered higher herbivory rates. Coley (1983) argued that the lack of correlation between herbivory and chemical defences might indicate differences in leaf ontogeny in relation to defence investment, because herbivory is measured as an accumulative trait while defence is measured at a particular point in time during leaf development. However, it may also be the case that better-defended species might suffer similar herbivory rates for other reasons, such as having generally higher palatability due to higher water content.

Herbivory was negatively related to leaf lifespan, for both mistletoes and hosts, in agreement with the hypothesis that long-lived leaves must be well defended because leaf damage increases with time (Coley 1983; Bryant *et al.* 1985; Coley 1988; Williams, Field & Mooney 1989). Several studies also show that leaves that suffer lower herbivory tend to live for longer periods of time (Stanton 1975; Coley 1980; Chabot & Hicks 1982). In addition, LL was negatively related to N_{mass} , P_{mass} and SLA for hosts, and mistletoes follow similar trends (Fig. 6), thus species with lower SLA and lower leaf nutrient concentrations tended to have longer LL. Therefore, our results support the prediction that mistletoes can also be placed along the continuum running between the two extreme evolutionary strategies (Chabot & Hicks 1982) of either constructing long-lived, low palatability leaves (with higher defence

investment or low protein content), or constructing short-lived leaves with higher N and potentially higher photosynthetic capacity to offset the shorter life span.

SLA and LL are expected to be correlated because structural reinforcements confer physical protection against herbivores and other physical hazards (Reich *et al.* 1991; Reich, Walters & Ellsworth 1992; Ryser 1996; Wright & Cannon 2001; Wright, Westoby & Reich 2002). However, the LL-SLA relationship was shifted in mistletoes in relation to their hosts: mistletoes achieved shorter LL at a given SLA (Fig. 6a). We believe this pattern is most likely a result of softer leaves in mistletoes for a given SLA (i.e, leaves with lower tissue density and higher moisture content), because leaf structure is closely linked to leaf lifespan (Reich 1993; Ryser 1996). Even though leaf toughness and DMC were not measured in all pairs considered in this study, our results indicate clear differences in leaf physical properties, with mistletoes showing on average half the DMC and leaf toughness of host leaves, and 2-fold higher leaf thickness. Moreover, the higher leaf water content and succulence is a well-reported characteristic trait for mistletoes (Popp *et al.* 1995; Canyon & Hill 1997; Glatzel & Geils 2009; Burns, Cunningham & Watson 2011).

Influences of soil P-impoverishment and aluminium accumulation on defence traits

Australian soils tend to be notably P-impoverished (Beadle 1966), and resource limitations tend to select for conservative strategies, such as higher leaf toughness and low nutritive value, resulting in traits conferring lower palatability (Coley, Bryant & Chapin III 1985; Diaz *et al.* 2004). In fact, we found that hosts and mistletoes from Australian savanna and closed woodland sites, which were associated with very low N_{mass} , showed the highest ratios of Ph : N_{mass} compared to the other two sites (Table 2). Moreover, these sites also showed the highest proportion of alkaloid-positive host species. In N-deficient environments, we would expect

that N use would be maximised and preferentially allocated to primary functions, such as photosynthesis rather than to defence (Herms & Mattson 1992), because alkaloids are rich in N and expensive to build (Waller, Nowacki & Edmund 1978). However, despite the expectations that C-based defences should generally be favoured in low-nutrient environments (resource availability hypothesis; Coley, Bryant and Chapin III (1985)), we found in these low-P sites that N-based defences were more predominant, without any C-based defence being substantially different from other sites. Under experimental manipulations, deficiency of P can cause increases in soluble nitrogen compound levels, which may provide alkaloid synthesis substrate (Stewart, Larher & Miflin 1980; White 1984; Rabe & Lovatt 1986). Our results suggest that P limitation might be the selective limitation in these sites and there should be very strong selective pressure to minimise nutrient replacement costs by constructing leaves with high probability of long lifespan, likely achieved in this case by investing heavily in N-based defences.

Another difference between sites worth noting is that 60% of all host species in the cerrado site are aluminium (Al) hyperaccumulators (Vochysiaceae and Melastomataceae families, Table 1). Hyperaccumulation of Al was demonstrated to act as anti-herbivore defence and suggested to be the physiological function driving the natural selection for hyperaccumulation of heavy metals in plants from tropical environments with high Al soil content (Pollard 2000; Jansen *et al.* 2002). It is argued that a metal-based defence might trade-off with other organic defences (Poschenrieder, Tolrà & Barceló 2006). Interestingly, the Al-accumulator hosts showed the lowest $Ta : N_{mass}$ of all hosts (*Miconia albicans* parasitised by *P. robustus* and *P. ovata* showed almost nil tannin in their leaves), mirrored in the mistletoes species (Fig. 2), indicating a possible trade-off between metal and C-based defence. Scalon, Haridasan and Franco (2013) suggested that Al might have a still unexplored important physiological role for the mistletoe *P. robustus*, because there is evidence for active

translocation of Al through the mistletoe phloem. If the function of Al in mistletoes is also related to anti-herbivore defence, then mistletoes accessing higher concentrations of Al in the host xylem and accumulating it in the leaves might also gain an advantage against herbivores, and hyperaccumulation of Al might be involved in host selection by mistletoes.

Batesian mimicry in Australian mistletoes

Host selection is a very important evolutionary process for mistletoes, which might gain protection against herbivores by mimicking host foliage or “choosing” the host with highest potential to transfer effective secondary compounds. Parasitic angiosperms can select for hosts by selective germination of seeds, meaning that the germination process only triggers when the seed is placed in a suitable hosts; or by selective vegetative growth, for mistletoes with auxiliary roots growing across different host plants (Marquardt & Pennings 2010). In the case of Australian mistletoes, mimicry of the host leaves morphological characteristics can be directly involved in host selection. By looking similar to their hosts, mimic mistletoes should be able to avoid vertebrate herbivory (Barlow & Wiens 1977; Ehleringer *et al.* 1986; Bannister 1989).

The confusion in the literature regarding the application of the terms “mimicry” and “crypsis” in cases of mistletoe and host leaf resemblance (Vane-Wright 1980) was brought up in Chapter 2. The difference between crypsis and mimicry is subtle; however, it has important implications for understanding the evolutionary drivers and ecological consequences for the species involved. As discussed in Chapter 2, protective crypsis (Endler 1981) implies that mistletoes should have traits that otherwise would make their leaves more attractive to herbivores, such as higher N_{mass} . However, if the advantage of resembling host leaves is to prevent herbivory damage by herbivores that actively avoid leaves from a specific host, it would constitute an example of Batesian mimicry (Vane-Wright 1980). In other words, if it is

a case of Batesian mimicry, mimic mistletoes could lack the ability to sequester host defence compounds, while non-mimic mistletoes would have to invest in chemical defences themselves or rely on host-derived toxins. In terms of C-based defences, mimic mistletoes were similar or even better defended than non-mimic ones (Fig. S2). However, Table S1 illustrates evidence of Batesian mimicry by showing that none of the highly-mimic mistletoe species tested positive for alkaloids, while all associated hosts did. Therefore, not needing to invest in an expensive N-rich compound such as alkaloids by morphologically resembling leaves of hosts with alkaloids, consists of a clear fitness advantage and might be involved in the evolution of mimicry in Australian mistletoes.

Conclusion

We investigated anti-herbivore defence in 35 mistletoes-host pairs across four different sites in Australia and Brazil, partially fulfilling the need for more field studies of parasitic plants in natural communities noted by Pennings and Callaway (2002). In fact, our results showed that very few of our initial expectations were supported and investment in anti-herbivore defence could indeed be involved in the evolutionary processes of many different aspects for parasitic angiosperms, such as host selection and functional strategies. Mistletoes had generally higher C-based defence investment, although suffering marginally higher herbivory rates compared to their hosts. We also showed evidence of tannins being transferred from hosts, with other classes of phenols and alkaloids possibly being excluded. There were differences in defence syndromes and trade-offs between mistletoes and hosts, but a similar negative relationship between herbivory rates and leaf lifespan, in agreement with the hypothesis that long-lived leaves must be well defended. Within Australian mistletoes-host pairs, we found a clear pattern of highly-mimic mistletoe species testing negative for alkaloids, while all associated hosts tested positive, illustrating evidence of Batesian mimicry.

Acknowledgements

We acknowledge CNPq for financial support and Australian National Parks and RECOR/IBGE offices for permits and logistic support. We also thank Izabelly Sant’Ana, Allyson Eller, Julieta Garcia-Russell, Julia Cooke and Fabricius Domingos for the valuable help in the field; Sara Cristina Caldas de Oliveira for the help with the chemical analyses; and F. Domingos, Emma Gray and Raquel Miatto for reviewing early versions of this manuscript.

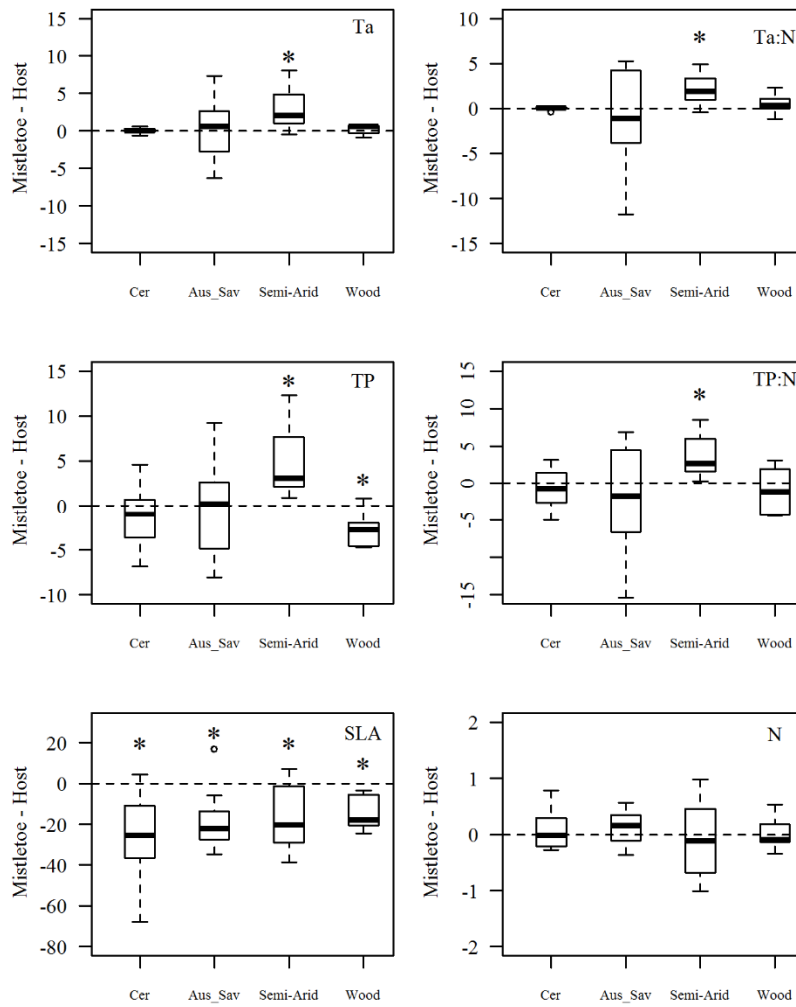


Figure 1. Pair-wise comparison between mistletoe to host difference in tannin concentration (Ta), tannin to nitrogen ratio (Ta : N), total phenol concentration (TP), total phenol to nitrogen ratio (TP : N), specific leaf area (SLA) and leaf nitrogen concentration per mass (N), across the four different sites: Brazilian Cerrado (Cer), Australian Savanna (Aus_Sav), Australian semi-arid woodland (Semi- Arid), and Australian closed woodland (Wood). The continuous line within the box shows the median, error bars show 10th and 90th percentiles. The symbol *denotes significant differences between the mistletoes and hosts at each site (paired t-tests, $P < 0.05$).

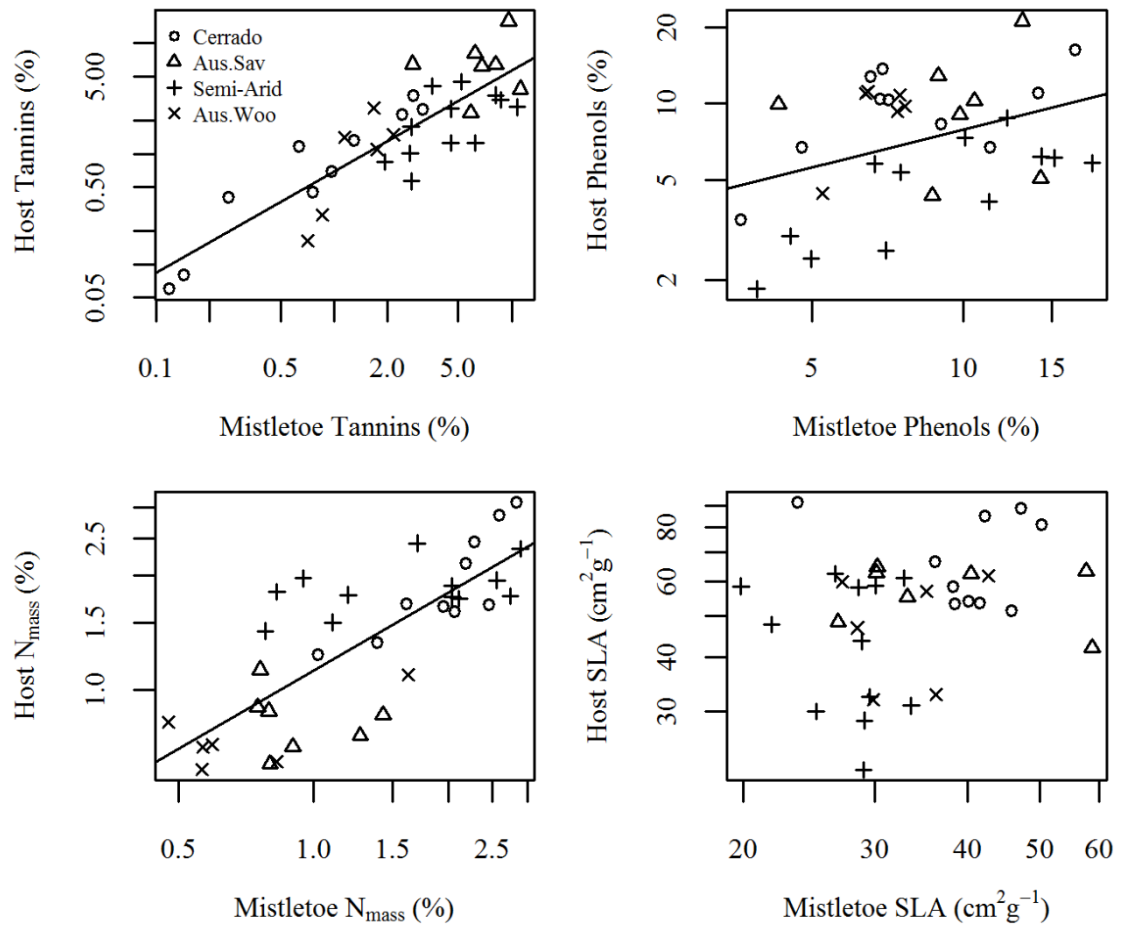


Figure 2. Ordinary least squares (OLS) relationships between mistletoes and hosts across four different sites for tannin concentration (Tannins: $r^2 = 0.71$, $P < 0.001$), total phenol concentration (Phenols: $r^2 = 0.14$, $P = 0.026$), leaf nitrogen concentration per mass (N_{mass} : $r^2 = 0.61$, $P < 0.001$), and specific leaf area (SLA, $P > 0.1$).

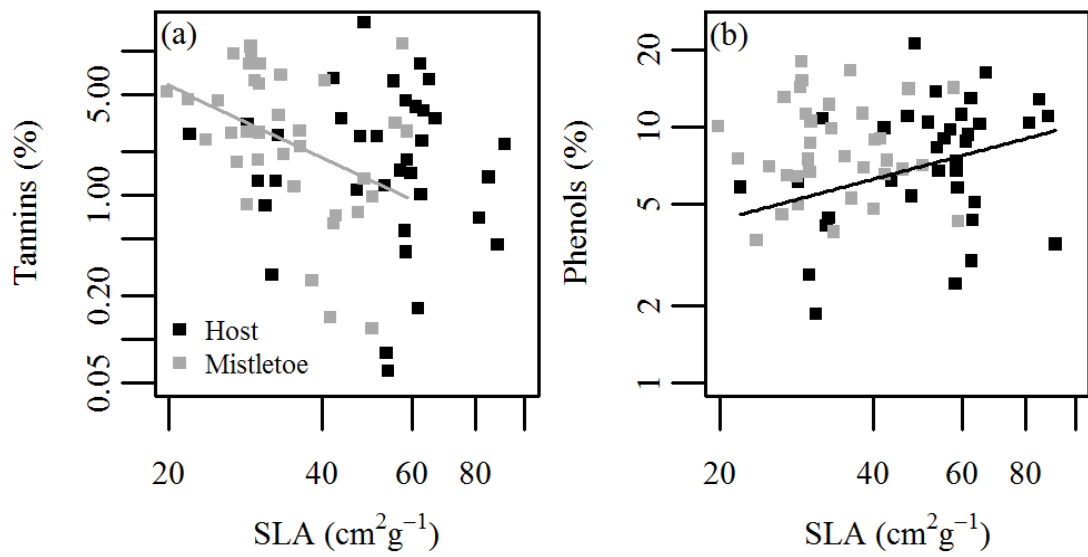


Figure 3. OLS relationships between specific leaf area (SLA) and tannin concentration (Tannins %), total phenol concentration (Phenols %), and leaf nitrogen concentration (N_{mass}) for 35 mistletoes (grey squares and lines) and their hosts (black squares and lines). Continuous lines represent significant relationships ($P < 0.05$). Correlation statistics are given in Table 3.

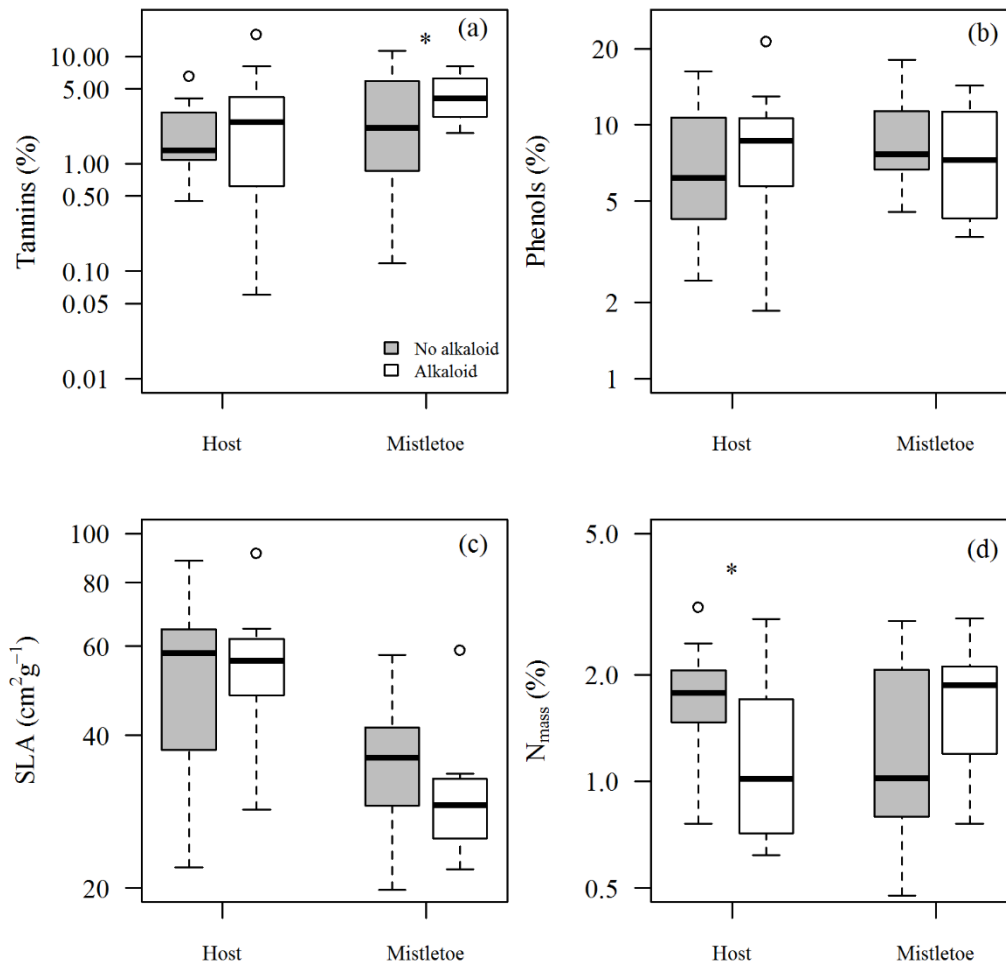


Figure 4. Host and mistletoe pair-wise comparison between alkaloid-positive (grey boxes) and alkaloid-negative (white boxes) species for tannin concentration (Tannins), total phenol concentration (Phenols), specific leaf area (SLA) and leaf nitrogen concentration per mass (N_{mass}). The continuous line within the box shows the median, error bars show 10th and 90th percentiles; and open circles represent outliers. The symbol * denotes significant differences between the alkaloid-positive and alkaloid-negative species for mistletoes or hosts (paired t-tests, $P < 0.05$).

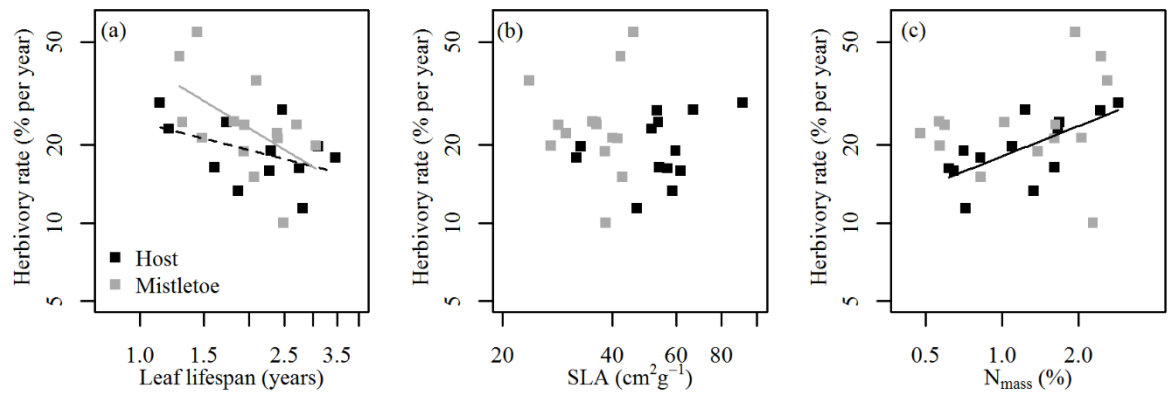


Figure 5. OLS relationships between herbivory rate and leaf lifespan (LL), specific leaf area (SLA) and leaf nitrogen concentration (N_{mass}) for 14 mistletoes-host pairs. Symbols and lines as in Figure 3 and marginally significant tendencies ($0.10 < P > 0.05$) are represented by dashed lines. Correlation statistics are given in Table 3.

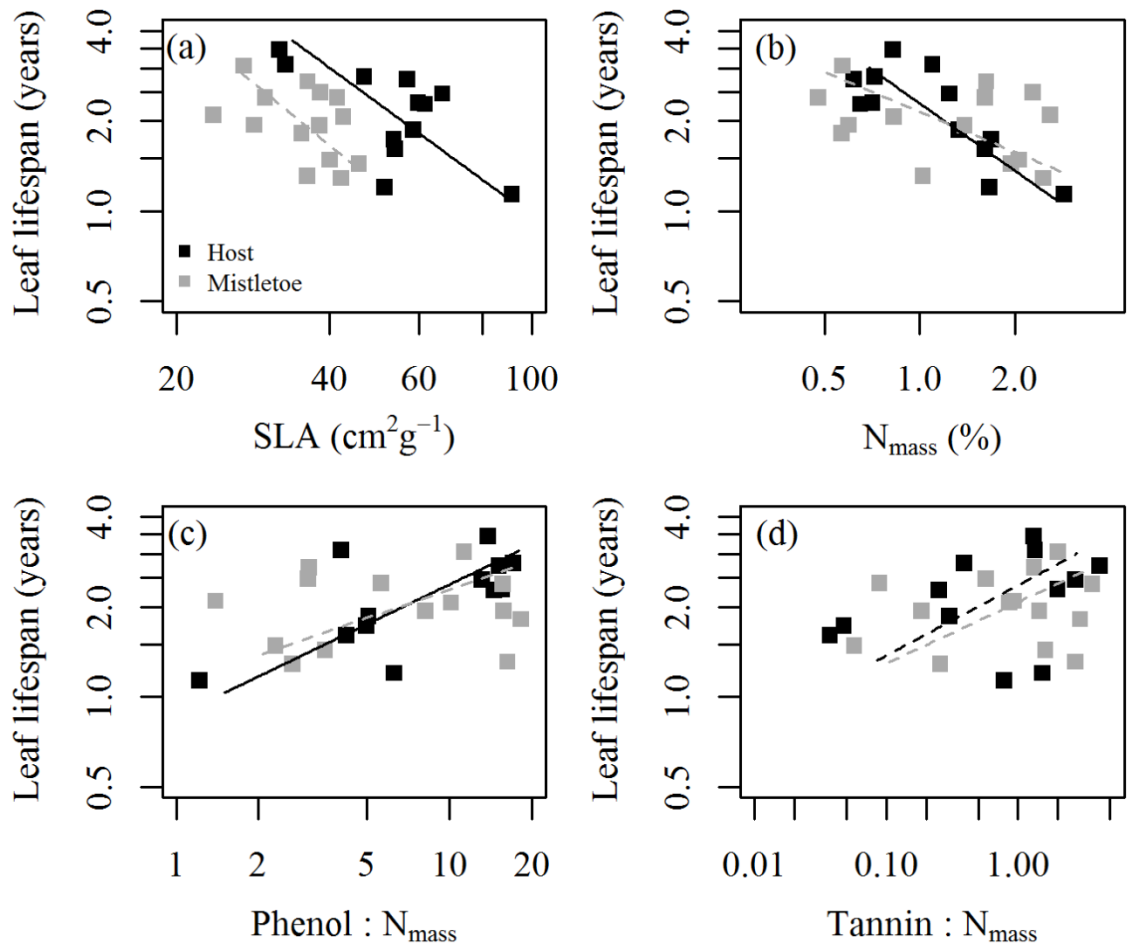


Figure 6. Standardised major axis (SMA) relationships between leaf lifespan (LL) and defence traits for 14 mistletoes (grey squares and lines) and 13 hosts (black squares and lines). Continuous lines represent significant relationships ($P < 0.05$), and marginally significant tendencies ($0.10 < P > 0.05$) are represented by dashed lines. Correlation statistics are given in Table 3. (a) LL on SLA. Common fitted slope, $\beta = -0.69$ ($-0.98, -0.50$). (b) LL on N_{mass} . Common fitted slope, $\beta = -1.58$ ($-2.30, -1.14$). (c) LL on TP : N_{mass} . Common fitted slope, $\beta = 2.59$ ($1.79, 3.83$). (d) LL on Ta : N_{mass} . Common fitted slope, $\beta = 4.56$ ($2.99, 6.96$).

Table S1. Presence (Yes) or absence (No) of alkaloids for resembling and non-resembling mistletoe-host pairs in Australia.

Mistletoe	Alkaloids	Host	Alkaloids
Highly mimic (n = 9)			
<i>Muellerina eucalyptoides</i>	No	<i>Eucalyptus hemastoma</i>	Yes
<i>Muellerina eucalyptoides</i>	No	<i>Eucalyptus moluccana</i>	Yes
<i>Muellerina eucalyptoides</i>	No	<i>Eucalyptus</i> spp.	Yes
<i>Amyema sanguinea</i>	No	<i>Corymbia porrecta</i>	Yes
<i>Amyema sanguinea</i>	No	<i>Eucalyptus miniata</i>	Yes
<i>Amyema sanguinea</i>	No	<i>Eucalyptus tetradonta</i>	Yes
<i>Amyema sanguinea</i>	No	<i>Corymbia blesseri</i>	Yes
<i>Amyema miquelli</i>	No	<i>Eucalyptus miniata</i>	Yes
<i>Amyema lucasii</i>	No	<i>Flindersia maculosa</i>	Yes
Mimic (n = 6)			
<i>Dendrophthoe vitellina</i>	No	<i>Eucalyptus</i> sp.	Yes
<i>Dendrophthoe vitellina</i>	No	<i>Angophora costata</i>	Yes
<i>Amyema preissii</i>	No	<i>Acacia aneura</i>	Yes
<i>Amyema maidenii</i>	No	<i>Acacia aneura</i>	Yes
<i>Lysiana linearifolia</i>	Yes	<i>Eremophila mitchellii</i>	No
<i>Lysiana exocarp</i>	Yes	<i>Alectryon oleifolius</i>	Yes
Non-mimic (n = 10)			
<i>Amyema congener</i>	No	<i>Allocasuarina littoralis</i>	No
<i>Dendrophthoe odontocalyx</i>	Yes	<i>Grevillea pteridifolia</i>	No
<i>Decaisnina signata</i>	Yes	<i>Xanthostemon paradoxus</i>	Yes
<i>Amyema miraculosa</i>	Yes	<i>Myoporum platycarpum</i>	No
<i>Amyema miraculosa</i>	Yes	<i>Eremophila mitchellii</i>	No
<i>Amyema preissii</i>	No	<i>Flindersia maculosa</i>	Yes
<i>Amyema maidenii</i>	No	<i>Acacia harpophylla</i>	No
<i>Lysiana exocarp</i>	Yes	<i>Acacia aneura</i>	Yes
<i>Amyema miraculosa</i>	Yes	<i>Eremophila longifolia</i>	No
<i>Amyema preissii</i>	No	<i>Senna eremophila</i>	No

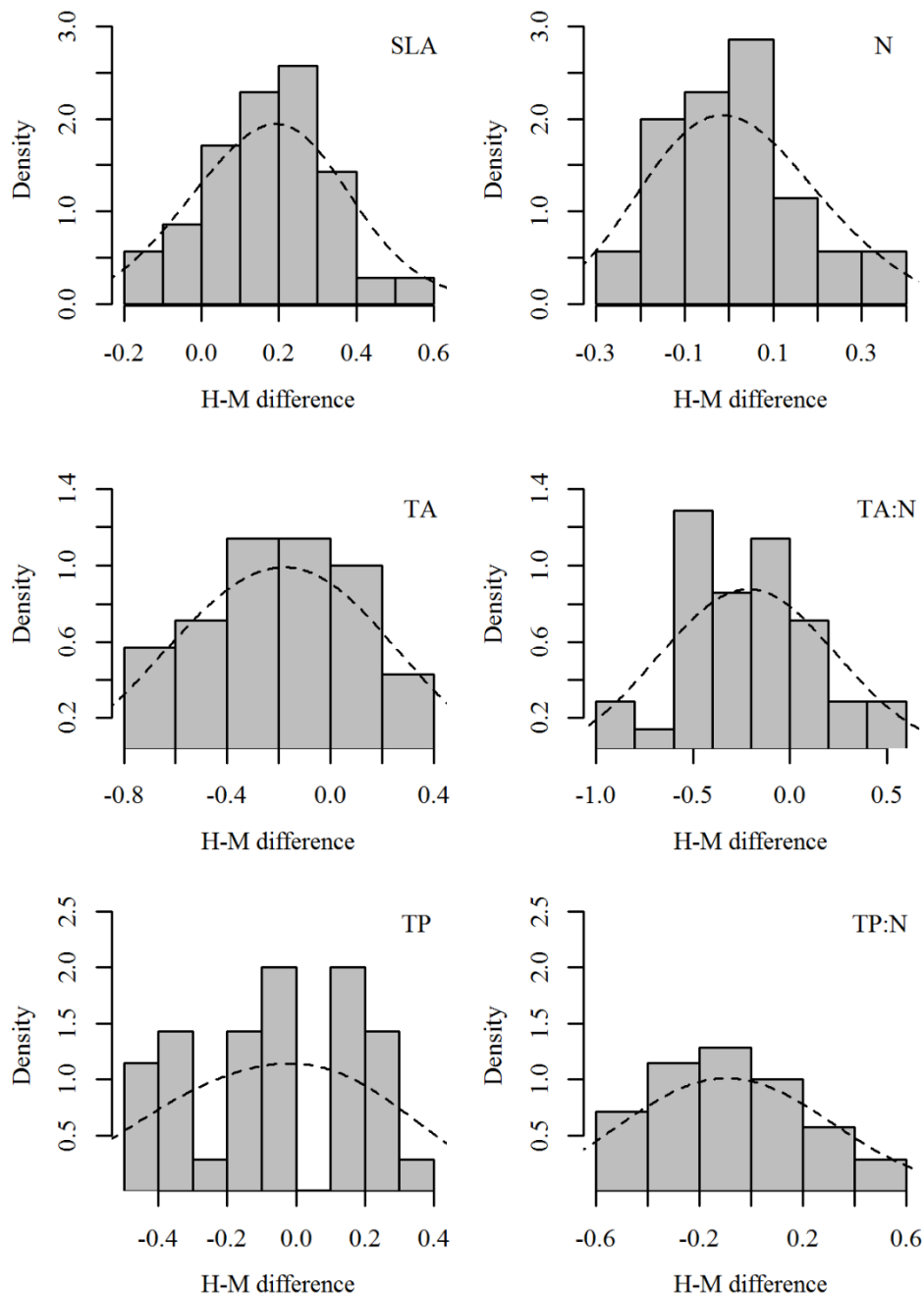


Figure S1. Frequency distribution of the differences between hosts and mistletoes in specific leaf area (SLA), leaf nitrogen concentration per mass (N), tannin concentration (Ta), tannin to nitrogen ratio (Ta : N), total phenol concentration (TP), and total phenol to nitrogen ration (TP : N).

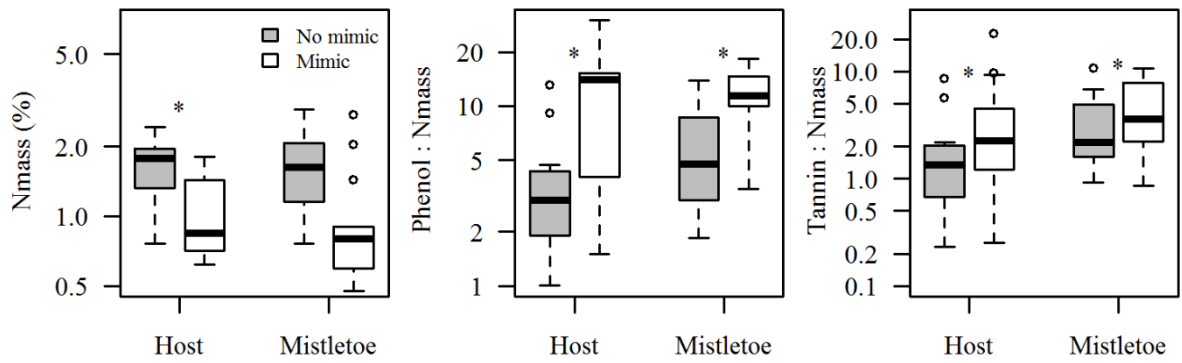


Figure S2. Boxplot of N_{mass} , $\text{Ph} : N_{\text{mass}}$ and $\text{Ta} : N_{\text{mass}}$ for mistletoes and hosts in a non-mimic relationship (grey boxes) and mimicking relationship (white box). The continuous line within the box shows the median and error bars show 10th and 90th percentiles. Outliers are represented by small open circles. The symbol * denotes significant differences between non-mimics and mimics (paired t-tests, $P < 0.05$).

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Chapter 6

General Discussion

General Discussion

Since the early days of plant physiology and ecology, mistletoes have been considered a fascinating model for investigating plant resource relations. The mechanism of water and nutrient partitioning between hosts and these hemiparasitic angiosperms is a particularly interesting physiological aspect of this relationship. Previous studies have suggested that mistletoes are profligate water users, and control water loss only minimally or not at all (e.g. (Hollinger 1983; Schulze, Turner & Glatzel 1984; Ehleringer, Cook & Tieszen 1986; El-Sharkawy, Cock & Hernandez 1986; Whittington & Sinclair 1988; Davidson, True & Pate 1989). They were also thought to be inefficient nutrient users, showing generally high concentrations of most macronutrients, while exhibiting very low rates of carbon gain (Lamont & Southall 1982; Glatzel 1983; Schulze, Turner & Glatzel 1984; Ehleringer *et al.* 1985). This thesis was largely motivated by the consideration of this unique system where, for mistletoes, the unit-costs are very low for accessing basic resources that otherwise are essentially limiting and energetically expensive to acquire. Another fundamental aim was to investigate whether these key aspects of the mistletoe-host relationship differ across different habitats and climate zones.

Prior to this thesis, much of our understanding of mistletoe-host relationships was based on studies of host-parasite physiology, typically local-scale studies of only one or just a few mistletoe species (but see Ehleringer *et al.* 1985; Ullmann *et al.* 1985). Essential aspects of mistletoe resource economics had also gone understudied, such as herbivory rates, leaf lifespan, nutrient resorption and anti-herbivory defence investment. Here I employed a comparative, “functional ecology” approach, using a broad range of mistletoe species on an intercontinental scale, to address these knowledge gaps. The purpose of this approach is to search for robust generalities among species.

In this chapter, I summarise and integrate key results from the four “data” chapters of my PhD thesis. The findings are related to three main themes in the literature: (1) the leaf economics spectrum, with regard to the evolution of functional trait adaptations; (2) the N-parasitism hypothesis, and also the relevance of phosphorus; and (3) the mimicry hypothesis and, more generally, host selection by mistletoes.

Functional trait trade-offs and adaptations

Mistletoes are unique plants and are expected to differ greatly from autotrophic plants, particularly considering the efficiency with which they use nitrogen and water in photosynthesis. I found consistent differences between mistletoes and hosts, largely regarding water use and leaf morphological structure, and the consequences of these differences for photosynthetic carbon gain.

Results from Chapters 2 and 3 showed that mistletoes exhibit higher g_s , higher instantaneous internal to external CO₂ concentrations ($c_i : c_a$), and more negative $\delta^{13}C$ (as a proxy for long-term $c_i : c_a$). As noted by Stewart and Press (1990) and confirmed here, the water and carbon economy of parasitic plants do not conform (to the same extent as their hosts) with the optimization hypothesis for the maximisation of daily water use efficiency (Cowan & Farquhar 1976; Cowan 1977). Mistletoes achieved a lower photosynthetic rate at a given g_s compared to their hosts (Chapter 3), which could be a result of higher intercellular or mesophyll-driven resistance to the diffusion of CO₂. It is notable that their leaves show a considerable level of succulence (with very low dry matter content: Chapter 5), because increasing leaf succulence has been associated with increasing mesophyll resistance (Griffiths *et al.* 2008; Griffiths 2013; Ripley *et al.* 2013).

Stomata operate to ensure the balance between CO₂ uptake and water losses (Wong, Cowan & Farquhar 1979), and mesophyll-driven signals coordinate photosynthesis with

stomatal behaviour. This relation is well documented, but a complete understanding of the underlying mechanisms involved is still lacking (Lawson 2009; Lawson *et al.* 2014). The concentration of CO₂ inside the leaf (c_i) is determined not only by the stomatal aperture, but also by the consumption of CO₂ for photosynthesis (Lawson *et al.* 2008). However, there is some evidence that K⁺ and Cl⁻ ion channels in the guard cell might be at least as important in controlling stomatal aperture as c_i (Pandey, Zhang & Assmann 2007). Indeed, K was found in high concentrations in mistletoe leaves, and it was even partially resorbed, while N was not (Chapter 4), suggesting that K might have further importance for these plants.

In relation to respiration, its relatively high rate compared to photosynthesis in mistletoes (Chapter 3) implies high maintenance costs for leaves and lower gross carbon gain. However, the high respiration may be necessitated by the energetic costs of using heterotrophic carbon transferred through the host xylem, and the maintenance of supposedly larger ion gradients between cellular compartments.

Another marked difference I found between mistletoes and hosts was the lower SLA in mistletoes (Chapter 3 and Chapter 5). Lower SLA can be a result of higher tissue density (mass per volume) or higher thickness. In general, species with low SLA have lower N_{mass} and photosynthetic rates (Reich, Walters & Ellsworth 1991; Reich, Walters & Ellsworth 1992; Reich, Walters & Ellsworth 1997; Wright *et al.* 2004), implying that SLA acts modulating the variation in photosynthesis with leaf N (Reich, Ellsworth & Walters 1998b). The reasons that SLA affects photosynthesis-N relationship include differential allocation of N (Evans 1989; Poorter & Evans 1998); limitations caused by internal self-shading (Terashima & Hikosaka 1995); and slow intercellular diffusion of CO₂ (Niinemets 1999). Photosynthesis scales with SLA because lower SLA is associated with greater allocation to structural biomass, rather than metabolic components (Vitousek, Field & Matson 1990; Reich, Ellsworth & Walters 1998a), imposing a physical limitation to achieve higher photosynthetic rates. Here I showed

evidence that the lower SLA in mistletoes could be a result of having thicker, but softer, leaves (Chapter 5). This accords with the finding that they achieved shorter LL for a given SLA (Chapter 5) which, interestingly, previous work has shown to be generally true of species from low rainfall regions (Wright & Westoby 2002).

Taken together, these results highlight the important implications of the hemiparasitic lifestyle on leaf structure for mistletoes, limiting the carbon gain and modifying the relationship between key traits (such as photosynthesis-N, photosynthesis-dark respiration, photosynthesis-g_s, and LL-SLA). More interestingly, this pattern is maintained across multiple mistletoe species and between different habitats (Chapter 3). Understanding the effect of variation in leaf structure has the potential to explain inconsistencies between leaf structure, nitrogen and carbon relations (Reich, Ellsworth & Walters 1998b; Reich *et al.* 1999). The unique traits of mistletoes provide key insights into plant trait relations, allowing for a more mechanistic interpretation of the limitations and drivers of these relationships, and thereby to the improvement of the estimation of scaling leaf traits, globally.

Trait adaptations and environmental constraints

Regarding nutrient use efficiency, I found that mistletoes generally employed ecological strategies to optimise nutrient use, such as extending leaf lifespan to enhance nutrient proficiency (Chapter 4), investing in defence syndrome combinations (Chapter 5), and resorbing some level of essential nutrients like P and K (Chapter 4).

Trait variability can be particularly intriguing considering both mistletoes and hosts, because mistletoes may experience more relaxed selective pressure to optimise the use of resources. Surprisingly, I found very strong evidence that mistletoes are responding similarly to their hosts to environmental constraints. I showed this with respect to water availability in Chapter 2, where I found that mistletoes become more conservative in their water use as

aridity increases globally, as do host plants. On a smaller scale, when I compared wet and dry sites in Chapter 3, I also found mistletoes exhibiting water saving strategies in dry sites, suggesting that mistletoes are not completely inefficient in regulating water loss, as had once been assumed (Hollinger 1983; Schulze, Turner & Glatzel 1984; Ehleringer, Cook & Tieszen 1986; El-Sharkawy, Cock & Hernandez 1986; Whittington & Sinclair 1988; Davidson, True & Pate 1989).

Universal parasitic theories, such as the optimal virulence hypothesis, suggest that, for obligatory parasites, there should be a balance of the virulence (i.e., reduction in host fitness) to avoid host death and keep the parasite alive for longer periods of time (Levin & Pimentel 1981; Anderson & May 1982). To summarise, despite showing clear differences in general traits to their hosts, mistletoes still adopt resource-conservative ecological strategies, possibly in order to avoid driving their hosts and themselves to death, particularly in cases where hosts are experiencing environmental constraints, such as water stress.

Implications for the N-parasitism hypothesis, and P-impooverishment

The N-parasitism hypothesis (Schulze, Turner & Glatzel 1984) posits that mistletoes are most strongly limited by access to nitrogen. Hence, they should exhibit very high transpiration rates in order to acquire sufficient N from the host xylem stream. This would result in lower water use efficiency (WUE) in relation to their hosts, particularly when N concentration in the host xylem is low. However, in the global analysis in Chapter 2, mistletoes growing on hosts with higher N_{mass} , or those growing on N-fixing hosts (which is proxy indicator of higher xylem nitrogen concentration) did not show more similar WUE to their hosts than mistletoes growing on low-N hosts. This suggests that higher N in the host xylem does not influence WUE in mistletoes, and provides evidence against the N-parasitism hypothesis.

Although N and P resorption efficiency is normally coupled (Aerts 1996), nutrient resorption patterns are a good indication of nutrient availability and limitation. In low-P soils, plant species tend to resorb P more efficiently than N (Wright & Westoby 2003), while for plants with higher P than the normal range, P resorption is reduced (Chapin III & Moilanen 1991). Therefore, the fact that mistletoes did not show N resorption, but did show a considerable degree of P resorption (Chapter 4) is a very strong indication that, at least for Australian and Brazilian low-P soils, N is not the most limiting nutrient for these plants. In addition, it raises the possibility that the most limiting nutrient is in fact P – as evidenced by its uncoupled resorption with N (Chapter 4). Indeed, perhaps on these low-P soils, it could be the need for P (along with heterotrophic C) that largely drives the high transpiration rates in mistletoes, hence the concept of “P-parasitism” (rather than “N-parasitism”) should be considered. More broadly, mistletoes as well as hosts from low-rainfall sites showed increased P on both an area and mass basis (Chapter 3), suggesting a potential role for leaf P in enhancing water use efficiency. However, this topic remains poorly understood and further work is required, particularly considering future climate change scenarios in P-impooverished soils.

Implications for the mimicry hypothesis

The evolution of mimicry in natural systems has puzzled scientists for centuries (Bates 1862; Müller 1879; Pasteur 1982), providing some of the best examples of natural selection. Resemblance of parasite leaves to host leaves was an old idea (Drummond 1840; Hemsley 1896) brought back into light by Barlow and Wiens (1977). By mimicking their hosts, mistletoes could afford to have higher leaf N concentration and avoid vertebrate herbivory (Barlow & Wiens 1977; Ehleringer *et al.* 1986; Bannister 1989). However, some small scale studies have raised some concerns regarding this hypothesis, particularly whether mimicry

has any fitness benefit for the mistletoe (Canyon & Hill 1997; Schaefer & Ruxton 2009), or even whether mimicry truly exists (Blick, Burns & Moles 2012), and no convincingly systematic test has been performed to demonstrate its validity.

In Chapter 2, I showed that mimicry might be associated with N-fixing hosts, as mimic mistletoes only showed higher N concentration than their hosts when growing on N-fixing hosts. I suggested that it might be a result of N-fixing hosts investing more heavily in N-based anti-herbivore defences, fulfilling the conditions of Batesian mimicry (i.e., the palatable mimic modelled on the unpalatable host). Indeed, in Chapter 5 I found that highly-mimic mistletoes were associated with alkaloid-positive hosts, suggesting that mimicry in Australian mistletoes makes it unnecessary to invest heavily in N-expensive defences, such as alkaloids.

Future directions and the relevance of understanding mistletoe functioning

Parasitic plants are widely ignored in the study of plant communities, despite constituting a substantial part of it. The literature on parasitic plants is extensive but largely focused on the relatively small number of hemiparasites that have some sort of economic importance (Bell & Adams 2011), such as the root parasite Sandalwood (*Santalum* sp.), used in the cosmetic industry; and the mistletoe *Viscum album*, used to treat symptoms of cancer. However, parasitic angiosperms offer an array of potential applications in varied fields that are still evolving, and the understanding of mistletoe functioning should continue to generate insights into unsolved issues in general plant ecophysiology. Although this thesis certainly contributes to this enterprise, various additional aspects of the mistletoe-host system could usefully be considered in future work. For example, from a leaf-level perspective, mistletoes can be valuable models to explore:

- The role of mesophyll resistance and leaf morphological traits in photosynthetic limitations;
- Leaf venation, succulence and the implications of leaf hydraulic architecture for water relations;
- The effect of potential access to host-derived secondary metabolites;
- Maintenance costs of cellular ionic gradients, in relation to dark respiration;
- Limitation of nutrients and the mechanisms behind a plant ability to cope with nutrient imbalance or accumulation of heavy metals;
- The function of K^+ in stomatal aperture control;
- Heterotrophic carbon gain;
- Nitrogen allocation to different functions.

The contribution of mistletoes to the vegetative community can vary, and studies are needed to verify the impact of mistletoes on hosts, and on ecosystems. There is a need to understand the significance of mistletoes for the plant communities, particularly regarding their contribution to biomass, nutrient cycling and population dynamics, and their effects on the economy of hosts. Mistletoes are known to affect host foliage area, hydraulic conductivity, sap mineral concentration (Tennakoon & Pate 1996), growth, reproduction, allometry (Silva & del Rio 1996; Press & Phoenix 2005), and ratio of sapwood to foliage area (Meinzer, Woodruff & Shaw 2004). In addition, mistletoes can affect host respiration and photosynthetic rates (Wanner & Tinnin 1986; Sala, Carey & Callaway 2001; Meinzer, Woodruff & Shaw 2004). Hosts can compensate for the extra sink resulting from hemiparasites by altering carbon allocation, increasing Rubisco content, increasing leaf area, or even delaying leaf senescence (Watling & Press 2001). Further investigations are essential for understanding how mistletoes influence host trait relationships, and if these effects vary

predictably across environments. At a broad ecosystem scale, mistletoes provide insight into different topics, such as:

- The effect of xylem-tapping parasites on carbon, nutrient and water relations of hosts, and the impact of this on the plant community's structure and composition;
- The influence of mistletoes on soil and ecosystem processes (Press 1998; Press & Phoenix 2005), such as impacts on nutrient cycling, soil moisture, and host's arbuscular mycorrhiza colonisation. Particularly, the impact of quality and quantity of litter in nutrient cycling of natural ecosystems;
- The response of parasitic plants and their hosts to climate change;
- General parasitology theories of host selection and host resistance.

General Conclusion

In this thesis, I investigated several aspects of the mistletoe-host system, partially fulfilling the need for more field studies of parasitic plants in natural communities, noted by Pennings and Callaway (2002). Little support was found for the N-parasitism hypothesis in a global context, and in three P-impooverished sites, where the lack of N resorption suggests that N is not a limiting nutrient for mistletoes. Moreover, natural selection may have favoured P resorption processes in mistletoes occurring in P-impooverished habitats. My thesis shows, for the first time, that mistletoes and hosts have similar responses to precipitation and moisture index gradients, by considering water and nitrogen use in a global context, and by comparing photosynthetic trait adaptations in wet and dry sites. I also showed evidence that tannins are transferred from hosts, as well as differences in defence syndromes and trait trade-offs between mistletoes and hosts. I provided evidence that the evolution of mimicry in Australian mistletoes could be associated with higher N availability in the hosts and with alkaloid-positive hosts, illustrating a case of Batesian mimicry. In summary, I provided insights into

functional trait adaptation to aridity, N and P function and limitation, nutrient resorption patterns, and the fundamental role that secondary compounds play in host selection, especially in the case of mimic mistletoes in Australia.

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