



## **COPYRIGHT AND USE OF THIS THESIS**

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author's moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author's reputation

For further information contact the University's Copyright Service.

**[sydney.edu.au/copyright](http://sydney.edu.au/copyright)**

# PLANT DRIVERS OF FORAGING BEHAVIOUR IN A GENERALIST BROWSER



**Rebecca S. Stutz**

A thesis submitted in fulfilment of the requirements for the degree of  
Doctor of Philosophy

School of Biological Sciences, Faculty of Science



THE UNIVERSITY OF  
**SYDNEY**

2016

## Declaration

The material presented in this thesis is my own original work except where otherwise duly acknowledged. It does not contain material which has been previously accepted for a degree or diploma by the University of Sydney or any other institution.

This thesis may be made available for loan and limited copying in accordance with the *Copyright Act* (1968).

Rebecca S. Stutz

## Preface

With the exception of the General Introduction and Discussion, this thesis is presented as a series of stand-alone manuscripts that have either been published or submitted for review in international peer-reviewed journals. As a consequence, there is some unavoidable repetition, particularly of the study site description and some statistical methods.

I conceived most of the ideas presented in this thesis, and otherwise contributed substantially to developing and conducting the research under the guidance of my academic supervisors, including experimental design, data collection and analysis, and manuscript writing.

I am the primary author of all the manuscripts included in this thesis. I have acknowledged the contributions of all co-authors by listing their names at the beginning of each chapter as they appear in the published/submitted manuscripts. Due to joint authorship, all research is described using the pronoun “we” instead of “I”.

All research was approved by The University of Sydney Animal Ethics Committee (L04/2-2012/3/5696) and Australian National Parks (BDR12/00001). Funding was provided by Booderee National Park, The Norman Wettenhall Foundation and The University of Sydney.

## Acknowledgements

The production of this thesis would not have been possible without the support of a special group of people that helped me tackle the challenges of PhD research. To them I offer my immense gratitude!

Firstly, I would like to thank my supervisory team at the University of Sydney. My primary supervisor Clare McArthur spent countless hours teaching me the nuances of the statistical package SAS, labored through the many “versions” of my manuscripts, indulged (some of) my crazy proposals for foraging experiments and guided me through the evolving ideas of this thesis. Best of all, she took me along to South Africa to see white rhinos in action! My co-supervisor Peter Banks is one of the most widely read people I've met and could always come up with new ideas for experiments from his vast mental stock of reviewed research (over a piccolo latte, of course). He was an excellent resource for coming up with the right angle from which to frame my manuscripts.

Special thanks go to Nick Dexter of Booderee National Park for initiating the project, providing funding, housing and lots of seedlings, and giving me helpful advice throughout. I am greatly indebted to Stig Pederson of Booderee Botanic Gardens for collecting local seeds and growing thousands of plants for my experiments – all with the knowledge that they would face off with his greatest enemy, the swamp wallaby. I would also like to thank Martin Fortescue for approving the project, and Matt Hudson, Chris McGregor, and Sharon and Narelle in the front office for their support and good humour.

I would like to thank Nick Proschogo from the School of Chemistry for patiently showing me – a complete novice – the ropes in his mass spectrometry lab, and accommodating the horde of seedlings that accompanied me.

My fellow McArthur lab members, particularly Valentina Mella and Miguel Bedoya Perez, enriched my PhD experience. From Day 1, Miguel – and his collection of spreadsheets –

answered all my annoying questions from admin to wallabies. Valentina's sense of humour and encouragement were a critical element to getting through the challenges of research. Her friendship and advice are much appreciated. I am grateful also to the ever-changing residents of Room 408/409 with whom I've shared the PhD battle, especially Kerry Gibbons, Petah Low and Ryan Leonard. Special thanks go to Mathew Crowther for allowing me to invade his office and take up his time on many occasions to run my statistical analyses past him!

I was also lucky enough to share 'the house' at Booderee with some lovely PhD students, in particular Claire Foster (ANU) – it was good to have a human to talk to and share meals with at the end of long days amongst the ferns (and to rescue me when the keys were inside the car and I was not)! Finding volunteers to come to beautiful Jervis Bay was surprisingly difficult (I wonder if it had anything to do with the wall of bracken fern they would face?) so I am very grateful to Elyce Coluccio and Ayesha Siti Abdul-Rahman for assisting me on a field trip!

Finally, I would like to express my gratitude to my family for supporting me through my studies. My parents, Elisabeth and Markus Stutz, have always encouraged me to pursue my interests and helped me greatly to achieve my goals. I may be slightly biased, but all evidence suggests that they are the best parents in the world ( $p < 0.05$ ). My dad spent two weeks of his holidays helping me set up my final field experiment – I'm sure he returned to Perth completely exhausted! My brother, Michael, helped me find a home when I came to Sydney, cooked me lots of fancy meals, and shared many laughs (usually about journal impact factors in ecology). Last but not least, my partner, Ben Croak, has helped me immensely with all facets of the PhD, from fighting through the ferns with me on many field trips, to proof-reading my manuscripts, to drying my tears when things went wrong and encouraging me to persevere. I'm very lucky to have someone in my corner that has already been through a PhD and knows how good it feels to finish it in the end!

# Table of Contents

<b>Declaration</b> .....	<b>ii</b>
<b>Preface</b> .....	<b>iii</b>
<b>Acknowledgements</b> .....	<b>iv</b>
<b>Table of Contents</b> .....	<b>vi</b>
<b>Thesis Summary</b> .....	<b>1</b>
<b>Chapter 1: General Introduction</b> .....	<b>5</b>
Herbivory: ecological and evolutionary consequences .....	6
The foraging process .....	7
<i>Decisions at a hierarchy of scales</i> .....	7
<i>Choosing plants amongst the vegetation matrix</i> .....	8
<i>Neighbours matter</i> .....	9
<i>Herbivore modifiers of plant-driven foraging</i> .....	11
Plants as drivers of foraging behaviour: a holistic approach to herbivory .....	12
<i>Study system</i> .....	14
<i>Thesis structure</i> .....	16
<b>Chapter 2: Associational Refuge in Practice: Can Existing Vegetation Facilitate Woodland Restoration?</b> .....	<b>30</b>
Abstract.....	31
Introduction.....	32
Materials and methods .....	37
<i>Study site</i> .....	37
<i>Study design</i> .....	37
<i>Statistical analyses</i> .....	40
Results .....	43
<i>Associational effects at large and small patch scales</i> .....	44
<i>Foraging by different herbivore species</i> .....	47
Discussion .....	48
<i>Associational effects at large and small patch scales</i> .....	49
<i>Foraging by different herbivore species</i> .....	52
<i>Implications for management</i> .....	53
Acknowledgements.....	54
References .....	55
<b>Chapter 3: Herbivore Search Behaviour Drives Associational Plant Refuge</b> .....	<b>61</b>

Abstract.....	62
Introduction.....	63
Materials and methods .....	66
<i>Study site</i> .....	66
<i>Study design</i> .....	66
<i>Herbivore behavioural response to patch treatments</i> .....	67
<i>Short- and longer-term effectiveness of associational plant refuge</i> .....	70
Results .....	70
<i>Herbivore behavioural response to patch treatments</i> .....	70
<i>Short- and longer-term effectiveness of associational refuge</i> .....	72
Discussion .....	74
<i>Foraging decisions are influenced by patch neighbours</i> .....	75
<i>Associational plant refuge results in tolerance to herbivory</i> .....	77
<i>Theoretical and practical implications</i> .....	77
Acknowledgements.....	79
References .....	80
<b>Chapter 4: Follow Your Nose: A Browsing Mammal Uses Leaf Odour as a Foraging Cue</b> .....	<b>84</b>
Abstract.....	85
Introduction.....	86
Materials and methods .....	87
<i>Study site</i> .....	87
<i>Experiment 1: Random vs. cue-based foraging</i> .....	88
<i>Availability of odour cues from whole seedlings</i> .....	88
<i>Experiment 2: Use of odour in the absence of visual seedling cues</i> .....	89
<i>Experiment 3: Use of odour to find whole plants</i> .....	90
<i>Statistical analyses</i> .....	90
Results .....	91
<i>Experiment 1: Random vs. cue-based foraging</i> .....	91
<i>Availability of odour cues from whole seedlings</i> .....	91
<i>Experiment 2: Use of odour in the absence of visual seedling cues</i> .....	93
<i>Experiment 3: Use of odour to find whole plants</i> .....	95
Discussion .....	96
References .....	98



Supplementary Information.....	101
<b>Chapter 5: Olfactory and Visual Plant Cues as Drivers of Selective Browsing .....</b>	<b>103</b>
Abstract.....	104
Introduction.....	105
Methods .....	108
<i>Study site</i> .....	108
<i>Seedling nutrient treatments</i> .....	109
<i>Seedling characteristics</i> .....	109
<i>Experiment 1: Behavioural response to seedling odour</i> .....	110
<i>Experiment 2: Browsing patterns as a function of seedling odour and visual cues</i> .....	111
Results .....	113
<i>Seedling characteristics</i> .....	113
<i>Experiment 1: Behavioural response to seedling odour</i> .....	115
<i>Experiment 2: Browsing patterns as a function of seedling odour and visual cues</i> .....	117
Discussion .....	119
<i>Differential behavioural response to plant olfactory cues</i> .....	120
<i>The influence of olfactory and visual cues on browsing patterns</i> .....	121
<i>A matter of cue detectability, not plant quality</i> .....	123
Acknowledgements.....	124
References .....	125
Supporting Information .....	132
<b>Chapter 6: General Discussion .....</b>	<b>133</b>
Research findings and implications.....	134
<i>Existing vegetation can disrupt the foraging process</i> .....	134
<i>A behavioural mechanism for associational plant refuge</i> .....	135
<i>Olfaction can play a critical role in plant detection</i> .....	136
<i>Fertilizer regimes can influence detection of plants by herbivores</i> .....	137
Future directions.....	138
<i>Integrating herbivore behaviour into associational plant effect theory</i> .....	139
<i>Plant quality indicators for browsers</i> .....	139
<i>Detection of visual plant cues: chromatic vs achromatic contrasts</i> .....	140
<i>Large-scale changes to plant-herbivore interactions</i> .....	140
References .....	143

# Thesis Summary

Herbivores can significantly alter the composition and structure of plant communities, and this has potentially far-reaching consequences for other organisms via trophic cascades. Understanding the factors influencing browsing patterns of free-ranging herbivores has fundamental ecological significance and is also key to strategic management of herbivory. Herbivore foraging decisions culminating in the consumption of any particular plant are complex and multi-faceted, shaped both by the plant itself and by its neighbours. My overarching aim was to understand the ways in which plants influence foraging behaviours in a generalist browser. I explored this aim using free-ranging swamp wallabies (*Wallabia bicolor*) as a model and examined the plant drivers leading to their consumption of palatable focal plants (native tree seedlings, *Eucalyptus pilularis*). My specific objectives were to:

- (1) identify the characteristics of neighbouring plants that affect browsing of focal plants;
  - (2) quantify the behavioural response of the herbivore to neighbouring plants when neighbours protect focal plants from browsing, and when they fail as refuges;
  - (3) assess the role of leaf odour in palatable plant detection amongst interspecific neighbours;
- and
- (4) test whether herbivores use olfactory and visual plant cues to browse differentially on intraspecific plants varying in nutritional quality.

Associational plant refuge arises when plant neighbours reduce focal plant susceptibility to herbivory. The key to understanding when and why associational refuge works, and when it breaks down, is to define which patch variables, at multiple spatial scales, are effective against herbivores. For **Objective (1)**, I tested the capacity of existing vegetation to act as associational refuge for planted eucalypt seedlings by disrupting the foraging process (e.g. search, detection and consumption decisions), and to influence foraging patterns in terms of habitat selection. At the large patch scale (100 m<sup>2</sup>), seedlings were browsed later in vegetation

patches with less canopy cover and fewer browsed plant species, and in fern- over grass-dominated patches. At the small patch scale (4 m<sup>2</sup>), browsing was delayed with greater cover of understorey vegetation. Lower canopy cover may have delayed browsing of seedlings by lowering patch quality and hence motivation to visit, for example through higher thermal costs (reduced shade) or higher perceived predation risk. All other variables were consistent with associational plant refuge, acting by impeding the search process and seedling detection. Understanding how plant patch variables influence browsing patterns could inform strategic placement of seedlings amongst existing vegetation to optimise their chances of escape from herbivory, although neighbouring plants may eventually fail to provide refuge if herbivore pressure is high.

The key to understanding why associational plant refuge works or fails is to quantify the specific foraging behaviours generating such refuges. For **Objective (2)**, I endeavoured to integrate herbivore foraging and associational plant refuge theories by linking foraging decisions directly to browsing outcomes on focal plants and their neighbours. I tested the hypotheses that obstructive, unpalatable neighbours would lead to associational refuge for palatable focal plants by reducing the number of patch visits and/or disrupting the search process. I quantified swamp wallaby behaviour in patches comprising a central eucalypt seedling with neighbours of control (existing grasses or ferns) or manipulated vegetation (tall, unpalatable native daisy, *Coronidium elatum*). I found that associational plant refuge in this system was driven by reduced investment in search behaviour during visits to manipulated patches. While this refuge eventually broke down, with all focal plants being browsed after one year, the manipulated patch facilitated tolerance to herbivory – surviving focal plants were found only amongst manipulated plants. By quantifying how neighbouring vegetation influences foraging behaviour in the short-term and focal plant survival in the longer-term, we can understand why associational refuge fails, and how palatable plants may persist in the presence of abundant herbivores.

My experiments examining associational plant refuges for Objectives (1) and (2) yielded qualitative evidence that swamp wallabies use plant cues to find eucalypt seedlings. How herbivores detect specific plants amongst an array using visual and/or olfactory cues is central to understanding how they make efficient choices between plant patches. By enabling selective feeding among and within vegetation patches, effective use of resource cues should influence herbivore fitness and may alter plant communities. Volatile organic compounds in leaves of many trees and shrubs are toxic, but conversely herbivores may use them as foraging cues. For **Objective (3)**, I tested whether swamp wallabies used leaf odour as a cue to find food plants. Swamp wallabies used cut seedling odour as a food cue, visiting patches containing vials with cut eucalypt seedlings earlier than those with empty vials. They also visited and investigated patches with seedling odour more often. Grey kangaroos, which also occurred at the site and consume grasses and forbs almost exclusively, did not differentiate between the two patch treatments. In a second experiment, I manipulated the available seedling cues and compared the time to browsing. Seedlings with reduced visual cues (pinned to the ground) and seedlings with enhanced olfactory cues (upright but planted together with a pinned down seedling) were browsed as fast as seedlings with normal cues (single upright). My results experimentally demonstrate the use of leaf odour as a cue for finding food plants by a mammalian browser, and suggest that the threshold for odour detection is low (a single plant).

For **Objective (4)**, I tested the hypothesis that swamp wallabies could use odour cues to differentiate between plants of different nutritional quality. Swamp wallabies visited and investigated patches containing vials of high-nutrient cut seedlings more often than those with low-nutrient cut seedlings, which in turn were visited and investigated more often than empty vials. Grey kangaroos (grazers) showed no differences among treatments. I then tested whether the ability to differentiate plants of differing nutritional quality, based on odour, would translate into different patterns of browsing for seedlings. It did, but not as expected; wallabies browsed low-nutrient seedlings earlier than high-nutrient seedlings. This difference

disappeared when long-range visual cues were reduced by pinning seedlings to the ground. My results suggest that visual cues were important for finding upright seedlings, with low-nutrient seedlings being more apparent due to their visual contrast against background vegetation. In a realistic context where multiple sensory cues were available, the outcome was more complex than that predicted from odour alone. We suggest that foraging decisions were based on net cue apparency rather than plant quality, a strategy that may favour food quantity over relatively fine-scale differences in quality.

My study identified vegetation characteristics providing associational refuge at two spatial scales consistent with disrupted search and detection of focal seedlings by herbivores. I demonstrated that neighbouring vegetation reduced investment in searching behaviour by wallabies during visits to manipulated patches and this drove associational plant refuge. At the patch level, foraging decisions were influenced both by odour and visual cues of plants, allowing wallabies to find food plants and to differentiate between plants of differing nutritional quality. Plant neighbours and cues emitted by plants themselves can influence foraging decisions and thus drive patterns of herbivory.

Understanding the role of odour for a mammalian browser in detecting focal plants raises the question of how associational plant refuge works. Associational refuges are often assumed to work through palatability, physical barriers to access, and visual obstruction – but here I have shown that while these may play a role, if a mammalian herbivore can use its nose to find focal plants, then these obstacles may not be as effective. To protect focal plants from such herbivores may require obstruction of olfactory plant cues. My results also demonstrate the importance of quantifying foraging decisions in order to explain the refuge outcome and to understand the underlying mechanisms driving it from the perspectives of both herbivore and plant. From the fundamental ecology viewpoint, my work links herbivore foraging ecology to plant-plant facilitation by considering the animal behavioural response to plants. It also explains why associational plant refuge may break down in the longer term, and this may have implications for management decisions in an applied ecology context.

# CHAPTER 1

---

## General Introduction



Images: R.S. Stutz

## **Herbivory: ecological and evolutionary consequences**

Herbivores consume approximately one fifth of the earth's annual biomass production, making herbivory the major pathway for photosynthesised energy to enter the food web (Agrawal 2011). Herbivores can therefore exert top-down control on plant densities. But they can also influence plant community composition and structure because they feed selectively based on plant traits, and because the plants they consume differ in their ability to recover from damage (Brown & Stuth 1993; Rosenthal & Kotanen 1994; Anderson & Briske 1995; Augustine & McNaughton 1998).

Herbivory can have ecosystem-scale implications. Herbivores can control invasions of exotic plant species and their distributions (Becerra & Bustamante 2008; Cushman, Lortie & Christian 2011; Dexter *et al.* 2013) and influence the persistence of browse sensitive plant species (Hidding, Tremblay & Côté 2012). Herbivory is also the most common cause of mortality in seedlings (Moles & Westoby 2004), affecting recruitment into later growth stages. This may delay natural forest regeneration and reduce the success of restoration efforts, altering canopy structure (Lange & Graham 1983; Western & Maitumo 2004; Long, Pendergast & Carson 2007; Parsons *et al.* 2007; Smit *et al.* 2015). Herbivory can therefore have cascading effects on other organisms utilizing vegetation, and on their predators (Hobbs 1996; Côté *et al.* 2004; Nuttle *et al.* 2011; Borzak, O'Reilly-Wapstra & Potts 2015).

Anthropogenic disturbances have resulted in an increasing number of ecosystems requiring restoration, including re-establishment of plant components (e.g. Paschke, DeLeo & Redente 2000; Tordoff, Baker & Willis 2000). At the same time, reduced top-down pressure on herbivores in some systems – for example, by removing apex predators – has resulted in the dilemma of planting in areas where levels of herbivory are high (Ruhren & Handel 2003; Koch, Richardson & Lamont 2004). The field of foraging ecology is often focussed on individual herbivores and the food-finding process, while restoration of degraded systems considers the outcomes of foraging on plants. To successfully manage herbivory in the restoration context,

we need to unite these two fields and examine the problem from the dual perspectives of the herbivore and the plant, linking foraging behaviours to browsing outcomes.

## **The foraging process**

### *Decisions at a hierarchy of scales*

Consumption of a plant results from a series of foraging decisions made across a hierarchy of scales; these decisions can be made at multiple scales simultaneously and be driven by a complex array of factors at each scale (Kotliar & Wiens 1990). The foraging process for herbivores involves search, detection and consumption decisions (*sensu* predator foraging, Endler 1991). For a large herbivore, this may involve choices at spatial scales varying continuously from landscapes to plant communities, plant patches, individual plants and plant parts (Senft *et al.* 1987). Selection between units at each scale may be related to characteristics of the available food as well as extrinsic factors that may be mediated by vegetation, including competition, predation risk, and climatic conditions (Wywiałowski 1987; Reimoser & Gossow 1996; Dexter 1998; Van Beest *et al.* 2010).

Various theoretical frameworks have been developed to describe this complex foraging calculus. The most influential of these is the Optimal Foraging Theory (OFT), which states that the need to maximise energy gain – and thus ultimately improve fitness – drives foraging decisions (Emlen 1966; MacArthur & Pianka 1966). An extension of the optimality model, the classic Marginal Value Theorem (MVT), describes the problem of diminishing returns: a forager should leave a patch when the energy available there is diminished to a level lower than the energy available in another patch (Charnov 1976). At the core of this theory is the foraging patch, a concept that is particularly difficult to define in practice. Broadly, patches represent spatial aggregations of resources that are hierarchical in nature, where decisions at larger patch scales influence decisions at smaller ones, and thus all spatial scales from the plant part to the landscape can be viewed as nested patches (Kotliar & Wiens 1990; Searle *et*



*al.* 2006; Searle & Shipley 2008). Different approaches exist for determining the boundaries of patches, based either on the characteristics of the vegetation (e.g. physical differences from the surroundings; Kotliar & Wiens 1990) or the behaviour of the herbivore (e.g. where the rate of intake or movement changes; Senft *et al.* 1987; Bailey *et al.* 1996). Despite these difficulties in defining patches, and criticisms about the applicability of the OFT and MVT outside the laboratory, these theories have provided the foundations for more complex models modified to better describe the foraging process in various systems (Pyke, Pulliam & Charnov 1977; Mangel & Clark 1986; Stephens & Krebs 1986; Schoener 1987). These models take into account some of the variables outlined in the following discussion, including optimization for variables other than energy gain. Belovsky (1984a) classified the derivations of the OFT as contingency models (e.g. Owen-Smith & Novellie 1982; Farnsworth & Illius 1998), linear program models (e.g. Westoby 1974; Belovsky 1984b; Belovsky 1986), and models based on the net energy content of food weighted by its abundance (e.g. Stenseth & Hansson 1979). In addition, OFT has provided a point of contrast for other models predicting foraging selection. For example, the geometric framework of nutrition has proven to be very valuable for understanding diet selection by animals, and the influence of ingested foods on subsequent food selection (Raubenheimer, Simpson & Mayntz 2009; Simpson *et al.* 2010).

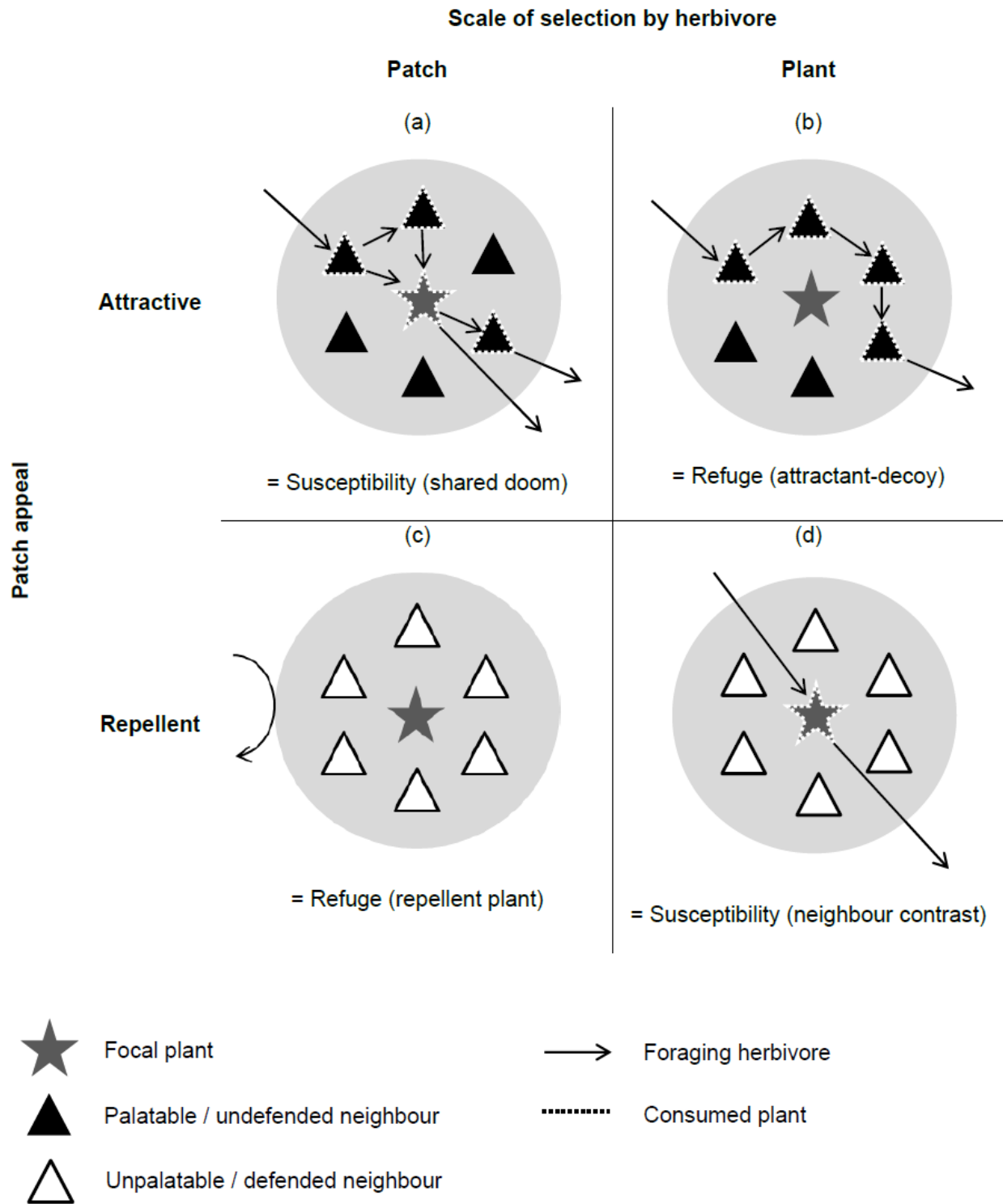
### *Choosing plants amongst the vegetation matrix*

To forage efficiently, herbivores should consume the most profitable plants at the minimum cost of obtaining them (MacArthur & Pianka 1966). But what determines the profitability of feeding on plants and what are the associated costs? The most obvious factors affecting plant profitability relate to the characteristics of the plant itself. Plants vary in energy and nutrient content and in the presence of physical and chemical anti-herbivore defences (Carmona, Lajeunesse & Johnson 2011). Physical defences include thorns, spines, and fibrous leaves or stems (Hanley *et al.* 2007; Johnson 2011), while chemical defences comprise plant secondary compounds that deter herbivores by acting as toxins or digestibility reducers (Foley & McArthur 1994; Foley, Iason & McArthur 1999). However, some herbivores have developed

counter-adaptations to particular plant defences. Specialist herbivores have developed physiological mechanisms to process the similar defensive chemicals in their narrow dietary niches (Boyle *et al.* 1999; Sorensen, McLister & Dearing 2005). Meanwhile, generalist herbivores may use dietary mixing and temporal switching to prevent overloading the detoxification pathways of the same or similar secondary metabolites (Bernays *et al.* 1994; Dearing & Cork 1999; Wiggins, McArthur & Davies 2006). Herbivores may also manipulate physical defences to facilitate plant consumption, for example, removal of cactus spines by white-throated woodrats *Neotoma albigua* (Kohl, Miller & Dearing 2015). Thus there are temporal and energetic costs to foraging involved in searching for palatable plants and dealing with plant defences.

### *Neighbours matter*

Neighbouring plants in a patch can also influence the vulnerability of a particular plant to herbivory, a phenomenon known as an associational or neighbourhood effect (Tahvanainen & Root 1972; Atsatt & O'Dowd 1976). Neighbouring plants may render a focal plant more or less vulnerable to herbivory, termed associational susceptibility or refuge, respectively. The outcome depends on two factors; the dominant scale of selection used by the herbivore, i.e. between patches or between individual plants within patches and second, the relative patch palatability or level of defence (Fig. 1). For example, a herbivore choosing between patches may reject or fail to locate a plant because neighbouring plants provide physical or chemical barriers such as spines, visual masking, toxins or odours. This has been termed the repellent plant mechanism (Atsatt & O'Dowd 1976). Such barriers may be deterrent or simply interfere with visual or olfactory signals of plants, thereby reducing their apparency to herbivores (Miller, Kinnaird & Cummins 1982; McAuliffe 1986; Hambäck, Agren & Ericson 2000). Meanwhile, where the herbivore chooses between individual plants within patches, defended or unpalatable neighbours may render a focal plant more vulnerable to herbivory because it is of higher relative quality. This is termed neighbour contrast susceptibility (Bergvall *et al.* 2006). Undefended or palatable neighbours can result in consumption of the focal plant if the



**Figure 1.** The sum of the behavioural interactions between foraging herbivores and focal and neighbouring plants leads to observed associational plant effects. The type of plant-herbivore interaction depends on the quality of neighbouring relative to focal plants (patch appeal), and whether the herbivore predominantly selects between patches or individual plants within a patch (scale of selection).

herbivore selects between patches (shared doom; Wahl & Hay 1995) or protection of the focal plant if the herbivore selects between plants within the patch (attractant-decoy; Atsatt & O'Dowd 1976). Selection at the patch-level does not preclude subsequent plant-level selection within the patch; and several studies have illustrated that herbivores select at multiple scales (Hjältén, Danell & Lundberg 1993; Bergman, Iason & Hester 2005; Baraza, Zamora & Hódar 2006).

#### *Herbivore modifiers of plant-driven foraging*

The characteristics of the herbivore influence decisions at all stages of the foraging process, from where and how long to search for palatable plants, the ability to detect them, and whether or not to consume them. For example, herbivores may choose to forage amongst structurally and compositionally different vegetation depending on their position on the grazer-browser continuum (i.e. a diet dominated by grasses or forbs, shrubs and trees; Spret & McArthur 2002) and their level of specialisation for feeding on particular plants (generalists vs specialists). The decision to consume a particular plant defines diet breadth, and may be influenced by characteristics that vary temporally such as satiation, nutrient status and toxin load (Dearing & Cork 1999; Alm, Birgersson & Leimar 2002; Milchunas & Noy-Meir 2002). Where herbivores forage in groups, consumption may also be influenced by competition with other individuals and the use of social cues to make patch-leaving decisions (Bergvall *et al.* 2006).

The perception of sensory information by animals is critical to the foraging process; it reduces uncertainty about the environment and therefore increases foraging efficiency (Dall *et al.* 2005; Schmidt, Dall & Van Gils 2010). Herbivores differ in the scales at which they are able to perceive heterogeneity and thus make foraging decisions (Kotliar & Wiens 1990; Schmidt & Brown 1996). This is physiologically determined by the sensory apparatus to see, smell, taste, touch and hear (Schaefer & Ruxton 2011), but can be modified by behavioural factors such as travel speed (Hirsch 2010) and environmental conditions including vegetation structure,

temperature and wind (Schooley & Wiens 2003; Prevedello, Forero-Medina & Vieira 2011; Munoz & Blumstein 2012). To understand how plants influence the foraging process, it is therefore critical to assess how they may modify perception of plant cues, and conversely, whether herbivores may subvert deterrent cues such as toxic odours or spines to detect defended but palatable plants (Bedoya-Pérez *et al.* 2014b; Kohl, Miller & Dearing 2015).

Previous experiences can also play an important role in foraging success (Kamil & Roitblat 1985). The use of spatial memory can significantly enhance search efficiency and therefore increase food intake (Benhamou 1994; Janson 1998). This extends to dynamic environments where it may be advantageous to learn either to return to previously rewarding sites or to avoid them because they have been depleted (Burke *et al.* 2002; Winter & Stich 2005). On the large scale, spatial memory can lead to migrations to areas where food is known to occur seasonally (Benhamou, Sauvé & Bovet 1990). Positive and negative consequences associated with a food or food cue can also influence acceptance and rejection decisions at the consumption stage (Early & Provenza 1998; Provenza, Kimball & Villalba 2000). Thus learning and memory can play a role in determining where and when food resources are available, as well as in deciding whether or not to consume a plant or plant part.

### **Plants as drivers of foraging behaviour: a holistic approach to herbivory**

Consumption of any particular plant is context-dependent. A major criticism of many plant-herbivore studies has been that they focus on binary interactions between a herbivore and a plant. This approach does not consider the complex background against which foraging decisions are made in nature (Hay *et al.* 2004; Agrawal, Lau & Hambäck 2006). This is particularly important for addressing foraging behaviours in free-ranging animals that are influenced by factors beyond the individual plant such as associational effects.

Firstly, herbivore foraging behaviours are a result of decisions occurring at multiple scales, and thus experimental designs that preclude larger-scale decisions may not be representative

of foraging patterns in the field. The need to consider both the scale(s) of selection and opportunity for choice was demonstrated with red-bellied pademelons *Thylogale billardierii* and seedlings of *Eucalyptus nitens*. The initial study, presenting a plant-level choice, showed that seedlings were less vulnerable to browsing among vegetation of higher palatability, abundance and height (Miller, McArthur & Smethurst 2007). This was consistent with associational refuge both via the attractant-decoy mechanism, whereby the more palatable and abundant neighbouring plants were preferred to the focal seedlings, and reduced apparency of seedlings amongst taller vegetation. However, when offered choice at the patch and plant scales, seedlings were less vulnerable in low-quality patches, following the repellent plant model (Miller, McArthur & Smethurst 2009).

Secondly, the complex background against which interactions occur can affect the detection of cues from preferred plants by herbivores. Herbivores must be able to discriminate cues of their preferred plants or plant parts from those of the surrounding vegetation, and this should be easier as the differences between them increase; for example, plants or plant parts that look or smell distinctly different from their surroundings should be easier to find (Bradbury & Vehrencamp 1998; Schaefer & Ruxton 2011). The cue-to-noise ratio influences the ability of an animal to distinguish preferred food cues from other cues in the environment and thus 'noisy' environments can render food more difficult to locate (Ruxton 2009; Carthey, Bytheway & Banks 2011; Price & Banks 2012). This in turn affects the efficiency of the search process, and therefore the rate of detection and ultimately consumption of preferred plants by herbivores.

Here, my overall aim was to take a holistic approach to understanding the plant drivers of mammalian herbivore foraging behaviour. I conducted all my experiments in the field, testing the foraging responses of free-ranging animals to native plant species. My research is thus of direct ecological relevance because it was carried out against the complex backdrop of a real foraging environment and thus incorporates the effects of decisions made across multiple

scales. From an applied perspective, such an understanding would inform restoration strategies in degraded areas under herbivore pressure. My specific avenues of enquiry were:

(1) Which characteristics of neighbouring plants, at multiple scales, affect browsing of focal plants?

(2) How do neighbouring plants alter herbivore foraging behaviour when neighbours protect focal plants from consumption?

(3) Do herbivores use leaf odour to detect palatable plants amongst their interspecific neighbours?

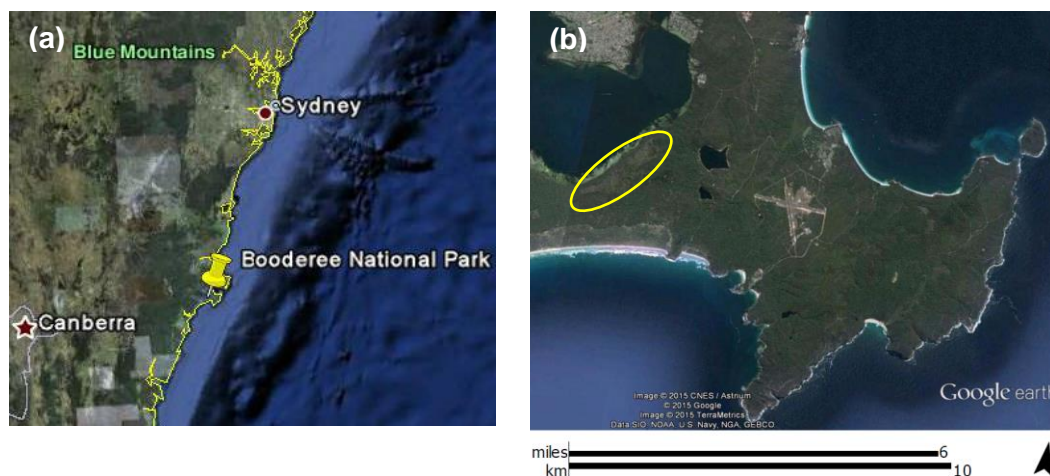
(4) How do herbivores respond to differences in olfactory and visual plant cues driven by intraspecific variation in plant nutritional content?

#### *Study system*

I examined foraging behaviour in the swamp wallaby *Wallabia bicolor*, as it interacted with native tree seedlings and as affected by other plants. Swamp wallabies are macropodid marsupials weighing 10 – 23 kg, and are common in temperate regions of eastern Australia (Menkhorst & Knight 2001). They have small over-lapping home ranges but are essentially solitary animals (Jarman & Coulson 1989; Troy & Coulson 1993). They are active both day and night, preferring to forage in areas with lateral cover during the day (Lentle *et al.* 2005; Di Stefano *et al.* 2009; Swan *et al.* 2009). Swamp wallabies are generalist browsers, consuming a wide range of plants including forbs, shrubs, trees, ferns, grasses, sedges, rushes and vines, as well as fungi (Hollis, Robertshaw & Harden 1986; Osawa 1990; Davis, Coulson & Forsyth 2008). This exposes them to a wide range of plant defences. While their diet is broad, swamp wallabies exhibit foraging selectivity and do not simply consume plants in direct proportion to availability (Di Stefano & Newell 2008). In trials using food pellets, swamp wallabies have been shown to trade-off between nutrients and the toxic terpene 1,8-cineole (Bedoya-Pérez *et al.*

2014a); the latter, while acting as a toxin by reducing consumption, was also used as a cue for finding food pellets in a matrix (Bedoya-Pérez *et al.* 2014b).

My study site was located in a degraded woodland in Booderee National Park on the south east coast of Australia (35°08'30"S, 150°39'10"E; Fig. 2). The site was once a Monterey pine *Pinus radiata* plantation but most pine trees were removed approximately 20 years ago with the goal of restoring the native eucalypt canopy. The vegetation at the time of my field research (2011-2015) comprised a mosaic of dense understorey (mostly bracken fern *Pteridium esculentum*) with intermittent patches of open grassed areas (Fig. 3). Canopy trees were sparsely distributed and the success of natural regeneration was poor, with recruitment failure of tree seedlings attributed to high herbivore pressure (Dexter *et al.* 2013). Lethal baiting of the invasive red fox *Vulpes vulpes* within the park has led to significant increases in mammal abundance, particularly of swamp wallabies, which comprise the largest component of fox diets in adjacent unbaited areas (Roberts *et al.* 2006; Dexter *et al.* 2013). Swamp wallabies are also known to consume eucalypt seedlings in other areas of Australia, leading to conflict with commercial forestry activities (Di Stefano 2003; Di Stefano 2004). Restoration of canopy trees in the presence of abundant browsers therefore required an understanding of the factors affecting their foraging decisions and the escape of tree seedlings from herbivory. I used seedlings of the native canopy tree *Eucalyptus pilularis* to test the foraging responses of free-ranging swamp wallabies and the browsing consequences for plants.



**Figure 2.** Location of (a) Booderee National Park and (b) study area within the park.

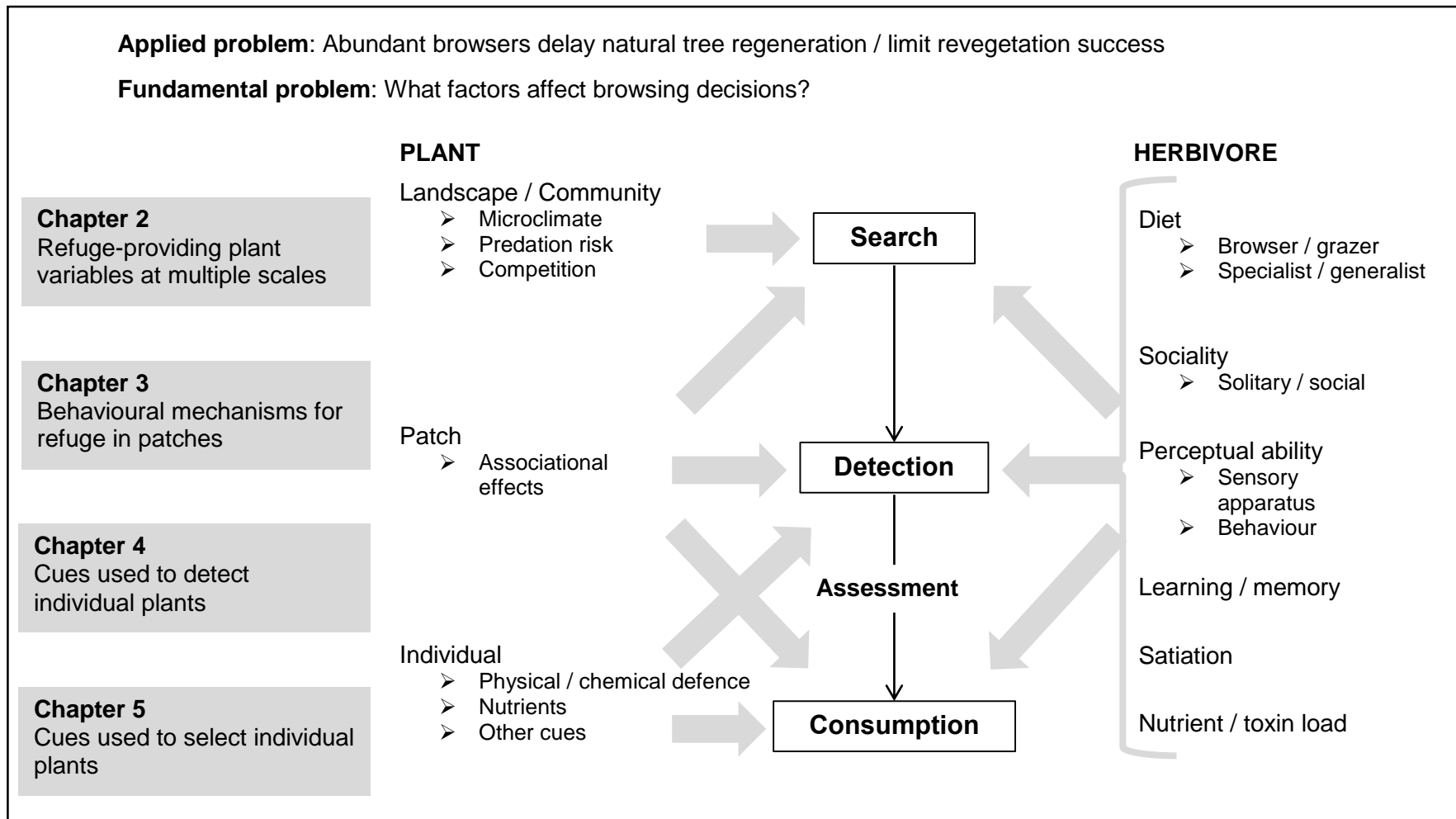




**Figure 3.** Mosaic vegetation in ‘pine paddocks’, Booderee National Park, Jervis Bay Territory, Australia.

### *Thesis structure*

My research considered the plant-herbivore interactions driving foraging decisions at multiple scales and at different stages of the foraging processes (Fig. 4). In Chapter 2, I tested the influence of existing vegetation characteristics on herbivory of planted seedlings – i.e. capacity to provide associational refuge and more generally influence herbivore foraging patterns. On the basis of established refuge-providing characteristics, I manipulated plant neighbourhoods in Chapter 3 to assess which stage(s) of the herbivore foraging process that refuge-providing



**Figure 4.** Overview of thesis chapters in the context of the plant-herbivore interactions driving foraging decisions. The stages of foraging are affected by characteristics of plants at multiple scales, influencing where herbivores choose to search, how long they search, how easily they can detect palatable plants, and how they assess palatability and therefore decide to consume or reject plants. This is modified by herbivore characteristics acting at different stages of the foraging process. My thesis considers the plant drivers of foraging behaviour in a generalist browser, the swamp wallaby *Wallabia bicolor*.

neighbours disrupt. I observed that swamp wallabies foraging in experimental patches exhibited distinct sniffing behaviour. In Chapter 4, I therefore tested the role of plant odour in detection of palatable food by swamp wallabies. I then examined the influences of olfactory and visual cues in detection and browsing of seedlings varying in nutritional quality, i.e. as drivers of intra-specific selective foraging, in Chapter 5. In Chapter 6, I synthesised the knowledge gained from my research and used it to suggest directions for future studies aiming to understand mammalian foraging ecology from a mechanistic viewpoint.

## References

- Agrawal, A.A. (2011) Current trends in the evolutionary ecology of plant defence. *Functional Ecology*, **25**, 420-432.
- Agrawal, A.A., Lau, Jennifer A. & Hambäck, Peter A. (2006) Community heterogeneity and the evolution of interactions between plants and insect herbivores. *The Quarterly Review of Biology*, **81**, 349-376.
- Alm, U., Birgersson, B. & Leimar, O. (2002) The effect of food quality and relative abundance on food choice in fallow deer. *Animal Behaviour*, **64**, 439-445.
- Anderson, V.J. & Briske, D.D. (1995) Herbivore-induced species replacement in grasslands: is it driven by herbivory tolerance or avoidance? *Ecological Applications*, **5**, 1014-1024.
- Atsatt, P.R. & O'Dowd, D.J. (1976) Plant defense guilds. *Science*, **193**, 24-29.
- Augustine, D.J. & McNaughton, S.J. (1998) Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *The Journal of Wildlife Management*, **62**, 1165-1183.
- Bailey, D.W., Gross, J.E., Laca, E.A., Rittenhouse, L.R., Coughenour, M.B., Swift, D.M. & Sims, P.L. (1996) Mechanisms that result in large herbivore grazing distributions. *Journal of Range Management*, **49**, 386 - 400.
- Baraza, E., Zamora, R. & Hódar, J.A. (2006) Conditional outcomes in plant–herbivore interactions: neighbours matter. *Oikos*, **113**, 148-156.
- Becerra, P.I. & Bustamante, R.O. (2008) The effect of herbivory on seedling survival of the invasive exotic species *Pinus radiata* and *Eucalyptus globulus* in a Mediterranean ecosystem of Central Chile. *Forest Ecology and Management*, **256**, 1573-1578.
- Bedoya-Pérez, M., Issa, D., Banks, P. & McArthur, C. (2014a) Quantifying the response of free-ranging mammalian herbivores to the interplay between plant defense and nutrient concentrations. *Oecologia*, **175**, 1167-1177.
- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014b) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.

- Belovsky, G.E. (1984a) Herbivore optimal foraging: a comparative test of three models. *The American Naturalist*, **124**, 97-115.
- Belovsky, G.E. (1984b) Summer diet optimization by beaver. *American Midland Naturalist*, **111**, 209-222.
- Belovsky, G.E. (1986) Optimal foraging and community structure: implications for a guild of generalist grassland herbivores. *Oecologia*, **70**, 35-52.
- Benhamou, S. (1994) Spatial memory and searching efficiency. *Animal Behaviour*, **47**, 1423-1433.
- Benhamou, S., Sauvé, J.-P. & Bovet, P. (1990) Spatial memory in large scale movements: efficiency and limitation of the egocentric coding process. *Journal of Theoretical Biology*, **145**, 1-12.
- Bergman, M., Iason, G.R. & Hester, A.J. (2005) Feeding patterns by roe deer and rabbits on pine, willow and birch in relation to spatial arrangement. *Oikos*, **109**, 513-520.
- Bergvall, U.A., Rautio, P., Kesti, K., Tuomi, J. & Leimar, O. (2006) Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. *Oecologia*, **147**, 253-260.
- Bernays, E.A., Bright, K.L., Gonzalez, N. & Angel, J. (1994) Dietary mixing in a generalist herbivore: tests of two hypotheses. *Ecology*, **75**, 1997-2006.
- Borzak, C.L., O'Reilly-Wapstra, J.M. & Potts, B.M. (2015) Direct and indirect effects of marsupial browsing on a foundation tree species. *Oikos*, **124**, 515-524.
- Boyle, R., McLean, S., Foley, W. & Davies, N. (1999) Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. *Journal of Chemical Ecology*, **25**, 2109-2126.
- Bradbury, J. & Vehrencamp, S. (1998) *Principles of animal communication*. Sinauer Associates, Sunderland, MA.
- Brown, J.R. & Stuth, J.W. (1993) How herbivory affects grazing tolerant and sensitive grasses in a central Texas grassland: integrating plant response across hierarchical levels. *Oikos*, **67**, 291-297.

- Burke, D., Cieplucha, C., Cass, J., Russell, F. & Fry, G. (2002) Win-shift and win-stay learning in the short-beaked echidna (*Tachyglossus aculeatus*). *Animal Cognition*, **5**, 79-84.
- Carmona, D., Lajeunesse, M.J. & Johnson, M.T.J. (2011) Plant traits that predict resistance to herbivores. *Functional Ecology*, **25**, 358-367.
- Carthey, A.J.R., Bytheway, J.P. & Banks, P.B. (2011) Negotiating a noisy, information-rich environment in search of cryptic prey: olfactory predators need patchiness in prey cues. *Journal of Animal Ecology*, **80**, 742-752.
- Charnov, E.L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, **9**, 129-136.
- Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C. & Waller, D.M. (2004) Ecological impacts of deer overabundance. *Annual Review of Ecology Evolution and Systematics*, **35**, 113-147.
- Cushman, J.H., Lortie, C.J. & Christian, C.E. (2011) Native herbivores and plant facilitation mediate the performance and distribution of an invasive exotic grass. *Journal of Ecology*, **99**, 524-531.
- Dall, S.R.X., Giraldeau, L.-A., Olsson, O., McNamara, J.M. & Stephens, D.W. (2005) Information and its use by animals in evolutionary ecology. *Trends in Ecology & Evolution*, **20**, 187-193.
- Davis, N.E., Coulson, G. & Forsyth, D.M. (2008) Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research*, **35**, 684-694.
- Dearing, M.D. & Cork, S. (1999) Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula*. *Journal of Chemical Ecology*, **25**, 1205-1219.
- Dexter, N. (1998) The influence of pasture distribution and temperature on habitat selection by feral pigs in a semi-arid environment. *Wildlife Research*, **25**, 547-559.

- Dexter, N., Hudson, M., James, S., MacGregor, C. & Lindenmayer, D.B. (2013) Unintended consequences of invasive predator control in an Australian forest: overabundant wallabies and vegetation change. *PLoS ONE*, **8**, e69087.
- Di Stefano, J. (2003) Mammalian browsing at the Mount Cole State forest: defining a critical browsing level and assessing the effect of multiple browsing events. *Australian Forestry*, **66**, 287 - 293.
- Di Stefano, J. (2004) The importance of ecological research for ecosystem management: The case of browsing by swamp wallabies (*Wallabia bicolor*) in commercially harvested native forests. *Ecological Management & Restoration*, **5**, 61-67.
- Di Stefano, J. & Newell, G.R. (2008) Diet selection by the swamp wallaby (*Wallabia bicolor*): feeding strategies under conditions of changed food availability. *Journal of Mammalogy*, **89**, 1540-1549.
- Di Stefano, J., York, A., Swan, M., Greenfield, A. & Coulson, G. (2009) Habitat selection by the swamp wallaby (*Wallabia bicolor*) in relation to diel period, food and shelter. *Austral Ecology*, **34**, 143-155.
- Early, D.M. & Provenza, F.D. (1998) Food flavor and nutritional characteristics alter dynamics of food preference in lambs. *Journal of Animal Science*, **76**, 728-734.
- Emlen, J.M. (1966) The role of time and energy in food preference. *The American Naturalist*, **100**, 611-617.
- Endler, J.A. (1991) Interactions between predators and prey. *Behavioural ecology: an evolutionary approach* (eds J.R. Krebs & N.B. Davies), pp. 169-196. Blackwell Scientific Publishing, Oxford.
- Farnsworth, K.D. & Illius, A.W. (1998) Optimal diet choice for large herbivores: an extended contingency model. *Functional Ecology*, **12**, 74-81.
- Foley, W.J., Iason, G.R. & McArthur, C. (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? *Nutritional ecology of herbivores: proceedings of the Vth International Symposium on*

- the Nutrition of Herbivores* (eds H.-J. Jung & G. Fahey), pp. 130–209. American Society of Animal Science, Savoy, Illinois.
- Foley, W.J. & McArthur, C. (1994) The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective. *The Digestive System in Mammals: Food, form and function* (eds D.J. Chivers & P. Langer), pp. 370-391. Cambridge University Press, Cambridge.
- Hambäck, P.A., Agren, J. & Ericson, L. (2000) Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, **81**, 1784-1794.
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, **8**, 157-178.
- Hay, M.E., Parker, J.D., Burkepile, D.E., Caudill, C.C., Wilson, A.E., Hallinan, Z.P. & Chequer, A.D. (2004) Mutualisms and aquatic community structure: the enemy of my enemy is my friend. *Annual Review of Ecology Evolution and Systematics*, **35**, 175-197.
- Hidding, B., Tremblay, J.-P. & Côté, S.D. (2012) Survival and growth of balsam fir seedlings and saplings under multiple controlled ungulate densities. *Forest Ecology and Management*, **276**, 96-103.
- Hirsch, B.T. (2010) Tradeoff between travel speed and olfactory food detection in ring-tailed coatis (*Nasua nasua*). *Ethology*, **116**, 671-679.
- Hjältén, J., Danell, K. & Lundberg, P. (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. *Oikos*, **68**, 125-131.
- Hobbs, N.T. (1996) Modification of ecosystems by ungulates. *The Journal of Wildlife Management*, **60**, 695-713.
- Hollis, C., Robertshaw, J. & Harden, R. (1986) Ecology of the swamp wallaby (*Wallabia bicolor*) in northeastern New-South-Wales .1. Diet. *Wildlife Research*, **13**, 355-365.
- Janson, C.H. (1998) Experimental evidence for spatial memory in foraging wild capuchin monkeys, *Cebus apella*. *Animal Behaviour*, **55**, 1229-1243.



- Jarman, P. & Coulson, G. (1989) Dynamics and adaptiveness of grouping in macropods. *Kangaroos, wallabies and rat-kangaroos*, pp. 527-547.
- Johnson, M.T.J. (2011) Evolutionary ecology of plant defences against herbivores. *Functional Ecology*, **25**, 305-311.
- Kamil, A.C. & Roitblat, H.L. (1985) The ecology of foraging behavior: implications for animal learning and memory. *Annual Review of Psychology*, **36**, 141-169.
- Koch, J.M., Richardson, J. & Lamont, B.B. (2004) Grazing by kangaroos limits the establishment of the grass trees *Xanthorrhoea gracilis* and *X. preissii* in restored bauxite mines in eucalypt forest of southwestern Australia. *Restoration Ecology*, **12**, 297-305.
- Kohl, K.D., Miller, A.W. & Dearing, M.D. (2015) Evolutionary irony: evidence that 'defensive' plant spines act as a proximate cue to attract a mammalian herbivore. *Oikos*, **124**, 835-841.
- Kotliar, N.B. & Wiens, J.A. (1990) Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos*, **59**, 253-260.
- Lange, R.T. & Graham, C.R. (1983) Rabbits and the failure of regeneration in Australian arid zone *Acacia*. *Australian Journal of Ecology*, **8**, 377-381.
- Lentle, R.G., Hume, I.D., Stafford, K.J., Kennedy, M., Springett, B.P. & Haslett, S. (2005) The temporal organisation of the feeding behaviour of four species of wallaby: examining chronobiological and homeostatic influences. *Australian Journal of Zoology*, **53**, 117-129.
- Long, Z.T., Pendergast, T.H. & Carson, W.P. (2007) The impact of deer on relationships between tree growth and mortality in an old-growth beech-maple forest. *Forest Ecology and Management*, **252**, 230-238.
- MacArthur, R.H. & Pianka, E.R. (1966) On optimal use of a patchy environment. *The American Naturalist*, **100**, 603-609.
- Mangel, M. & Clark, C.W. (1986) Towards a unified foraging theory. *Ecology*, **67**, 1127-1138.

- McAuliffe, J.R. (1986) Herbivore-limited establishment of a Sonoran Desert tree, *Cercidium microphyllum*. *Ecology*, **67**, 276-280.
- Menkhorst, P. & Knight, F. (2001) *A Field Guide to the Mammals of Australia*. Oxford University Press, Melbourne.
- Milchunas, D.G. & Noy-Meir, I. (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, **99**, 113-130.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2007) Effects of within-patch characteristics on the vulnerability of a plant to herbivory. *Oikos*, **116**, 41-52.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2009) Spatial scale and opportunities for choice influence browsing and associational refuges of focal plants. *Journal of Animal Ecology*, **78**, 1134-1142.
- Miller, G.R., Kinnaird, J.W. & Cummins, R.P. (1982) Liability of saplings to browsing on a red deer range in the Scottish Highlands. *Journal of Applied Ecology*, **19**, 941-951.
- Moles, A.T. & Westoby, M. (2004) What do seedlings die from and what are the implications for evolution of seed size? *Oikos*, **106**, 193-199.
- Munoz, N.E. & Blumstein, D.T. (2012) Multisensory perception in uncertain environments. *Behavioral Ecology*, **23**, 457-462.
- Nuttle, T., Yerger, E.H., Stoleson, S.H. & Ristau, T.E. (2011) Legacy of top-down herbivore pressure ricochets back up multiple trophic levels in forest canopies over 30 years. *Ecosphere*, **2**, art4.
- Osawa, R. (1990) Feeding strategies of the swamp wallaby, *Wallabia Bicolor*, on North Stradbroke Island, Queensland. I: Composition of diets. *Wildlife Research*, **17**, 615-621.
- Owen-Smith, N. & Novellie, P. (1982) What should a clever ungulate eat? *The American Naturalist*, **119**, 151-178.
- Parsons, M.H., Lamont, B.B., Koch, J.M. & Dods, K. (2007) Disentangling competition, herbivory, and seasonal effects on young plants in newly restored communities. *Restoration Ecology*, **15**, 250-262.

- Paschke, M.W., DeLeo, C. & Redente, E.F. (2000) Revegetation of roadcut slopes in Mesa Verde National Park, U.S.A. *Restoration Ecology*, **8**, 276-282.
- Prevedello, J.A., Forero-Medina, G. & Vieira, M.V. (2011) Does land use affect perceptual range? Evidence from two marsupials of the Atlantic Forest. *Journal of Zoology*, **284**, 53-59.
- Price, C.J. & Banks, P.B. (2012) Exploiting olfactory learning in alien rats to protect birds' eggs. *Proceedings of the National Academy of Sciences*, **109**, 19304-19309.
- Provenza, F.D., Kimball, B.A. & Villalba, J.J. (2000) Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. *Oikos*, **88**, 424-432.
- Pyke, G.H., Pulliam, H.R. & Charnov, E.L. (1977) Optimal foraging: a selective review of theory and tests. *The Quarterly Review of Biology*, **52**, 137-154.
- Raubenheimer, D., Simpson, S.J. & Mayntz, D. (2009) Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology*, **23**, 4-16.
- Reimoser, F. & Gossow, H. (1996) Impact of ungulates on forest vegetation and its dependence on the silvicultural system. *Forest Ecology and Management*, **88**, 107-119.
- Roberts, M.W., Dexter, N., Meek, P.D., Hudson, M. & Buttemer, W.A. (2006) Does baiting influence the relative composition of the diet of foxes? *Wildlife Research*, **33**, 481-488.
- Rosenthal, J.P. & Kotanen, P.M. (1994) Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution*, **9**, 145-148.
- Ruhren, S. & Handel, S.N. (2003) Herbivory constrains survival, reproduction and mutualisms when restoring nine temperate forest herbs. *Journal of the Torrey Botanical Society*, **130**, 34-42.
- Ruxton, G.D. (2009) Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 549-557.
- Schaefer, H.M. & Ruxton, G.D. (2011) *Plant-animal communication*. Oxford University Press, Oxford, U.K.

- Schmidt, K.A. & Brown, J.S. (1996) Patch assessment in fox squirrels: the role of resource density, patch size, and patch boundaries. *American Naturalist*, **147**, 360-380.
- Schmidt, K.A., Dall, S.R.X. & Van Gils, J.A. (2010) The ecology of information: an overview on the ecological significance of making informed decisions. *Oikos*, **119**, 304-316.
- Schoener, T. (1987) A brief history of optimal foraging ecology. *Foraging Behavior* (eds A. Kamil, J. Krebs & H.R. Pulliam), pp. 5-67. Springer US.
- Schooley, R.L. & Wiens, J.A. (2003) Finding habitat patches and directional connectivity. *Oikos*, **102**, 559-570.
- Searle, K. & Shipley, L. (2008) The comparative feeding behaviour of large browsing and grazing herbivores. *The Ecology of Browsing and Grazing* (eds I. Gordon & H.T. Prins), pp. 117-148. Springer Berlin Heidelberg.
- Searle, K.R., Vandervelde, T., Hobbs, N.T., Shipley, L.A. & Wunder, B.A. (2006) Spatial context influences patch residence time in foraging hierarchies. *Oecologia*, **148**, 710-719.
- Senft, R.L., Coughenour, M.B., Bailey, D.W., Rittenhouse, L.R., Sala, O.E. & Swift, D.M. (1987) Large herbivore foraging and ecological hierarchies. *Bioscience*, **37**, 789-799.
- Simpson, S.J., Raubenheimer, D., Charleston, M.A. & Clissold, F.J. (2010) Modelling nutritional interactions: from individuals to communities. *Trends in Ecology & Evolution*, **25**, 53-60.
- Smit, C., Ruifrok, J.L., van Klink, R. & Olf, H. (2015) Rewilding with large herbivores: the importance of grazing refuges for sapling establishment and wood-pasture formation. *Biological Conservation*, **182**, 134-142.
- Sorensen, J.S., McLister, J.D. & Dearing, M.D. (2005) Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. *Ecology*, **86**, 125-139.
- Sprent, J.A. & McArthur, C. (2002) Diet and diet selection of two species in the macropodid browser-grazer continuum: do they eat what they 'should'? *Australian Journal of Zoology*, **50**, 183-192.

- Stenseth, N.C. & Hansson, L. (1979) Optimal food selection: a graphic model. *The American Naturalist*, **113**, 373-389.
- Stephens, D.W. & Krebs, J.R. (1986) *Foraging theory*. Princeton University Press, New Jersey.
- Swan, M., Di Stefano, J., Greenfield, A. & Coulson, G. (2009) Fine-scale habitat selection by adult female swamp wallabies (*Wallabia bicolor*). *Australian Journal of Zoology*, **56**, 305-309.
- Tahvanainen, J.O. & Root, R.B. (1972) The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia*, **10**, 321-346.
- Tordoff, G.M., Baker, A.J.M. & Willis, A.J. (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*, **41**, 219-228.
- Troy, S. & Coulson, G. (1993) Home range of the swamp wallaby, *Wallabia bicolor*. *Wildlife Research*, **20**, 571-575.
- Van Beest, F.M., Mysterud, A., Loe, L.E. & Milner, J.M. (2010) Forage quantity, quality and depletion as scale-dependent mechanisms driving habitat selection of a large browsing herbivore. *Journal of Animal Ecology*, **79**, 910-922.
- Wahl, M. & Hay, M.E. (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia*, **102**, 329-340.
- Western, D. & Maitumo, D. (2004) Woodland loss and restoration in a savanna park: a 20-year experiment. *African Journal of Ecology*, **42**, 111-121.
- Westoby, M. (1974) An analysis of diet selection by large generalist herbivores. *The American Naturalist*, **108**, 290-304.
- Wiggins, N.L., McArthur, C. & Davies, N.W. (2006) Diet switching in a generalist mammalian folivore: fundamental to maximising intake. *Oecologia*, **147**, 650-657.
- Winter, Y. & Stich, K.P. (2005) Foraging in a complex naturalistic environment: capacity of spatial working memory in flower bats. *The Journal of Experimental Biology*, **208**, 539-548.

Wywiałowski, A.P. (1987) Habitat structure and predators: choices and consequences for rodent habitat specialists and generalists. *Oecologia*, **72**, 39-45.

## CHAPTER 2

---

### Associational Refuge in Practice:

### Can Existing Vegetation Facilitate Woodland Restoration?

Published as: Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015) Associational refuge in practice: can existing vegetation facilitate woodland restoration? *Oikos*, **124**, 571–580.



Swamp wallaby (*Wallabia bicolor*) handling an experimental tree seedling (*Eucalyptus pilularis*) amongst dense neighbouring vegetation.

## Abstract

Herbivores can dramatically diminish revegetation success, but associational refuge theory predicts that neighbouring plants could hinder browsing of planted seedlings. The key to strategic restoration using associational refuge is to define which patch variables are effective against the appropriate herbivores, at multiple scales, and to understand which stages of the foraging process these variables disrupt. Our study aimed to test the capacity of existing vegetation to act as associational refuge for planted seedlings by affecting search, detection and consumption decisions, and more generally influence herbivore foraging patterns. We conducted a field trial with free-ranging, mammalian herbivores and nursery-raised, native tree seedlings. We quantified seedling browsing damage over time in relation to a suite of existing patch variables at two spatial scales (100 m<sup>2</sup> and 4 m<sup>2</sup>). After 2 months, 78% of seedlings were browsed, suffering mean foliage loss of 90.5%. Focal seedlings were almost exclusively consumed by swamp wallabies (*Wallabia bicolor*), an abundant generalist browser. Once a swamp wallaby investigated a seedling, the probability of consumption was high (86%). At the large scale, browsing of seedlings was delayed in patches with lower canopy cover and fewer browsed plant species. Seedlings in fern-dominated patches escaped browsing for longer than those in grass-dominated patches. At the small scale, browsing was delayed with higher cover of understorey vegetation. Associational refuge was provided by vegetation with characteristics, and at spatial scales, consistent with disrupted search and detection of focal seedlings by herbivores. Thus strategic placement of seedlings in existing vegetation — based on understanding which herbivore species is responsible and how it responds to vegetation — can take advantage of associational refuge during restoration. However, given rapid seedling detection by herbivores, associational refuge may be inadequate in the long-term under high browsing pressure unless high absolute numbers of seedlings are planted among refuge.



## Introduction

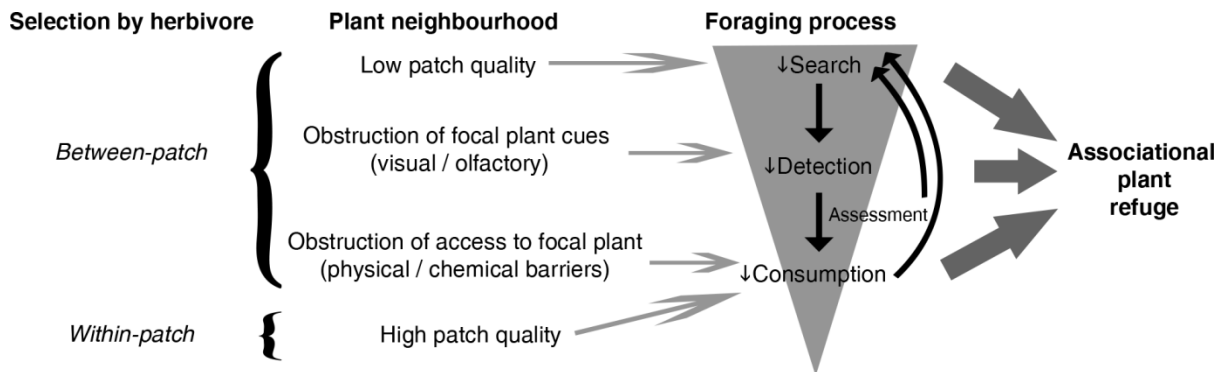
Widespread habitat degradation through anthropogenic activities has necessitated effective restoration to better conserve biodiversity (Hobbs & Norton 1996). This often involves revegetating large areas to restore structural and compositional complexity of an ecosystem. Survival of revegetation plantings, however, is highly variable. Where herbivore density is high, feeding pressure may reduce seedling growth and survival, altering vegetation structure and potentially influencing other ecosystem components through trophic cascades (Estes *et al.* 2011). Culling animals, particularly native herbivores, to reduce browsing pressure can carry significant costs in relation to conservation, ethics and public perception (Di Stefano 2004; Baker *et al.* 2007). Solutions such as fencing or individual plant guards are often expensive, and may be detrimental to resident biota (Jensen, Götmark & Löff 2012). An alternative is to exploit traits of the vegetation itself; but for this to work, it is critical to understand the foraging ecology of herbivores.

For a generalist herbivore, the foraging process involves several stages. Animals move through the environment, searching for patches with potentially palatable food; they must then detect the food and assess whether to consume or reject it (*sensu* predator foraging process, Endler 1991). This process is thus iterative and occurs at a hierarchy of spatial scales from regional systems to landscapes, plant communities, patches, individual plants and plant parts (Senft *et al.* 1987; Kotliar & Wiens 1990). Selecting where to forage at larger spatial scales, such as habitat selection within home ranges and patch selection within habitats is influenced by a range of biotic and abiotic factors. These factors include those associated specifically with food quality, such as the chemistry and physical characteristics of food plants, as well as those affecting foraging more generally, such as perceived predation risk, inter- and intra-specific competition, and (micro-) climate. From the plant perspective, the influence all these factors have on herbivore foraging in turn shapes the vulnerability of any particular plant to being consumed.

At the patch level, vulnerability of a particular plant to herbivory may also be shaped by associational effects of neighbouring vegetation (i.e. refuge or susceptibility; Tahvanainen & Root 1972; Atsatt & O'Dowd 1976; Palmer *et al.* 2003) based on whether the surrounding vegetation matrix is attractive or repellent relative to the focal plant or other patches, and whether the herbivore selects predominantly between or within patches (Fig. 1; Baraza, Zamora & Hódar 2006; Bergvall *et al.* 2006; Barbosa *et al.* 2009). For example, a focal plant may receive refuge from herbivory if it occurs in a patch of low perceived quality and the herbivore selects between patches (the repellent plant mechanism; Atsatt & O'Dowd 1976), but suffer susceptibility if the herbivore selects between plants within the patch (the neighbour contrast mechanism; Bergvall *et al.* 2006). Conversely, if the herbivore selects between plants in a patch, a focal plant in a high quality patch may receive refuge via its neighbours acting as sacrificial plants (the attractant-decoy mechanism; Atsatt & O'Dowd 1976), but a herbivore selecting between patches would prefer the high quality patch and the focal plant within it would be more susceptible as a result (the shared doom mechanism; Wahl & Hay 1995). Understanding the scale at which the herbivore makes foraging decisions and the factors driving this process is critical to the application of associational plant theory.

The strength of an associational effect depends on characteristics of both the vegetation (nutrient and secondary chemistry, physical barriers such as spines, visual masking, or odours) and the herbivore (use of visual, olfactory and/or taste cues, diet specialisation and nutritional state), as they both affect the foraging process (Milchunas & Noy-Meir 2002). Thus plant associations can influence each stage of the foraging process (Fig. 1), from where to search for food, whether a focal plant is detected, to the final decision of whether to consume it or not. From an applied perspective, understanding the stage(s) in the foraging process affected by plant associations allows strategic management to maintain or enhance existing associational refuge effects. While some of the literature on associational effects has alluded to the importance of understanding the foraging process (e.g. Miller *et al.* 2006; Emerson *et*

*al.* 2012), we are not aware of any study that has explicitly considered the stages of foraging to explain the mechanisms behind associational refuge and susceptibility.



**Figure 1.** Conceptual diagram depicting the relationship between plant neighbourhood characteristics and the stages of the foraging process (occurring at diminishing spatial scales) resulting in associational refuge for a focal plant. The effect of the plant neighbourhood depends on whether the herbivore selects between or within patches. For example, between-patch selection results in refuge for a focal plant in a low quality patch as the herbivore will avoid or reduce search effort in the patch, while within-patch selection results in refuge when patch quality is high relative to the focal plant and the herbivore thus consumes alternative plants. A decrease at any stage of the foraging process can lead to reduced consumption of focal plants and therefore result in associational refuge.

In an applied context, the capacity of neighbours to protect planted vegetation has been examined mostly in highly modified production settings; for example, as refuge from herbivory for tree seedlings in forestry plantations (e.g. Miller, McArthur & Smethurst 2006; Harmer *et al.* 2010) and in livestock grazing areas (e.g. Callaway *et al.* 2005; Smit, Den Ouden & Müller-Schärer 2006; Van Uytvanck *et al.* 2008). These production systems generally allow greater scope than most restoration projects for manipulating neighbouring vegetation to optimize refuge effects. However, a much greater final density of focal plants may be needed in production systems to define success than when restoring plant communities to lower but

natural densities. Thus it is important to assess associational refuges in the relevant context. In vegetation restoration, studies of associational refuge effects have focused on particular plant associations selected *a priori* from the overall plant community rather than assessing a suite of potential refuge-providing variables; for example, shrubs as associational refuges for tree seedlings (García & Obeso 2003; Gómez-Aparicio *et al.* 2004; Jensen, Götmark & Löff 2012), grasses for tree seedlings (Anthelme & Michalet 2009; Good *et al.* 2014), and grasses for shrubs (Soliveres *et al.* 2011). Selecting a subset, in this way, precludes a broader evaluation of potentially useful associations from other vegetation types.

While vegetation is central to understanding associational refuges, the herbivores—and how they respond to vegetation—are also critical. The foraging responses of herbivores in studies of associational effects have been examined using captive animal trials or, if conducted in the field, inferred from inspection of plant damage (e.g. Pietrzykowski *et al.* 2003). These captive animal trials can help untangle specific responses of different herbivores, but they may not reflect responses of free-ranging animals exposed to diverse biotic and abiotic influences. Inferring foraging responses from plant damage in the field is adequate if there is only one herbivore species or herbivores can be identified by examining bite marks (e.g. Hjältén, Danell & Lundberg 1993); however, in multi-herbivore systems where this is not the case, it may misrepresent the response of each species, particularly where there are multiple herbivores browsing a single plant, leading to ill-targeted strategies to reduce damage.

Here, our aim was to test the capacity of existing vegetation to act (1) as associational refuge and (2) more generally in influencing foraging patterns of herbivores in a restoration context with a multi-species herbivore community. Our focal plants were nursery-raised eucalypt seedlings planted within a structurally heterogeneous woodland. We assessed a suite of neighbouring vegetation variables in patches at two spatial scales (100 m<sup>2</sup> and 4 m<sup>2</sup>). We chose variables specifically for their potential to provide associational refuge via their influence on various stages of the foraging process. We also included several variables that could influence foraging more generally via effects on perceived predation risk and shelter for

herbivores. We made novel use of video cameras to monitor the foraging responses of each species of mammalian herbivore in this community, allowing us to differentiate between by-standers and browsers of focal seedlings, and to reveal the foraging patterns that underpinned the relationships between patch characteristics and seedling browsing.

We predicted that the fate of focal plants would be influenced by patch variables related to neighbours and therefore associational effects at both patch scales, as well as those determining patterns of foraging more generally and outside the associational domain. Specifically, we hypothesized that focal plants would experience associational refuge where (1) patch quality was low and thus search effort reduced, (2) the plant neighbourhood created a barrier to the visual and/or olfactory cues of the focal plant, reducing detection rates, or (3) the plant neighbourhood restricted herbivore capacity to access the focal plant, increasing decisions to reject it once detected (Fig. 1). These fall under the repellent plant mechanism of associational refuge, with herbivore selections between- rather than within-patches dominating the outcomes. Our nursery-raised plants were well fertilised and thus of high nutritional quality relative to the existing vegetation neighbourhood, hence we expected them to be consumed once found. We therefore did not expect to find attractant-decoy refuge resulting from within-patch selection. The patch variables predicted to affect foraging more generally were those with the potential to influence habitat selection in terms of perceived predation risk and micro-climate.

The mammalian herbivore assemblage consisted of swamp wallabies *Wallabia bicolor*, eastern grey kangaroos *Macropus giganteus*, red-necked wallabies *M. rufogriseus*, and non-native European rabbits *Oryctolagus cuniculus*. We expected swamp wallabies to have the greatest browsing impact on planted seedlings given their high abundance (accounting for 77% of spotlighting survey sightings in 2009; Dexter *et al.* 2013), small overlapping home ranges (up to  $16.0 \pm 2.5$  ha in Victorian eucalypt forest; Troy & Coulson 1993), and foraging strategy toward the browser end of the browser-grazer continuum (Hollis, Robertshaw & Harden 1986). Although also abundant at the study site, the kangaroo is a grazer (Davis,

Coulson & Forsyth 2008), as is the rarer red-necked wallaby (Sprent & McArthur 2002). The relative abundance of the rabbit, a mixed feeder (Davis, Coulson & Forsyth 2008), was unknown but expected to be low and patchy. We predicted that the frequency per patch of foraging visits by the dominant browser and the subset during which they browsed focal plants, would be consistent with patterns of herbivory related to patch variables.

## **Materials and methods**

### *Study site*

We conducted our study in a 50 ha area of open woodland in Booderee National Park (BNP), Jervis Bay Territory, on the south east coast of Australia (35°08'30"S, 150°39'10"E). The park experiences a temperate maritime climate with warm summers and cooler winters, and rainfall spread throughout the year. The understorey vegetation was a patchwork of dense bracken fern *Pteridium esculentum*, shortly-cropped introduced grasses, sedge-like grasses (*Lomandra* spp.) and small scrubby trees (*Monotoca elliptica*). The sparse overstorey consisted of native eucalypts (*Eucalyptus pilularis* and *E. botryoides*), *Acacia implexa* and a few *Pinus radiata* (remaining from a small plantation removed approximately 20 years prior). Park managers were seeking to restore the eucalypt canopy, however natural recruitment of eucalypt seedlings was very low (pers. obs.). A local post-fire herbivore-exclusion trial indicated that mammal herbivory significantly reduced eucalypt seedling abundance (Dexter *et al.* 2013).

### *Study design*

In January 2012 (summer), we placed 81 commercially sourced *E. pilularis* tube-stock seedlings (592 mm  $\pm$  SE 9) in a 9 x 9 grid across the study area, with inter-seedling distances of 40 m (total grid size = 320 x 320 m). We chose these distances so that one seedling was not visible from the next, and the grid design was ideal for using spatial autocorrelation

techniques to test for independence of seedling browsing between sites. The maximum temperature ranged from 18°C to 30°C over the study period with a mean of 9.2 mm daily rainfall (Point Perpendicular, Jervis Bay; Bureau of Meteorology 2012). All seedlings were removed at the end of the study to avoid genetic contamination of the local stock. We kept seedlings in plastic pots buried to 5 cm above ground level, filled with sand, and held down with coarse plastic mesh secured with two tent pegs. We added one teaspoon of Scotts Osmocote Native Gardens fertilizer (NPK 17.9 : 0.8 : 7.3, iron and trace elements) to each seedling and watered it in. To observe herbivore foraging behaviour in the field, every second seedling was monitored via infra-red motion-sensored camera (ScoutGuard SG550, Hunting Cam Online, Gadsden SC, USA) fixed to a wooden post at 0.6 m height and 1.5 m horizontal distance from the seedling and set to record 60 s videos. We recorded seedling height, leaf number, and visually estimated leaf area lost to herbivory (following Pietrzykowski *et al.* 2003) weekly for 5 weeks, and again after 8 weeks. We diagnosed leaf damage as mammalian or invertebrate based on distinctive bite marks and damage patterns.

To quantify the vegetation community we measured a suite of plant characteristics at two scales by using large (100 m<sup>2</sup>) and small (4 m<sup>2</sup>) concentric quadrats around each seedling (hereafter, patches). We selected patch sizes to represent two different potential scales at which foraging decisions could be made by medium- to large-bodied mammalian herbivores. Studies considering herbivores filling similar ecological niches have detected significant associational plant effects by measuring patch characteristics at the 100 m<sup>2</sup> scale (e.g. red deer *Cervus elaphus*; Bee *et al.* 2009). The 4 m<sup>2</sup> scale is comparable to feeding stations used in food selection trials (e.g. fallow deer *Dama dama*, Bergvall *et al.* 2006; swamp wallabies, Bedoya-Pérez *et al.* 2014) and the 'microhabitat' scale used by Baraza, Zamora & Hódar (2006) to assess associational refuge from ungulate browsing. At both patch scales, we visually estimated understorey cover (sum of projected cover of the three most common understorey lifeforms, classified as tree, shrub, fern, forb, vine or grass) and cover of canopy trees. For ease of estimation, we subdivided each patch into four sub-quadrats, and calculated

mean covers for the whole patch. We also counted the number of adult (> 10 cm DBH) and juvenile (< 10 cm DBH) trees and tree species, and the number of plant species with clear signs of mammal herbivory at the end of the trial, in both the 100 m<sup>2</sup> and 4 m<sup>2</sup> patches. These variables may affect the searching stage by altering time spent foraging in a patch (and therefore seedling encounter rate) or directly affect detection by impacting on seedling conspicuousness. Some (e.g. number of browsed plant species) may also directly affect the nutritional state of the herbivore and therefore indirectly influence future consumption decisions. We assessed the number of browsed plant species after the conclusion of the trial to provide a crude measure of patch palatability; we considered the number of plant species browsed as a better measure of patch palatability than absolute plant quantities given that browsers optimise their intake by diet switching (Wiggins, McArthur & Davies 2006). We acknowledge the limitations of this method: browsers may have consumed some neighbouring plants incidentally whilst consuming the focal seedling. However, it is unlikely that incidental browsing would significantly influence overall patterns, and thus patches with a high number of browsed species relative to other patches could reasonably be deemed to indicate high patch palatability. We measured relative lateral visibility (potentially affecting seedling detection) using a white cylinder (H 30 cm x D 15 cm) placed over the seedling, and recording estimates of the proportion visible at eight evenly-spaced points at 2 m radius (estimating visibility at larger distances was impractical). We made estimates from a squatting position (~70 cm to eye-level) to approximate the view of a swamp wallaby. We used an ordinal system for all estimates: 0%, 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, 96-100%, converted to median values for analyses (following Pietrzykowski *et al.* 2003). Distances from each seedling to the nearest (unsealed) driving track were measured in ArcGIS 10 (ESRI 2010).

We used the camera footage to determine the foraging responses of the resident mammalian herbivores. It allowed us to quantify which herbivore was responsible for most browsing damage to focal plants, whether this was related to foraging frequency within patches, and whether patterns of foraging frequency were related to patch type. At each patch, we



quantified the number of general foraging visits per patch by each herbivore species (searching, handling and/or consumption of any plant) and the subset of visits during which the focal seedling was browsed. This allowed us to determine whether browsing by a particular herbivore species was related only to the herbivore's apparent abundance in the study area (and therefore probability of encountering focal seedlings) or also to its feeding strategy (from grazer to browser). To determine whether decisions leading to seedling detection were more critical than those resulting in consumption once found (as predicted), we also calculated the proportion of foraging visits in which swamp wallabies (the dominant browsers, see results) investigated focal seedlings (i.e. by sniffing and/or handling) and the resulting browsing decision (consumption or rejection). We defined a 'visit' as an independent foraging bout, excluding videos taken less than 20 minutes after the last unless they were of distinctly different individuals. Multiple individuals of the same species in the same footage were recorded as a single visit.

### *Statistical analyses*

Due to the high percentage of foliage removed during each browsing visit, we treated seedlings as either browsed or not browsed by mammals, with browsing delay (time after planting when browsing first occurred) as our response variable. We ensured that browsing delay (weeks) and browsing severity (percentage of leaf area lost) by the end of the trial were closely related using Pearson's correlation analysis (CORR procedure, SAS 9.3, SAS Institute Inc., Cary, NC, USA). Change in seedling height was not a useful measure of browsing because browsers behaved inconsistently with regard to stem consumption, in some cases removing all the foliage without affecting stem height, and in others removing the entire stem.

First, we examined the spatial pattern of browsing delay to determine whether browsing at each focal plant was spatially independent. We tested for clumping in browsing delay (i.e. spatial autocorrelation) using a distance-based non-binary weighting scheme with the VARIOGRAM procedure (SAS 9.3) and assuming normality. The resulting test statistics,

Moran's I and Geary's c, determine the probability of spatial autocorrelation in the data (i.e. clustered or over-dispersed pattern of browsing delay relative to a spatially random distribution) and both statistics are useful in quantifying spatial patterns in ecological data (Perry *et al.* 2002).

To test whether any of our patch variables could explain the focal plant browsing observed, we performed nonparametric survival (failure time) analyses. Specifically, we examined the relationships between browsing delay (event time) and patch variables (covariates) using the Kaplan-Meier (product-limit) method (LIFETEST procedure, SAS 9.3) where seedlings that were not browsed during the trial were right-censored. Pearson's correlation analyses (CORR procedure, SAS 9.3) were used to test for correlations between 17 explanatory variables: canopy cover, understorey cover, number of adult tree species, number of juvenile tree species, number of adult trees, number of juvenile trees, and number of plant species with evidence of mammalian herbivory at both the large and small patch scale; mean and standard deviation of lateral visibility at 2 m from the seedling; and distance to the nearest track. Where a correlation exceeded  $r = 0.7$  (criterion suggested by Tabachnick & Fidell 1989), we excluded the explanatory variable that exhibited the lowest correlation with the response variable (browsing delay). Thus, we excluded two covariates at the 4 m<sup>2</sup> level: number of adult trees (correlated with adult tree species richness,  $r = 0.82$ ,  $P < 0.0001$ ) and number of juvenile trees (correlated with juvenile tree species richness,  $r = 0.86$ ,  $P < 0.0001$ ). We also calculated the dominant vegetation type (grass, fern or other) by foliage cover for each patch at large and small scales. We tested these as strata, excluding continuous variables, in a separate analysis for each patch scale. 'Grass' comprised mixed introduced grasses less than 0.1 m in height, while 'fern' comprised swards of *P. esculentum* between 0.5 m and 1.2 m in height. 'Other' vegetation types were sedges (*Lomandra* spp.), small scrubby trees (*M. elliptica*) or vines. These were excluded from the analysis due to low sample size (at the large scale, sedge  $n = 2$  and tree  $n = 4$  patches; at the small scale, sedge  $n = 6$ , tree  $n = 7$ , and vine = 1 patches). Grass and fern patches were spatially interspersed across the study grid. We also tested for

the effect of the presence of the camera on browsing delay using the LIFETEST procedure with camera presence / absence (1 / 0) as strata.

To further explore the differences in browsing delay between fern- and grass-dominated patches at the large scale, we used the NLIN procedure (SAS 9.3) to fit a Gompertz function (a sigmoidal function where the approaches to the left and right asymptotes are asymmetrical) to the frequency of browsed seedlings in fern and grass patches over time. Following Pietrzykowski *et al.* (2003), we included a parameter that allowed us to compare browsing delay between the strata of vegetation type. There were 40 seedlings in fern and 35 in grass, and we therefore randomly removed five seedlings in fern from the analysis to achieve a balanced dataset before fitting the function.

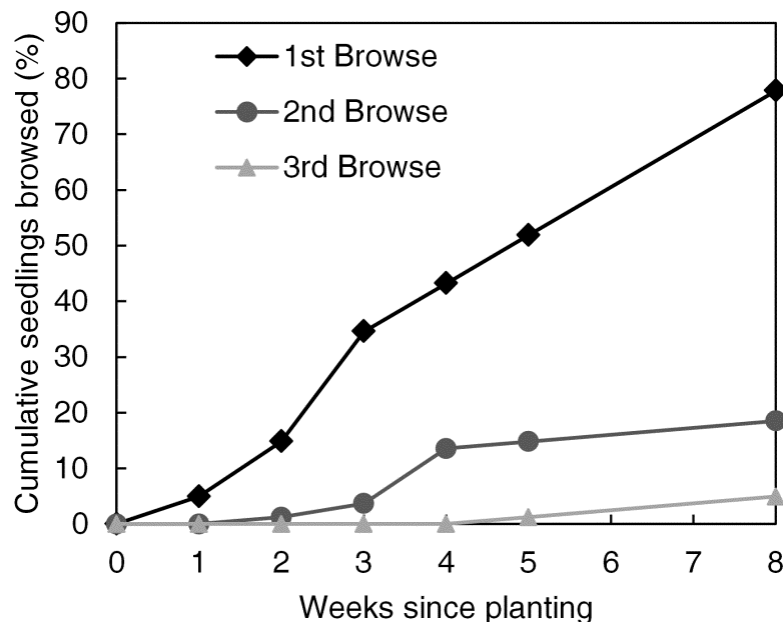
To determine if fern- and grass-dominated patches (classified at the large scale) differed in the characteristics that we found to be important overall (from survival analysis: canopy cover and number of browsed plant species in large patches; mean lateral visibility at 2m, understorey cover in small patches), we performed one-way analyses of variance comparing these characteristics between them (PROC ANOVA with Tukey's correction for multiple comparisons, SAS 9.3). The number of browsed species was square root transformed to achieve normality.

We determined the relative importance of the different mammalian herbivores to focal plant browsing, by assessing differences in the number of foraging visits per patch recorded on cameras (both general foraging within the patch and browsing of focal seedlings) between species (swamp wallabies, kangaroos, red-necked wallabies, rabbits). We used generalised linear mixed models fitted to a Poisson distribution with a log link function (log-linear models) with grid location as a random factor (GLIMMIX procedure, SAS 9.3).

## Results

Mammalian browsing of the focal seedlings was high, with mean  $68.2\% \pm \text{SE } 4.7$  leaf area loss by the end of the trial. However, if a seedling was browsed, it was usually browsed severely by the trial's end, with mean  $90.5\% \pm \text{SE } 17.8$  of leaf area lost. This resulted in essentially binary data (browsed / not browsed) for each seedling. Browsing delay (weeks until first browse) and browsing severity by the end of the trial (leaf area lost) were strongly negatively correlated ( $r = -0.84$ ,  $P < 0.0001$ ,  $n = 63$ ). Invertebrate herbivory contributed very little to overall browsing damage (mean  $7.3\% \pm \text{SE } 1.1$  leaf area loss in 8 weeks).

The rate of browsing (number of seedlings browsed per week) increased markedly in the first three weeks, and thereafter remained relatively constant (Fig. 2). At the end of the trial (eight weeks after planting), 78% of the seedlings had been browsed, 19% browsed a second time and 5% a third time. There was no evidence for spatial autocorrelation in browsing delay of focal seedlings (Moran's  $I = -0.011$ ,  $P = 0.74$ ; Geary's  $c = 0.996$ ,  $P = 0.87$ ).



**Figure 2.** Cumulative percentage of seedlings browsed for the first, second and third time at the end of each week after planting.

### Associational effects at large and small patch scales

Survival analysis showed a significant effect of three continuous patch variables on browsing delay (Table 1). At the large patch scale, canopy cover and number of browsed plant species were negatively related to browsing delay (i.e. seedlings were browsed earlier with greater canopy cover and with browsed neighbours). At the small patch scale, understorey vegetation cover was positively related to browsing delay (i.e. seedlings escaped browsing for longer with greater cover). Mean lateral visibility at the small patch scale exhibited a marginal negative relationship with browsing delay (i.e. seedlings of lower visibility escaped browsing for longer). Browsing delay was not significantly affected by the presence / absence of the camera ( $\chi^2_1 = 0.081$ ,  $P = 0.776$ ).

**Table 1.** Wilcoxon rank test for effects of covariates in small (4 m<sup>2</sup>) and large (100 m<sup>2</sup>) patches on browsing delay of focal seedlings (DF = 1). Significant effects are in bold.

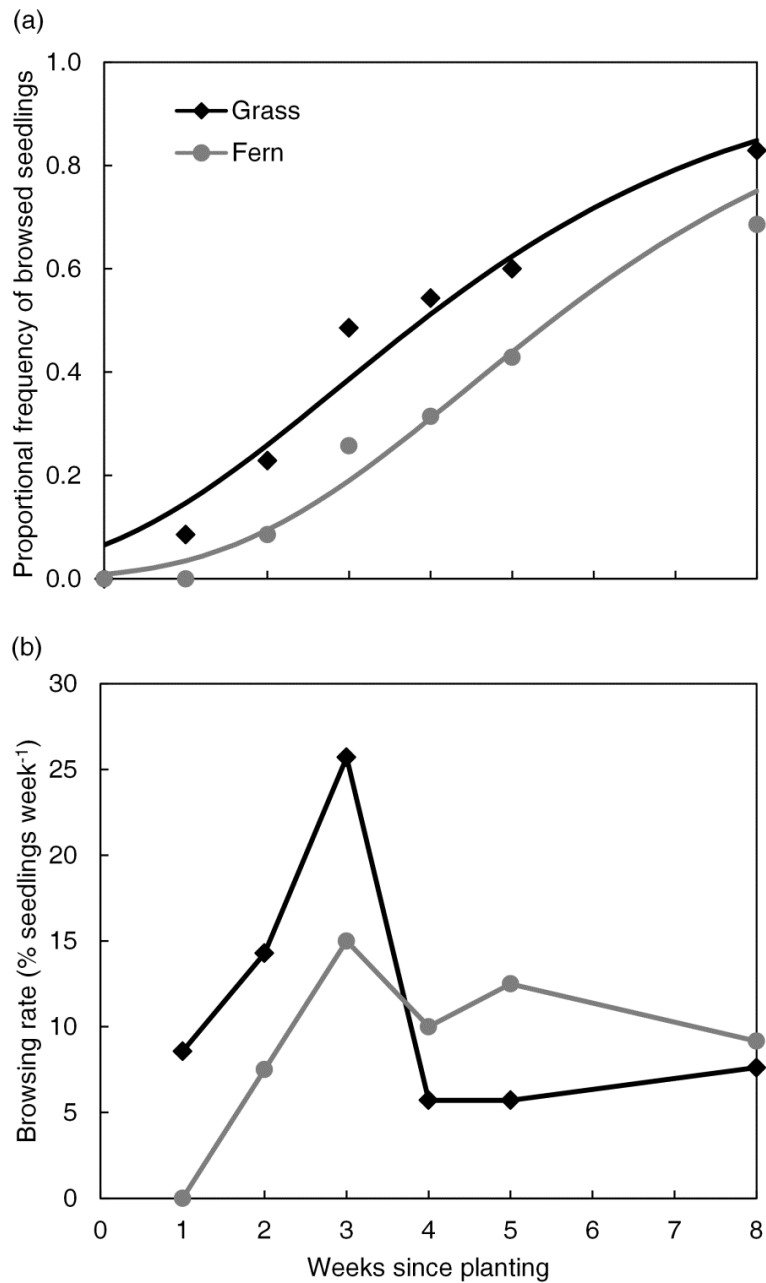
Variable	Patch size	T	SE	$\chi^2$	P
# Adult tree spp.	Large	-7.89	5.51	2.05	0.152
	Small	4.22	2.37	3.17	0.075
# Adult trees	Large	7.11	35.39	0.04	0.841
Canopy cover (%)	Large	-192.60	81.88	5.53	<b>0.019</b>
	Small	-173.20	146.70	1.39	0.238
# Browsed plant spp.	Large	-17.98	8.87	4.10	<b>0.043</b>
	Small	2.44	5.96	0.17	0.683
# Juvenile tree spp.	Large	-2.78	3.53	0.62	0.430
	Small	-0.16	1.85	0.01	0.932
# Juvenile trees	Large	-0.65	10.90	0.00	0.953
Understorey cover	Large	215.10	121.70	3.12	0.077
	Small	304.10	150.90	4.06	<b>0.044</b>
Lateral visibility	<Large >Small	-176.70	92.42	3.66	0.056
Variation in visibility (SD)	<Large >Small	26.72	40.45	0.44	0.509
Distance to track (m)	> Large	47.98	227.80	0.04	0.833

Browsing delay was lower in grass- than fern-dominated patches at the large scale ( $\chi^2_1 = 4.35$ ,  $P = 0.037$ ) but not at the small scale ( $\chi^2_1 = 1.11$ ,  $P = 0.293$ ). By the end of the trial, 83% of focal seedlings had been browsed in grass-dominated patches but only 69% in fern-dominated patches. The Gompertz function (Eq. 1) adequately described the proportional frequency ( $f$ ) of browsed seedlings over time in fern- and grass-dominated large patches (Fig. 3a):

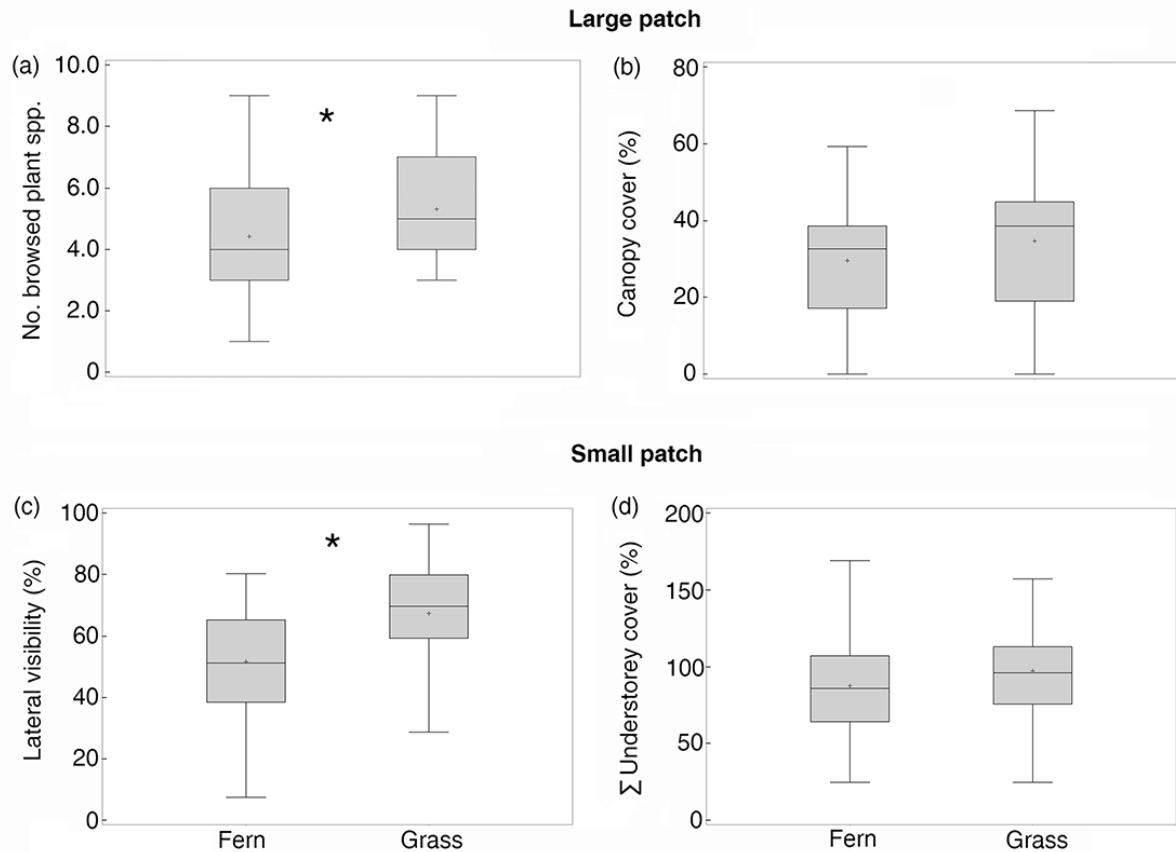
$$f = e^{-e^{-\beta_1(\text{weeks} - \beta_2 - \beta_3 * \text{type})}} \quad (\text{Eq. 1})$$

Where  $\beta_1 = 0.35$  (SE 0.031),  $\beta_2 = 2.86$  (SE 0.20),  $\beta_3 = 1.59$  (SE 0.30), weeks = weeks since planting, and type = vegetation type (grass = 0, fern = 1). The curve for the rate of increase in browsing frequency in both grass- and fern-dominated patches were therefore similar but delayed by 1.6 weeks (~11 days) in fern-dominated plots. The rate of seedling browsing was higher in grass than fern plots in the first three weeks after planting (Fig. 3b) but then dropped below the rate in fern patches from week four, coinciding with the reduction in availability of seedlings and overall reduction in browsing rate observed in Fig. 2.

Two of the significant continuous patch variables that affected seedling browsing delay differed significantly between fern- and grass-dominated large patches; (1) more browsed plant species at the large scale in grass than fern patches ( $F_{1,73} = 5.28$ ,  $P = 0.024$ ; Fig. 4a) and (2) greater mean lateral visibility at 2 m in grass than fern patches ( $F_{1,73} = 16.14$ ,  $P = 0.0001$ ; Fig. 4c). There were no significant differences between fern and grass patches in canopy cover in large patches ( $F_{1,73} = 1.87$ ,  $P = 0.18$ ; Fig. 4b) or understorey vegetation cover in small patches ( $F_{1,73} = 1.85$ ,  $P = 0.18$ ; Fig. 4d).



**Figure 3.** (a) Gompertz function describing the relationship between browsing delay of seedlings planted in grass- versus fern-dominated large patches. (b) Weekly rates of seedling browsing in grass- versus fern-dominated large patches.



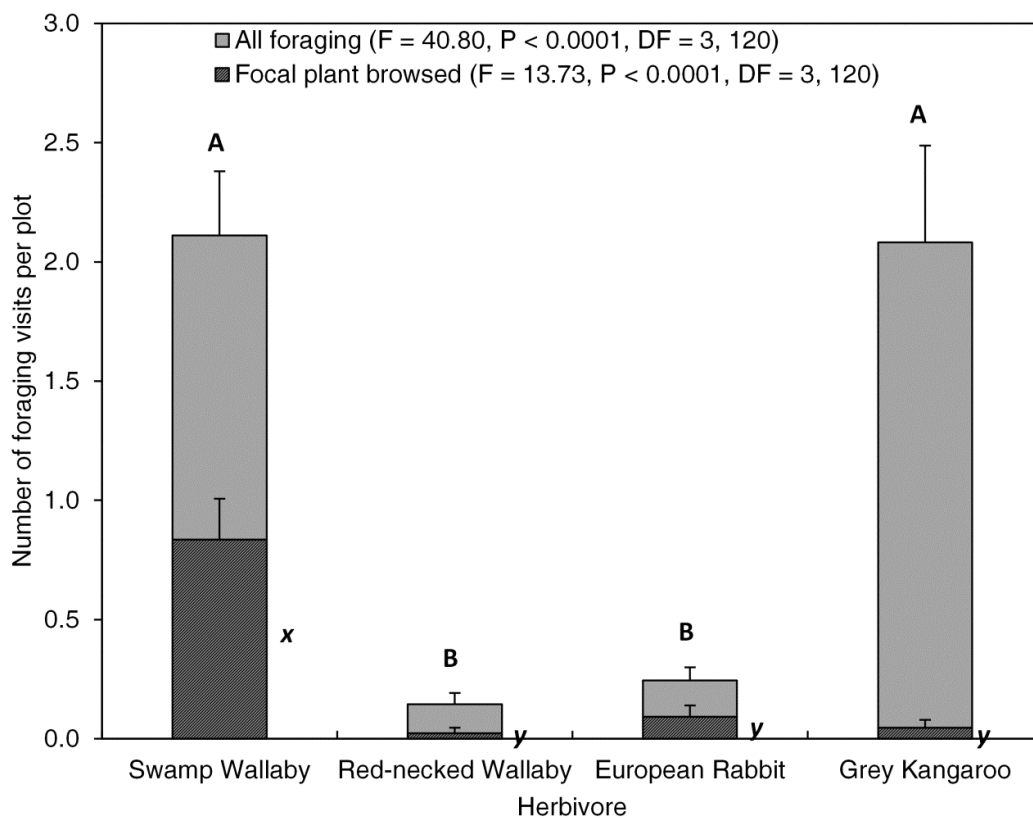
**Figure 4.** Box-and-whisker plots of (a) number of browsed plant species and (b) canopy cover in large patches, (c) lateral visibility and (d) understorey cover in small patches, as a function of vegetation type (at the large patch scale). An asterisk marks a significant pair-wise difference ( $\alpha = 0.05$ ).

#### *Foraging by different herbivore species*

Of the 283 foraging visits captured by motion-triggered cameras, 43 (15%) resulted in consumption of the focal seedling. The number of visits of both general foraging and browsing of focal seedlings differed significantly among herbivore species (Fig. 5). Swamp wallabies foraged significantly more frequently than red-necked wallabies and rabbits ( $P < 0.0001$ ) but not kangaroos ( $P = 0.32$ , Fig. 5). Swamp wallabies were responsible for 84% of browsing visits on focal seedlings, browsing them more frequently than any other herbivore ( $P < 0.0006$ ). The proportion of all foraging visits by a particular species, in which the focal seedling was browsed, was similar for swamp wallabies (30%) and rabbits (29%), but with few observations



of rabbits (swamp wallabies = 120 visits, rabbits = 14 visits). The proportion of all foraging visits by both kangaroos and red-necked wallabies, in which the focal seedling was browsed, was low (1% and 11% respectively; from 136 and 9 visits respectively). Swamp wallabies investigated the focal seedlings in 36% of foraging visits, and once investigated, consumed the seedling 86% of the time.



**Figure 5.** Mean number of video observations per plot of all foraging visits and the subset in which focal seedlings were browsed by mammalian herbivore species (L-S mean + SE). Capital letters (A, B) indicate significant differences between numbers of all foraging visits, while lower-case letters (x, y) represent differences between numbers of focal seedling browsing visits.

## Discussion

Here, we identified variables of existing vegetation patches, at different patch scales, that significantly affected escape from herbivory of eucalypt seedlings in a degraded woodland

system. This is the first study to find support for associational plant refuge by quantifying a suite of existing patch variables and determining the effectiveness of each in providing refuge for seedlings from free-ranging mammalian herbivores. Patch variables of canopy cover, number of browsed plant species, and dominant vegetation type at the large patch scale, and cover of understorey vegetation at the small patch scale, affected time to first browsing. Canopy cover affects herbivory via motivations to forage in a patch not directly related to associational refuge. All other variables fall within the domain of associational refuge and appeared to generate refuge via reductions in the search and detection phases of foraging. Within the multi-herbivore assemblage, we identified the species responsible for browsing damage (swamp wallaby) and importantly, identified one other species (European rabbit) that could significantly alter seedling escape from herbivory should population dynamics alter in the future. By defining the patch characteristics that delayed browsing and by quantifying the foraging response of the browser, we provide key steps towards developing a mechanistic understanding of the foraging patterns we observed. This is critical for developing a predictive model of browsing of focal plants in relation to neighbouring vegetation and, from an applied perspective, for developing effective restoration strategies for these focal plants.

#### *Associational effects at large and small patch scales*

At the large patch scale (100 m<sup>2</sup>), browsing of eucalypt seedlings was delayed with lower canopy cover, as fewer neighbouring plant species were browsed, and the dominant vegetation type was fern rather than grass, each presumably affecting the amount of time spent foraging in a patch and hence seedling encounter rate. While all of these variables can be linked to herbivore decisions, only the latter two can be linked to the provision of associational refuge.

The association of browsing delay with canopy cover could arise from several biotic and abiotic interactions with the herbivores. First, herbivores may selectively forage under canopy if

seedlings are more likely under adult trees (due to high seed drop directly below). Second, canopy cover may lower the risk of predation by raptors (e.g. wedge-tailed eagle *Aquila audax*, Leopole & Wolfe 1970), a pattern seen with other species (e.g. rodents, Longland & Price 1991). Third, canopy cover may provide protection from the elements. The high mid-summer temperatures (maximum temperatures up to 30°C) exacerbate water loss and thermoregulation costs known to affect diurnal movement patterns (e.g. kangaroos, Dawson 1972; feral pigs, Dexter 1999).

Focal plants escaped browsing for longer where fewer plant species were browsed at the large patch scale. This indicates associational refuge via the repellent plant mechanism, arising from between-patch selection based on perceived patch quality. We suggest that swamp wallabies abandon patches with few species worth browsing early, reducing search effort and therefore the probability of detecting and consuming the focal plant. Another browsing macropod, the red-bellied pademelon *Thylogale billardieri*, also spends less time foraging in low quality patches (Miller, McArthur & Smethurst 2009).

The study we present was limited to two months in the Australian summer, and would need to be repeated in winter to assess any seasonal effects. For example, while canopy may act as shelter from high summer temperatures, such patterns may not be seen in winter; equally, seasonal plant availability may affect the relative palatability of patches and thus alter associational effects (as documented by Miranda *et al.* 2011). Our results suggest that, at larger scales (i.e. ~100 m<sup>2</sup>), restoration will be more successful where canopy cover is low (conveniently, this is where tree planting is most needed) and where existing vegetation is less palatable.

Browsing delay could also be predicted from broad vegetation types at the large patch scale. The greater browsing delay of seedlings in fern- than in grass-dominated large patches is consistent with patterns established in artificial (forestry plantation) systems with different mammalian herbivores elsewhere in Australia (Bulinski & McArthur 2003; Pietrzykowski *et al.*

2003) and is associated with greater abundance of browsed plant species in grass patches (affecting the searching stage) and the greater lateral visibility (affecting focal seedling conspicuousness and hence detection). Planting seedlings in large fern-dominated patches (and avoiding pre-clearing) may therefore be a simple strategy for optimizing eucalypt restoration efforts as a result of reduced foraging visits from swamp wallabies.

At the small patch scale, browsing of eucalypt seedlings was delayed by higher understorey cover. We suggest, for several reasons, that higher understorey cover acted as a repellent plant refuge by reducing the conspicuousness of focal plants to herbivores and hence lowering the rate of detection, rather than by providing physical barriers to access resulting in a higher rejection rate of detected focal plants. First, if high understorey cover were an impediment to foraging, it should also have been a significant variable at the large scale. Second, swamp wallabies easily navigate through thick understorey and often rest within it during the day; they also consume and move through spiny plants, none of which form woody thickets (pers. obs.). Physical interference of neighbouring plant structures affecting detection and/or access and therefore consumption has been demonstrated in other studies of tree seedling herbivory by mammals (Pietrzykowski *et al.* 2003; Harmer *et al.* 2010; Castagneyrol *et al.* 2013). For swamp wallabies (the predominant browser), odour may play a large role in food detection (Bedoya-Pérez *et al.* 2014). If so, the denser vegetation may act by confounding olfactory cues (affecting the signal-to-noise ratio) or disrupting the flow of cues that otherwise facilitate the following of plumes (infotaxis; Vergassola, Villermaux & Shraiman 2007). Restoration strategies to optimise seedling retention and recruitment could therefore include planting tree seedlings in a relatively dense vegetation neighbourhood at the small scale, within a patch of poor quality plants and low canopy cover at the large scale. The competitive effects of planting seedlings in a high density vegetation neighbourhood need to be considered. We predict that any detrimental effects on growth and survival would likely be outweighed by associational refuge effects in a system under high browsing pressure such as this. Apart from associational refuge effects, we would expect no effect of neighbouring plant species richness on browsing

of focal plants, and a positive effect on growth of lower canopy cover through increased light availability.

Swamp wallabies are highly mobile animals and home range estimates (e.g. Troy & Coulson 1993) are greater than the total size of our experimental grid. However, the lack of spatial patterning to browsing of focal seedlings implies that the distance of 40 m between seedlings was sufficient to inhibit detection of the next closest seedling, and that the factors affecting differential browsing were operating at a smaller scale (< 40 m). While many studies discuss associational effects at different scales (e.g. Gómez-Aparicio *et al.* 2005; Bergvall *et al.* 2008; Rautio *et al.* 2008; Miller, McArthur & Smethurst 2009; Miranda *et al.* 2011), none have tested the independence of focal plants or plots of plants at the scales at which browsing damage was examined. Given that the swamp wallaby population density in the area is very high, that individuals occupy small, non-exclusive home ranges, and that there was no spatial patterning to browsing delay, it is highly unlikely that only one or two individuals were responsible for all the browsing in our study. Further, the monitoring equipment we used (cameras on wooden stakes) did not affect browsing delay. This is important because if herbivores did use such features to enhance their foraging strategy, any effects of natural refuge may be overridden.

#### *Foraging by different herbivore species*

Camera monitoring confirmed our prediction that the abundant browsing swamp wallaby would cause most damage to the focal eucalypt seedlings. We also found that the proportion of seedling browsing visits to overall foraging visits by rabbits was similar to that of swamp wallabies, suggesting that rabbits have the potential to significantly damage seedling stock (e.g. O'Reilly & McArthur 2000) if rabbit density rises. These results clearly elucidate which species should be targeted currently (swamp wallabies) or potentially (rabbits) when designing a restoration strategy, demonstrating the effectiveness of camera monitoring in multi-herbivore systems. Importantly, swamp wallabies consumed focal seedlings in most cases once they had investigated them (i.e. the rejection rate was low), confirming our prediction that the earlier

foraging stages, search and detection, rather than the decision to consume or reject, were the most important stages determining whether seedlings were browsed or not. Associational refuge was therefore likely driven by (1) a reduction in patch appeal (low patch quality) leading to decreased search effort, and (2) greater obstruction to visual or olfactory focal plant cues resulting in a lower rate of focal plant detection; this is in contrast to associational refuge by increased obstruction to focal plant access, increasing rejection rates of detected focal plants.

### *Implications for management*

We have shown that natural patch characteristics can provide associational refuge for planted seedlings in a restoration system with multiple herbivore species, at least in the short term. Given the nature of the factors providing refuge, the search and detection stages of the foraging process appear to be most disrupted, as opposed to the decision to consume a focal plant once found. This, then, is the key for future management strategies.

Choosing the best strategies to manage herbivory that will allow for seedling growth and recruitment to later developmental stages is context dependent. The aim of restoration in open woodlands versus forestry plantations, for example, is clearly different. In the former, often only a small number of trees need be recruited into the ecosystem, whereas forestry depends economically on maximising production by minimal loss on a tree count and biomass basis. Thus, although the difference in delay and proportion of browsed tree seedlings that we found here may be considered unsuccessful in forestry terms, it is potentially adequate for restoration, particularly if the absolute number of seedlings planted in strategic vegetation patches (here, for example, those dominated by fern) throughout the landscape is high. The success of such a strategy would depend on the robustness of the associational refuge to an increase focal plant density, but since only a relatively low density of trees is required in this natural system (as distinct from a production forestry plantation, for example), we suspect density could be low enough that refuge would still be effective. Additionally, the associational refuge provided by neighbours must outweigh any competitive effects detrimental to focal plant

growth, recruitment and survival in the long-term. For example, bracken fern, which we found provided refuge over 2 months, has been shown to reduce eucalypt seedling survival at 10 months post-planting through competition for light (Tolhurst & Turvey 1992). However, in systems such as in this study, where high browsing pressure is the major limitation to seedling survival and recruitment, prioritizing restoration actions to reduce herbivory are likely to improve outcomes despite potential competitive effects in the long-term. These are clearly important steps that must be resolved in future studies.

Finally, by identifying which species — within a multi-species herbivore community — is responsible for the damage, management strategies can be targeted to that species. Without such a targeted approach, resources may be wasted. In our system, understanding the foraging ecology of the swamp wallaby, specifically, is critical for future restoration success.

### **Acknowledgements**

Booderee National Park provided housing and financial support for this project. We thank Benjamin Croak and Elyce Coluccio for volunteering in the field, Mathew Crowther for advice on spatial statistics, and Park staff for facilitating site access. The University of Sydney Animal Ethics Committee approved all procedures (L04/2-2012/3/5696). All research was conducted under a National Parks Permit (BDR12/00001).

## References

- Anthelme, F. & Michalet, R. (2009) Grass-to-tree facilitation in an arid grazed environment (Air Mountains, Sahara). *Basic and Applied Ecology*, **10**, 437-446.
- Atsatt, P.R. & O'Dowd, D.J. (1976) Plant defense guilds. *Science*, **193**, 24-29.
- Baker, S.E., Johnson, P.J., Slater, D., Watkins, R.W. & Macdonald, D.W. (2007) Learned food aversion with and without an odour cue for protecting untreated baits from wild mammal foraging. *Applied Animal Behaviour Science*, **102**, 410-428.
- Baraza, E., Zamora, R. & Hódar, J.A. (2006) Conditional outcomes in plant–herbivore interactions: neighbours matter. *Oikos*, **113**, 148-156.
- Barbosa, P., Hines, J., Kaplan, I., Martinson, H., Szczepaniec, A. & Szendrei, Z. (2009) Associational resistance and associational susceptibility: having right or wrong neighbors. *Annual Review of Ecology Evolution and Systematics*, **40**, 1-20.
- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.
- Bee, J.N., Tanentzap, A.J., Lee, W.G., Lavers, R.B., Mark, A.F., Mills, J.A. & Coomes, D.A. (2009) The benefits of being in a bad neighbourhood: plant community composition influences red deer foraging decisions. *Oikos*, **118**, 18-24.
- Bergvall, U.A., Rautio, P., Kesti, K., Tuomi, J. & Leimar, O. (2006) Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. *Oecologia*, **147**, 253-260.
- Bergvall, U.A., Rautio, P., Sirén, H., Tuomi, J. & Leimar, O. (2008) The effect of spatial scale on plant associational defences against mammalian herbivores. *Ecoscience*, **15**, 343-348.



- Bulinski, J. & McArthur, C. (2003) Identifying factors related to the severity of mammalian browsing damage in eucalypt plantations. *Forest Ecology and Management*, **183**, 239-247.
- Bureau of Meteorology (2012) Climate Data Online. Commonwealth of Australia.
- Callaway, R.M., Kikodze, D., Chiboshvili, M. & Khetsuriani, L. (2005) Unpalatable plants protect neighbors from grazing and increase plant community diversity. *Ecology*, **86**, 1856-1862.
- Castagneyrol, B., Giffard, B., Péré, C. & Jactel, H. (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology*, **101**, 418-429.
- Davis, N.E., Coulson, G. & Forsyth, D.M. (2008) Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research*, **35**, 684-694.
- Dawson, T. (1972) Likely effects of standing and lying on the radiating heat load experienced by a resting kangaroo on a summer day. *Australian Journal of Zoology*, **20**, 17-22.
- Dexter, N. (1999) The influence of pasture distribution, temperature and sex on home-range size of feral pigs in a semi-arid environment. *Wildlife Research*, **26**, 755-762.
- Dexter, N., Hudson, M., James, S., MacGregor, C. & Lindenmayer, D.B. (2013) Unintended consequences of invasive predator control in an Australian forest: overabundant wallabies and vegetation change. *PLoS ONE*, **8**, e69087.
- Di Stefano, J. (2004) The importance of ecological research for ecosystem management: The case of browsing by swamp wallabies (*Wallabia bicolor*) in commercially harvested native forests. *Ecological Management & Restoration*, **5**, 61-67.
- Emerson, S., Brown, J., Whelan, C. & Schmidt, K. (2012) Scale-dependent neighborhood effects: shared doom and associational refuge. *Oecologia*, **168**, 659-670.
- Endler, J.A. (1991) Interactions between predators and prey. *Behavioural ecology: an evolutionary approach* (eds J.R. Krebs & N.B. Davies), pp. 169-196. Blackwell Scientific Publishing, Oxford.

- Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pickett, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011) Trophic downgrading of planet Earth. *Science*, **333**, 301-306.
- García, D. & Obeso, J.R. (2003) Facilitation by herbivore-mediated nurse plants in a threatened tree, *Taxus baccata*: local effects and landscape level consistency. *Ecography*, **26**, 739-750.
- Gómez-Aparicio, L., Valladares, F., Zamora, R. & Luis Quero, J. (2005) Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. *Ecography*, **28**, 757-768.
- Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, **14**, 1128-1138.
- Good, M.K., Clarke, P.J., Price, J.N. & Reid, N. (2014) Seasonality and facilitation drive tree establishment in a semi-arid floodplain savanna. *Oecologia*, **175**, 261-271.
- Harmer, R., Kiewitt, A., Morgan, G. & Gill, R. (2010) Does the development of bramble (*Rubus fruticosus* L. agg.) facilitate the growth and establishment of tree seedlings in woodlands by reducing deer browsing damage? *Forestry*, **83**, 93-102.
- Hjältén, J., Danell, K. & Lundberg, P. (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. *Oikos*, **68**, 125-131.
- Hobbs, R.J. & Norton, D.A. (1996) Towards a conceptual framework for restoration ecology. *Restoration Ecology*, **4**, 93-110.
- Hollis, C., Robertshaw, J. & Harden, R. (1986) Ecology of the swamp wallaby (*Wallabia bicolor*) in northeastern New-South-Wales .1. Diet. *Wildlife Research*, **13**, 355-365.
- Jensen, A.M., Götmark, F. & Löf, M. (2012) Shrubs protect oak seedlings against ungulate browsing in temperate broadleaved forests of conservation interest: a field experiment. *Forest Ecology and Management*, **266**, 187-193.

- Kotliar, N.B. & Wiens, J.A. (1990) Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos*, **59**, 253-260.
- Leopole, A. & Wolfe, T. (1970) Food habits of nesting wedge-tailed eagles, *Aquila audax*, in south-eastern Australia. *Wildlife Research*, **15**, 1-17.
- Longland, W.S. & Price, M.V. (1991) Direct observations of owls and heteromyid rodents: can predation risk explain microhabitat use? *Ecology*, **72**, 2261-2273.
- Milchunas, D.G. & Noy-Meir, I. (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, **99**, 113-130.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2006) Characteristics of tree seedlings and neighbouring vegetation have an additive influence on browsing by generalist herbivores. *Forest Ecology and Management*, **228**, 197-205.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2009) Spatial scale and opportunities for choice influence browsing and associational refuges of focal plants. *Journal of Animal Ecology*, **78**, 1134-1142.
- Miller, A.M., McArthur, C., Smethurst, P.J. & Wang, S.F. (2006) Preferences of two mammalian herbivores for tree seedlings and potential cover crops in plantation forestry. *Australian Forestry*, **69**, 114-121.
- Miranda, M., Díaz, L., Sicilia, M., Cristóbal, I. & Cassinello, J. (2011) Seasonality and edge effect determine herbivory risk according to different plant association models. *Plant Biology*, **13**, 160-168.
- O'Reilly, J.M. & McArthur, C. (2000) Damage to and intake of plantation seedlings by captive European rabbits (*Oryctolagus cuniculus*). *Australian Forestry*, **63**, 1-6.
- Palmer, S.C.F., Hester, A.J., Elston, D.A., Gordon, I.J. & Hartley, S.E. (2003) The perils of having tasty neighbors: grazing impacts of large herbivores at vegetation boundaries. *Ecology*, **84**, 2877-2890.
- Perry, J.N., Liebhold, A.M., Rosenberg, M.S., Dungan, J., Miriti, M., Jakomulska, A. & Citron-Pousty, S. (2002) Illustrations and guidelines for selecting statistical methods for quantifying spatial pattern in ecological data. *Ecography*, **25**, 578-600.

- Pietrzykowski, E., McArthur, C., Fitzgerald, H. & Goodwin, A.N. (2003) Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. *Journal of Applied Ecology*, **40**, 458-469.
- Rautio, P., Kesti, K., Bergvall, U.A., Tuomi, J. & Leimar, O. (2008) Spatial scales of foraging in fallow deer: implications for associational effects in plant defences. *Acta Oecologica*, **34**, 12-20.
- Senft, R.L., Coughenour, M.B., Bailey, D.W., Rittenhouse, L.R., Sala, O.E. & Swift, D.M. (1987) Large herbivore foraging and ecological hierarchies. *Bioscience*, **37**, 789-799.
- Smit, C., Den Ouden, J.A.N. & Müller-Schärer, H. (2006) Unpalatable plants facilitate tree sapling survival in wooded pastures. *Journal of Applied Ecology*, **43**, 305-312.
- Soliveres, S., García-Palacios, P., Castillo-Monroy, A.P., Maestre, F.T., Escudero, A. & Valladares, F. (2011) Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass–shrub interaction in a semi-arid ecosystem. *Oikos*, **120**, 710-719.
- Sprent, J.A. & McArthur, C. (2002) Diet and diet selection of two species in the macropodid browser-grazer continuum: do they eat what they 'should'? *Australian Journal of Zoology*, **50**, 183-192.
- Tabachnick, B.G. & Fidell, L.S. (1989) *Using Multivariate Statistics*, 2nd edn. Harper Collins Publishers Inc., New York.
- Tahvanainen, J.O. & Root, R.B. (1972) The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia*, **10**, 321-346.
- Tolhurst, K.G. & Turvey, N.D. (1992) Effects of bracken (*Pteridium esculentum* (Forst. f.) Cockayne) on eucalypt regeneration in west-central Victoria. *Forest Ecology and Management*, **54**, 45-67.
- Troy, S. & Coulson, G. (1993) Home range of the swamp wallaby, *Wallabia bicolor*. *Wildlife Research*, **20**, 571-575.

- Van Uytvanck, J., Maes, D., Vandenhaute, D. & Hoffmann, M. (2008) Restoration of woodpasture on former agricultural land: The importance of safe sites and time gaps before grazing for tree seedlings. *Biological Conservation*, **141**, 78-88.
- Vergassola, M., Villermaux, E. & Shraiman, B.I. (2007) 'Infotaxis' as a strategy for searching without gradients. *Nature*, **445**, 406-409.
- Wahl, M. & Hay, M.E. (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia*, **102**, 329-340.
- Wiggins, N.L., McArthur, C. & Davies, N.W. (2006) Diet switching in a generalist mammalian folivore: fundamental to maximising intake. *Oecologia*, **147**, 650-657.

## CHAPTER 3

---

### Herbivore Search Behaviour Drives Associational Plant Refuge

Published as: Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015) Herbivore search behaviour drives associational plant refuge. *Acta Oecologica*, **67**, 1–7.



Swamp wallaby searching for palatable seedling (with red terminal leaves) at the centre of a manipulated patch (*Coronidium elatum* neighbours).

## Abstract

Herbivore foraging decisions leading to consumption of a plant are complex and multi-faceted, shaped both by the plant itself and by its neighbours. Associational plant refuge arises when neighbours reduce focal plant susceptibility to herbivory. The specific foraging behaviours generating refuge patterns have rarely been examined in free-ranging systems, yet these are key to understanding why such refuge works or fails. We aimed to integrate herbivore foraging and associational plant refuge theories by linking foraging decisions directly to browsing outcomes on focal plants and their neighbours. We tested whether obstructive, unpalatable neighbours reduce the number of patch visits and/or interrupt searching, leading to associational refuge of focal plants. We compared visits by and behaviours of free-ranging mammalian browsers, swamp wallabies (*Wallabia bicolor*), in control and manipulated plant patches using cameras. Patches (7 m<sup>2</sup>) comprised a central focal plant (palatable native tree seedling, *Eucalyptus pilularis*) with neighbours of either existing or manipulated vegetation (unpalatable native daisy, *Coronidium elatum*). Wallabies made fewer visits to control than manipulated patches, but always browsed the focal plant during the first visit to a control patch. In contrast, wallabies often visited manipulated patches multiple times before browsing the focal plant. These 'futile' visits were both shorter and involved less searching time than visits when the focal plant was browsed. Focal plants escaped browsing for longer in manipulated than in control patches, and although none had escaped browsing after one year, survival was significantly greater in manipulated patches. We demonstrate that reduced investment in searching during visits to manipulated patches drove the associational plant refuge, but this refuge was eventually surmountable. Understanding the behaviours underpinning refuges allows better prediction of outcomes, and explains why refuge can collapse. By shaping foraging behaviour in patches, neighbouring vegetation can increase the probability that palatable plants persist despite high herbivore pressure.

## Introduction

From the herbivore perspective, plants occur in a landscape of vegetation patches varying in quality amongst an inedible matrix. An underlying assumption of optimal foraging theory is that animals forage to optimize their net energy intake per unit time (MacArthur & Pianka 1966) and for herbivores, such foraging involves searching among and within patches to find and consume plants that provide maximum net benefit. As herbivores deplete the food resources in a patch, returns diminish; and patch quitting is predicted when the cost of staying is greater than the cost of travelling to the next exploitable patch (marginal value theorem, Charnov 1976). Since the plant neighbourhood affects the cost-to-gain ratio of foraging in a patch, foraging decisions by herbivores are influenced not only by any particular plant's own chemical and physical characteristics, but also those of its neighbours, and of plants in other patches (Stephens & Krebs 1986). Neighbours can change the perceived profitability of a patch, and therefore how much time herbivores invest in searching for and consuming patch resources (Stephens 2008).

Where neighbouring plants protect focal plants from herbivory, the phenomenon is termed associational resistance or associational plant refuge (Tahvanainen & Root 1972; Atsatt & O'Dowd 1976; Pfister & Hay 1988). There are several mechanisms of associational plant refuge from vertebrate herbivores. Refuge can be generated when focal plants occur in patches of unpalatable or defended neighbours and herbivores select predominantly between rather than within patches (repellent plant defence, Atsatt & O'Dowd 1976; McNaughton 1978). This is the most commonly documented mechanism of associational plant refuge from vertebrate herbivory (Milchunas & Noy-Meir 2002). Alternatively, refuge may occur when neighbours are more palatable or undefended, so that they are consumed preferentially and thus allow focal plants to escape herbivores selecting between plants within patches (attractant-decoy or neighbour contrast defence; Atsatt & O'Dowd 1976; Bergvall *et al.* 2006). Neighbours can also protect a focal plant from herbivory by reducing its apparency, and



therefore its probability of detection by herbivores (Hambäck, Agren & Ericson 2000; Miller, McArthur & Smethurst 2007; Castagneyrol *et al.* 2013).

Associational plant refuges arise, therefore, from foraging decisions made by herbivores in relation to plant patches and individual plants within them. Most studies of associational plant refuge, however, focus on the plant perspective, quantifying outcomes of herbivory such as plant survival, size or damage, or depletion of artificial food in patches of differing quality while inferring the underlying foraging decisions from these outcomes. Baraza *et al.* (2006), for example, demonstrated that shrubs with greater physical defence (spines) and lower relative palatability provided better protection to saplings from ungulates. From this, they inferred that ungulates selected predominantly between patches rather than between plants within patches – the repellent plant mechanism of associational plant refuge. This inferential approach may tell us the scale of selection dominating herbivore foraging decisions, but we cannot determine how behaviours during visits to patches change as a result of the neighbouring vegetation.

We are aware of only two studies that have directly quantified the behaviour of vertebrate herbivores coupled with associational refuge, and these have assessed animals in captivity. Captive bred fallow deer *Dama dama* showed no difference in the amount of time spent or the number of individuals visiting patches of different quality, but ate less high tannin (low quality) pellets in patches dominated by low tannin (high quality) pellets – i.e. associational refuge by neighbour contrast defence (Bergvall *et al.* 2006). In contrast, captive pademelons *Thylogale billarierii* spent significantly less total time and time consuming neighbouring plants in constructed patches of low quality (herbicide) than high quality (grass), providing refuge for eucalypt seedlings via the repellent plant mechanism (Miller, McArthur & Smethurst 2009).

Our main aim was to compare the browsing behaviour of free-ranging herbivores in response to a focal plant and its neighbours in vegetation patches, and to quantify the browsing outcomes. In doing so, we unite concepts of foraging theory (the animal perspective) with associational refuge theory (the plant perspective), to provide a mechanistic understanding of

the differences in foraging outcomes for focal plants arising from behavioural responses to plant neighbours.

Our study system was a partially degraded area of a national park lacking much of its native eucalypt overstorey, in which native *Eucalyptus pilularis* tree seedlings were planted as part of restoration efforts. In Stutz et al. (2015), we found that browsing of these highly palatable eucalypt seedlings by free-ranging swamp wallabies *Wallabia bicolor* was delayed in control vegetation patches with higher cover of understorey vegetation and fewer browsed plant species (as associational refuge) and lower canopy cover (influencing habitat selection). Here, we manipulated vegetation patches with the aim of influencing wallaby foraging behaviour to enhance the associational plant refuge. We used field cameras to directly observe the browsing behaviour of wallabies in control and manipulated vegetation patches with *E. pilularis* seedlings as the focal plants. Observing behaviour allowed us to directly quantify and elucidate the foraging decisions underpinning any associational plant effects.

Manipulated neighbourhoods were predicted to lower the patch value in two ways; (1) by reducing the perceived net patch quality as a food source, and (2) by masking visual and olfactory cues from the focal plant, thus decreasing the cue-to-noise ratio (Schmidt, Dall & Van Gils 2010; Carthey, Bytheway & Banks 2011) and so reducing the capacity of wallabies to detect the focal plant. We therefore predicted that refuge in manipulated patches would be driven either by fewer visits to patches and/or by reduced time spent in a patch once there, specifically with less time spent searching. We expected the former if swamp wallabies perceive patch quality at a distance (deciding to avoid manipulated patches more often), and the latter if they assess patch quality once at a patch (deciding to leave manipulated patches early). Alternatively, if manipulated neighbours did not change the patch value, we predicted that refuge in manipulated patches would be driven by reduced search efficiency in patches; i.e. obstruction by neighbours leading to lower frequency of finding and consuming focal plants for the same absolute visit duration or search time.

## Materials and methods

### *Study site*

Our study site was a 50 ha area in the western part of Booderee National Park (BNP), Jervis Bay Territory, on the south coast of New South Wales, Australia (35°08'30"S, 150°39'10"E). The area was reclaimed from pine *Pinus radiata* plantation more than 20 years ago, with current vegetation comprising a mosaic of dense bracken fern *Pteridium esculentum*, shortly-cropped introduced grasses, sedge-like herbs *Lomandra* spp. and small scrubby trees *Monotoca elliptica*, with a sparse overstorey of remnant introduced *P. radiata*, and native *E. pilularis*, *E. botryoides* and *Acacia implexa*.

We have previously established that the abundant swamp wallaby was the most significant browser of *E. pilularis* seedlings at the site (Stutz *et al.* 2015). Swamp wallabies have a generalist diet, consuming a mixture of forbs, shrubs, ferns and grasses (Hollis, Robertshaw & Harden 1986; Osawa 1990), and are essentially solitary (Jarman & Coulson 1989). Other mammalian herbivores, including eastern grey kangaroos *Macropus giganteus*, red-necked wallabies *Macropus rufogriseus* and European rabbits *Oryctolagus cuniculus*, as well as herbivorous invertebrates, contributed little to eucalypt browsing (Stutz *et al.* 2015).

### *Study design*

We examined swamp wallaby behaviour in response to patches manipulated to create refuge for eucalypt seedlings versus control patches with existing vegetation. We selected patch sites from a grid of points that, in our previous study (Stutz *et al.* 2015), exhibited median browsing delays (three to five weeks) and therefore were expected to have patch characteristics that confer intermediate potential for wallabies to detect and browse seedlings. We planted one *E. pilularis* seedling (height = 525 ± 12 mm) at each of the 28 selected sites in June 2012 (winter). Shortly cropped introduced grasses dominated half of the sites, with the rest dominated by

bracken fern up to 0.5 m in height. We allocated control and manipulated treatments evenly between grass- and fern-dominated sites. In manipulated patches (n = 14), we surrounded each seedling with six plants of white paper daisy (*Coronidium elatum*) within a radius of 0.25 m (mean height approximately equal to *E. pilularis*). *C. elatum* is a local native species that is relatively unpalatable to swamp wallabies. It is one of a small cohort of plant species not browsed by wallabies in Booderee Botanic Gardens, an 80 ha area of cultivated and natural bushland within the park (pilot study and S. Pedersen, pers. comm.). It has a distinct odour, is broad-leaved and covered in hairs. All plants were raised from local seed stock at Booderee Botanic Gardens.

Patches were monitored for five weeks using motion-triggered infra-red trail cameras (ScoutGuard SG550, Hunting Cam Online, Gadsden SC, USA), set to record 60 s videos with instant re-trigger, and fastened to wooden posts (camera height = 0.6 m, distance to seedling = 1.5 m). We also visually inspected *E. pilularis* seedlings and *C. elatum* plants for browsing damage at one, two, three and five weeks after planting. We estimated the amount of foliage lost from each *E. pilularis* seedling and the cohort of *C. elatum* at each patch using an ordinal system following Pietrzykowski et al. (2003): 0 %, 1 – 5 %, 6 – 25 %, 26 – 50 %, 51 – 75 %, 76 – 95 %, 96 – 100 % (converted to mid-point values for analyses). We visually re-assessed seedlings for survival at one year post-planting.

#### *Herbivore behavioural response to patch treatments*

Swamp wallabies were responsible for all browsing behaviour involving the focal plant and we therefore excluded observations of other herbivores. We considered visits up to and including the first browsing of the focal plants. After the first browse, focal plants were considerably altered (partially or entirely consumed), and therefore no longer relevant for assessing potential refuge effects. Observations were delimited by a 1.5 m radius around the focal plant, equivalent to the distance between the camera and the focal plant, allowing us to observe behaviours in the area proximal to that defined by manipulated vegetation.

We quantified the number of visits per patch by swamp wallabies over the first five weeks after planting. To reduce any potential dependence of visits, we included only visits that occurred at least 20 min apart. Comparison of camera and visual inspection data indicated that the cameras did not capture all visits. We included only patches where cameras recorded visits (7 control and 13 manipulated patches) to avoid making assumptions about the number of visits where they did not. To test for the effect of patch treatment (control vs. manipulated) on the number of visits per patch, we used a log-linear model (GLIMMIX procedure, SAS 9.3, SAS Institute Inc., Cary, NC, USA). We also included existing vegetation (grass- vs. fern-dominated) and its interaction with patch treatment as fixed effects; they were not significant at  $\alpha = 0.25$  (criterion suggested by Winer, Brown & Michels 1991) and were thus removed in the final model.

We quantified time spent exhibiting a suite of behaviours in patches using JWatcher 1.0 ([www.jwatcher.ucla.edu](http://www.jwatcher.ucla.edu)). To test our hypothesis that patch treatment would lead to associational refuge by altering visit duration, we first compared the duration of visits as a function of patch type. However, wallabies browsed the focal plant at all first visits to control patches, but often visited manipulated patches before browsing the focal plant (see results). We therefore quantified the duration of visits between three treatment-outcome levels: (1) control patches when the focal plant was browsed, (2) manipulated patches when the focal plant was not browsed and (3) manipulated patches when the focal plant was finally browsed. We compared visit duration between the three treatment-outcome levels using a generalised linear mixed model with a negative binomial error distribution and patch identity as a random factor (GLIMMIX procedure, SAS 9.3). We also included existing vegetation type and its interaction with patch treatment as fixed effects; these effects were not significant but retained in the model because the interaction could not be rejected at  $\alpha = 0.25$  (see results).

We scored time allocated to searching and to other behaviours (vigilance, locomotion, resting, grooming, browsing focal plant, and browsing neighbouring plants) using a mutually exclusive ethogram for each independent visit (see Video 1 for examples of behaviours;

<http://dx.doi.org/10.1016/j.actao.2015.05.004>). Searching comprised sniffing while rearing the forelimbs or standing quadrupedally; vigilance constituted an abrupt pause in any previous behaviour accompanied by sudden movements of the head and ears; locomotion was defined as any forward motion that did not include any sniffing behaviour; grooming included coat and pouch cleaning using tongue and forelimbs; resting included any period of inactivity. Locomotion, grooming and resting together accounted for only 9.4% of the time spent over all visits and thus we excluded them from the analysis.

To elucidate differences in foraging behaviour between visits resulting in the different outcomes, we next compared the three dominant behaviours between patch treatments—time spent per visit searching, vigilant, and browsing plant neighbours—between the three treatment-outcome levels. For each behaviour, we ran a generalized linear mixed model with a negative binomial error distribution, treatment-outcomes and vegetation type as fixed effects and patch identity as a random effect (GLIMMIX procedure, SAS 9.3). The time spent browsing focal plants could only be compared between control and manipulated patches during visits where the focal plant was browsed, i.e. two levels of treatment, (1) and (3) above. We did not include patch identity as a random effect in this model since we considered only the first focal plant browsing visit at a patch, and thus there was only one visit per patch in this subset of the data. Vegetation type and its interaction with treatment-outcome were not significant at  $\alpha = 0.25$  for any of the behaviours and thus we present only the results of the reduced models.

We tested whether browsing of *C. elatum* was positively associated with focal plant browsing in manipulated patches, i.e. the collapse of the refuge. We compared the percentage of *C. elatum* foliage consumed (i) before and (ii) during the week in which the focal plant was browsed. We therefore only included patches where the focal plant was browsed in the first 5 weeks ( $n = 10$ , see results). We used the nonparametric Wilcoxon's signed rank test (UNIVARIATE procedure, SAS 9.3) because data were paired and not normally distributed.

### *Short- and longer-term effectiveness of associational plant refuge*

We considered browsing damage as either browsed or unbrowsed because swamp wallabies always consumed more than 80% of a focal plant's foliage when browsed ( $96.9 \pm 0.75\%$ ), consistent with our previous study (Stutz *et al.* 2015). To examine the effect of patch treatment and vegetation type (and their interaction) on the short-term temporal browsing pattern of seedlings, we performed nonparametric survival analysis on browsing delay (time to first browsing) using a Cox regression model (PHREG procedure, SAS 9.3). This method takes into account the right-censored event times, i.e. for seedlings that were not browsed after 5 weeks (Gardiner 2010). We selected the EXACT method for handling ties in browsing times (recommended by Allison 2010). The patch treatment by vegetation type interaction was not significant at  $\alpha = 0.25$  and was thus removed in the final model.

All focal plants had been browsed by one year post-planting and only some survived, i.e. with living root systems and signs of new growth. We therefore quantified and compared the number of seedlings surviving one year post-planting between patch treatments. To determine whether focal plant survival was related to the presence of *C. elatum* after one year, we compared the number of *C. elatum* plants remaining in patches (as a percentage of the six planted) across the two levels of the class factor (focal plant survived or not) using a Wilcoxon's exact test (NPAR1WAY procedure, SAS 9.3).

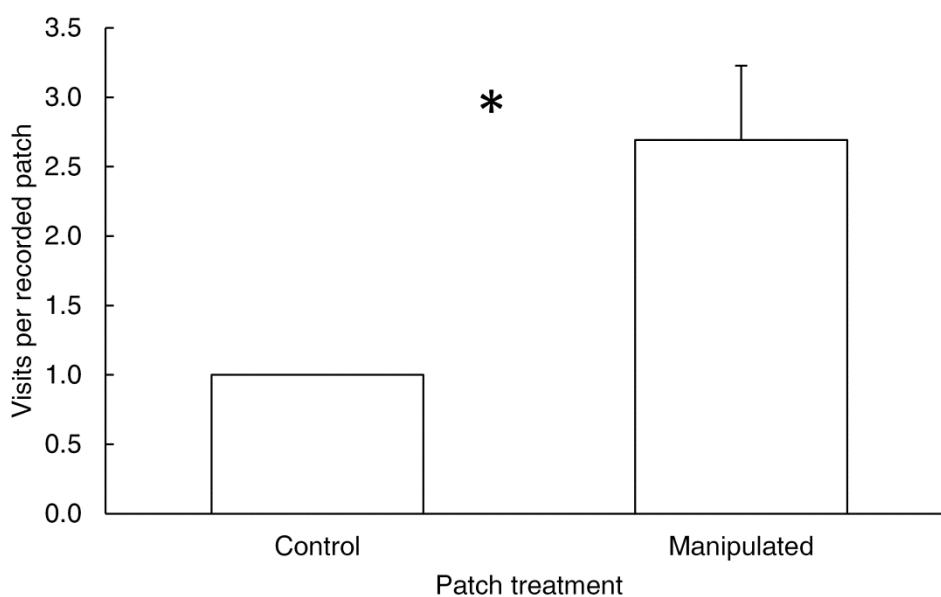
## **Results**

### *Herbivore behavioural response to patch treatments*

Motion-triggered cameras recorded 78 visits in total over the five-week period following planting. Swamp wallabies accounted for most of these (51 visits) and were responsible for all visits where the focal plant was browsed. European rabbits, eastern grey kangaroos and red-necked wallabies made the remaining visits. Of the visits by swamp wallabies, 42 occurred

before or during browsing of the focal plant. Both swamp wallabies and rabbits browsed *C. elatum* in manipulated patches (during 14 and 17 recorded visits, respectively).

Swamp wallabies visited control patches less often than manipulated patches ( $F_{1, 18} = 5.72$ ,  $P = 0.028$ , Fig. 1). Swamp wallabies always browsed the focal plant when they visited control patches. 'Futile' visits during which focal plants were not browsed occurred only in manipulated patches. Swamp wallabies made up to six visits to a manipulated patch without browsing the focal plant over the 5-week period ( $2.2 \pm 0.6$  futile visits patch<sup>-1</sup>).



**Figure 1.** Number of swamp wallaby visits per patch (mean + SE) in control and manipulated patches where cameras recorded visits (control  $n = 7$  patches, manipulated  $n = 13$  patches). Each control patch was visited only once (thus  $SE = 0$ ). Visits comprise those up to and including the visit during which the focal plant was first browsed. Asterisk denotes significant difference ( $P < 0.05$ ).

Swamp wallabies spent more time in manipulated patches when they browsed the focal plant than during 'futile' visits ( $F_{2, 20} = 9.24$ ,  $P = 0.0014$ , Fig. 2a). When the focal plant was browsed, visit duration did not differ significantly between patch treatments. The effect of existing vegetation type ( $F_{1, 20} = 0.19$ ,  $P = 0.66$ ) and its interaction with treatment ( $F_{2, 20} = 2.79$ ,  $P = 0.086$ ) were not significant. During 'futile' visits to manipulated patches, wallabies spent significantly less time searching than during visits to either patch treatment when they browsed

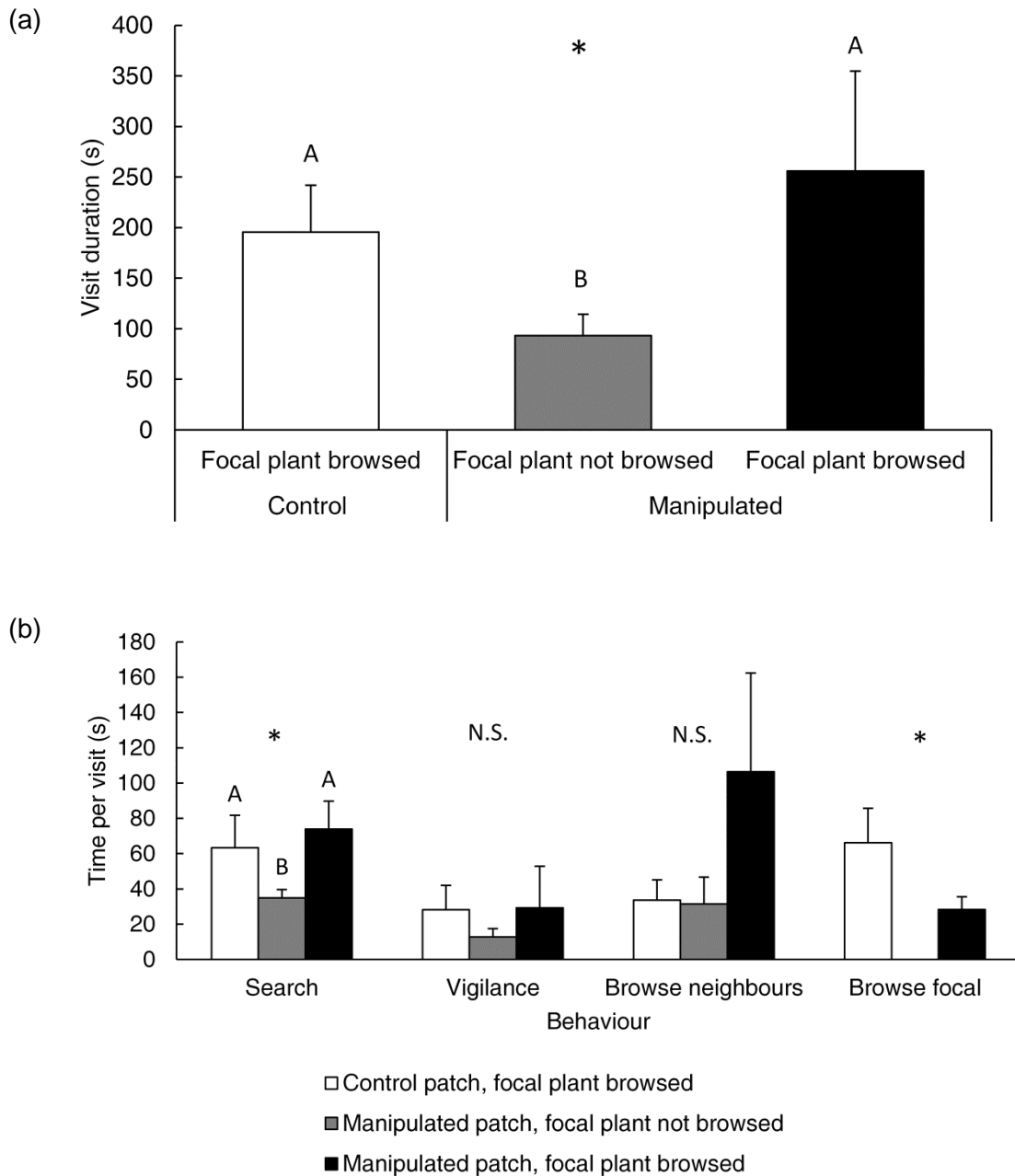


the focal plant ( $F_{2, 21} = 4.32$ ,  $P = 0.027$ , Fig. 2b). Time spent searching did not differ between control and manipulated patches when the focal plant was browsed. There was no significant difference in the time spent vigilant ( $F_{2, 21} = 0.45$ ,  $P = 0.64$ , Fig. 2b) or browsing neighbours ( $F_{2, 21} = 0.78$ ,  $P = 0.47$ , Fig. 2b). Wallabies spent more time browsing the focal plant in control than manipulated patches ( $F_{1, 12} = 5.19$ ,  $P = 0.042$ , Fig. 2b).

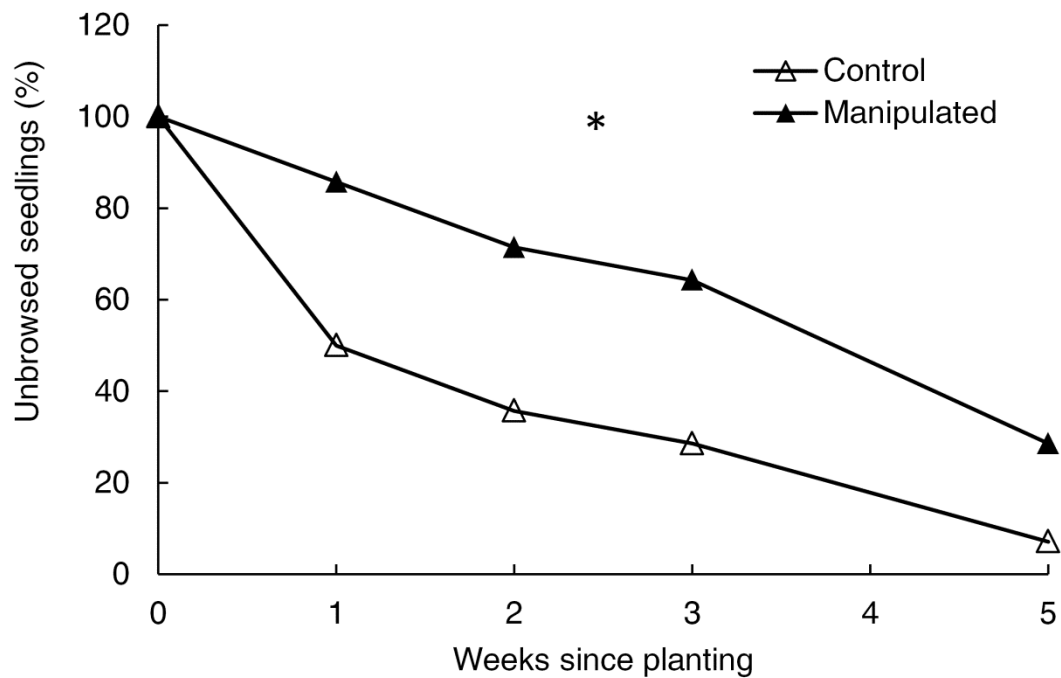
Most of the time spent browsing neighbours in manipulated patches involved consumption of *C. elatum* ( $32 \pm 13$  s visit<sup>-1</sup>), with relatively little time spent consuming other neighbouring vegetation ( $15 \pm 11$  s visit<sup>-1</sup>). Swamp wallabies consumed *C. elatum* foliage in 60 % of the manipulated patches in which they browsed the focal plant. They consumed significantly more *C. elatum* foliage in the week they browsed the focal plant ( $29.1 \pm 12.7$  %) than in previous weeks when they did not browse the focal plant ( $2.4 \pm 1.5$  %; Wilcoxon's signed rank test,  $S = 10.5$ ,  $P = 0.031$ ).

#### *Short- and longer-term effectiveness of associational refuge*

Focal plants in manipulated patches escaped herbivory for significantly longer than those in control patches in the first five weeks (Wald's  $\chi^2_1 = 4.71$ ,  $P = 0.030$ , Fig. 3). Vegetation type did not have a significant effect on time to browsing of focal plants (Wald's  $\chi^2_1 = 3.35$ ,  $P = 0.067$ ). The proportion of focal plants that escaped browsing after five weeks was greater in manipulated (29 %) than control patches (7 %).



**Figure 2.** Comparison of swamp wallaby (a) visit duration and (b) time allocation to search, vigilance, neighbour plant and focal plant browsing, by patch treatment when they browsed the focal plant or left it intact (mean + SE, asterisk = significant differences, letters = pairwise differences, N.S. = no significant differences). All observed visits to control patches resulted in focal plant browsing. The time spent browsing focal plants was compared only between visits to control and manipulated patches when the focal plant was browsed.



**Figure 3.** Cumulative percentage of focal plants in control and manipulated patches remaining unbrowsed at the end of each week after planting. Asterisk denotes significant difference ( $P < 0.05$ ).

All focal plants surviving after 1 year were heavily browsed and shorter than at planting ( $< 100$  mm compared to  $525 \pm 12$  mm at planting). Focal plant survival was greater in manipulated patches after one year, with five of 14 focal plants alive in manipulated patches but none in control patches. None of these five live plants had been browsed in the first two weeks after planting, and two had continued to escape browsing at the end of week 5. The percentage of *C. elatum* neighbours remaining was also greater in patches where focal plants had survived to 1 year than where they had not ( $86.7 \pm 9.7$  % compared to  $53.7 \pm 3.0$  %; Wilcoxon's exact test,  $S = 50.0$ , one-sided  $P = 0.053$ ).

## Discussion

Our study demonstrated that the presence of less palatable neighbours provided refuge for focal plants by altering the foraging behaviour of a generalist browser in food patches. Swamp

wallabies, the sole browsers of the focal eucalypt seedlings, visited manipulated patches more often than control patches. However most of the visits to manipulated patches were 'futile' and may have been a response to the higher degree of soil disturbance in constructing manipulated patches, rather than the plants themselves. That is, the visits were relatively short, involved less time spent searching within the patch, little if any browsing of the neighbour plants, and no browsing of the focal plant. In contrast, the first visit to control patches always resulted in focal plant browsing. The failure to locate focal plants in early visits to manipulated patches resulted in focal plants escaping herbivory for significantly longer than in control patches in the first five weeks after planting, and this was consistent with the pattern of focal plant survival after one year. When refuge in manipulated patches broke down, visit durations and search times did not differ from those in control patches. From this, it is clear that search behaviour by foraging wallabies was key to understanding associational plant refuge. Motivations that altered this search behaviour are, in turn, key for its ultimate failure.

#### *Foraging decisions are influenced by patch neighbours*

In manipulated patches, swamp wallabies reduced their search effort consistent with the predicted response to lower perceived patch quality. We suggest that this arose in part from their failure to detect focal plant cues, reducing their capacity to find and hence consume the focal plant. Rejection at the patch-level is commonly documented in the associational plant refuge literature on mammalian herbivores (e.g. Rebollo, Milchunas & Noy-Meir 2005; Parker, Caudill & Hay 2007; Miller, McArthur & Smethurst 2009). Here, we provide a behavioural and mechanistic basis to this common response.

The manipulated neighbourhood may have increased the difficulty of reaching the focal plant, causing swamp wallabies to consume it faster when browsing – swamp wallabies spent less time browsing the focal plant in manipulated than control patches. However, given that our manipulated neighbouring plants *C. elatum* were structurally dominant but not physically defended (e.g. no spines), physical impediment to foraging was an unlikely driver of the refuge

observed. Rather, we suggest *C. elatum* impeded detection of focal plant cues (olfactory or visual) and thus reduced plant apparency. Videos of the searching behaviour showed wallabies often sniffed and moved their heads in a way suggesting they used olfactory cues to locate the focal plant (see Video 1). This search behaviour is consistent with their ecology – olfactory cue-use is necessary to find the subterranean fungi that forms part of their diet (Hollis, Robertshaw & Harden 1986; Claridge & May 1994) – and with previous trials showing smell to be important in detecting artificial food pellets at feeding stations (Bedoya-Pérez *et al.* 2014). Reduced plant apparency has led to associational refuge in other systems, for example, reduction in leaf miner infestation on oak saplings *Quercus robur* hidden by tall neighbouring trees (Castagneyrol *et al.* 2013).

When swamp wallabies did finally find and consume the focal plant in manipulated patches, they also ate more *C. elatum* foliage (29.1%) than in previous visits (2.4%). We suggest that browsing of *C. elatum* alone, during these previous visits, constituted sampling and rejection rather than significant food intake. This supports patch-level selection by swamp wallabies and a repellent plant mechanism of refuge for the focal plants. The higher consumption of *C. elatum* foliage when the focal plant was browsed suggests enhanced herbivory of the neighbour during the focal plant browsing process (i.e. associational susceptibility of the neighbour via the 'shared doom' mechanism; Wahl & Hay 1995). This has been documented for other large herbivores feeding in mixed vegetation, for example, red deer and sheep at heather-grass boundaries (Palmer *et al.* 2003) and American bison in complex swards (Courant & Fortin 2010).

There is also evidence of some plant-level selection because when wallabies browsed the focal plant, they did not always consume the less preferred *C. elatum* foliage, and removed proportionally less of the available foliage from *C. elatum* than focal plants. Similarly, other mammalian herbivores including red-bellied pademelons, mountain hares *Lepus timidus* and voles *Microtus agrestis* have been shown to select at multiple scales despite demonstrating overall patterns consistent with repellent plant refuge (Hjältén, Danell & Lundberg 1993; Miller,

McArthur & Smethurst 2009). We suggest that refuge in manipulated patches eventually failed because swamp wallabies selected between plants within patches. Repellent plant refuge has been shown to fail when the scale of herbivore selection was too fine (McNaughton 1978) and this, in turn, may be related to proximity of both the focal plant to its neighbours (Bergvall *et al.* 2008) and one patch to the next (Rautio *et al.* 2008).

#### *Associational plant refuge results in tolerance to herbivory*

Associational plant refuge eventually failed in manipulated patches to the extent that all seedlings were eventually detected and consumed. However, the plant neighbours still facilitated tolerance to herbivory – reducing the effect of browsing on plant fitness (Strauss & Agrawal 1999) – and ultimately aiding the survival of focal plants. We suggest tolerance was facilitated via extrinsic mechanisms, rather than improving intrinsic physiological tolerance mechanisms of the focal plant itself (Rosenthal & Kotanen 1994), given that more *C. elatum* plants remained in manipulated patches where seedlings survived than where seedlings did not. One extrinsic mechanism is to increase the interval for recovery between browsing events, i.e. reducing browse frequency, consistent with our short-term results. Reduced frequency of damage has been linked to greater probability of plant survival in a suite of savannah tree species (Mundim *et al.* 2012). A second mechanism is to facilitate regeneration after browsing damage, for example, by providing favourable micro-environmental conditions (Gómez-Aparicio *et al.* 2005; Soliveres *et al.* 2011). This mechanism may have facilitated the new growth we observed on browsed seedlings amongst *C. elatum* neighbours.

#### *Theoretical and practical implications*

Our study is the first to link the provision of associational plant refuge to a specific behaviour – reduced investment in foraging search within patches – in free-ranging vertebrate herbivores. Understanding this behaviour also helped to explain why such refuge effects can break down. Manipulated vegetation was not an impenetrable barrier to herbivory; during some visits, a swamp wallaby browsed the focal plant regardless of the manipulated

vegetation. During such visits, we propose that wallabies were able to detect focal plant cues despite the manipulated vegetation, or were more motivated to respond to poor cues, and therefore invested as much time in searching as in control patches.

However, an associational plant refuge is specific to the particular combination of focal plant, neighbouring plants and herbivore species, and can vary enormously between different systems. In contrast to the short-term repellent plant refuge that we demonstrated, some studies show no reduction in palatable plant browsing amongst unpalatable neighbours (Milligan & Koricheva 2013), while in others, neighbours protected palatable plants from herbivores in the long-term (Parker, Caudill & Hay 2007). The level of protection from herbivory provided by neighbours can also be reduced under altered conditions in the same system. High herbivore pressure has been shown to diminish the effectiveness of associational plant refuge by increasing damage to neighbours and therefore reducing their protective capacity (Smit *et al.* 2007) or by increasing the net foraging effort so that focal plants are found despite the presence of neighbours (Brooker *et al.* 2006). We do not yet understand the patch-level behavioural mechanisms responsible for these changes. Herbivore foraging decisions drive associational plant refuge and its collapse; refuge therefore needs to be examined from the behavioural perspective to be fully understood (Le Bagousse-Pinguet, Gross & Straile 2012) and effectively manipulated.

Exploitation of associational refuges in ecological management will depend on how they function in altering herbivore foraging decisions. We found that refuge was achieved not by reducing the number of visits to a patch but the investment in search once in a patch, and therefore indirectly, the success in finding the focal plant. The behavioural response to plant neighbours may differ between herbivore species and therefore require a varied approach to creating refuge for focal plants among ecological communities. Understanding the behavioural mechanisms behind associational plant refuge may help explain circumstances when refuge works and when it fails. An important next step is to determine whether associational plant

refuge that herbivores penetrate can nevertheless facilitate focal plant tolerance over timescales necessary for tree establishment.

### **Acknowledgements**

Booderee National Park provided housing and financial support for this project. We thank Booderee Botanic Gardens, particularly Stig Pedersen, for collecting seeds and raising the plants used. The University of Sydney Animal Ethics Committee approved all procedures (L04/2-2012/3/5696). All research was conducted under a National Parks Permit (BDR12/00001).



## References

- Allison, P.D. (2010) *Survival analysis using SAS: a practical guide*, 2nd edn. SAS Institute Inc, Cary, NC.
- Atsatt, P.R. & O'Dowd, D.J. (1976) Plant defense guilds. *Science*, **193**, 24-29.
- Baraza, E., Zamora, R. & Hódar, J.A. (2006) Conditional outcomes in plant–herbivore interactions: neighbours matter. *Oikos*, **113**, 148-156.
- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.
- Bergvall, U.A., Rautio, P., Kesti, K., Tuomi, J. & Leimar, O. (2006) Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbour contrast susceptibility and defence. *Oecologia*, **147**, 253-260.
- Bergvall, U.A., Rautio, P., Sirén, H., Tuomi, J. & Leimar, O. (2008) The effect of spatial scale on plant associational defences against mammalian herbivores. *Ecoscience*, **15**, 343-348.
- Brooker, R.W., Scott, D., Palmer, S.C.F. & Swaine, E. (2006) Transient facilitative effects of heather on Scots pine along a grazing disturbance gradient in Scottish moorland. *Journal of Ecology*, **94**, 637-645.
- Carthey, A.J.R., Bytheway, J.P. & Banks, P.B. (2011) Negotiating a noisy, information-rich environment in search of cryptic prey: olfactory predators need patchiness in prey cues. *Journal of Animal Ecology*, **80**, 742-752.
- Castagneyrol, B., Giffard, B., Péré, C. & Jactel, H. (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology*, **101**, 418-429.
- Charnov, E.L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, **9**, 129-136.

- Claridge, A.W. & May, T.W. (1994) Mycophagy among Australian mammals. *Australian Journal of Ecology*, **19**, 251-275.
- Courant, S. & Fortin, D. (2010) Foraging decisions of bison for rapid energy gains can explain the relative risk to neighboring plants in complex swards. *Ecology*, **91**, 1841-1849.
- Gardiner, J.C. (2010) Survival analysis: overview of parametric, nonparametric and semiparametric approaches and new developments. *SAS Global Forum*.
- Gómez-Aparicio, L., Valladares, F., Zamora, R. & Luis Quero, J. (2005) Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. *Ecography*, **28**, 757-768.
- Hambäck, P.A., Agren, J. & Ericson, L. (2000) Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology*, **81**, 1784-1794.
- Hjältén, J., Danell, K. & Lundberg, P. (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. *Oikos*, **68**, 125-131.
- Hollis, C., Robertshaw, J. & Harden, R. (1986) Ecology of the swamp wallaby (*Wallabia bicolor*) in northeastern New-South-Wales .1. Diet. *Wildlife Research*, **13**, 355-365.
- Jarman, P. & Coulson, G. (1989) Dynamics and adaptiveness of grouping in macropods. *Kangaroos, wallabies and rat-kangaroos*, pp. 527-547.
- Le Bagousse-Pinguet, Y., Gross, E.M. & Stralle, D. (2012) Release from competition and protection determine the outcome of plant interactions along a grazing gradient. *Oikos*, **121**, 95-101.
- MacArthur, R.H. & Pianka, E.R. (1966) On optimal use of a patchy environment. *The American Naturalist*, **100**, 603-609.
- McNaughton, S.J. (1978) Serengeti ungulates: feeding selectivity influences the effectiveness of plant defense guilds. *Science*, **199**, 806-807.
- Milchunas, D.G. & Noy-Meir, I. (2002) Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos*, **99**, 113-130.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2007) Effects of within-patch characteristics on the vulnerability of a plant to herbivory. *Oikos*, **116**, 41-52.

- Miller, A.M., McArthur, C. & Smethurst, P.J. (2009) Spatial scale and opportunities for choice influence browsing and associational refuges of focal plants. *Journal of Animal Ecology*, **78**, 1134-1142.
- Milligan, H.T. & Koricheva, J. (2013) Effects of tree species richness and composition on moose winter browsing damage and foraging selectivity: an experimental study. *Journal of Animal Ecology*, **82**, 739-748.
- Mundim, F., Bruna, E., Vieira-Neto, E.M. & Vasconcelos, H. (2012) Attack frequency and the tolerance to herbivory of Neotropical savanna trees. *Oecologia*, **168**, 405-414.
- Osawa, R. (1990) Feeding strategies of the swamp wallaby, *Wallabia Bicolor*, on North Stradbroke Island, Queensland. I: Composition of diets. *Wildlife Research*, **17**, 615-621.
- Palmer, S.C.F., Hester, A.J., Elston, D.A., Gordon, I.J. & Hartley, S.E. (2003) The perils of having tasty neighbors: grazing impacts of large herbivores at vegetation boundaries. *Ecology*, **84**, 2877-2890.
- Parker, J., Caudill, C. & Hay, M. (2007) Beaver herbivory on aquatic plants. *Oecologia*, **151**, 616-625.
- Pietrzykowski, E., McArthur, C., Fitzgerald, H. & Goodwin, A.N. (2003) Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. *Journal of Applied Ecology*, **40**, 458-469.
- Rautio, P., Kesti, K., Bergvall, U.A., Tuomi, J. & Leimar, O. (2008) Spatial scales of foraging in fallow deer: implications for associational effects in plant defences. *Acta Oecologica*, **34**, 12-20.
- Rebollo, S., Milchunas, D.G. & Noy-Meir, I. (2005) Refuge effects of a cactus in grazed short-grass steppe. *Journal of Vegetation Science*, **16**, 85-92.
- Rosenthal, J.P. & Kotanen, P.M. (1994) Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution*, **9**, 145-148.
- Schmidt, K.A., Dall, S.R.X. & Van Gils, J.A. (2010) The ecology of information: an overview on the ecological significance of making informed decisions. *Oikos*, **119**, 304-316.

- Smit, C., Vandenberghe, C., Ouden, J.d. & Müller-Schärer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265-273.
- Soliveres, S., García-Palacios, P., Castillo-Monroy, A.P., Maestre, F.T., Escudero, A. & Valladares, F. (2011) Temporal dynamics of herbivory and water availability interactively modulate the outcome of a grass–shrub interaction in a semi-arid ecosystem. *Oikos*, **120**, 710-719.
- Stephens, D. (2008) Decision ecology: foraging and the ecology of animal decision making. *Cognitive, Affective, & Behavioral Neuroscience*, **8**, 475-484.
- Stephens, D.W. & Krebs, J.R. (1986) *Foraging theory*. Princeton University Press, New Jersey.
- Strauss, S.Y. & Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution*, **14**, 179-185.
- Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015) Associational refuge in practice: can existing vegetation facilitate woodland restoration? *Oikos*, **124**, 571–580.
- Tahvanainen, J.O. & Root, R.B. (1972) The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia*, **10**, 321-346.
- Wahl, M. & Hay, M.E. (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia*, **102**, 329-340.
- Winer, B.J., Brown, D.R. & Michels, K.M. (1991) *Statistical principles in experimental design*, 3rd edn. McGraw-Hill, New York.

## CHAPTER 4

---

### Follow Your Nose:

### A Browsing Mammal Uses Leaf Odour as a Foraging Cue

Submitted as: Stutz, R.S., Banks, P.B., Proschogo, N. & McArthur, C. (in review) Follow your nose: a browsing mammal uses leaf odour as a foraging cue.



Swamp wallaby investigating a vial containing a *Eucalyptus pilularis* seedling.

## Abstract

Studies of odour-driven foraging by mammals focus on attractant cues emitted by flowers, fruits and fungi. Yet the leaves of many plant species worldwide produce odour, which could act as a cue for foraging mammalian herbivores. Leaf odour may thus improve foraging efficiency for such herbivores in many ecosystems, by reducing search time, particularly, but not only, for plants that are visually obscured. We tested the use of leaf odour by a free-ranging mammalian browser, the swamp wallaby *Wallabia bicolor*, to find and browse palatable tree seedlings *Eucalyptus pilularis*. Wallabies used leaf odour (cut seedlings in vials) to find patches earlier, and visited and investigated them more often than control patches (empty vials), supporting the hypothesis that wallabies use seedling odour to enhance search efficiency. In contrast, the grey kangaroo *Macropus giganteus*, a grazer, showed no response to seedling odour. To distinguish the effects of visual and olfactory cues on browsing, we quantified browsing delay for whole seedlings in three treatments: upright (normal cues), pinned to the ground (reduced visual cues), and upright plus pinned seedlings (double olfactory cues). Wallabies browsed seedlings equally quickly in all treatments. We conclude that odour cues play a critical role in food-finding by swamp wallabies, and that these animals are finely tuned to detecting these cues with their threshold for detection reached by odours from only a single plant. The global significance of leaf odour in foraging by mammalian herbivores consuming conifers, eucalypts and other odour-rich species requires greater attention.

## Introduction

Plants emit odour as volatile organic compounds (VOCs) that disseminate across the landscape. Many of these odours act as signals to service-providing animals; the perfume of flowers attracts pollinators (Hoballah *et al.* 2005) and the smell of ripe fruits attracts seed dispersers (Hodgkison *et al.* 2007). But leaves also emit odour and, if exploited by herbivores to find food, may be a cue with costs to plants (Fraenkel 1959). We have limited understanding of the food-finding mechanisms used by vertebrate herbivores, particularly the role played by leaf odour. Yet leaf odour could be particularly important ecologically, as a cue that is effective from a distance or when a plant is visually cryptic, thereby increasing foraging efficiency as herbivores face the challenge of finding palatable plants amongst an array of vegetation.

The largest group of compounds responsible for plant odour are the terpenes (Langenheim 1994). Terpenes are not only VOCs but are also (usually mildly) toxic; thus they can act as a deterrent signal to herbivores by indicating a post-ingestive cost (Langenheim 1994). But browsers come into contact with toxins in most foods they ingest (Foley, Iason & McArthur 1999), and have evolved physiological and behavioural means to reduce the cost of consuming them (McArthur, Hagerman & Robbins 1991). Browsers, as specialists of toxic food, may therefore hijack the deterrent VOCs emitted by leaves and use them as cues for finding food (Bedoya-Pérez *et al.* 2014). The 'evolutionary irony' resulting from the arms race between plants and their herbivores is a well-established phenomenon, including the classic example of specialist invertebrates using toxic plant odours to target their plant hosts (Fraenkel 1959; Ehrlich & Raven 1964), and more recently, specialist vertebrates identifying palatable plants by the presence of physical defences (Kohl, Miller & Dearing 2015). Experimental evidence for the use of leaf odour by vertebrates as a cue to find food is lacking.

Here we test whether a free-ranging generalist browser uses leaf odour cues to detect plants. Our system comprised the swamp wallaby *Wallabia bicolor* and *Eucalyptus pilularis* seedlings. In a previous study, swamp wallabies used 1,8-cineole, a toxic VOC found in many eucalypt

species, to locate artificial food pellets in an inedible matrix at feeding stations (Bedoya-Pérez *et al.* 2014). *Eucalyptus pilularis* produces a suite of VOCs including terpenes (Molangui *et al.* 1997), and its seedlings are a highly preferred food resource for wallabies (Stutz *et al.* 2015). By targeting this early growth stage, wallabies often need to detect seedlings hidden among other vegetation.

We first confirmed that visits to patches by swamp wallabies were not random encounters but driven by seedling cues, and that these seedlings emitted VOCs that could act as cues. We then tested herbivore responses to seedling odour in the absence of any visual cues using buried vials that contained cut seedlings or were empty. We compared the responses of wallabies with eastern grey kangaroos *Macropus giganteus*; the latter feed mainly on grasses and forbs (Davis, Coulson & Forsyth 2008), and while some of these plants also emit VOCs, we expected that their odour would differ from that of eucalypt seedlings. Kangaroos were therefore not expected to be attracted to seedling odour and served as a procedural control. To test the use of odour cues in finding whole plants, we then quantified the browsing response of wallabies to seedlings with reduced visual or enhanced odour cues. We predicted that if wallabies used odour to find plants, they would browse visually cryptic seedlings as quickly as control seedlings, and a quantitative increase in odour cues would lead to earlier browsing.

## **Materials and methods**

### *Study site*

We conducted our study in 50 ha of degraded coastal woodland in Booderee National Park, Jervis Bay Territory, Australia (35°08'30"S, 150°39'10"E). The area is a mosaic of dense bracken fern (*Pteridium esculentum*), introduced grasses, herbs (*Lomandra* spp.) and small scrubby trees (*Monotoca elliptica*). The overstorey is sparse, comprising remnant introduced *Pinus radiata*, and native *E. pilularis*, *E. botryoides* and *Acacia implexa*.



### *Experiment 1: Random vs. cue-based foraging*

To determine whether swamp wallabies encountered patches at random or used plant cues to find rewarding patches, we compared the time taken by wallabies to visit patches varying in the presence and quantity of a food reward (palatable *E. pilularis* seedlings). We used a paired design, consisting of one patch with either one or three seedlings and one patch without a seedling. We constructed a transect 10 m into the woodland from a gravel track, with patches in each pair separated by 30 m, and at least 60 m to the next pair (see Appendix 1). To record the first visits to patches, we filmed each patch using a motion-triggered infra-red camera (ScoutGuard SG550, Hunting Cam Online, Gadsden, SC, USA) fixed to a shelf at 1 m height, and set to record 60 s videos with instant re-trigger.

### *Availability of odour cues from whole seedlings*

To confirm that odour cues were available from whole seedlings, we quantified the VOCs in the headspaces of intact *Eucalyptus pilularis* seedlings ( $n = 10$ ). We inserted each seedling upside-down into a 250 mL glass cylinder ( $h = 30$  cm) and held it in place using a clamp and retort stand (see Fig. S1). The whole seedling including all the foliage was held inside the cylinder, with only a small section of the stem (~2 cm) protruding from the top, as well as the plastic forestry tube containing the roots and soil. We sealed the top of the cylinder by wrapping aluminium foil around the stem of the seedling and securing the foil around the rim of the cylinder with rubber bands. We introduced a Solid Phase Micro-Extraction (SPME) fibre (red 100 $\mu$ m polydimethylsiloxane; Supelco, Bellefonte, PA, USA) through the foil and allowed it to extract the headspace VOCs for 20 min. The VOCs are adsorbed onto the fibre until equilibrium is reached between the headspace and fibre, allowing for direct comparison of relative quantities of VOCs between samples prepared under the same conditions (Vas & Vékey 2004); this solvent-free technique is commonly used to assess the composition and quantities of plant VOC emissions in ecological studies (Hanley *et al.* 2013; Low *et al.* 2014).

We analysed samples by combined gas chromatography/ion-trap mass spectrometry (GC-ITMS). We used a ThermoQuest Trace GC Ultra-Finnigan PolarisQ ion trap mass

spectrometer (Austin, TX, USA) with a Zebron ZB-5MS column (5 % phenyl, 95 % dimethylpolysiloxane; 30 m x 0.25 mm i.d., 0.25 µm film thickness; Phenomex Inc., Torrance, CA, USA) under ionization energy of 70 eV. We inserted the SPME fibre into the injector operated in splitless mode at 200 °C. The carrier gas was helium at a constant flow rate of 1.5 mL min<sup>-1</sup>. The oven temperature was held at 40 °C for 2 min, increased at 10 °C min<sup>-1</sup> to 60 °C, then 30 °C min<sup>-1</sup> to 180 °C, and then held for 5 min. We preconditioned the SPME fibre at 220 °C for 5 min before exposing it to the headspace of each seedling. The mass spectrometer scanned from mass 50 to 650 and data were processed by Xcalibur software (Thermo Fisher Scientific Inc., Waltham, MA, USA). We compared spectra with those in the NIST 2002 database to tentatively identify some of the VOC peaks.

#### *Experiment 2: Use of odour in the absence of visual seedling cues*

We tested whether herbivores were attracted to *E. pilularis* odour by quantifying behaviours in patches with and without seedling odour. We used plastic vials (50 mL) with punctured lids that either contained a cut seedling or were empty (control). We used vials rather than whole plants to separate herbivore responses to seedling odour from visual seedling cues. We cut seedlings into 1 cm pieces to compensate for the inhibitory effect of the vial on VOC diffusion compared to whole seedlings in the open. It is common practice to use mechanically damaged plant material for testing odour perception (Hanley & Sykes 2009; Hanley *et al.* 2013), and eucalypts are not known to exhibit induced defence responses in response to damage (Rapley *et al.* 2007; Henery *et al.* 2008).

We constructed a 360 m transect running approximately parallel to an unsealed driving track (minimum distance to track = 30m; see Fig. S2). Every 40m along the transect, we set up a pair of vials at 5 m perpendicular to either side of the transect line. Each pair of vials constituted one of 10 treatment blocks, with treatments within each block assigned systematically along the transect. We buried the vials flush with the ground and secured them with tent pegs. We replaced cut seedlings weekly to maintain the odour cue. To test whether the presence of

seedling odour affected patch selection, we filmed each patch as described for Experiment 2. We quantified the time in days to first visit and first investigation (visit where the nose touched the vial), and the number of visits and investigations, by wallabies and kangaroos at each patch over three weeks. To maintain sample independence, we excluded visits that occurred less than 20 min after the last.

### *Experiment 3: Use of odour to find whole plants*

We quantified the effect of visual and olfactory cues on time taken for swamp wallabies to browse *E. pilularis* seedlings (i.e. browsing delay) in three cue treatments (see Fig. S3): (1) an upright seedling (control), (2) a seedling pinned down with coarse plastic mesh (reduced visual and normal olfactory cues), and (3) an upright seedling next to a pinned down seedling (normal visual and double olfactory cues). To control for any effect of the plastic mesh, we also pinned a mesh next to upright seedlings in treatment (1); we filmed all replicates as described above to test whether animals interacted with the mesh (i.e. potentially using it as a food cue). Seedlings were grown from local seed at Booderee Botanic Gardens and were between 150 mm and 200 mm in height. We planted seedling treatments in four sets of three-by-three latin-square grids, with 40 m between treatments within a grid and 80 m between grids. Each treatment was thus assigned systematically and replicated 12 times in total. We quantified browsing delay by weekly visual inspection for six weeks, when all replicates had been browsed (see results).

### *Statistical analyses*

We tested for treatment differences in all time-to-event data using Cox regression models with the EXACT ties handling option (PHREG procedure, SAS 9.3, SAS Institute Inc., Cary, NC, USA). For Experiment 1, we first tested for differences in time to first visit to patches with or without seedlings using the marginal model with block (pair) as the cluster identity (the frailty model did not converge). We then compared first visits for low- and high-density seedling patches – equivalent to first browse as all first visits resulted in browsing. The latter analysis did not involve paired data and thus we did not include the cluster identity.

We tested for differences in time to first visit to vials between treatments in Experiment 2, analysing wallabies and kangaroos separately. We used the frailty model and included block as a random factor to account for any spatial effects. To test whether the frequency of visits and investigations by each herbivore species differed between treatments, we used log-linear models, including block as a random factor (GLIMMIX procedure, SAS 9.3).

For Experiment 3, we also used frailty models to test for differences in time to first browse between treatments, but did not include a random factor as the design did not include blocks. We report the Wald statistics for all Cox models.

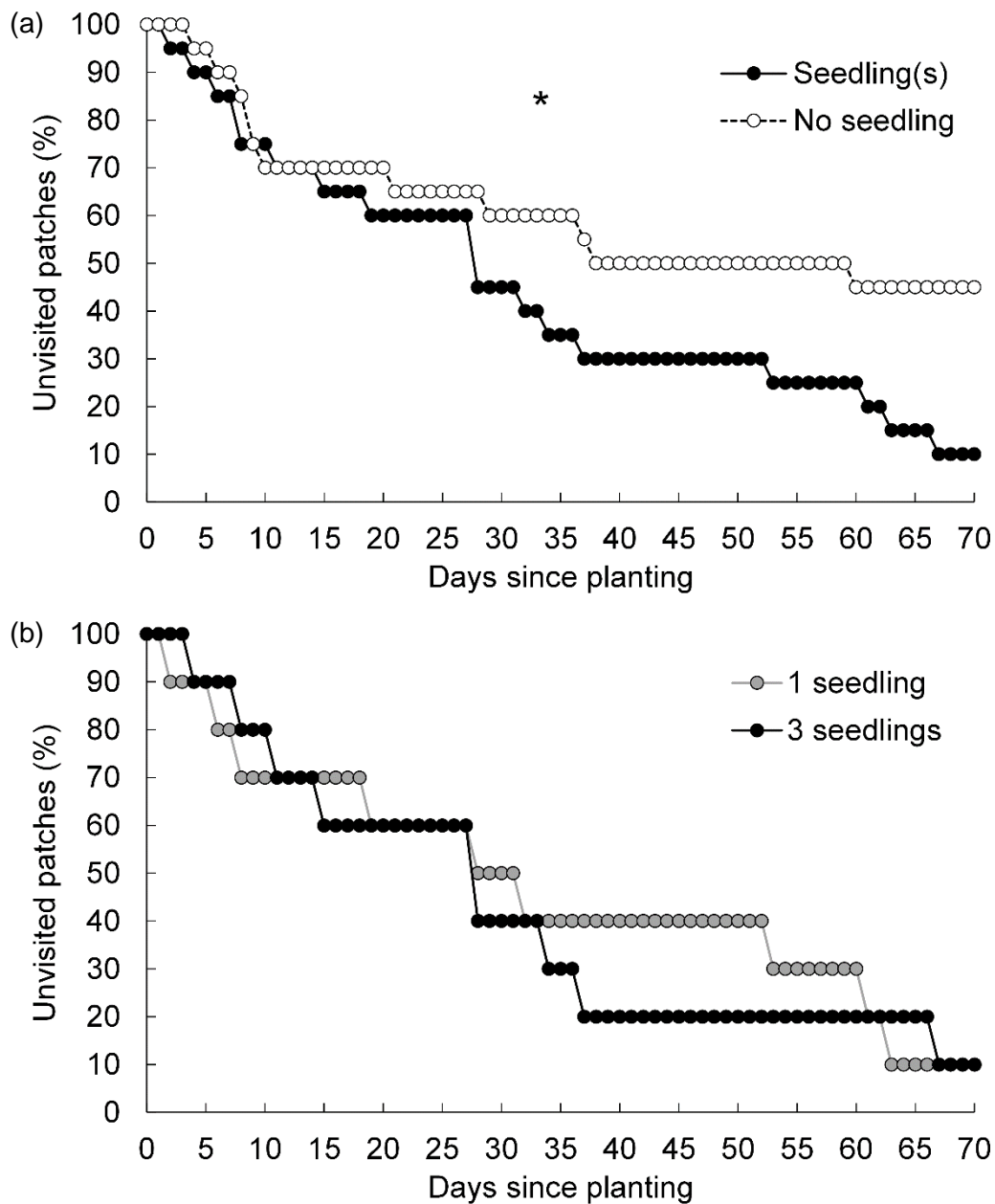
## Results

### *Experiment 1: Random vs. cue-based foraging*

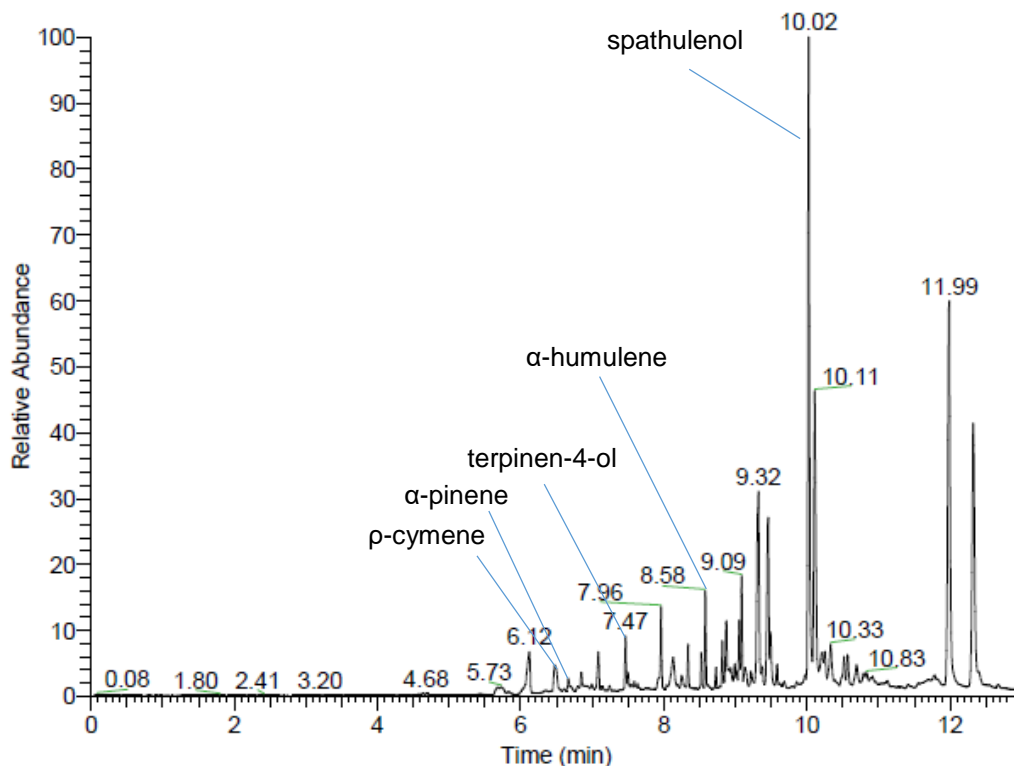
Swamp wallabies visited patches with seedlings significantly earlier than those without (Fig. 1a; sandwich  $\chi^2_1 = 4.47$ ,  $P = 0.034$ ). Patches with a seedling reward were 2.16 times more likely to be visited than patches without a seedling reward (95% CI 1.06 – 4.40). There was no difference in time to visit (equivalent to browse) between patches that contained one or three seedlings (Fig. 1b;  $\chi^2_1 = 0.0085$ ,  $P = 0.93$ ).

### *Availability of odour cues from whole seedlings*

We detected VOCs in the headspaces of all seedlings; a representative chromatogram is shown in Fig. 2. Using mass spectral comparisons, we tentatively identified five terpene VOCs: spathulenol, p-cymene, terpinen-4-ol,  $\alpha$ -pinene and  $\alpha$ -humulene.



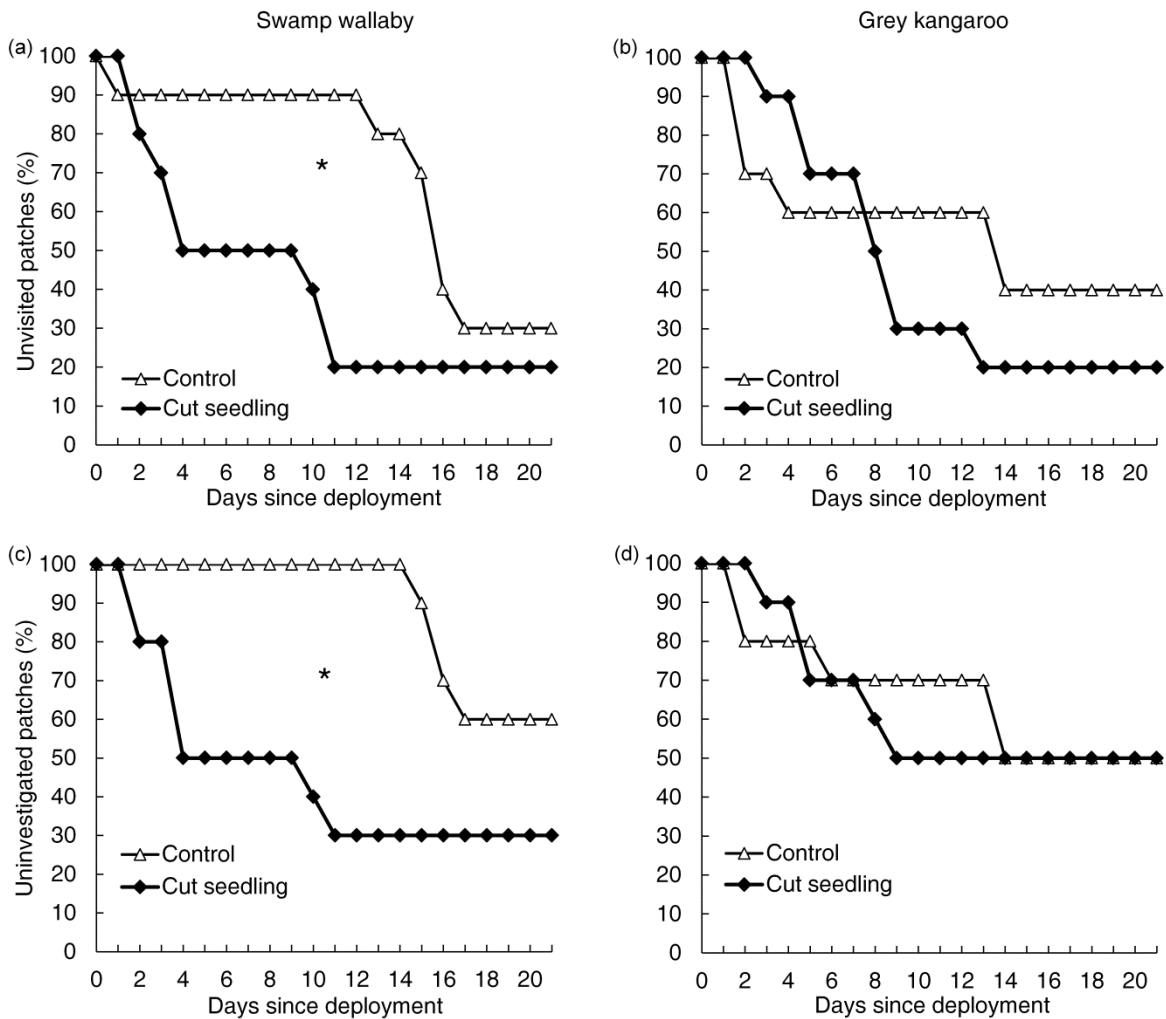
**Figure 1.** Patches remaining unvisited by presence and quantity of *E. pilularis* seedlings: (a) with or without seedlings, and (b) with one or three seedlings.



**Figure 2.** Representative chromatogram showing VOC peaks in the headspace of a *Eucalyptus pilularis*. Labelled peaks are tentative identifications based on spectral comparisons with the NIST 2002 spectral database.

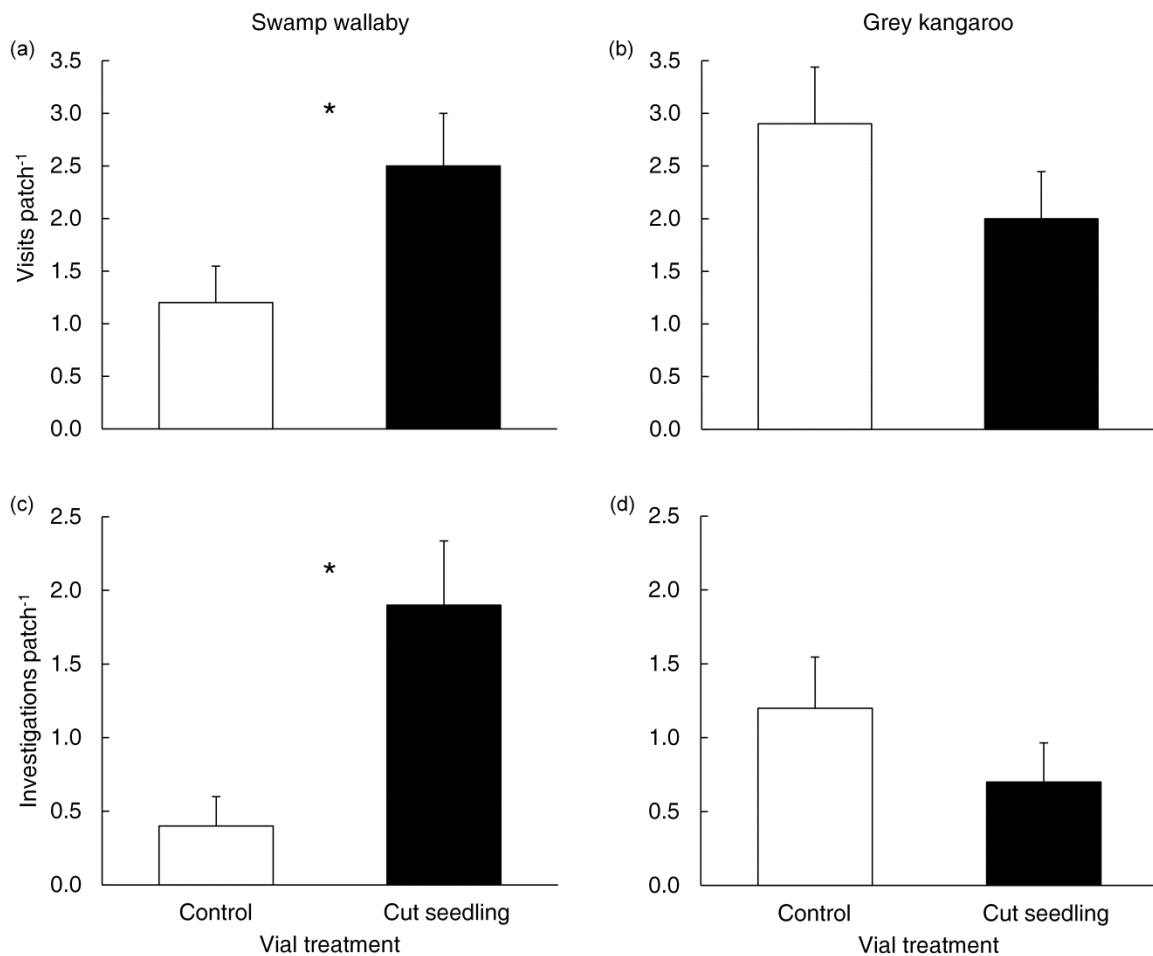
### *Experiment 2: Use of odour in the absence of visual seedling cues*

Wallabies first visited patches with seedling odour significantly earlier than control patches ( $\chi^2_{0.90} = 4.54$ ,  $P = 0.028$ , Fig. 3a); patches with seedling odour were 3.90 times more likely to have been visited during the trial than control patches (95% hazard ratio CI = 1.12 – 13.64). In contrast, there was no difference between treatments in the time taken by kangaroos to first visit patches ( $\chi^2_{0.96} = 0.28$ ,  $P = 0.58$ , Fig. 3b). Treatment differences in the time to first investigation by swamp wallabies followed the same but more marked pattern as visits ( $\chi^2_{0.86} = 8.38$ ,  $P = 0.0029$ , Fig. 3c); patches with seedling odour were 11.67 times more likely to have been investigated during the trial than control patches (95% hazard ratio CI = 2.21 – 61.65). Time to first investigation by kangaroos did not differ between odour treatments ( $\chi^2_{0.97} = 0.045$ ,  $P = 0.82$ , Fig. 3d).



**Figure 3.** Effect of treatment (control, seedling odour) on percentage of patches remaining unvisited and uninvestigated over time by swamp wallabies (a, c) and eastern grey kangaroos (b, d), respectively (\* $P < 0.05$ ; Experiment 2).

Wallabies made more visits to patches with seedling odour than to control patches ( $F_{1, 18} = 4.37$ ,  $P = 0.051$ , Fig. 4a), while kangaroos visited patches in both treatments similarly ( $F_{1, 18} = 1.63$ ,  $P = 0.22$ , Fig. 4b). Similarly, wallabies made more investigations in patches with seedling odour than in control patches ( $F_{1, 18} = 8.02$ ,  $P = 0.011$ , Fig. 4c), with no treatment differences in the number of investigations by kangaroos ( $F_{1, 18} = 1.28$ ,  $P = 0.27$ , Fig. 4d).

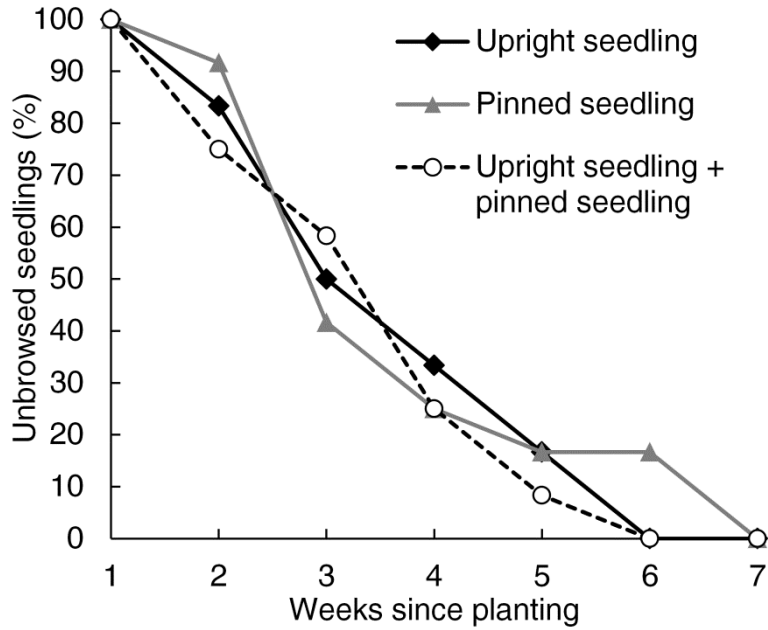


**Figure 4.** Effect of odour treatment on number of visits and investigations per patch by swamp wallabies (a,c) and eastern grey kangaroos (b, d), respectively (mean + SE, \* $P < 0.05$ ; Experiment 1).

### *Experiment 3: Use of odour to find whole plants*

Swamp wallabies removed at least 90 % of the available foliage when they browsed a seedling. There was no difference in time to first browse among seedling treatments ( $\chi^2_2 = 0.42$ ,  $P = 0.81$ , Fig. 5). Swamp wallabies did not interact with the plastic mesh in the control treatment. They browsed pinned seedlings by pulling them through the mesh with their teeth. In the upright plus pinned seedling treatment, the upright seedling was always eaten first in videos ( $n = 9$ ).





**Figure 5.** Seedlings remaining unbrowsed over time by treatment (Experiment 3).

## Discussion

Our study experimentally demonstrates that a browsing mammal, the swamp wallaby, exploits patches non-randomly and uses leaf odour cues to find food plants, exploiting the deterrent or inadvertent odour cues that plants emit. Swamp wallabies visited and investigated patches with cut *E. pilularis* seedlings in buried vials both earlier and more frequently than control patches with empty vials, patterns not observed in the grazing grey kangaroo. The presence of terpenes in the headspace of *E. pilularis* seedlings confirmed the cue was available for exploitation. Wallaby encounters with patches were therefore driven by odour cues from their food plant rather than a result of random movement through the environment. We showed that reducing the visual cues, by pinning the seedling to the ground, did not affect browsing delay, suggesting that odour cues were sufficient for seedling detection. Additional odour cues did not, however, help wallabies to detect and browse seedlings earlier. We suggest that wallabies incorporate information from odour cues in finding seedlings, but that the minimum threshold

is low – odour from a single plant gave as much information as odour from two plants ('all-or-nothing' sensu Schaefer & Ruxton 2011).

The attraction of herbivores resulting in loss of foliage, and thus reducing the area available for photosynthesis, is clearly disadvantageous to plants. This is in contrast to flowers and fruits that produce attractant cues for seed-dispersers and pollinators. Our results thus indicate that wallabies have hijacked olfactory cues that were not produced to attract leaf-eating herbivores, such as the volatile by-products of plant metabolic processes, or those produced for specific functions (Langenheim 1994; Peñuelas & Llusà 2004). The latter includes toxic terpenes that may be an anti-herbivory signal – for the terpenes themselves (Bedoya-Pérez *et al.* 2014) or for other toxins (Lawler *et al.* 1999) – and wallabies may have used this signal as a foraging cue to find rather than avoid plants. Thus while toxic terpenes within plants can limit consumption by mammals, volatilized terpenes may also act as a cue for finding the plants in the first place, replacing or enhancing visual information in structurally complex vegetation and improving foraging efficiency. However, to decouple the effects of toxic terpenes from other VOCs comprising the headspace odour, further experiments should test whether wallabies differentially investigate artificial odour blends (in natural concentrations) comprising only the terpenes and those comprising the full VOC complement.

Many mammals consume VOC-producing plants; it is therefore likely that herbivorous mammals in other parts of the world, particularly browsers, also use VOCs for finding food, including ecologically and/or economically important plant species. Sitka spruce *Picea sitchensis* (Duncan, Hartley & Iason 1994), ponderosa pine *Pinus ponderosa* (Snyder 1992), and balsam poplar *Populus balsamifera* (Reichardt *et al.* 1990) are just three examples. Our findings therefore have significant implications for predicting herbivore browsing behaviour and the persistence of their food plants. By ignoring the potential for leaf VOCs to act as food cues, we may significantly underestimate the foraging efficiency of browsers in many ecosystems.

## References

- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.
- Davis, N.E., Coulson, G. & Forsyth, D.M. (2008) Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research*, **35**, 684-694.
- Duncan, A.J., Hartley, S.E. & Iason, G.R. (1994) The effect of monoterpene concentrations in Sitka spruce (*Picea sitchensis*) on the browsing behaviour of red deer (*Cervus elaphus*). *Canadian Journal of Zoology*, **72**, 1715-1720.
- Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants - a study in coevolution. *Evolution*, **18**, 586-608.
- Foley, W.J., Iason, G.R. & McArthur, C. (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? *Nutritional ecology of herbivores: proceedings of the Vth International Symposium on the Nutrition of Herbivores* (eds H.-J. Jung & G. Fahey), pp. 130–209. American Society of Animal Science, Savoy, Illinois.
- Fraenkel, G.S. (1959) The raison d'être of secondary plant substances. *Science*, **129**, 1466-1470.
- Hanley, M.E., Girling, R.D., Felix, A.E., Olliff, E.D., Newland, P.L. & Poppy, G.M. (2013) Olfactory selection of *Plantago lanceolata* by snails declines with seedling age. *Annals of Botany*, **112**, 671-676.
- Hanley, M.E. & Sykes, R.J. (2009) Impacts of seedling herbivory on plant competition and implications for species coexistence. *Annals of Botany*, **103**, 1347-1353.
- Henery, M.L., Wallis, I.R., Stone, C. & Foley, W.J. (2008) Methyl jasmonate does not induce changes in *Eucalyptus grandis* leaves that alter the effect of constitutive defences on larvae of a specialist herbivore. *Oecologia*, **156**, 847-859.

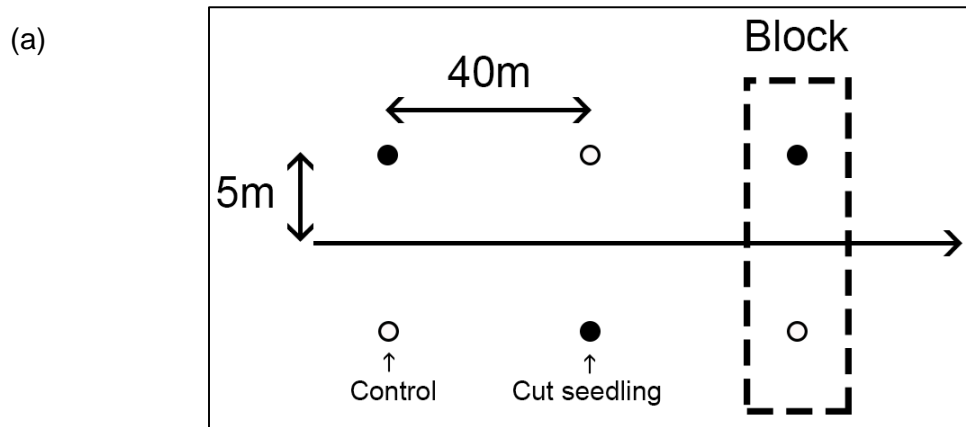
- Hoballah, M., Stuurman, J., Turlings, T.J., Guerin, P., Connétable, S. & Kuhlemeier, C. (2005) The composition and timing of flower odour emission by wild *Petunia axillaris* coincide with the antennal perception and nocturnal activity of the pollinator *Manduca sexta*. *Planta*, **222**, 141-150.
- Hodgkison, R., Ayasse, M., Kalko, E.V., Häberlein, C., Schulz, S., Mustapha, W., Zubaid, A. & Kunz, T. (2007) Chemical ecology of fruit bat foraging behavior in relation to the fruit odors of two species of paleotropical bat-dispersed figs (*Ficus hispida* and *Ficus scortechinii*). *Journal of Chemical Ecology*, **33**, 2097-2110.
- Kohl, K.D., Miller, A.W. & Dearing, M.D. (2015) Evolutionary irony: evidence that 'defensive' plant spines act as a proximate cue to attract a mammalian herbivore. *Oikos*, **124**, 835-841.
- Langenheim, J.H. (1994) Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, **20**, 1223-1280.
- Lawler, I.R., Stapley, J., Foley, W.J. & Eschler, B.M. (1999) Ecological example of conditioned flavor aversion in plant–herbivore interactions: effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology*, **25**, 401-415.
- Low, P.A., McArthur, C., Fisher, K. & Hochuli, D.F. (2014) Elevated volatile concentrations in high-nutrient plants: do insect herbivores pay a high price for good food? *Ecological Entomology*, **39**, 480-491.
- McArthur, C., Hagerman, A.E. & Robbins, C.T. (1991) Physiological strategies of mammalian herbivores against plant defenses. *Plant Defenses Against Mammalian Herbivory* (eds R.T. Palo & C.T. Robbins), pp. 103-114. CRC Press, Boca Raton, Florida.
- Molangui, T., Menut, C., Bouchet, P., Bessièrè, J.-M. & Habimana, J.-B. (1997) Aromatic plants of tropical Central Africa. Part XXX. Studies on volatile leaf oils of 10 species of *Eucalyptus* naturalized in Rwanda. *Flavour and Fragrance Journal*, **12**, 433-437.

- Peñuelas, J. & Llusà, J. (2004) Plant VOC emissions: making use of the unavoidable. *Trends in Ecology & Evolution*, **19**, 402-404.
- Rapley, L., Allen, G., Potts, B. & Davies, N. (2007) Constitutive or induced defences - how does *Eucalyptus globulus* defend itself from larval feeding? *Chemoecology*, **17**, 235-243.
- Reichardt, P.B., Bryant, J.P., Mattes, B.R., Clausen, T.P., Chapin, F.S., III & Meyer, M. (1990) Winter chemical defense of Alaskan balsam poplar against snowshoe hares. *Journal of Chemical Ecology*, **16**, 1941-1959.
- Schaefer, H.M. & Ruxton, G.D. (2011) *Plant-animal communication*. Oxford University Press, Oxford, U.K.
- Snyder, M.A. (1992) Selective herbivory by Abert's squirrel mediated by chemical variability in ponderosa pine. *Ecology*, **73**, 1730-1741.
- Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015) Associational refuge in practice: can existing vegetation facilitate woodland restoration? *Oikos*, **124**, 571–580.
- Vas, G. & Vékey, K. (2004) Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry*, **39**, 233-254.

## Supplementary Information



**Figure S1.** Experimental set-up used to extract headspace volatiles from *Eucalyptus pilularis* seedlings.



**Figure S2.** (a) Schematic of design for Experiment 2 and (b) photographs of camera and vial set-up.



**Figure S3.** Photographs of seedling treatments tested in Experiment 3: (a) upright, (b) pinned down, and (c) upright plus pinned down.



## CHAPTER 5

---

### Olfactory and Visual Plant Cues as Drivers of Selective Browsing

Submitted as: Stutz, R.S., Croak, B.M, Banks, P.B., Proschogo, N. & McArthur, C. (in review)

Olfactory and visual plant cues as drivers of selective browsing.



Swamp wallaby finding a low-nutrient *Eucalyptus pilularis* seedling pinned to the ground.



## Abstract

Food quality is an important consideration in the foraging strategy of all animals, including herbivores. Those that can detect and assess the nutritional value of plants from afar, using senses such as smell and sight, can forage more efficiently than those that must assess food quality by taste. Selective foraging not only affects herbivore fitness but can influence the structure and composition of plant communities, yet little is known about how olfactory and visual cues help herbivores to find preferred plants. We tested the ability of a free-ranging, generalist mammalian browser, the swamp wallaby (*Wallabia bicolor* Desmarest), to use olfactory and visual plant cues to find and/or browse differentially on *Eucalyptus pilularis* Sm. seedlings grown under different nutrient conditions. Low-nutrient seedlings differed visually, physically and chemically from high-nutrient seedlings. In the absence of visual cues, wallabies used odour to differentiate seedlings. They visited and investigated patches with high-nutrient seedling odour most, followed by patches with low-nutrient seedling odour, and patches with no added odour least. However, when visual and olfactory cues of seedlings were present, wallabies reversed their foraging response and were more likely to browse low- than high-nutrient seedlings. This browsing difference, in turn, disappeared when long-range visual cues were reduced by pinning seedlings horizontal to the ground. Our study shows that herbivores can respond to odours of higher nutrient plants. But in ecologically realistic scenarios they use a variety of visual and olfactory cues, with a context-dependent outcome that is not always selection of high nutrient food. Our results demonstrate the importance of testing the sensory abilities of herbivores in realistic multi-sensory settings to understand their ecological function in selective foraging.

## Introduction

For herbivores, the nutritional quality of plants varies among species, among individuals of the same species, and between different parts of a plant (O'Reilly-Wapstra, McArthur & Potts 2004; Loney *et al.* 2006). Herbivores, including large generalist browsers, contend with this inter- and intra-specific variation in quality by feeding selectively (Augustine & McNaughton 1998; Sprent & McArthur 2002; Shrader *et al.* 2012) and thus they must assess the quality of the food they find. This food assessment involves associating plant cues perceived via smell, sight and taste, with positive or negative post-ingestive feedback (Foley, Iason & McArthur 1999; Provenza, Kimball & Villalba 2000; Duncan *et al.* 2006). The use of plant cues to forage is important ecologically, and not only from the herbivore perspective because selective browsing can significantly affect the composition and structure of plant communities (Wahungu, Catterall & Olsen 1999; Côté *et al.* 2004; Rooney 2009). Thus, the role of visual and olfactory plant cues in facilitating selective browsing has implications for ecosystems beyond herbivores and even beyond herbivore-plant interactions.

The odour, visual appearance and taste of plants or plant parts are linked to their chemical and physical composition, and thus may be used as cues to detect the palatability and nutritional quality of food items (Jachmann 1989; Sumner & Mollon 2000a; Close, Beadle & Hovenden 2001). For foragers, the advantage of odour and visual cues is that foraging decisions can be made from a distance. The use of taste, in contrast, involves approaching and handling the plant – costly if the plant is subsequently rejected. Odour and visual cues may therefore play a particularly important role in enhancing foraging efficiency and in diet selection (Warburton & Mason 2003; Goff & Klee 2006). Olfactory plant cues may be particularly useful for detecting palatable plants amongst complex vegetation where visual discrimination is difficult.

Plant odour arises from a highly diverse group of volatile organic compounds (VOCs), many of which are potentially toxic if ingested (Langenheim 1994; Peñuelas & Llusà 2004). Such

VOCs may act as feeding deterrents warning herbivores of negative post-ingestive consequences. But many browsing herbivores have evolved physiological and behavioural mechanisms to deal with toxins (McArthur, Hagerman & Robbins 1991; Dearing, Foley & McLean 2005; Iason & Villalba 2006). Browsers may therefore use VOCs, not to avoid, but rather to actually find food plants as well as assess their quality once found (Halitschke *et al.* 2008; Bedoya-Pérez *et al.* 2014).

Plants raised under different fertilizer regimes have been shown to emit different amounts of VOCs, both absolute and relative to biomass (Gouinguéné & Turlings 2002; Holopainen & Gershenzon 2010; Low *et al.* 2014). It is therefore plausible that browsers not only use plant VOCs to locate plants, but also to assess their relative quality. The ability to use odour alone to differentiate between seedlings of plant species varying in palatability, and between low- and high-nutrient algae, has been demonstrated in simplified laboratory experiments with gastropods (Hanley, Collins & Swann 2011; Moelzner & Fink 2014). However, studies on vertebrates have primarily been concerned with the toxic role of terpene VOCs in food once ingested, or as cues for other more toxic substances such as formylated phloroglucinol compounds (FPCs; i.e. conditioned flavour aversions, Lawler *et al.* 1999; Provenza, Kimball & Villalba 2000; Moore *et al.* 2004; O'Reilly-Wapstra, McArthur & Potts 2004), rather than as foraging cues for finding plants.

Visual plant cues including plant size, leaf shape, brightness and colour may also indicate suitability as a food resource. Contrasts in these factors relative to a plant's surrounds can influence its visual apparency to herbivores and thus its susceptibility to browsing (Schaefer & Ruxton 2011; Castagneyrol *et al.* 2013). For example, greater nutrient availability can result in larger plants that have a higher probability of being browsed (Hartley *et al.* 1997), while changes in fruit colour signal ripeness for frugivores (Sumner & Mollon 2000b). Conversely, red colouration in leaves can serve as a warning to herbivores of poor nutritional quality or the

presence of toxic compounds, and thus lead to preferential consumption of green leaves (Gould 2004; Karageorgou & Manetas 2006; Karageorgou, Buschmann & Manetas 2008).

For many animals, foraging involves the use of multiple cues over different sensory modalities to maximise efficiency in the search and detection of food, as well assessment once found (Sztefi *et al.* 2003; Duistermars & Frye 2008; Munoz & Blumstein 2012). Sensory modes provide information that operates at different scales and may be affected differently by environmental variables. For example, wind and temperature affect odours, while habitat structure affects visual cues. If cues in one sensory mode are absent or difficult to perceive, a forager may use other cues to locate and assess food or habitat patches (Bicca-Marques & Garber 2004; Prevedello, Forero-Medina & Vieira 2011). Understanding the mechanisms for finding food is critical for determining how environmental conditions influence detectability, yet the way in which olfactory and visual cues help herbivores to find preferred plants is not well understood.

Here, our overall aim was to determine whether a free-ranging generalist browser uses odour and visual cues to forage selectively, based on intra-specific differences in plant chemical and physical characteristics. Our model system was a mammalian browser, the swamp wallaby (*Wallabia bicolor* Desmarest), and *Eucalyptus pilularis* seedlings, a highly preferred food source for wallabies (Stutz *et al.* 2015a). We generated high- and low- nutrient seedlings using fertilizer. Higher-nutrient eucalypt seedlings also have higher concentrations of terpenes, FPCs and fibre, but are nevertheless preferred by marsupial herbivores (Close *et al.* 2003; O'Reilly-Wapstra *et al.* 2005; Loney *et al.* 2006; Miller, McArthur & Smethurst 2007; Miller, McArthur & Smethurst 2009).

In Experiment 1, we tested whether wallabies could use odour cues alone to differentiate low- and high-nutrient seedlings, in the absence of any visual seedling cues. We quantified behavioural responses of wallabies to patches under three odour treatments: buried vials that were empty, or that contained a cut *E. pilularis* seedling grown under either low- or high-

nutrient conditions. We compared the responses of wallabies and eastern grey kangaroos (*Macropus giganteus* Shaw), the latter as a procedural control. Grey kangaroos are grazers (Davis, Coulson & Forsyth 2008), and are not attracted to seedling odour (Chapter 4). We therefore expected wallabies, but not kangaroos, to respond positively to odours associated with a higher quality browse resource (i.e. high-nutrient > low-nutrient > no seedling).

In Experiment 2, our aim was to quantify and compare how olfactory and visual plant cues together influenced browsing of intact low- and high-nutrient seedlings. We could not provide odourless seedlings, yet we could not be certain that artificial seedlings would appear realistic to wallabies (in terms of shape and colour), given the lack of knowledge about wallaby vision. To disentangle the role of olfaction and vision, we therefore presented seedlings from both nutrient treatments either in the normal upright position or pinned to the ground, the latter to reduce long-range visual cues. We predicted that if odour was the predominant foraging cue used by wallabies, then wallabies would find and browse high-nutrient seedlings earlier than low-nutrient ones.

## **Methods**

### *Study site*

Our study site was a 50 ha area of degraded coastal woodland in Booderee National Park, Jervis Bay Territory, Australia (35°08'30"S, 150°39'10"E). The vegetation structure was patchy, comprising areas of dense bracken fern (*Pteridium esculentum*), introduced grasses, herbs (*Lomandra* spp.) and small scrubby trees (*Monotoca elliptica*), and with a sparse overstorey of remnant introduced *Pinus radiata*, and native *Eucalyptus pilularis*, *E. botryoides* and *Acacia implexa*.

### *Seedling nutrient treatments*

We sourced 300 tubestock seedlings of *E. pilularis* from Cumberland State Forest Nursery (West Pennant Hills, NSW, Australia), that had been grown outdoors for 6 months with slow release fertilizer. We removed the fertilized soil from each seedling by washing the roots in water, and planted them in an inert medium of coarse perlite (Grade C, Exfoliators, Dandenong, VIC, Australia) in 1 L plastic pots with drainage. We kept seedlings on elevated drainage benches in an unregulated greenhouse for six weeks from mid-March 2014. The greenhouse was naturally illuminated, humidity was uncontrolled, and the temperature ranged from 10 °C to 35 °C.

We systematically allocated rows of seedlings to either low- or high-nutrient treatments such that treatments were evenly dispersed throughout the greenhouse. On the first day, we hand-watered seedlings to saturation. For the high-nutrient treatment, we then applied 100 mL of low-phosphate fertilizer solution three times per week for 6 weeks at 1 g L<sup>-1</sup> (Peters® CalMag Finisher, Scotts Australia, Bella Vista, NSW, Australia, N:P:K = 13:2:17 + 5Ca). For seedlings in the low-nutrient treatment we applied an equivalent amount of water (100 mL) three times per week.

### *Seedling characteristics*

After six weeks, we randomly selected 10 seedlings from each treatment to assess differences in physical and chemical characteristics. We quantified seedling height and number of leaf nodes, and placed the above-substrate portion in a drying oven at 60 °C for 72 h to obtain dry weights and ground them in a ball mill. Subsamples of ~ 0.1 g were used to quantify nitrogen content by combustion using a Leco FP-428 nitrogen/protein analyser (M.C. Franklin Laboratory, Camden, NSW, Australia). We used whole seedlings including the stem because wallabies often consumed seedlings in their entirety (Stutz *et al.* 2015a). We expected the high-nutrient treatment to produce seedlings with greater height, number of leaf nodes, weight and nitrogen content than the low-

nutrient treatment. We compared seedling characteristics between treatments using a MANOVA (GLM procedure, SAS 9.3).

We also tested whether the amount of odour cue available differed between low- and high-nutrient seedlings ( $n = 10$  per treatment). We extracted the VOCs from the headspaces of *E. pilularis* seedlings using Solid Phase Micro-Extraction (SPME) and analysed the samples using Gas Chromatography coupled with Ion Trap Mass Spectrometry (GC-ITMS). The details of the procedure are as described in Stutz *et al.* (in review, Chapter 4), but here we used a shorter oven programme: 40 °C for 2 min, increasing at 10 °C min<sup>-1</sup> to 60 °C, then 30 °C min<sup>-1</sup> to 180 °C, and then held for 1 min. We processed the data using Xcalibur software (Thermo Fisher Scientific Inc., Waltham, MA, USA) and compared spectra with those in the NIST 2002 database to tentatively identify some of the VOC peaks. To test whether high-seedlings emitted more VOCs in total than low-nutrient seedlings, we conducted a non-parametric Wilcoxon's exact test (NPAR1WAY procedure, SAS 9.3).

#### *Experiment 1: Behavioural response to seedling odour*

In this experiment, we tested the behavioural response of swamp wallabies (browsers) and grey kangaroos (grazers) to seedling odour. We used plastic vials (50 mL) with perforated lids that were either: (1) empty (procedural control), (2) contained a low-nutrient seedling, or (3) contained a high-nutrient seedling. It is common practice to use macerated leaf material for testing odour perception (Hanley & Sykes 2009; Hanley *et al.* 2013). To be more ecologically relevant, we did not macerate the seedlings but rather cut them into 1 cm sections. This balanced our wish to retain leaf structure against the need to aid the emission of volatiles from the vials. Eucalypts are not known to exhibit induced defence responses, such as the increased synthesis of terpenes or FPCs, in response to damage by herbivores (Rapley *et al.* 2007; Henery *et al.* 2008), as seen in many other plants. We replenished the cue by replacing the cut seedling in the vial each week over the 4 weeks of the experiment. Each treatment was represented once per block in seven blocks. Treatments within a block were 10 m apart,

with 40 m between blocks. To monitor patch visits, we positioned motion-triggered, infra-red cameras 1 m above each vial, and set it to record 60 s videos with instant re-trigger (ScoutGuard SG550v, Hunting Cam Online, Gadsden, SC, USA). We defined a patch as the area in the camera field of view.

To determine whether the herbivores responded differently to odour treatments, we counted the number of days to the first patch visit and investigation by wallabies and kangaroos. We recorded a visit when an individual entered a patch, and defined investigations as visits during which the individual's nose touched the vial. We tested the effect of odour treatment on the time to first patch visit and investigation by wallabies and kangaroos using separate Cox regression models (PHREG procedure, SAS 9.3). This method takes into account right-censored data, i.e. patches that the herbivore did not visit during the experiment (Allison 2010). We selected the EXACT method for dealing with ties, and included block as a random factor. We report the Wald statistics for all Cox models.

We also tested the use of seedling odour cues to differentiate patches by quantifying the number of visits and number of investigations per patch by wallabies and kangaroos over 4 weeks. To maintain sample independence, visits that were within 20 min of the last were excluded from analysis. To test whether the frequency of visits and investigations by each herbivore species differed among odour treatments, we used log-linear models with block as a random factor (Poisson response distribution with log link function, GLIMMIX procedure, SAS 9.3), and Tukey's post-hoc adjustment to account for multiple pairwise comparisons between odour treatments.

### *Experiment 2: Browsing patterns as a function of seedling odour and visual cues*

In this experiment, we determined the relative importance of odour and visual cues in detecting and browsing seedlings varying in nutrient content. Here, we reduced the available long-range visual cues in half of the seedlings in each nutrient treatment by pinning them horizontal to the ground using tent pegs; the seedlings had thin and flexible stems allowing them to be pinned down without breaking. We used a two-factor randomised block design, with two levels per



factor: nutrient treatment (low/high) by position (upright/pinned). We planted seedlings in 40 blocks, each comprising the four seedling treatments arranged in random order along a line perpendicular to an unsealed driving track (160 seedlings in total). The four seedlings in a block were 20 m apart, and blocks were separated by 30 – 60 m. We visually inspected seedlings for browsing damage weekly for 6 weeks and estimated leaf area browsed as in Stutz *et al.* (2015a).

To test the effect of nutrient treatment and position on the time taken by wallabies to browse seedlings, we used a Cox regression model including nutrient treatment, position, and their interaction as fixed effects (PHREG procedure, SAS 9.3). We did not include kangaroos in this analysis because wallabies were responsible for all seedling browsing (based on bite marks, recorded videos, and previous work). Patches with seedlings that wallabies did not browse during the experiment were right-censored. We again dealt with ties using the EXACT method and included block as a random factor.

We tested for a difference in percentage foliage removed in seedlings browsed by week 6 between treatments (nutrient treatment, position, and their interaction) using a generalized linear mixed model (GLMM) with a lognormal error distribution, identity link function, and block as a random factor (GLIMMIX procedure, SAS 9.3).

To determine whether patch visits always led to browsing, we filmed one treatment in each block ( $n = 10$  per treatment, total filmed = 40), positioned above the seedling as described for vials in Experiment 1. We did not quantify the number of visits and investigations to seedlings (as we had in Experiment 1), because browsed seedlings were not replaced, and the cues had therefore altered (i.e. been reduced substantially once browsed). As both visual and olfactory cues of seedlings could be affected by light (Peñuelas & Llusià 2001; Kelber & Lind 2010), we also determined whether filmed seedlings were browsed during the day or night. We classified coloured videos as day and grey-scale videos as night, as the latter resulted from an automatically deployed red lens under low light conditions. We compared the number

of seedlings browsed during the day and night between the four treatment combinations of nutrient (low, high) and position (upright, pinned down) using a Fisher's exact test (FREQUENCY procedure, SAS 9.3).

## Results

### *Seedling characteristics*

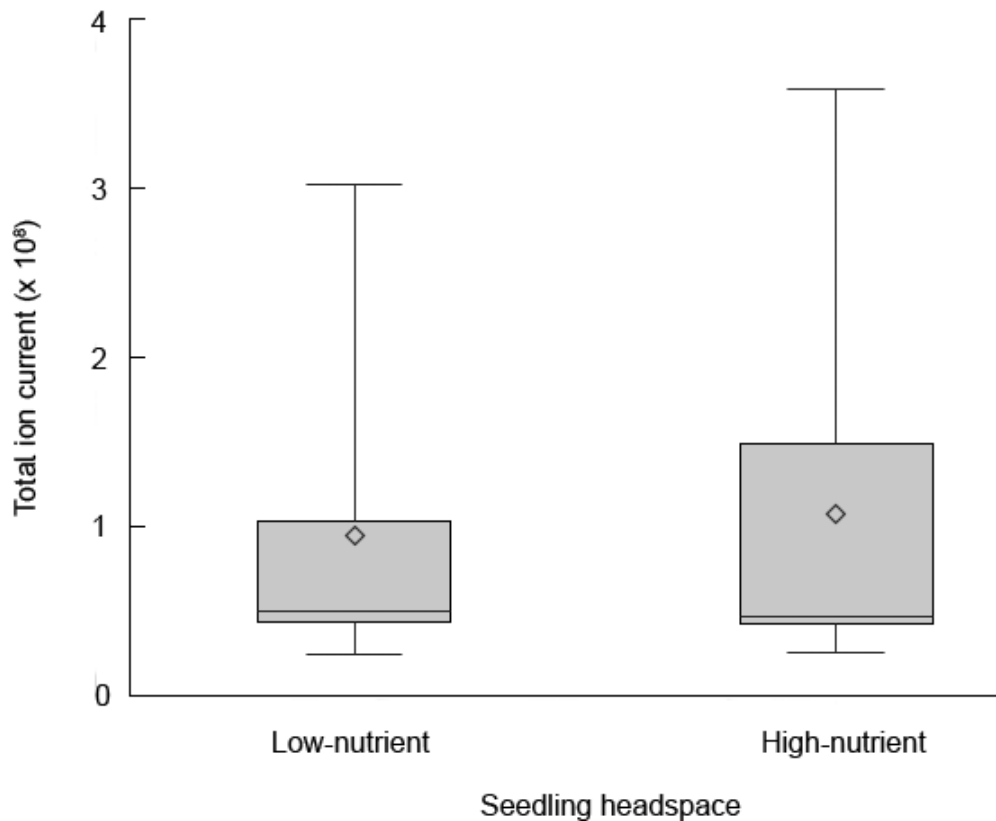
Nutrient treatment had a significant effect on seedling characteristics (Wilks'  $\Lambda = 0.025$ ,  $F_{4, 15} = 144.18$ ,  $P < 0.0001$ ). The nitrogen concentration of seedlings was over three times greater in the high- than low-nutrient treatment (Table 1). High-nutrient seedlings also had 1.6 times more leaf node pairs and 1.3 times greater dry weight. Seedling height did not differ significantly between treatments. Low-nutrient seedlings had red stems with light green leaves while high-nutrient seedlings had dark green stems and leaves (Fig. 1). While there was a trend towards high-nutrient seedlings emitting a greater amount of total VOCs (indicated by total ion current) than low-nutrient seedlings, variation between plants was large and this difference was not statistically significant ( $S = 107.00$ , one-sided  $P = 0.46$ , Fig. 2). We tentatively identified five terpenes in the headspaces of seedlings in both treatments:  $\alpha$ -phellandrene,  $p$ -cymene,  $\alpha$ -pinene, terpinen-4-ol and  $\alpha$ -humulene (see Fig. S1).

**Table 1.** Differences in chemical and physical characteristics of *E. pilularis* seedlings after 6 weeks under low- and high-nutrient treatments. Bold type denotes significant effects at  $\alpha = 0.05$ .

Variable	Low-nutrient (mean $\pm$ SE)	High-nutrient (mean $\pm$ SE)	F <sub>1, 18</sub>	P
Nitrogen (%)	0.55 $\pm$ 0.03	1.70 $\pm$ 0.05	463.55	<b>&lt; 0.0001</b>
No. of leaf nodes	14.60 $\pm$ 3.25	23.20 $\pm$ 3.04	13.45	<b>0.0018</b>
Dry weight (g)	3.69 $\pm$ 0.58	4.90 $\pm$ 0.58	5.37	<b>0.032</b>
Height (m)	0.52 $\pm$ 0.02	0.57 $\pm$ 0.02	2.40	0.14



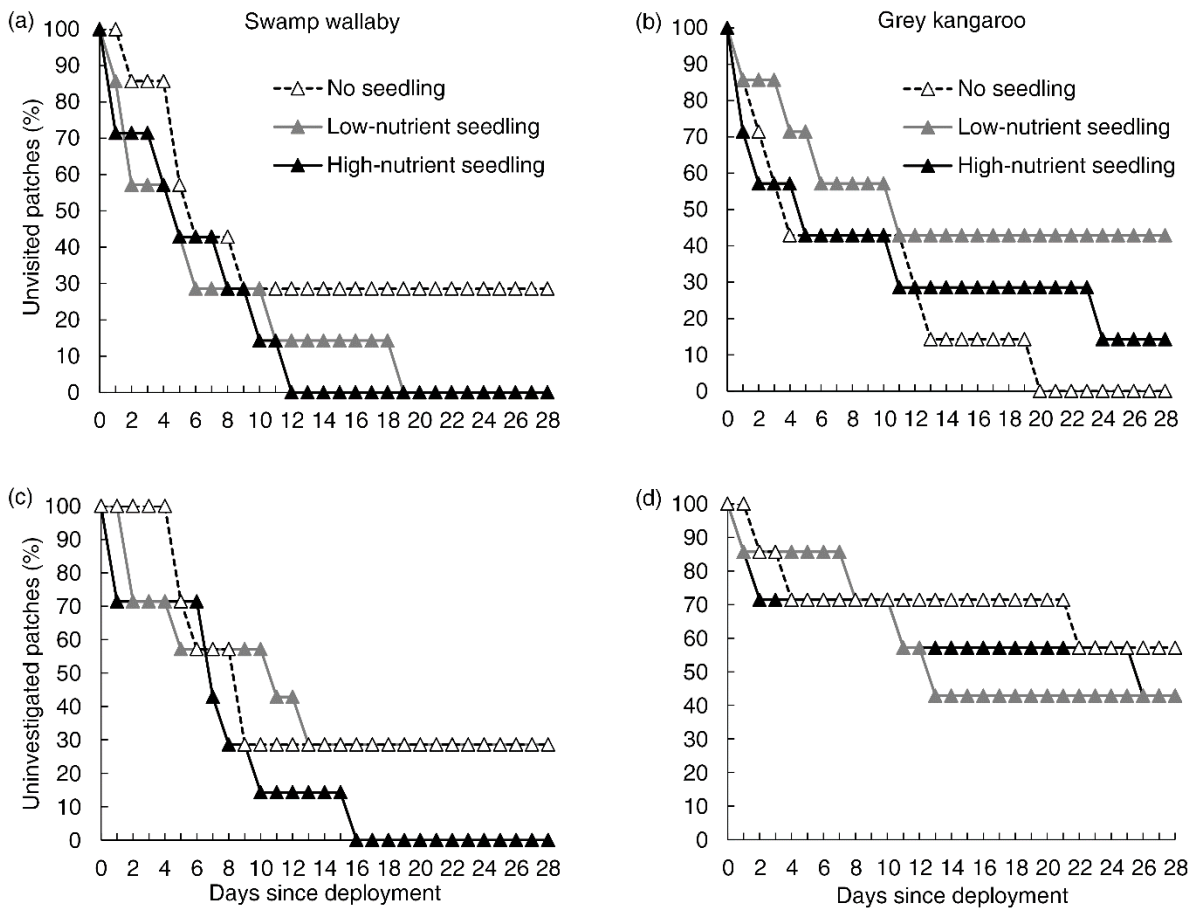
**Figure 1.** Colour difference between *E. pilularis* seedlings grown under low-nutrient (left) and high-nutrient (right) fertilizer regimes. Photo credit: R. Stutz.



**Figure 2.** Box-and-whisker plot of relative total ion currents detected in headspace samples of low- and high-nutrient *E. pilularis* seedlings.

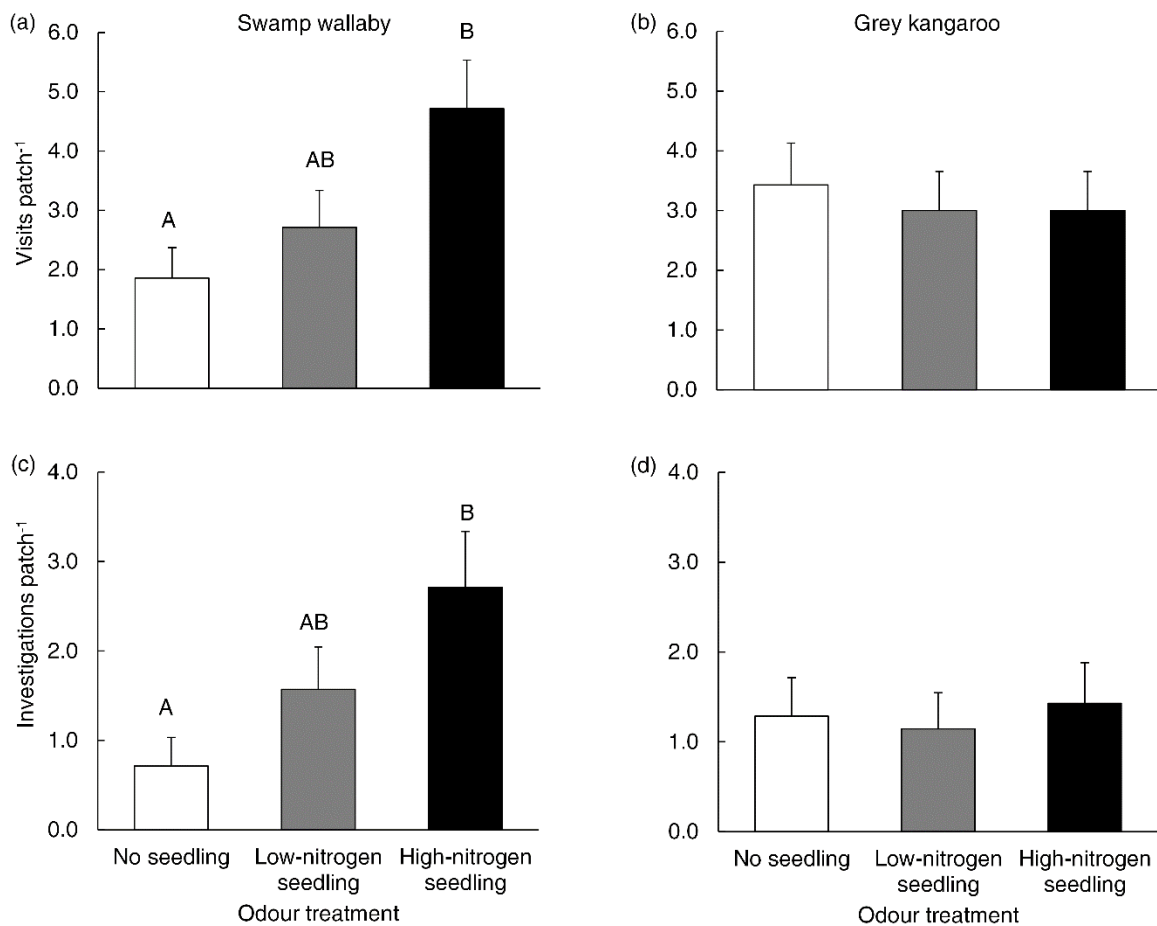
*Experiment 1: Behavioural response to seedling odour*

Swamp wallabies had visited all patches with high-nutrient seedling odour by Day 12 and low-nutrient seedling odour by Day 19; the only patches remaining unvisited by wallabies at the end of the experiment (Day 28) were those with no seedling odour (Fig. 3a). In contrast, grey kangaroos visited all patches with empty vials by Day 20 but not all patches with cut seedling vials in either nutrient treatment by Day 28 (Fig. 3b). However, there was no statistically significant difference between odour treatments in the time to first visit by swamp wallabies ( $X^2_2 = 1.82$ ,  $P = 0.38$ ) or grey kangaroos ( $X^2_2 = 2.62$ ,  $P = 0.25$ ). The time to patch investigation followed similar non-significant patterns for both herbivores (wallaby:  $X^2_2 = 1.64$ ,  $P = 0.44$ , Fig. 3c; kangaroo:  $X^2_2 = 0.32$ ,  $P = 0.85$ , Fig. 3d).



**Figure 3.** Effect of odour treatment on percentage of patches remaining unvisited and uninvestigated over time by swamp wallabies (a, c) and grey kangaroos (b, d), respectively (Experiment 1).

There was a significant difference in the number of visits to patches between treatments by wallabies ( $F_{2, 12} = 4.61$ ,  $P = 0.033$ ). Visits were mostly to patches with high-nutrient seedling odour, then to patches with low-nutrient seedling odour and least to patches with no odour (Fig. 4a). Kangaroos did not differentiate among patch treatments ( $F_{2, 12} = 0.14$ ,  $P = 0.87$ , Fig. 4b). The number of investigations by wallabies differed among treatments as for visits ( $F_{2, 12} = 3.80$ ,  $P = 0.053$ , Fig. 4c), but again, kangaroos did not differentiate treatments ( $F_{2, 12} = 0.11$ ,  $P = 0.90$ ; Fig. 4d).



**Figure 4.** Effect of odour treatment on number of visits and investigations per patch by swamp wallabies (a, c) and grey kangaroos (b,d), respectively (mean + SE; Experiment 1). Different letters (A, B) indicate significant pairwise differences after Tukey's post-hoc adjustments.

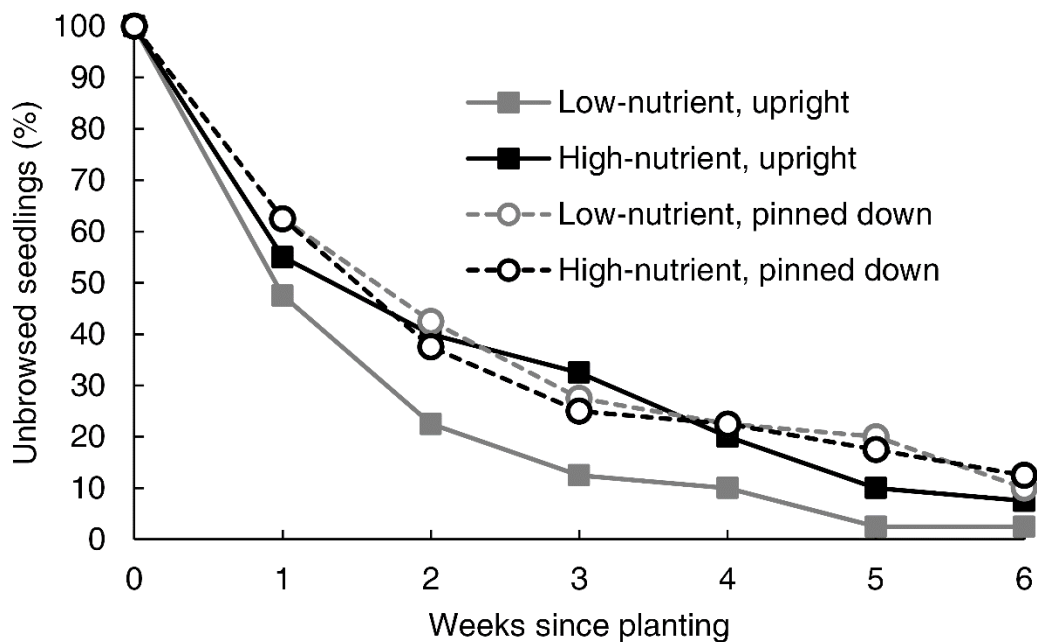
*Experiment 2: Browsing patterns as a function of seedling odour and visual cues*

Low-nutrient seedlings were browsed earlier than high-nutrient seedlings, upright seedlings earlier than those pinned down, and there was also a significant block effect (Fig. 5, Table 2a). In the upright position, the hazard of being browsed for high-nutrient seedlings was less than (i.e. 61 %) that for low-nutrient seedlings, but when seedlings were pinned down, nutrient treatment had no effect (Table 2b). Among low-nutrient seedlings, the browsing hazard was

almost halved (54 %) when they were pinned down instead of upright, but for high-nutrient seedlings position had no effect (Table 2b).

A greater percentage of seedling leaf area was browsed from upright ( $87.6 \pm 2.6$  %) than pinned down seedlings ( $70.7 \pm 4.2$  %;  $F_{1, 117} = 8.23$ ,  $P = 0.0049$ ), with no significant difference between nutrient treatments ( $F_{1, 117} = 0.39$ ,  $P = 0.54$ ) and no significant interaction between nutrient and position treatments ( $F_{1, 117} = 0.46$ ,  $P = 0.50$ ).

Of the 40 seedlings filmed, there were wallaby videos for 34 and no videos for six: three not browsed and three browsed. Patch visits usually resulted in seedling browsing, with 91% of filmed seedlings browsed during the first wallaby visit, and the remaining 9% browsed during the second visit. Wallabies browsed 47% of filmed seedlings during the day and 53% at night, with no significant differences between the four nutrient-by-position treatments (Fisher's exact test,  $P = 0.16$ ,  $N = 34$ ).



**Figure 5.** Experiment 2, seedlings remaining unbrowsed (%) over time by treatment (nutrient treatment and position;  $N = 160$ ).

**Table 2.** Experiment 2 (a) tests of fixed factors (nutrient treatment: 2 levels = low, high; position treatment: 2 levels = upright, pinned down) and random factor (block: 40 levels) on time to first browsing of *E. pilularis* seedlings. (b) Hazard ratio estimates and 95% confidence intervals (CI) for nutrient and position treatments retaining the interaction. Reference levels for effects (treatments) are low-nutrient and upright. Positive and negative parameter estimates indicate that the browsing hazard is higher or lower in the reference level, respectively. A hazard ratio < 1 indicates higher hazard in the reference level, 1 indicates equal hazard, and > 1 indicates lower hazard in the reference level. Bold type denotes significant effects at  $\alpha = 0.05$ .

(a) Factor	Estimate $\pm$ SE	$\chi^2$	DF	P	Adjusted DF	Adjusted P
Nutrient	-0.49 $\pm$ 0.25	3.81	1	0.051	0.96	<b>0.048</b>
Position	-0.62 $\pm$ 0.25	6.12	1	0.013	0.96	<b>0.012</b>
Nutrient x Position	0.49 $\pm$ 0.36	1.88	1	0.17	0.95	0.16
Block	-	37.90	-	-	20.53	<b>0.011</b>

(b) Factor	Interaction	Hazard Ratio Estimate	95% CI
Nutrient (high:low)	Upright	<b>0.61</b>	<b>0.38 – 1.00</b>
	Pinned down	1.00	0.61 – 1.65
Position (pinned down:upright)	Low-nutrient	<b>0.54</b>	<b>0.32 – 0.88</b>
	High-nutrient	0.88	0.53 – 1.44

## Discussion

Our first experiment demonstrated the ability of a browsing mammalian herbivore, the swamp wallaby, to detect olfactory cues emitted by cut seedlings in vials. Importantly, wallabies most often visited and investigated the odour arising from cut high-nutrient seedlings. Our second



experiment showed that, once visual cues were also provided, wallabies browsed low-nutrient seedlings earliest provided that they were upright. Wallabies almost always consumed seedlings once found, thus this result corresponds to differential detection rather than selection once found. So, while wallabies responded most strongly to the odour of cut high-nutrient seedlings in Experiment 1, the upright low-nutrient seedlings were more easily detected in Experiment 2. It may be that high-nutrient seedlings emitted more VOCs than low-nutrient seedlings only when cut, disturbing the plant cell structures; indeed, we only demonstrated a non-significant trend towards greater VOC emissions from high-nutrient seedlings. However, this does not preclude the possibility that high-nutrient seedlings may have differed qualitatively or in the quantity of key VOCs in the odour bouquet (e.g. *p*-cymene in the headspace of *E. nitens*; Low *et al.* 2014). Another plausible explanation for our results is that visual cues over-rode the response to odour alone in Experiment 2. The greater visual apparency of upright low-nutrient seedlings was likely a function of their colour and/or brightness in contrast to background vegetation combined with their upright position enabling relatively long-range detection. Low-nutrient seedlings had red stems and light green leaves, while the neighbouring vegetation consisted mostly of darker green ferns. Together, our results show that free-ranging wallabies demonstrate a labile response to plant cues during foraging, using different sensory modes depending on those proffered by the resource. To draw ecologically relevant conclusions for both herbivore and plant, it is therefore important to test sensory abilities in a realistic multi-sensory foraging context with whole plants. Experiments testing single sensory cues (Hanley, Collins & Swann 2011; Moelzner & Fink 2014) demonstrate capacity to differentiate using a single cue, but it is clear from our results that the browsing outcome has a more complex basis.

#### *Differential behavioural response to plant olfactory cues*

Free-ranging swamp wallabies differentiated nutrient treatments by plant odours, indicating that they perceived and responded to qualitative and/or quantitative differences in the emitted VOCs associated with cut high-nutrient seedlings. This occurred amongst a complex

vegetation community that provided a “noisy” olfactory background, including an overstorey of pine and eucalypt that are known to produce an array of VOCs (Cool & Zavarin 1992; Molangui *et al.* 1997). Despite this complexity, the results reflect those for the gastropod *Lymnea stagnalis* in simplified laboratory trials, which demonstrated a preference for VOCs extracted from nutrient-rich versus nutrient-poor algae (Moelzner & Fink 2014). Our study also showed that eastern grey kangaroos, which feed mainly on grasses (Davis, Coulson & Forsyth 2008), did not exhibit a differential response toward seedling odour treatments. This is consistent with a foraging strategy that does not involve searching for browse plants such as tree seedlings, and supports the inference that wallabies responded to seedling odour as a cue for food.

Neither wallabies nor kangaroos demonstrated a statistically significant difference in time to first patch visit or investigation between odour treatments. We treat these results with caution given that we designed Experiment 1 to test for differences in count variables (number of visits and investigations) and our power to test time-to-event data was thus limited. Broadly, wallabies first visited patches as would be expected if they were selecting odours of the highest nutrient-value resources. This again contrasted with kangaroos that visited all control patches but not all patches with seedling odour. As grass-specialists, kangaroos appeared to actually avoid plants containing toxic VOCs as do other macropodid grazers (e.g. Jones *et al.* 2003).

#### *The influence of olfactory and visual cues on browsing patterns*

In contrast to our expectations for odour-driven plant selection, low-nutrient seedlings were browsed earlier than high-nutrient seedlings (Experiment 2). This pattern was driven by seedlings in the upright “normal” position. Our results therefore do not support the hypothesis that wallabies use plant odours to preferentially detect and consume higher nutrient plants. We conclude that seedling colour or brightness was the most important cue, given that seedling height did not differ and biomass was actually lower in low-nutrient seedlings. While the visual system in swamp wallabies is unknown, there is evidence supporting the capacity

to differentiate shades of green in another macropod, the tammar wallaby *Macropus eugenii* (Hemmi & Grünert 1999; Ebeling, Natoli & Hemmi 2010; Ebeling & Hemmi 2014). Thus contrasts in colour and/or brightness against the background may play an important role in food detection by swamp wallabies as has been demonstrated in other foragers (Schaefer *et al.* 2006; Verdeny-Vilalta, Aluja & Casas 2015). The number of filmed seedlings browsed during the day and night did not differ significantly between treatments, and thus colour vision may be important for foraging wallabies even in low light conditions, as in several other taxa (Kelber & Roth 2006; Gomez *et al.* 2009; Kelber & Lind 2010; Veilleux, Louis & Bolnick 2013). Conversely, the reduced visual apparency of high-nutrient seedlings against neighbouring plants may act as an associational refuge from browsing (Hjältén, Danell & Lundberg 1993; Castagneyrol *et al.* 2013), although in our system the effect was short-lived.

Wallabies consumed seedlings during the first visit to most patches. This is consistent with previous research (Stutz *et al.* 2015b), and suggests that consumption of *E. pilularis* seedlings is limited by the detection phase of foraging and not by assessment and consumptive decision-making once the seedlings have been detected. The percentage of leaf area removed from browsed seedlings was very high irrespective of nutrient treatment, thus the food was essentially exhausted in one visit. Given that herbivores usually consume more from high- than low-nutrient plants under *ad libitum* conditions (Villalba, Provenza & Bryant 2002; Close *et al.* 2004; Loney *et al.* 2006), wallabies may have eaten a greater percentage from high- than low-nutrient seedlings if the seedlings had been larger. However wallabies did consume proportionally more from upright than pinned down seedlings. Thus, pinning seedlings to the ground did not impede swamp wallabies in locating seedlings but it did interfere with browsing of the foliage. Leaves may have been more difficult to handle or see against the ground cover.

### *A matter of cue detectability, not plant quality*

The behavioural patterns in Experiment 1 were consistent with better detection of odours associated with the (cut) higher-nutrient seedlings, but the inverse was true for browsing patterns of upright seedlings in Experiment 2. This result is only contradictory if interpreted on the basis of a single sensory cue (odour). From a multi-sensory perspective (vision and olfaction), we suggest that wallabies detected upright low-nutrient seedlings earlier than high-nutrient seedlings because they integrated the visual and olfactory cues from the plants. Visual apparency of the former was a stronger cue than odour from the latter, and it was the net strength of the resource cues that drove preferential browsing of low-nutrient seedlings.

While the detection of high quality food can contribute to foraging efficiently, failure to detect and consume the best quality food is not necessarily maladaptive. Foragers have limited time to meet their intake requirements and thus must make a trade-off between finding high quality food and consuming the maximum quantity of food (Senft *et al.* 1987). Given that most plants are nutritionally poor, a strategy to consume visually conspicuous low-nutrient seedlings rather than cryptic high-nutrient seedlings may therefore be more efficient. This may also explain the persistence of visual systems that result in the consumption of lower quality food in other foragers. For example, crows *Corvus ossifragus* can detect red (unripe) fruits from greater distances than black (ripe) fruits (Schaefer *et al.* 2006). Clearly – to a wallaby – even low-nutrient eucalypt seedlings were a worthwhile food source. So while the use of food cues may not result in greater detection of higher quality food, it nevertheless facilitates the detection and consumption of a certain quantity of preferred plant species. This may be as, or more, important than food quality.

## **Acknowledgements**

The University of Sydney Animal Ethics Committee approved all procedures (L04/2-2012/3/5696). All research was conducted under a National Parks Permit (BDR12/00001). A Small Environmental Grant from the Norman Wettenhall Foundation supported this research. Booderee National Park provided accommodation during fieldwork; we are grateful to Nicholas Dexter and Matthew Hudson for advice, and to Stig Pederson of Booderee Botanic Garden for collecting local seed and raising the plants used in a pilot study. We thank Markus Stutz for assistance in the field, Ajantha Horadagoda for conducting nitrogen analyses, and Charles Warren for use of his greenhouse at the University of Sydney.

## References

- Allison, P.D. (2010) *Survival analysis using SAS: a practical guide*, 2nd edn. SAS Institute Inc, Cary, NC.
- Augustine, D.J. & McNaughton, S.J. (1998) Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *The Journal of Wildlife Management*, **62**, 1165-1183.
- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.
- Bicca-Marques, J.C. & Garber, P.A. (2004) Use of spatial, visual, and olfactory information during foraging in wild nocturnal and diurnal anthropoids: a field experiment comparing *Aotus*, *Callicebus*, and *Saguinus*. *American Journal of Primatology*, **62**, 171-187.
- Castagneyrol, B., Giffard, B., Péré, C. & Jactel, H. (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology*, **101**, 418-429.
- Close, D., McArthur, C., Paterson, S., Fitzgerald, H., Walsh, A. & Kincade, T. (2003) Photoinhibition: a link between effects of the environment on eucalypt leaf chemistry and herbivory. *Ecology*, **84**, 2952-2966.
- Close, D.C., Beadle, C.L. & Hovenden, M.J. (2001) Cold-induced photoinhibition and foliar pigment dynamics of *Eucalyptus nitens* seedlings during establishment. *Australian Journal of Plant Physiology*, **28**, 1133-1141.
- Close, D.C., McArthur, C., Pietrzykowski, E., Fitzgerald, H. & Paterson, S. (2004) Evaluating effects of nursery and post-planting nutrient regimes on leaf chemistry and browsing of eucalypt seedlings in plantations. *Forest Ecology and Management*, **200**, 101-112.
- Cool, L.G. & Zavarin, E. (1992) Terpene variability of mainland *Pinus radiata*. *Biochemical Systematics and Ecology*, **20**, 133-144.

- Côté, S.D., Rooney, T.P., Tremblay, J.-P., Dussault, C. & Waller, D.M. (2004) Ecological impacts of deer overabundance. *Annual Review of Ecology Evolution and Systematics*, **35**, 113-147.
- Davis, N.E., Coulson, G. & Forsyth, D.M. (2008) Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research*, **35**, 684-694.
- Dearing, M.D., Foley, W.J. & McLean, S. (2005) The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 169-189.
- Duistermars, B.J. & Frye, M.A. (2008) Crossmodal visual input for odor tracking during fly flight. *Current Biology*, **18**, 270-275.
- Duncan, A.J., Ginane, C., Elston, D.A., Kunaver, A. & Gordon, I.J. (2006) How do herbivores trade-off the positive and negative consequences of diet selection decisions? *Animal Behaviour*, **71**, 93-99.
- Ebeling, W. & Hemmi, J.M. (2014) Dichromatic colour vision in wallabies as characterised by three behavioural paradigms. *PLoS ONE*, **9**, e86531.
- Ebeling, W., Natoli, R.C. & Hemmi, J.M. (2010) Diversity of color vision: not all Australian marsupials are trichromatic. *PLoS ONE*, **5**, e14231.
- Foley, W.J., Iason, G.R. & McArthur, C. (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? *Nutritional ecology of herbivores: proceedings of the Vth International Symposium on the Nutrition of Herbivores* (eds H.-J. Jung & G. Fahey), pp. 130–209. American Society of Animal Science, Savoy, Illinois.
- Goff, S.A. & Klee, H.J. (2006) Plant volatile compounds: sensory cues for health and nutritional value? *Science*, **311**, 815-819.
- Gomez, D., Richardson, C., Lengagne, T., Plenet, S., Joly, P., Léna, J.-P. & Théry, M. (2009) The role of nocturnal vision in mate choice: females prefer conspicuous males

in the European tree frog (*Hyla arborea*). *Proceedings of the Royal Society of London B: Biological Sciences*.

- Gouinguéné, S.P. & Turlings, T.C.J. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296-1307.
- Gould, K.S. (2004) Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *Journal of Biomedicine and Biotechnology*, 314-320.
- Halitschke, R., Stenberg, J.A., Kessler, D., Kessler, A. & Baldwin, I.T. (2008) Shared signals –‘alarm calls’ from plants increase apparency to herbivores and their enemies in nature. *Ecology Letters*, **11**, 24-34.
- Hanley, M.E., Collins, S.A. & Swann, C. (2011) Advertising acceptability: is mollusk olfaction important in seedling selection? *Plant Ecology*, **212**, 727-731.
- Hanley, M.E., Girling, R.D., Felix, A.E., Olliff, E.D., Newland, P.L. & Poppy, G.M. (2013) Olfactory selection of *Plantago lanceolata* by snails declines with seedling age. *Annals of Botany*, **112**, 671-676.
- Hanley, M.E. & Sykes, R.J. (2009) Impacts of seedling herbivory on plant competition and implications for species coexistence. *Annals of Botany*, **103**, 1347-1353.
- Hartley, S.E., Iason, G.R., Duncan, A.J. & Hitchcock, D. (1997) Feeding behaviour of red deer (*Cervus elaphus*) offered sitka spruce saplings (*Picea sitchensis*) grown under different light and nutrient regimes. *Functional Ecology*, **11**, 348-357.
- Hemmi, J.M. & Grünert, U. (1999) Distribution of photoreceptor types in the retina of a marsupial, the tammar wallaby (*Macropus eugenii*). *Visual Neuroscience*, **16**, 291-302.
- Henery, M.L., Wallis, I.R., Stone, C. & Foley, W.J. (2008) Methyl jasmonate does not induce changes in *Eucalyptus grandis* leaves that alter the effect of constitutive defences on larvae of a specialist herbivore. *Oecologia*, **156**, 847-859.
- Hjältén, J., Danell, K. & Lundberg, P. (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. *Oikos*, **68**, 125-131.



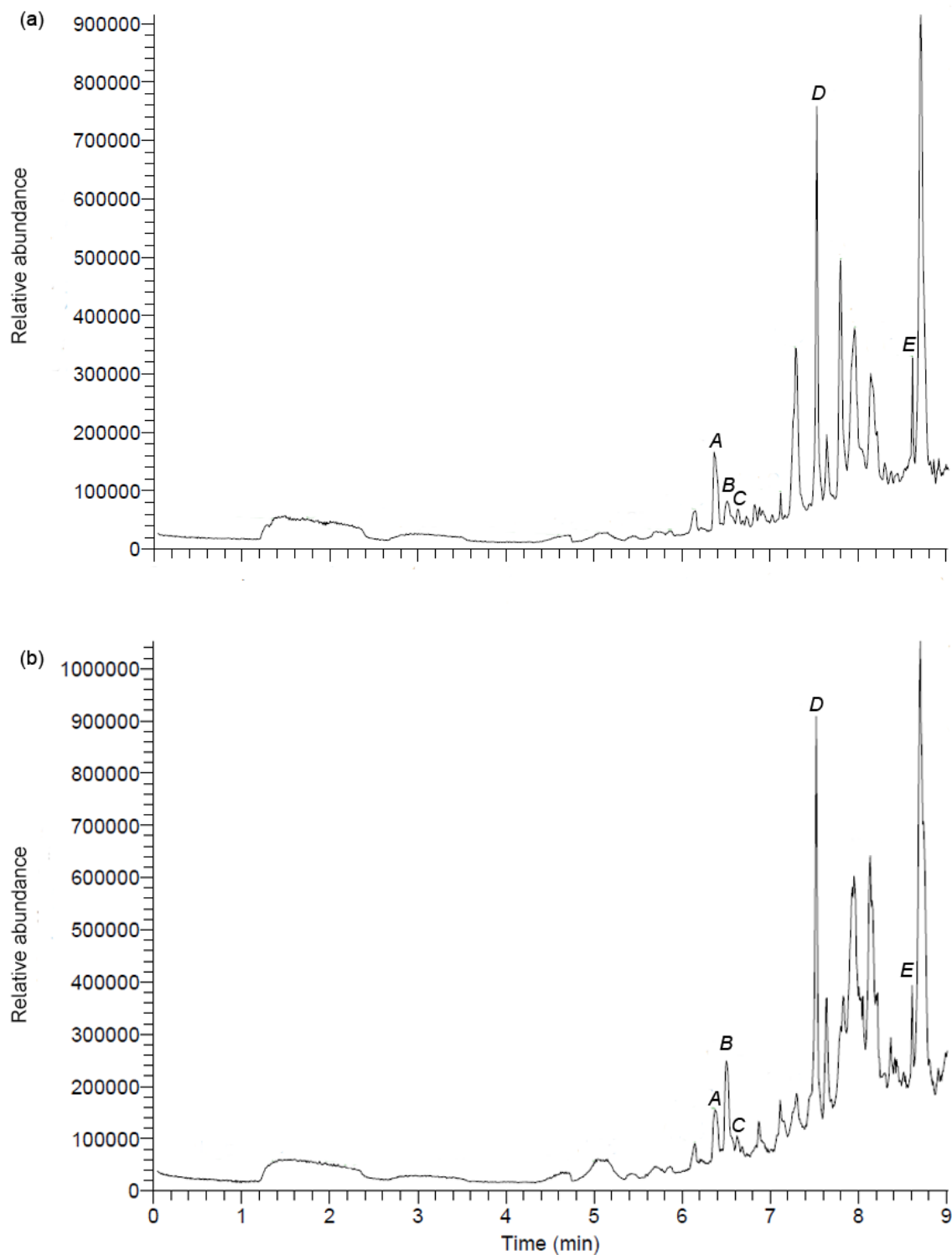
- Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, **15**, 176-184.
- Iason, G. & Villalba, J. (2006) Behavioral strategies of mammal herbivores against plant secondary metabolites: the avoidance–tolerance continuum. *Journal of Chemical Ecology*, **32**, 1115-1132.
- Jachmann, H. (1989) Food selection by elephants in the ‘miombo’ biome, in relation to leaf chemistry. *Biochemical Systematics and Ecology*, **17**, 15-24.
- Jones, A.S., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2003) Kangaroos avoid eating seedlings with or near others with volatile essential oils. *Journal of Chemical Ecology*, **29**, 2621-2635.
- Karageorgou, P., Buschmann, C. & Manetas, Y. (2008) Red leaf color as a warning signal against insect herbivory: Honest or mimetic? *Flora - Morphology, Distribution, Functional Ecology of Plants*, **203**, 648-652.
- Karageorgou, P. & Manetas, Y. (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. *Tree Physiology*, **26**, 613-621.
- Kelber, A. & Lind, O. (2010) Limits of colour vision in dim light. *Ophthalmic and Physiological Optics*, **30**, 454-459.
- Kelber, A. & Roth, L.S.V. (2006) Nocturnal colour vision – not as rare as we might think. *The Journal of Experimental Biology*, **209**, 781-788.
- Langenheim, J.H. (1994) Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, **20**, 1223-1280.
- Lawler, I.R., Stapley, J., Foley, W.J. & Eschler, B.M. (1999) Ecological example of conditioned flavor aversion in plant–herbivore interactions: effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology*, **25**, 401-415.

- Loney, P., McArthur, C., Sanson, G., Davies, N., Close, D. & Jordan, G. (2006) How do soil nutrients affect within-plant patterns of herbivory in seedlings of *Eucalyptus nitens*? *Oecologia*, **150**, 409-420.
- Low, P.A., McArthur, C., Fisher, K. & Hochuli, D.F. (2014) Elevated volatile concentrations in high-nutrient plants: do insect herbivores pay a high price for good food? *Ecological Entomology*, **39**, 480-491.
- McArthur, C., Hagerman, A.E. & Robbins, C.T. (1991) Physiological strategies of mammalian herbivores against plant defenses. *Plant Defenses Against Mammalian Herbivory* (eds R.T. Palo & C.T. Robbins), pp. 103-114. CRC Press, Boca Raton, Florida.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2007) Effects of within-patch characteristics on the vulnerability of a plant to herbivory. *Oikos*, **116**, 41-52.
- Miller, A.M., McArthur, C. & Smethurst, P.J. (2009) Spatial scale and opportunities for choice influence browsing and associational refuges of focal plants. *Journal of Animal Ecology*, **78**, 1134-1142.
- Moelzner, J. & Fink, P. (2014) The smell of good food: volatile infochemicals as resource quality indicators. *Journal of Animal Ecology*, **83**, 1007-1014.
- Molangui, T., Menut, C., Bouchet, P., Bessièrè, J.-M. & Habimana, J.-B. (1997) Aromatic plants of tropical Central Africa. Part XXX. Studies on volatile leaf oils of 10 species of *Eucalyptus* naturalized in Rwanda. *Flavour and Fragrance Journal*, **12**, 433-437.
- Moore, B.D., Wallis, I.R., Palá-Paúl, J., Brophy, J.J., Willis, R.H. & Foley, W.J. (2004) Antiherbivore chemistry of *Eucalyptus* - cues and deterrents for marsupial folivores. *Journal of Chemical Ecology*, **30**, 1743-1769.
- Munoz, N.E. & Blumstein, D.T. (2012) Multisensory perception in uncertain environments. *Behavioral Ecology*, **23**, 457-462.
- O'Reilly-Wapstra, J.M., McArthur, C. & Potts, B.M. (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Functional Ecology*, **18**, 677-684.

- O'Reilly-Wapstra, J., Potts, B., McArthur, C. & Davies, N. (2005) Effects of nutrient variability on the genetic-based resistance of *Eucalyptus globulus* to a mammalian herbivore and on plant defensive chemistry. *Oecologia*, **142**, 597-605.
- Peñuelas, J. & Llusà, J. (2001) The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum*, **44**, 481-487.
- Peñuelas, J. & Llusà, J. (2004) Plant VOC emissions: making use of the unavoidable. *Trends in Ecology & Evolution*, **19**, 402-404.
- Prevedello, J.A., Forero-Medina, G. & Vieira, M.V. (2011) Does land use affect perceptual range? Evidence from two marsupials of the Atlantic Forest. *Journal of Zoology*, **284**, 53-59.
- Provenza, F.D., Kimball, B.A. & Villalba, J.J. (2000) Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. *Oikos*, **88**, 424-432.
- Rapley, L., Allen, G., Potts, B. & Davies, N. (2007) Constitutive or induced defences - how does *Eucalyptus globulus* defend itself from larval feeding? *Chemoecology*, **17**, 235-243.
- Rooney, T. (2009) High white-tailed deer densities benefit graminoids and contribute to biotic homogenization of forest ground-layer vegetation. *Plant Ecology*, **202**, 103-111.
- Schaefer, H.M., Levey, D.J., Schaefer, V. & Avery, M.L. (2006) The role of chromatic and achromatic signals for fruit detection by birds. *Behavioral Ecology*, **17**, 784-789.
- Schaefer, H.M. & Ruxton, G.D. (2011) *Plant-animal communication*. Oxford University Press, Oxford, U.K.
- Senft, R.L., Coughenour, M.B., Bailey, D.W., Rittenhouse, L.R., Sala, O.E. & Swift, D.M. (1987) Large herbivore foraging and ecological hierarchies. *Bioscience*, **37**, 789-799.
- Shrader, A.M., Bell, C., Bertolli, L. & Ward, D. (2012) Forest or the trees: at what scale do elephants make foraging decisions? *Acta Oecologica*, **42**, 3-10.
- Sprent, J.A. & McArthur, C. (2002) Diet and diet selection of two species in the macropodid browser-grazer continuum: do they eat what they 'should'? *Australian Journal of Zoology*, **50**, 183-192.

- Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015a) Associational refuge in practice: can existing vegetation facilitate woodland restoration? *Oikos*, **124**, 571–580.
- Stutz, R.S., Banks, P.B., Dexter, N. & McArthur, C. (2015b) Herbivore search behaviour drives associational plant refuge. *Acta Oecologica*, **67**, 1-7.
- Sumner, P. & Mollon, J.D. (2000a) Catarrhine photopigments are optimized for detecting targets against a foliage background. *The Journal of Experimental Biology*, **203**, 1963-1986.
- Sumner, P. & Mollon, J.D. (2000b) Chromaticity as a signal of ripeness in fruits taken by primates. *The Journal of Experimental Biology*, **203**, 1987-2000.
- Szetei, V., Miklósi, Á., Topál, J. & Csányi, V. (2003) When dogs seem to lose their nose: an investigation on the use of visual and olfactory cues in communicative context between dog and owner. *Applied Animal Behaviour Science*, **83**, 141-152.
- Veilleux, C.C., Louis, E.E. & Bolnick, D.A. (2013) Nocturnal light environments influence color vision and signatures of selection on the OPN1SW opsin gene in nocturnal lemurs. *Molecular Biology and Evolution*, **30**, 1420-1437.
- Verdeny-Vilalta, O., Aluja, M. & Casas, J. (2015) Relative roles of resource stimulus and vegetation architecture on the paths of flies foraging for fruit. *Oikos*, **124**, 337-346.
- Villalba, J.J., Provenza, F.D. & Bryant, J.P. (2002) Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos*, **97**, 282-292.
- Wahungu, G.M., Catterall, C.P. & Olsen, M.F. (1999) Selective herbivory by red-necked pademelon *Thylogale thetis* at rainforest margins: factors affecting predation rates. *Australian Journal of Ecology*, **24**, 577-586.
- Warburton, H. & Mason, G. (2003) Is out of sight out of mind? The effects of resource cues on motivation in mink, *Mustela vison*. *Animal Behaviour*, **65**, 755-762.

## Supporting Information



**Figure S1.** Example chromatograms of VOC peaks in the headspaces of (a) low-nutrient and (b) high-nutrient *E. pilularis* seedlings. Letters denote terpenes tentatively identified through spectral comparisons with the NIST 2002 database: A =  $\alpha$ -phellandrene, B =  $p$ -cymene, C =  $\alpha$ -pinene, D = terpinen-4-ol and E =  $\alpha$ -humulene.

## CHAPTER 6

---

### General Discussion



## Research findings and implications

Using a series of field experiments, I examined foraging behaviour in a model mammalian generalist browser, the swamp wallaby *Wallabia bicolor*, and the consequences for seedlings of a native canopy tree *Eucalyptus pilularis*. I tested the influence of neighbouring plants on browsing patterns of focal tree seedlings to infer which stages of the foraging process were affected (Chapter 2), and the behavioural mechanism responsible for refuge-provision (Chapter 3). Associational plant refuge in my study system was short-lived; I therefore explored the plant cues used by wallabies to find focal seedlings amongst complex vegetation, allowing them to overcome any refuge-providing characteristics (Chapter 4). I then tested whether raising seedlings under differing nutrient availabilities could alter plant cues sufficiently to affect detection by wallabies (Chapter 5).

The antagonistic interaction between herbivores and plants is generally explored from a single perspective: foraging behaviour by herbivores or patterns of browsing damage on plants. My research explored the dual perspectives involved in plant-herbivore interactions by linking foraging behaviour to its browsing outcomes for plants. I examined the herbivore and plant perspectives of foraging in a free-ranging herbivore searching for a natural food source against the complex background of a real foraging environment. Thus my work is ecologically relevant and contributes both to the fundamental understanding of plant-herbivore interactions, and to applications of this understanding to restoration ecology.

### *Existing vegetation can disrupt the foraging process*

In Chapter 2, I tested the potential of a suite of vegetation variables to provide associational refuge for eucalypt seedlings from free-ranging herbivores. Browsing of focal seedlings was delayed in patches with higher cover of understorey vegetation at the small scale, and lower canopy cover, fewer browsed neighbouring plant species, and fern- rather than grass-dominated vegetation at the large scale. With the exception of canopy cover, all of these variables are consistent with the repellent plant mechanism of associational refuge, operating

by lowering patch appeal and/or providing a physical barrier to detection of plant cues. I suggested that browsing delay under low canopy cover may have resulted from preference for habitat offering higher probability of seedling encounter (seed drop under canopy), shelter from the elements and/or lower perceived risk of predation. Swamp wallabies also consumed the focal plant in most cases when they investigated it, and thus there appeared to be little scope for refuge once a focal plant had been detected (i.e. in deciding whether to consume it or not). To the best of my knowledge, this is the first study of associational plant effects to link specific vegetation variables to the phases of the foraging process they disrupt. From an applied perspective, my study suggests that it may be beneficial to retain unpalatable and/or visually obstructive plant neighbours when restoring systems under herbivore pressure, supporting retention of existing vegetation as an emerging strategy in restoration ecology (Gómez-Aparicio *et al.* 2004; Padilla & Pugnaire 2006; Gómez-Aparicio 2009).

#### *A behavioural mechanism for associational plant refuge*

Having determined that existing vegetation can provide associational refuge by influencing both the search and detection stages of foraging (Chapter 2), I aimed to understand the behavioural mechanisms behind this in Chapter 3. I compared wallaby foraging behaviours toward a central eucalypt seedling in patches of existing vegetation and in manipulated patches consisting of obstructive, unpalatable native plants. Despite the fact that wallabies visited manipulated patches more often, they provided refuge for focal plants by reducing investment in searching once there, while all first visits to control patches resulted in focal plant browsing. All seedlings were eventually browsed, but the only survivors after one year were those in manipulated patches, suggesting that these neighbours played a role in plant tolerance to herbivory (Stowe *et al.* 2000). By understanding how neighbours alter herbivore behaviour to provide refuge, we can explain the mechanism behind a functioning refuge, and why it may break down, and thus make better predictions about outcomes for plants.



In my study system, a relatively unpalatable and (visually / olfactorily) obstructive plant neighbourhood was effective at delaying browsing of palatable nursery-raised tree seedlings. Generalist browsers in other systems may also be effectively deterred from browsing planted vegetation in restoration areas using non-preferred structurally dominant neighbourhoods. However, the scale of the neighbourhood may vary significantly dependent on body size and forage selection strategy, as well as on the overall herbivore density (i.e. competition for resources). The effectiveness of associational plant refuge can be reduced under high herbivore densities because they can damage protective neighbours (Smit *et al.* 2007) or because the net foraging effort is high so that focal plants are found despite the presence of protective neighbours (Brooker *et al.* 2006). Thus the use of associational plant refuges for restoration in areas with abundant herbivores may need to be coupled with measures to control the herbivore population such as culling and/or contraception.

#### *Olfaction can play a critical role in plant detection*

Herbivores in my study system were eventually able to overcome refuge and browse eucalypt seedlings (Chapters 2 and 3), suggesting that they used one or more plant cues to detect seedlings from amongst their neighbours. Several lines of evidence supported the use of olfactory information, including observations of sniffing behaviours (Chapter 3) and previous research on swamp wallabies showing elevated intake of food pellets containing low levels of a volatile terpene amongst an inedible matrix (Bedoya-Pérez *et al.* 2014b). In Chapter 4, I demonstrated that wallabies have the ability to detect seedling volatile organic compounds (VOCs), visiting and investigating buried vials with cut seedlings both earlier and more often than control (empty) vials. I also tested browsing response to whole seedlings. Browsing was not delayed by reducing long-range visual cues (pinning seedlings to the ground), nor by enhancing odour but not visual cues (planting upright seedlings with an additional pinned down seedling). This suggests that wallabies were able to use odour to detect seedlings, and the threshold for detection was low.

The use of olfaction in detecting plants has important implications both for herbivore ecology and restoration strategies. The use of plant odour by leaf-eating herbivores to detect preferred plants is clearly a response to a cue that is not intended to attract herbivores – unlike signals to attract pollinators and seed-dispersers to fruits and flowers, there is no advantage for a plant to have its foliage consumed. Leaf-eaters may therefore be eavesdropping on VOCs that are by-products of plant metabolic processes or those that are produced for a specific function, such as plant defence, and using them to inform their search for food (Ehrlich & Raven 1964; Bedoya-Pérez *et al.* 2014b; Kohl, Miller & Dearing 2015). Restoration of palatable plants amongst herbivores employing olfaction to forage may therefore require strategies to subvert odour cues from plants. This may include creating neighbourhoods of unpalatable plants that emit VOCs themselves (i.e. decreasing the cue-to-noise ratio) or that physically obstruct the odour plume emitted by VOC-producing focal plants.

#### *Fertilizer regimes can influence detection of plants by herbivores*

Evidence supporting the use of VOCs to detect palatable eucalypt seedlings amongst complex vegetation (Chapter 4) prompted an exploration of whether plant cues could be manipulated to alter detection by wallabies. I maintained eucalypt seedlings under two extreme fertilizer regimes, creating two groups of seedlings that differed significantly in nitrogen content – a characteristic associated with differences in biomass, physical appearance and levels of VOCs (Chapter 5; Close *et al.* 2005; Low *et al.* 2014). Wallabies tended to visit and investigate vials containing cut high-nutrient seedlings most often, followed by cut low-nutrient seedlings and empty control vials, consistent with selection based on higher nitrogen content and foliage quantity. However, the pattern of browsing of whole seedlings was the inverse of what I expected from the vial experiment: low-nutrient seedlings were browsed earlier than high-nutrient seedlings. When long-range visual cues were reduced by pinning seedlings to the ground, the effect of nutrient treatment disappeared. This suggests that earlier browsing of low-nutrient seedlings was brought about by greater visual apparency. While herbivores can respond to odours of nutritionally higher quality plants, cue strength may be more important,

acting at the detection rather than the consumption stage. For browsers, vision and olfaction may therefore be used to detect palatable plants but perhaps not to assess fine-scale differentials in plant quality within the same plant species. Intra-specific differences in quality may not be as important as maximising food intake, favouring a strategy that prioritises quantity of specific plant species over quality. My study demonstrates that herbivores may have finer scale perceptual abilities than indicated by browsing outcomes. Importantly, this suggests that the ecological relevance of sensory cues tested in isolation may be more complex in realistic multisensory environments.

In terms of restoration strategies, these results indicate that attempts to reduce seedling appeal through nursery practices should consider how seedling cues may be affected. One reason why nursery-raised plants used in restoration or forestry operations may be particularly appealing to herbivores is their high nutrient content relative to natural vegetation. While nutrient-starved seedlings in my study were browsed earlier than well-fertilized seedlings, the former probably represents an extreme case. These effects may not be apparent using seedlings of other tree species or less severe fertilizer treatments. Meanwhile, any manipulations of individual focal plants (and thus the cues they emit), should consider the characteristics of the plant neighbourhood and the foraging strategy of the dominant herbivores in the system.

### **Future directions**

As with any study, the experimental approaches I used were limited both by time and resources. This means that my experiments were relatively short-term (three weeks to three months), which is substantially less than the time required for tree seedlings to escape the stage most vulnerable to browsing, much less establish a forest. I used externally-sourced seedlings in pots when locally-grown seedlings were not available (Chapters 2 and 5) and these needed to be removed at the completion of each experiment, thus for most experiments

I could not assess longer-term variables such as seedling survival and compensatory growth (except at one year post-planting in Chapter 3). In addition, the experiments were often conducted only in one season and thus I could not rule out seasonal effects on the patterns I observed. Certainly the browsing pressure appeared to be greater during some experiments than others – for example, vials with cut seedlings appeared to be investigated more rapidly in Chapter 5 than in Chapter 4 – and this may have been related to the quality and/or quantity of available forage as a result of climate or macropod population density. Unlike studies that take advantage of an existing restoration effort or forestry plantation, I planted seedlings specifically for my experiments and thus sample sizes were not large. Replicating experiments with larger samples may result in sufficient power to explore other factors affecting browsing patterns, including any differences in browsing behaviours between night and day that may have important implications for the use of olfactory and visual cues. Behaviours of individuals were recorded using cameras and thus over a small spatial scale. Marking individuals may enable more detailed observations of behaviours between patches.

#### *Integrating herbivore behaviour into associational plant effect theory*

In Chapters 2 and 3, I illustrated that an understanding of herbivore foraging behaviour leads to a better mechanistic understanding of the plant neighbourhood effects resulting in browsing patterns. Further, in Chapters 4 and 5, I demonstrated the role of olfaction and vision in detecting focal plants amongst the neighbouring vegetation, overcoming any refuge effects. Extending this approach to other systems would lead to a better understanding of why refuge works or fails, and thus appropriate strategies to create refuge in restoration systems.

#### *Plant quality indicators for browsers*

In a field study using food pellets, Bedoya-Pérez *et al.* (2014a) showed that swamp wallabies adjusted pellet consumption in response to the concentration of nitrogen and one terpene VOC (1,8-cineole). I manipulated the nitrogen content of eucalypt seedlings and thus, presumably, also the within-plant concentration of plant secondary metabolites, but found no

difference in the percentage of foliage consumed between high- and low-nutrient seedlings (Chapter 5). Clearly, browsers do not consume all plants available to them, and I suspect that if food items were larger, post-selection differences in consumption may occur, as with many other species (Hartley *et al.* 1997; Close *et al.* 2004; Loney *et al.* 2006). In my study, it was not possible to test this later stage in the foraging process because the focal plant (eucalypt seedling) comprised a short meal that was consumed in a few minutes at most, irrespective of whether it was of high- or low-nutrient content. Future studies could therefore use larger plants to assess whether swamp wallabies demonstrate post-selective differences in consumption, as seen in other plant-herbivore systems. It would also be valuable to consider the role of flavour and learned flavour aversions in selection of real plants by wild browsers, as has been studied in domestic and captive animals (Provenza, Kimball & Villalba 2000; Yearsley *et al.* 2006; Bergvall 2009).

#### *Detection of visual plant cues: chromatic vs achromatic contrasts*

In Chapter 5, I concluded that differential browsing was driven by visual cues provided by low-nutrient seedlings. The visual system in marsupials is not well understood, yet it appears that testing behavioural responses can be informative as to the functionality of vision for foraging. Further work could determine whether differential detection of visual cues in browsing mammals is due to chromatic (colour) or achromatic (brightness) contrasts. Sumner and Mollon (2000a; 2000b) suggest chromatic contrasts may be more useful against a background of vegetation because incident light can cause great variation in achromatic background cues. This may be true for frugivorous primates, but folivory may operate under a different paradigm, especially for groups of marsupials that may be at least functionally red-green colour-blind (Ebeling, Natoli & Hemmi 2010).

#### *Large-scale changes to plant-herbivore interactions*

In Chapter 2, I found that plant variables at two patch scales influenced browsing of focal plants by swamp wallabies. Choices made by herbivores at larger patch scales can influence

those made at smaller ones, and are not simply the sum of the small patch choices (Searle *et al.* 2006). I therefore suggest that we must consider the changes we have set in motion at coarse scales to be able to predict plant-herbivore interactions into the future and how they might contribute to shaping our world.

Climate change is expected to have physiological impacts on plants and herbivores, with potentially synergistic flow-on effects on their interactions that reverberate through the ecosystem (Ayres 1993). Higher temperatures, for example, may result in higher rates of plant odour emissions (Peñuelas & Llusà 2001; Gouinguené & Turlings 2002; Holopainen & Gershenson 2010), which in turn may alter detection rates by herbivores, depending on how cue:noise ratios are affected. Herbivores may also be directly affected by altered climatic conditions; climate change has already facilitated the range expansion of tropical marine herbivores into temperate waters, leading to marine deforestation in areas ill-adapted to functionally different herbivores (Vergés *et al.* 2014a; Vergés *et al.* 2014b). Such changes may influence higher order trophic interactions, particularly for prey that rely on habitat structure as refuge from predators (Farina *et al.* 2014).

The establishment of plants and herbivores in novel environments (e.g. deer in New Zealand; Husheer, Coomes & Robertson 2003) is likely to continue as human mobility increases. Meanwhile, intentional introductions aimed at mediating negative impacts will also require understanding of plant-herbivore interactions. Re-introductions of locally extinct herbivores have had unanticipated consequences – some have experienced dramatic increases in population numbers under conditions of low mortality, such as koalas (Menkhorst 2008) and deer (Nuttle *et al.* 2011), resulting in over-browsing. To regulate over-browsing by native herbivores, locally extinct apex predators such as wolves have been reintroduced to some areas, with a reduction in herbivore numbers resulting in profound changes in vegetation structure (Ripple & Beschta 2003). Similar reintroductions have been proposed for dingoes *Canis lupus dingo* in Australian ecosystems (Glen *et al.* 2007; Letnic, Ritchie & Dickman 2012), the major native predator of swamp wallabies (Robertshaw & Harden 1986). To better

predict the impacts of predator reintroductions, we will need to understand the interactions between their herbivorous prey and the plant community.

## References

- Ayres, M.P. (1993) Global change, plant defense, and herbivory. *Biotic interactions and global change* (eds P. Kareiva, J. Kingsolver & R. Huey). Sinauer Associates, Sunderland, MA.
- Bedoya-Pérez, M., Issa, D., Banks, P. & McArthur, C. (2014a) Quantifying the response of free-ranging mammalian herbivores to the interplay between plant defense and nutrient concentrations. *Oecologia*, **175**, 1167-1177.
- Bedoya-Pérez, M.A., Isler, I., Banks, P.B. & McArthur, C. (2014b) Roles of the volatile terpene, 1,8-cineole, in plant–herbivore interactions: a foraging odor cue as well as a toxin? *Oecologia*, **174**, 827-837.
- Bergvall, U.A. (2009) Development of feeding selectivity and consistency in food choice over 5 years in fallow deer. *Behavioural Processes*, **80**, 140-146.
- Brooker, R.W., Scott, D., Palmer, S.C.F. & Swaine, E. (2006) Transient facilitative effects of heather on Scots pine along a grazing disturbance gradient in Scottish moorland. *Journal of Ecology*, **94**, 637-645.
- Close, D.C., McArthur, C., Hagerman, A.E. & Fitzgerald, H. (2005) Differential distribution of leaf chemistry in eucalypt seedlings due to variation in whole-plant nutrient availability. *Phytochemistry*, **66**, 215-221.
- Close, D.C., McArthur, C., Pietrzykowski, E., Fitzgerald, H. & Paterson, S. (2004) Evaluating effects of nursery and post-planting nutrient regimes on leaf chemistry and browsing of eucalypt seedlings in plantations. *Forest Ecology and Management*, **200**, 101-112.
- Ebeling, W., Natoli, R.C. & Hemmi, J.M. (2010) Diversity of color vision: not all Australian marsupials are trichromatic. *PLoS ONE*, **5**, e14231.
- Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants - a study in coevolution. *Evolution*, **18**, 586-608.
- Farina, S., Arthur, R., Pagès, J.F., Prado, P., Romero, J., Vergés, A., Hyndes, G., Heck, K.L., Glenos, S. & Alcoverro, T. (2014) Differences in predator composition alter the



- direction of structure-mediated predation risk in macrophyte communities. *Oikos*, **123**, 1311-1322.
- Glen, A.S., Dickman, C.R., Soulé, M.E. & Mackey, B.G. (2007) Evaluating the role of the dingo as a trophic regulator in Australian ecosystems. *Austral Ecology*, **32**, 492-501.
- Gómez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**, 1202-1214.
- Gómez-Aparicio, L., Zamora, R., Gómez, J.M., Hódar, J.A., Castro, J. & Baraza, E. (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, **14**, 1128-1138.
- Gouinguéné, S.P. & Turlings, T.C.J. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296-1307.
- Hartley, S.E., Iason, G.R., Duncan, A.J. & Hitchcock, D. (1997) Feeding behaviour of red deer (*Cervus elaphus*) offered sitka spruce saplings (*Picea sitchensis*) grown under different light and nutrient regimes. *Functional Ecology*, **11**, 348-357.
- Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, **15**, 176-184.
- Husheer, S.W., Coomes, D.A. & Robertson, A.W. (2003) Long-term influences of introduced deer on the composition and structure of New Zealand *Nothofagus* forests. *Forest Ecology and Management*, **181**, 99-117.
- Kohl, K.D., Miller, A.W. & Dearing, M.D. (2015) Evolutionary irony: evidence that 'defensive' plant spines act as a proximate cue to attract a mammalian herbivore. *Oikos*, **124**, 835-841.
- Letnic, M., Ritchie, E.G. & Dickman, C.R. (2012) Top predators as biodiversity regulators: the dingo *Canis lupus dingo* as a case study. *Biological Reviews*, **87**, 390-413.
- Loney, P., McArthur, C., Sanson, G., Davies, N., Close, D. & Jordan, G. (2006) How do soil nutrients affect within-plant patterns of herbivory in seedlings of *Eucalyptus nitens*? *Oecologia*, **150**, 409-420.

- Low, P.A., McArthur, C., Fisher, K. & Hochuli, D.F. (2014) Elevated volatile concentrations in high-nutrient plants: do insect herbivores pay a high price for good food? *Ecological Entomology*, **39**, 480-491.
- Menkhorst, P. (2008) Hunted, marooned, re-introduced, contracepted: a history of Koala management in Victoria. *Too Close for Comfort: Contentious Issues in Human–Wildlife Encounters* (eds D. Lunney, A. Munn & W. Meikle), pp. 73-92.
- Nuttall, T., Yarger, E.H., Stoleson, S.H. & Ristau, T.E. (2011) Legacy of top-down herbivore pressure ricochets back up multiple trophic levels in forest canopies over 30 years. *Ecosphere*, **2**, art4.
- Padilla, F.M. & Pugnaire, F.I. (2006) The role of nurse plants in the restoration of degraded environments. *Frontiers in Ecology and the Environment*, **4**, 196-202.
- Peñuelas, J. & Llusià, J. (2001) The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum*, **44**, 481-487.
- Provenza, F.D., Kimball, B.A. & Villalba, J.J. (2000) Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. *Oikos*, **88**, 424-432.
- Ripple, W.J. & Beschta, R.L. (2003) Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. *Forest Ecology and Management*, **184**, 299-313.
- Robertshaw, J. & Harden, R. (1986) The ecology of the dingo in northeastern New South Wales .4. Prey selection by dingoes, and its effect on the major prey species, the swamp wallaby, *Wallabia bicolor* (Desmarest). *Wildlife Research*, **13**, 141-163.
- Searle, K.R., Vandervelde, T., Hobbs, N.T., Shipley, L.A. & Wunder, B.A. (2006) Spatial context influences patch residence time in foraging hierarchies. *Oecologia*, **148**, 710-719.
- Smit, C., Vandenberghe, C., Ouden, J.d. & Müller-Schärer, H. (2007) Nurse plants, tree saplings and grazing pressure: changes in facilitation along a biotic environmental gradient. *Oecologia*, **152**, 265-273.

- Stowe, K.A., Marquis, R.J., Hochwender, C.G. & Simms, E.L. (2000) The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics*, **31**, 565-595.
- Sumner, P. & Mollon, J.D. (2000a) Catarrhine photopigments are optimized for detecting targets against a foliage background. *The Journal of Experimental Biology*, **203**, 1963-1986.
- Sumner, P. & Mollon, J.D. (2000b) Chromaticity as a signal of ripeness in fruits taken by primates. *The Journal of Experimental Biology*, **203**, 1987-2000.
- Vergés, A., Steinberg, P.D., Hay, M.E., Poore, A.G.B., Campbell, A.H., Ballesteros, E., Heck, K.L., Booth, D.J., Coleman, M.A., Feary, D.A., Figueira, W., Langlois, T., Marzinelli, E.M., Mizerek, T., Mumby, P.J., Nakamura, Y., Roughan, M., van Sebille, E., Gupta, A.S., Smale, D.A., Tomas, F., Wernberg, T. & Wilson, S.K. (2014a) The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B*, **281**, 20140846.
- Vergés, A., Tomas, F., Cebrian, E., Ballesteros, E., Kizilkaya, Z., Dendrinos, P., Karamanlidis, A.A., Spiegel, D. & Sala, E. (2014b) Tropical rabbitfish and the deforestation of a warming temperate sea. *Journal of Ecology*, **102**, 1518-1527.
- Yearsley, Jonathan M., Villalba, Juan J., Gordon, Iain J., Kyriazakis, I., Speakman, John R., Tolkamp, Bert J., Illius, Andrew W. & Duncan, Alan J. (2006) A theory of associating food types with their postingestive consequences. *The American Naturalist*, **167**, 705-716.