The influences of plant secondary metabolites on the foraging behaviour and carrying capacities of the African elephant, *Loxodonta africana*

by

Melissa Holbrook Schmitt

Submitted in fulfilment of the academic requirements for the degree of

Doctor of Philosophy

In the Discipline of Ecology
School of Life Sciences
College of Agriculture, Engineering, and Science
University of KwaZulu-Natal
Pietermaritzburg, South Africa

As the candidate's supervisors, we have approved this thesis for submission.

Supervisors:

Dr. Adrian M. Shrader and Prof. David M. Ward

Signed: _____   Name: Dr. Adrian M. Shrader   Date: 20 February 2017

Signed: _____   Name: Prof. David M. Ward   Date: 20 February 2017

Abstract

Herbivore diet selection is influenced by plant quality and availability. For browsing and mixed-feeding herbivores, such as elephants, which consume trees and shrubs, plant quality is influenced by the concentrations of crude protein, fibre, and plant secondary metabolites (PSMs). To date, the driving factors behind elephant diet selection have not been well understood. The broad aim of this study was to better understand how pre-ingestive cues such as volatile organic compounds (VOCs) and postingestive cues such as the effects of plant secondary metabolites (PSMs) influenced the foraging behaviour of African elephants. I also examined the salivary tannin-binding proteins of elephants and how these influenced carrying capacities. I found that elephants were able to differentiate between plant odours in the form of VOCs across multiple spatial scales. This could help elephants to reduce their search time while foraging across a landscape. I also found that these pre-ingestive cues were a better indicator of diet preference than the tested postingestive cues such as digestibility, crude protein, tannins (a type of total polyphenol) and the ratio of palatable: unpalatable indices. The VOCs in *preferred* plant species had significantly lower concentrations and diversity of PSMs than avoided species, particularly with respect to monoterpenes, a known anti-herbivory VOC. In contrast, *avoided* plant species were more digestible and had lower levels of polyphenols and tannins. Ultimately, I found that terpenes play a stronger role in elephant diet choice than phenolics (such as tannins) and other postingestive feedback measures. This is likely because terpenes are a pre-ingestive cue that also incur postingestive costs, which the elephants can use prior to making foraging decisions, unlike other postingestive feedback measures. While it seems counterintuitive that *preferred* plants have high concentrations of tannins, which reduces the amount of crude protein available, I found that elephants have salivary tannin-binding proteins, which neutralize the influences of tannins by an average of 75%. This neutralization influences the amount of available crude protein, which would, in turn, influence the carrying-capacity for elephants. I constructed an optimal-foraging model for carrying capacity that incorporated the negative effects of tannins and the neutralization of these chemicals by the tannin-binding affinity of elephant saliva. I also included diet breadth and dietary contribution of browsed species, browse quality, and the available standing crop of browsed species. As a result, my model produced more plausible estimates of elephant carrying capacity than conventional standing-crop models.

COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE

Declaration 1: Plagiarism

I, Melissa Holbrook Schmitt, declare that

(i)   The research reported in this dissertation/thesis, except where otherwise indicated, is my original research

(ii)   This dissertation/thesis has not been submitted for any degree or examination at any other university

(iii)   This dissertation/thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv)   This dissertation/thesis does not contain other peoples' writing, unless specifically acknowledged as being sourced from other researchers.
Where other written sources have been quoted, then:

   a) Their words have been re-written but the general information attributed to them has been referenced;

   b) Where their exact words have been used, their writing has been placed inside quotation marks and referenced.

v)   Where I have reproduced a publication of which I am author, co-author or editor, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

Melissa Holbrook Schmitt
20 February 2017

Declaration 2: Publications

My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 5

1. Schmitt, M. H.*, Ward, D., & Shrader, A. M. (2016). Incorporating secondary metabolites, tannin-binding proteins, and diet breadth into carrying-capacity models for African elephants. *Ecological Modelling*, *332*, 8-18.

This publication reflects my own original work. I collected the data, formulated the mathematical model, and wrote the manuscript. My supervisors are listed as the co-authors to reflect their constructive input on the study as well as for providing comments on drafts.

Melissa Holbrook Schmitt
Student

Dr. Adrian Morgan Shrader
Supervisor

Professor David Mercer Ward
Co-supervisor

20 February 2017

Preface

The work described in this thesis was carried out at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa, from January 2013 to October 2016, under the supervision of Dr. Adrian Morgan Shrader and Professor David Mercer Ward.

This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, represents original work by the authors and has not been submitted in any form to another university. Where use of other work was made, it has been acknowledged in the document.

Melissa Holbrook Schmitt
Student

Dr. Adrian Morgan Shrader
Supervisor

Professor David Mercer Ward
Co-supervisor

20 February 2017

Acknowledgements

This study would not have been possible without the help of numerous people. I would like to give my sincere thanks to everyone who contributed his/her time to my thesis.

Firstly, I would like to thank both of my supervisors, Dr. Adrian M. Shrader and Prof. David M. Ward. From the first time we met to discuss the proposal of this study, I have been amazed at their vast knowledge and eagerness to impart this knowledge to others. They have given me numerous comments, critiques, as well as encouragement throughout the duration of my graduate career. Without their invaluable efforts, this project would not have been possible.

Secondly, I would like to thank the staff at Adventures with Elephants and the Rory Hensman Elephant Research Unit (RHERU) located outside Bela Bela, South Africa. Sean and Mike Hensman very graciously allowed me to conduct a significant portion of my research at Adventures with Elephants using their six semi-tame elephants. I cannot thank the Hensman family enough for their interest, support, and inspiration throughout the duration of my study. In addition, I would like to thank all of the elephant handlers from Adventures with Elephants, especially Francis Kamupambe and David Mupupu, for keeping me safe while I collected data as well as teaching me about the elephants. I would like to give particular thanks to Zacharia Sithole Muzotora for his support, enthusiasm and for personally guarding and guiding me throughout the duration of my fieldwork in Bela Bela. Without the generosity and support of the AWE staff, this project would not have been possible.

Thank you to Prof. Dean Goldring, Prof. Theresa Coetzer, and their students, particularly Robert Krause, Zainab Baig, and Kajal Mansi Fowder, for assistance with the gel electrophoresis procedures. I am eternally grateful for the patience and time spent teaching me how to run gels as well as the constructive feedback about my results.

Prof. Clare McArthur served as both an incredible role model as well as an invaluable resource to me. I am sincerely grateful for the time she invested discussing my current projects as well as ideas for future projects. The energy and time that she spent with me has furthered my knowledge and academic path and for that, I am extremely grateful.

Prior to this study, I was fairly unfamiliar with many of the details of the world of olfaction. To learn about these details, I turned to Dr. Adam Shuttleworth, who spent many long days (and weeks), tirelessly teaching me the nuances of the olfactory world and the techniques used to study odour. Without his help, many aspects of my study would not have been possible.

I would like to thank all of my labmates for their many hours of project discussion, statistical debates, draft editing, and moral support. I give particular recognition and thanks to Kayleigh Muller, Tamanna Patel, Doug Makin, Mark Summers, and Steph Lyle for assisting me in the lab as well as providing me with constructive criticism on all aspects of my project. Also, huge thanks to Dr. Sammy Davis for being my PhD sister and pillar of strength on the other side of the globe.

I thank the Stears family for being my second family here in South Africa. Without their love, support, and encouragement, I would never have been as successful as I have been. I am very proud to be a part of their family. I thank my family for encouraging me to chase my dreams and for supporting me along the way. I appreciate all of the time and effort they have put in to helping me achieve my goals. I am extremely proud to become the next "Dr. Holbrook/Schmitt" in our family. I would not be the person I am today without the lifetime of love, adventure, and zeal for science that my parents have given me. Their love, support, and sacrifices have only been rivalled by that of the Stears family.

My husband, Keenan Stears, has been my biggest cheerleader and has spent countless hours discussing my project, debating statistical models, editing drafts, making sure my

teacup is always full, and taking care of me when I forget to. I thank him for being the best husband and colleague that I could ever ask for.

Table of Contents

List of Tables

List of Figures

Thesis aims

The broad aim of my PhD was to better understand how volatile organic compounds (VOCs), plant secondary metabolites (PSMs), and salivary tannin-binding proteins influenced the foraging behaviour of African elephants (*Loxodonta africana*). In addition, I determined how the combination of several of these factors influenced elephant carrying capacities. My four main data chapters focused on the following objectives: (1) to determine if elephants use plant odour to make foraging decisions, (2) to establish to what extent postingestive feedback measures and pre-ingestive cues of available plants indicate elephant diet choice, (3) to explore the tannin-binding affinity of elephant saliva to understand how the presence of salivary tannin-binding proteins explains elephant diet choice, and (4) to build a carrying-capacity model that incorporates both dietary preferences and the ability of elephants to semi-neutralize tannins that they eat via salivary tannin-binding proteins.

Thesis layout and overview

All four of my data chapters are written as scientific papers, one of which has been published in *Ecological Modelling* (Chapter 5). Because of this, I have written these chapters using the pronoun "we" instead of "I". However, all the writing is my personal work, with my supervisors providing advice and editing only. Because each data chapter is written as a scientific paper, I have put a reference list, which is formatted for the journal *Functional Ecology,* at the end of each chapter. The only exception is in Chapter 5, which has its own reference list in the style of *Ecological Modelling*, because it has already been published there.

My thesis is comprised of six chapters: a literature review, four data chapters, and a conclusions chapter. Due to the structure and content of my data chapters, some repetition is inevitable, particularly in the Methods sections and reference lists. My literature review introduces aspects of foraging theory, plant secondary metabolites (PSMs), the mechanisms

that herbivores use to cope with PSMs, and relevant details about my study species, the African elephant.

My first data chapter explores the role of plant odour cues for foraging elephants. Prior studies have suggested the importance of odour cues for foraging mammalian herbivores (Bedoya-Pérez *et al.* 2014; Stutz *et al.* 2015; Stutz *et al.* 2016b), but no study has established whether elephants use odour to make foraging decisions across multiple spatial scales. To do this, I identified whether elephants use olfactory cues in the form of volatile organic compounds (VOCs) emitted from the plants in their environment to make feeding decisions, and the spatial scale they used to make these decisions. I showed that elephants select their preferred plant species across large and small spatial scales, potentially allowing elephants to reduce their search time by allowing them to target preferred plant species both within a feeding station and between patches.

My second data chapter focuses on the content of the odour cues emitted from preferred and avoided plant species. Previous attempts to determine what drives browser diet selection have typically focused on postingestive feedback measures (e.g. crude protein, digestibility, total polyphenols, including tannins: Pellew 1984; Foguekem *et al.* 2011; Ulappa *et al.* 2014), which are all aspects of vegetation that can only be assessed by herbivores after they commit to eating a particular item (Illius & Gordon 1993; Provenza 1995). An alternative, and potentially more effective, method is to use pre-ingestive cues in the form of VOCs emitted from plants. To determine the extent to which postingestive feedback measures and pre-ingestive cues predict elephant diet choices, I used a number of laboratory analyses on plant samples collected from the field. For the postingestive feedback measures, I determined the crude protein, digestibility, and total polyphenol (including tannins) concentrations of preferred and avoided plant species. For the pre-ingestive cues, I used a Gas Chromatograph-Mass Spectrometer (GC-MS) to identify VOCs using odour samples

collected in the field. I found that terpenes play a stronger role in elephant diet choice than phenolics (such as tannins) and other postingestive feedback measures, which is likely because terpenes are a pre-ingestive cue that also incur postingestive costs. Thus, elephants can use them as a cue prior to making foraging decisions, unlike other postingestive feedback measures. It is, however, possible that the elephants had linked olfactory cues of the different plants to postingestive feedback via conditioned learning prior to my study.

My third data chapter focuses on the tannin-binding affinity of elephant saliva and how this may help elephants cope with tannins. Non-ruminant, large-bodied herbivores may be forced to eat low-quality food to sustain their metabolic requirements (Bell 1969; Geist 1974; Jarman 1974). Because tannins reduce plant quality by decreasing the amount of available protein that herbivores can absorb, the presence of salivary tannin-binding proteins may allow herbivores greater access to the protein in their forage items. I found that elephants were able to reduce the activity of tannins using tannin-binding proteins in their saliva. This means that the estimated amount of available protein in browse material is higher than if the effects of tannins alone were included, which ultimately influences the carrying capacity of an area.

For my fourth data chapter, I constructed a carrying-capacity model for elephants based on Hobbs and Swift's (1985) carrying-capacity model of mammalian herbivores. Very few models have incorporated tannins to explore their effects on carrying capacity (Windels & Hewitt 2011). I used the tannin-binding affinity of elephant saliva as well as other factors including crude protein content, tannin content, and available biomass. This chapter has been published in *Ecological Modelling* (Volume 332, 24 July 2016, Pages 8–18). Finally, in my conclusions chapter (Chapter 6), I synthesize the results from the previous chapters and suggest future directions for studies.

Chapter One

Literature review

Foraging ecology

Food items are not homogeneously distributed throughout a landscape (Shipley 2007; Bell 2012). Moreover, nutrient availability varies spatially, temporally, across plant species, and even within a plant species (Mattson 1980). As a result, variability of available nutrients can pose a foraging challenge for herbivores. Thus, herbivores should make foraging decisions that allow them to meet their energetic requirements and maximise their fitness despite temporal and nutritional variation in food quality and availability. This can be an acute problem for large mammalian herbivores, especially those with an inefficient digestive system such as elephants, because they need to spend much of their day feeding (Demment & Van Soest 1985).

In an effort to understand how animals make foraging decisions within their environment, Optimal Foraging Theory models (OFT) were developed (Pyke *et al.* 1977; Stephens & Krebs 1986). To reduce energy loss, animals should make informed decisions about how and where to feed (Pyke *et al.* 1977). Moving from patch to patch at random likely would not yield as high an energy intake as moving among targeted patches that are composed of nutrient-rich, preferred food items (Charnov 1976). The Marginal Value Theorem, which is part of OFT, predicts optimal residence times at a given patch (Charnov 1976). This theorem suggests that foragers should employ a feeding strategy that maximises gain per unit time where resources decrease with time (Charnov 1976). However, for herbivores to do this, they need to use a certain currency (e.g. most high-quality food per unit time) and have an intimate knowledge of their environment in terms of this currency. As a result, herbivores must consider how long they should forage at a given patch, before expending energy and spending time searching for a new one (Charnov 1976; Klaassen *et al.*

2006). When making foraging decisions at spatial scales beyond an individual's body length, it is imperative that the individual forages in the most energy-efficient as well as nutritionally maximising manner to reduce energy wastage and increase fitness (Pyke *et al.* 1977; Houston & McNamara 2014).

Although energy consideration is important for herbivores, they have multiple requirements beyond energy. Thus, there have been numerous models formed based on a multi-currency framework to gain a better understanding of these requirements and how herbivores make foraging decisions based on them (Sterner & Elser 2002; Elser 2006; Raubenheimer *et al.* 2009). Hobbs and Swift's 1985 model for carrying-capacities of herbivores raises the point that herbivores do not use all resources similarly and that forage items must be explicitly incorporated into models. Additional models were then formed that were also diet-specific, such as the Geometric Framework (GF) approach. This approach graphically models the main relationships among important variables in nutritional ecology based on the logic of state–space geometry (Raubenheimer *et al.* 2009). According to this logic, important variables are expressed and related to each other within a geometric space defined by two or more relevant food components (Raubenheimer *et al.* 2009). Other models, such as the Ecological Stoichiometry approach, have been formed to examine the balance between multiple chemical elements and energy in ecological interactions (Elser 2006).

Factors influencing diet selection

Herbivores make dietary selections on the basis of *palatability* and *acceptability* (Heady 1964; Mentis & Tainton 1981; Owen-Smith & Cooper 1987). *Palatability* is the preference with which an item is consumed (GRSC 1989) determined by inherent features of the item itself (e.g. digestibility, nutrient levels, plant defence mechanisms) (Trollope *et al.* 1990), whereas *acceptability* is the overall attractiveness of an item to an animal (Tainton 1999).

For mammalian herbivores, it is important to gain information about the quality and availability of plants in their environment to determine the plant's palatability and acceptability. Herbivores can obtain this information using several methods, including visual examination of available plants, taste-testing, learning from relatives and peers, and assessing the olfactory cues emitted from plants (Krueger *et al.* 1974; Thorhallsdottir *et al.* 1990; Stutz *et al.* 2016b).

Plant chemical defences

Many species of plants have secondary metabolites (PSMs, also called secondary compounds because they are not used for photosynthesis (primary compounds)) which deter herbivory, usually by disrupting key biochemical processes in cells within the herbivore (McArthur *et al.* 1991; Shimada 2006; Kohl *et al.* 2015). Secondary metabolites are primarily found in woody plants (Rhoades & Cates 1976), and thus browsers and mixed-feeders are more affected than pure grazers. There are a wide array of PSMs, but some of the most well-known and documented PSMs for their disruptive properties for mammalian herbivores are tannins and monoterpenes (Shimada 2006; Barbehenn & Constabel 2011; Kohl *et al.* 2015).

There are two major types of tannins: condensed and hydrolysable, of which there are several subcategories (Salminen & Karonen 2011). Condensed tannins are compounds made of flavans linked by tight C-C bonds that are difficult to break apart, while hydrolysable tannins are esters of polyols, as well as phenolic acids, that are easily decomposed (Shimada 2006). Tannins have numerous negative side effects including reducing digestibility and effective protein intake (Robbins *et al.* 1987; Shimada & Saitoh 2003), and even, in more extreme cases, liver and kidney failure (Fowler & Richards 1965), endogenous nitrogen loss (Blytt *et al.* 1988; Shimada & Saitoh 2003), damage to the gastrointestinal mucosa (Blytt *et al.* 1988), and acute toxicity (Shimada 2006).

Monoterpenes are part of the terpenoid class of chemicals, which is the largest chemical class, comprised of 15-20,000 terpenoids (Harborne 1991). Monoterpenes are very numerous with more than 1000 individual monoterpenes identified (Seigler 1998). Monoterpenes have been documented in a wide range of plant species from algae to monocots and dicots (Seigler 1998) and are well documented for having anti-herbivory properties (Harborne 1991; Duncan *et al.* 1994; Vourc'h *et al.* 2002; Shipley *et al.* 2012; Frye *et al.* 2013; Ulappa *et al.* 2014; Kohl *et al.* 2015). Monoterpenes have been found to inhibit digestive enzymes (Kohl *et al.* 2015) and compromise energy budgets of mammals (Sorensen *et al.* 2005). Furthermore, monoterpenes are volatile organic compounds (VOCs) that are often very fragrant (Harborne 1991). VOCs are compounds that easily evaporate due to their light weight (between 100-200 amu) and high vapour pressure (Schoonhoven *et al.* 2005), which makes them easy to detect via olfaction (Harborne 1991). Moreover, herbivores are known to use odour cues to make dietary selections in an effort to avoid ingesting monoterpenes (Vourc'h *et al.* 2002; Frye *et al.* 2013; Nobler 2016).

To cope with PSMs, mammalian herbivores can either behaviourally reduce their intake by eating a mixed diet and/or by cyclic or intermittent ingestion, or by avoiding consumption of specific PSMs (Bernays *et al.* 1994; Villalba *et al.* 2004; Provenza *et al.* 2007). Behavioural avoidance can be driven by dietary learning and conditioned flavour aversions. Dietary learning, occurs when information is obtained via personal experience, or by learning from family members, regarding which plant species to consume or avoid (Bryant *et al.* 1991; Bates *et al.* 2008; Hart *et al.* 2008). Often young individuals watch and learn what food items to eat or avoid from older conspecifics (Provenza 1994). For example, lambs have exhibited learning in reference to their dietary selection (Mirza & Provenza 1990). Thorhallisdottir *et al.* (1990) found that lambs made dietary selections based on both their own trial and error as well as from watching the diet selection of their mother. Conditioned

flavour aversion, which occurs when an herbivore associates the flavour of a certain plant with postingestive costs, has been demonstrated in numerous herbivore species (Provenza *et al.* 1990; Lawler *et al.* 1999)**.** Provenza *et al.* (1990) found that goats learn to avoid blackbrush twigs because they contain high levels of condensed tannins. Additionally, brushtail possums (*Trichosurus vulpecula*) have learned to avoid consuming specific *Eucalyptus* plants because of elevated levels of diformylphloroglucinol compounds (DFPCs) (Lawler *et al.* 1999). Although the compounds are not directly detectable becasue they are not volatile, the possums have learned that the DFPC levels are correlated with certain volatile terpenes, which they can use as a cue to make diet selections (Lawler *et al.* 1999).

Herbivores can also use physiological mechanisms to neutralize the impacts of these compounds (Freeland & Janzen 1974). Arguably, the simplest way that mammalian herbivores can handle secondary compounds is by changing their feeding strategy to avoid species or plant parts that contain elevated levels of PSM. However, the ubiquitous distribution of PSMs makes this difficult.

Herbivores have evolved a range of physiological mechanisms to cope with PSMs in their diets (Freeland & Janzen 1974; McArthur *et al.* 1991; Launchbaugh *et al.* 2001). Tannins, most notably, form precipitates with tannin-binding proteins in the saliva of many mammalian species (Mehansho *et al.* 1987a; Shimada 2006; Schmitt *et al.* 2016). When salivary tannin-binding proteins bind to tannins, they form protein precipitates. Bound tannins can then pass through the digestive system without deleterious effects (Shimada 2006).

Numerous studies have revealed the existence of tannin-binding proteins in saliva across a wide array of omnivorous and herbivorous species of mammals that frequently encounter these items in their diets. These include ruminants (Austin *et al.* 1989; Juntheikki 1996; Schmitt *et al.* 2016), rodents and lagomorphs (Spielman & Bennick 1989; Mole *et al.* 1990; McArthur *et al.* 1995) and primates (Wrangham & Waterman 1981; Oppenheim *et al.*

1985; Ann & Lin 1993), including humans (Bennick & Connell 1971; Bacon & Rhodes 1998). Several studies have demonstrated that the production of tannin-binding proteins in some herbivore species is inducible (i.e. greater production when needed) (Mehansho *et al.* 1985; Mehansho *et al.* 1987b; Clauss *et al.* 2005a). Inducibility of salivary tannin-binding proteins suggests that these herbivore species are able to cope with changes in their environment with regard to plant quality/defences by either incorporating species with higher level of PSMs or individual plants of the same species that have higher PSM levels. This means that salivary tannin-binding proteins may play a large role in mammalian herbivore foraging ecology.

Chemical analyses

To gain an estimate of plant quality, one can use a number of chemical assays to determine various measures of forage quality including crude protein, digestibility, and the concentration of various PSMs. These assays can either provide a general or very specific picture of plant quality and are not necessarily perfect. There are numerous assays that are directed towards more general measures of plant quality such as the use of 6.25 as a factor to convert total nitrogen (N) to % crude protein (Van Soest 1994). This technique, which gives a general estimate of crude protein content, is not ideal because the factor is not universally applicable to all plant species (McArt *et al.* 2009; Windels & Hewitt 2011). To gain a more accurate estimate of available N, one could use an analysis for digestible N (as per (Wallis *et al.* 2010). Furthermore, the field of tannin analysis has expanded dramatically in recent years, with a large number of tannin-specific protocols available as well as better ways to estimate the actual anti-nutritional value of tannins (Barbehenn & Constabel 2011; Salminen & Karonen 2011). Dietary tannins distort the relationship between total protein and available protein (Windels & Hewitt 2011). Thus, one could also take this relationship into account to determine a more precise measure of available protein

(Robbins *et al.* 1987). Another general assay used frequently is that for total polyphenols (e.g. the Prussian Blue Analyses). Although these assays provide an estimate of the concentration of total polyphenols present in plant samples, they do not identify specific compounds. This is problematic because the profile of phenolic compounds in one plant species is unlikely to be the same as those in any other species. One could use more advanced technology such as liquid chromatography–mass spectrometry (LC-MS) to identify and quantify the individual compounds within vegetation (Rohloff 2015). However, despite these recent advances, I used the general approaches for estimating crude protein and tannins due to logistical constraints. It is important to note that using general chemical assays can be limiting. Future research should consider using more detailed analyses.

Olfaction

One cue that herbivores may use when making foraging decisions is the smell of the different plant species (Bell 2012). For terrestrial animals, odour molecules travel through the air and contact with their chemoreceptors, which in turn stimulate the receptors (Eisenberg & Kleiman 1972). These odour molecules are made up of volatile organic compounds (VOCs). Both mammals and insects are known to use VOCs in their environment for a range of purposes such as communication, detection of prey items, detection of predators, and to find locations to oviposit (Geervliet *et al.* 1998; Kluever *et al.* 2009; Mitko *et al.* 2016). However, for herbivorous mammals, olfaction has been primarily identified as a mechanism by which herbivores can locate and select which plant species to eat (Bedoya-Pérez *et al.* 2014; Stutz *et al.* 2016a; Stutz *et al.* 2016b).

Herbivores are able to use olfaction in plant choice because plants emit odours in the form of VOCs, many of which are green-leaf volatiles (Dudareva *et al.* 2004) as well as many plant secondary metabolites (PSMs) (Peñuelas & Llusià 2004). Plant VOCs can be released

from different parts of the plant (e.g. leaf, stem, or inflorescence) and can have different intentions (i.e. attractant vs. deterrent) (Tholl *et al.* 2006). For example, VOCs released from the inflorescence can attract pollinators (Pichersky & Gershenzon 2002; Shuttleworth & Johnson 2009; Johnson *et al.* 2011). Alternatively, injured or damaged plants can emit VOCs that deter further damage from herbivores or pathogens as well as signal to nearby plants about potential impending damage (Engelberth *et al.* 2004). Additionally, unlike tannins, PSM VOCs can be detected prior to committing to consumption of a plant (Tholl *et al.* 2006).

Similar to tannins, certain PSM VOCs, such as terpenes (e.g. monoterpenes and sesquiterpenes), are more effective as herbivory deterrents with increasing concentrations (Courtois *et al.* 2012). A basal level of certain terpenes and tannins can be acceptable to herbivores in their diets (Cooper & Owen-Smith 1985; Shipley *et al.* 2012; Camp *et al.* 2015). However, there will be a threshold at which the concentrations of these chemicals become too high for the herbivore, and it will ultimately avoid feeding on these plants (Cooper *et al.* 1988; Shipley *et al.* 2012; Camp *et al.* 2015). As a result, herbivores may use differences in scent profiles to avoid individual plants that contain high PSM levels (Peñuelas & Llusià 2004; Frye *et al.* 2013).

An additional advantage of olfaction is that plant odour can be detected from much greater distances than visual cues, as well as through visually obstructing barriers, because odour can travel well beyond the sight line of the odour-producing plant (Bell 2012; Stutz *et al.* 2015; Stutz *et al.* 2016b). While odour has the potential to be directed by the wind, and can be affected by temperature and light (Niinemets *et al.* 2004), it could still be a useful tool for herbivores to detect preferred plant species across multiple spatial scales (Bell 2012). Increasingly, research has suggested that herbivorous mammals can use the VOCs emitted from plants to make foraging decisions (Bedoya-Pérez *et al.* 2014; Stutz *et al.* 2015; Stutz *et al.* 2016a). However, the degree and scale to which mammalian herbivores use odours when

foraging is still largely unknown (Provenza & Balph 1987; Pietrzykowski *et al.* 2003; Bedoya-Pérez *et al.* 2014).

The African elephant

The African Elephant, *Loxodonta africana*, is the largest extant terrestrial mammal, weighing between 3000 kg (females) to 7000 kg (males) (Skinner & Chimimba 2005). They are native to most of sub-Saharan Africa and occur in a wide range of habitats with differing vegetation types (Skinner & Chimimba 2005). Both African and Asian elephants (*Elephas maximus*) have been found to use olfaction in controlled experimental trials (Plotnik *et al.* 2014; Miller *et al.* 2015). In the case of African elephants, a recent study suggested that their sense of smell is ~2 times greater than the domestic dog (*Canis lupus*) (Miller *et al.* 2015). For example, African elephants are able to detect 2,4,6-Trinitrotoluene (TNT), which is an explosive compound that is difficult to detect via olfaction due to poor volatilization, suggesting that African elephants have a high olfactory acuity (Miller *et al.* 2015). While Miller *et al.*'s (2015) study suggests that African elephants have a very sensitive sense of smell, to my knowledge, no study has explored their senses of smell under natural conditions, or on natural food plants.

Elephants are hindgut fermenters, which forces them to ingest greater amounts of low-quality high-fibre food than ruminants because fibre does not limit passage rate and retard daily intake (Demment & Van Soest 1985; Clauss *et al.* 2003). For elephants, the necessity to eat more is even higher than expected due to its body size, which is due to the low digestive efficiency that elephants achieve (Müller *et al.* 2013). Elephants show several physiological adaptations for digestion, including a decreased digestion time (i.e. faster passage rate of food) due to a shorter gastro-intestinal tract for faster throughput of plant matter, a reduced caecum, and a wider small intestine and colon than theoretically expected (Clauss *et al.* 2003).

Additionally, African elephants have a very limited ability to alter intake level to change digestion retention time (Clauss *et al.* 2007). These traits result in a faster passage rate but yield a lower nutritional gain (Clauss *et al.* 2003; Clauss *et al.* 2005b). These features allow elephants to use a wider range of forage items than ruminants (Owen-Smith & Chafota 2012).

Another factor that influences elephant diet selection is their body size. Body size influences mass-specific metabolic requirements (Kleiber 1947; Owen-Smith 1988), which results in larger animals requiring less energy per unit body mass than smaller animals. This means that larger animals can tolerate lower quality foods than smaller animals (Bell 1969; Jarman 1974), allowing them to feed on a wide range of food items. This relationship between body mass and digestive efficiency of herbivores is known as the Jarman-Bell principle (Geist 1974). As predicted by this principle (Bell 1969; Geist 1974; Jarman 1974), African elephants should be able to maintain their fitness by being non-selective and eating low-quality foods. Elephants are classified as mixed-feeders (Codron *et al.* 2006; Owen-Smith & Chafota 2012) and have a very wide dietary breadth that incorporates grasses as well as browse such as leaves of trees and shrubs and high-fibre forage such as twigs, bark, and roots (Bax & Sheldrick 1963; Cerling *et al.* 1999; Owen-Smith & Chafota 2012). However, the relative contribution of grass and browse to their diets varies both temporally as well as regionally (Owen-Smith 1988; van der Merwe *et al.* 1988; Codron *et al.* 2006). In some areas, elephants are reported to eat a diet comprised mostly of grass (Buss 1961), while other studies report that elephants heavily utilize grass after heavy rains (De Boer *et al.* 2000) and browse during the dry season when grass quality is poorest (Codron *et al.* 2006). Contrary to theoretical assumptions about elephant diets, recent findings suggest that elephants are more selective than once thought. For example, a number of studies have shown that elephants forage selectively and favour certain plant species over others (Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013; Ward *et al.* 2016). In particular, elephants have been shown

to minimize their intake of polyphenols and, particularly, tannins (Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013).

Because of their high total metabolic requirements due to their large body size, elephants eat between 100-200 kg of wet-matter vegetation per day (Wyatt & Eltringham 1974; Guy 1976). In addition to the large quantities of vegetation that they consume, they can also be destructive foragers (Ben-Shahar 1993; Conybeare 2004; Kerley & Landman 2006; Valeix *et al.* 2011). Elephants frequently knock down, up-root, or otherwise damage trees (Ben-Shahar 1993; Conybeare 2004; Kerley & Landman 2006; Valeix *et al.* 2011). Thus, within reserves, elephants can have negative impacts on vegetation structure and biodiversity (Kerley & Landman 2006). Because of the large impact that elephants have on shaping the structure and community composition of the vegetation in their environments, elephants have been labelled as ecosystem engineers (Jones *et al.* 1994; Valeix *et al.* 2011).

Southern African countries have a large number of reserves to preserve biodiversity across a range of biomes (Scholes & Biggs 2005). These reserves are usually fenced to minimize the potential danger to people and farmland caused by wild animals, and to reduce illegal entry of people (such as for poaching). Consequently, these countries face a unique problem of trying to manage expanding elephant populations within confined areas that are often small (Owen-Smith *et al.* 2006). As a result of living in a closed system, to minimize problems caused by inbreeding and to reduce negative impacts on the vegetation, elephant populations in southern Africa have often been removed and relocated to a number of reserves across Africa, albeit with varying degrees of success. A more drastic management technique to control elephant populations is culling. Historically, culling was the only viable option for controlling expanding populations (Buechner *et al.* 1963; Glover 1963). Typically, when culling elephants, the entire family herd is eliminated because of their complex social structure (Thomson 2003; Slotow *et al.* 2008). An alternative technique to control elephant

populations is the use of contraceptives (Pimm & van Aarde 2001; Kerley & Shrader 2007).

A key difference between culling and using contraceptives on population management of

elephants is that contraceptives do not have an immediate effect on reducing a current

population size. Many elephant-based management decisions regarding culling, removal of

individuals, and the administration of contraceptives are based on stocking-rate estimates

(Slotow *et al.* 2005). Estimating an ecologically accurate carrying capacity for an area is

critical for a sustainable management of a species (Leopold 1933; Morellet *et al.* 2007; Kuzyk

et al. 2009). However, models of carrying capacity that incorporate appropriate key variables

are lacking for elephant populations.

References

Ann, D.K. & Lin, H.H. (1993) Macaque salivary proline-rich protein: structure, evolution, and expression. *Critical Reviews in Oral Biology & Medicine,* **4**, 545–551.

Austin, P.J., Suchar, L.A., Robbins, C.T. & Hagerman, A.E. (1989) Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *Journal of Chemical Ecology,* **15**, 1335–1347.

Bacon, J.R. & Rhodes, M.J.C. (1998) Development of a competition assay for the evaluation of the binding of human parotid salivary proteins to dietary complex phenols and tannins using a peroxidase-labeled tannin. *Journal of Agricultural and Food Chemistry,* **46**, 5083–5088.

Barbehenn, R. & Constabel, P. (2011) Tannins in plant–herbivore interactions. *Phytochemistry,* **72**, 1551-1565.

Bates, L.A., Poole, J.H. & Byrne, R.W. (2008) Elephant cognition. *Current Biology,* **18**, 544–545.

Bax, P.N. & Sheldrick, D.L.W. (1963) Some preliminary observations on the food of elephant in the Tsavo Royal National Park (East) of Kenya. *African Journal of Ecology,* **1**, 40–51.

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.

Bell, R.H.V. (1969) The use of the herb layer by grazing ungulates in the Serengeti. *Animal Populations in Relation to their Food Resources* (ed. A. Watson), pp. 111–124. Blackwell, Oxford, United Kingdom.

Bell, W.J. (2012) *Searching Behaviour: The Behavioural Ecology of Finding Resources.* Springer Science & Business Media, Dordrecht, Netherlands.

Ben-Shahar, R. (1993) Patterns of elephant damage to vegetation in northern Botswana. *Biological Conservation,* **65**, 249–256.

Bennick, A. & Connell, G.E. (1971) Purification and partial characterization of four proteins from human parotid saliva. *Biochemical Journal,* **123**, 455–464.

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.

Blytt, H.J., Guscar, T.K. & Butler, L.G. (1988) Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. *Journal of Chemical Ecology,* **14**, 1455-1465.

Bryant, J.P., Provenza, F.D., Pastor, J., Reichardt, P.B., Clausen, T.P. & du Toit, J.T. (1991) Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics,* **22**, 431–446.

Buechner, H.K., Buss, I.O., Longhurst, W.M. & Brooks, A.C. (1963) Numbers and migration of elephants in Murchison Falls National Park, Uganda. *Journal of Wildlife Management,* **21**, 36–53.

Buss, I.O. (1961) Some observations on food habits and behavior of the African elephant. *Journal of Wildlife Management,* **25**, 131–148.

Camp, M.J., Shipley, L.A., Johnson, T.R., Forbey, J.S., Rachlow, J.L. & Crowell, M.M. (2015) Modeling trade-offs between plant fiber and toxins: A framework for quantifying risks perceived by foraging herbivores. *Ecology,* **96**, 3292–3302.

Cerling, T.E., Harris, J.M. & Leakey, M.G. (1999) Browsing and grazing in elephants: The isotope record of modern and fossil proboscideans. *Oecologia,* **120**, 364–374.

Charnov, E.L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology,* **9**, 129–136.

Clauss, M., Frey, R., Kiefer, B., Lechner-Doll, M., Loehlein, W., Polster, C., Rössner, G.E. & Streich, J.W. (2003) The maximum attainable body size of herbivorous mammals: Morphophysiological constraints on foregut, and adaptations of hindgut fermenters. *Oecologia,* **136**, 14–27.

Clauss, M., Gehrke, J., Hatt, J.-M., Dierenfeld, E.S., Flach, E.J., Hermes, R., Castell, J., Streich, J.W. & Fickel, J. (2005a) Tannin-binding salivary proteins in three captive rhinoceros species. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* **140**, 67–72.

Clauss, M., Robert, N., Walzer, C., Vitaud, C. & Hummel, J. (2005b) Testing predictions on body mass and gut contents: Dissection of an African elephant *Loxodonta africana* Blumenbach 1797. *European Journal of Wildlife Research,* **51**, 291–294.

Clauss, M., Steinmetz, H., Eulenberger, U., Ossent, P., Zingg, R., Hummel, J. & Hatt, J.-M. (2007) Observations on the length of the intestinal tract of African Loxodonta africana (Blumenbach 1797) and Asian elephants Elephas maximus (Linné 1735). *European Journal of Wildlife Research,* **53**, 68-72.

Codron, J., Lee-Thorp, J.A., Sponheimer, M., Codron, D., Grant, R.C. & de Ruiter, D.J. (2006) Elephant (*Loxodonta africana*) diets in Kruger National Park, South Africa: Spatial and landscape differences. *Journal of Mammalogy,* **87**, 27–34.

Conybeare, A.M. (2004) Elephant impacts on vegetation and other biodiversity in the broadleaved woodlands of S-Central Africa. *Biodiversity of the Four Corners Area: Technical Reviews.* (eds J.R. Timberlake & S.L. Childes), pp. 477–508. Biodiversity Foundation for Africa, Harare, Zimbabwe.

Cooper, S.M. & Owen-Smith, N. (1985) Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia,* **67**, 142–146.

Cooper, S.M., Owen-Smith, N. & Bryant, J.P. (1988) Foliage acceptability to browsing ruminants in relation to seasonal changes in the leaf chemistry of woody plants in a South African savanna. *Oecologia,* **75**, 336–342.

Courtois, E.A., Baraloto, C., Paine, C.T., Petronelli, P., Blandinieres, P.A., Stien, D., Höuel, E., Bessière, J.M. & Chave, J. (2012) Differences in volatile terpene composition between the bark and leaves of tropical tree species. *Phytochemistry,* **82**, 81–88.

De Boer, W.F., Ntumi, C.P., Correia, A.U. & Mafuca, J.M. (2000) Diet and distribution of elephant in the Maputo Elephant Reserve, Mozambique. *African Journal of Ecology,* **38**, 188–201.

Demment, M.W. & Van Soest, P.J. (1985) A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist,* **125**, 641–672.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004) Biochemistry of plant volatiles. *Plant Physiology,* **135**, 1893–1902.

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.

Eisenberg, J.F. & Kleiman, D.G. (1972) Olfactory Communication in Mammals. *Annual Review of Ecology and Systematics,* **3**, 1–32.

Elser, J. (2006) Biological stoichiometry: a chemical bridge between ecosystem ecology and evolutionary biology. *American Naturalist,* **168**, S25-S35.

Engelberth, J., Alborn, H.T., Schmelz, E.A. & Tumlinson, J.H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, 1781–1785.

Foguekem, D., Tchamba, M.N., Gonwouo, L.N., Ngassam, P. & Loomis, M. (2011) Nutritional status of forage plants and their use by elephant in Waza National Park, Cameroon. *Scientific Research and Essays,* **6**, 3577–3583.

Fowler, M.E. & Richards, W.P. (1965) Acorn poisoning in a cow and a sheep. *Journal of the American Veterinary Medical Association,* **147**, 1215-1220.

Freeland, W.J. & Janzen, D.H. (1974) Strategies in herbivory by mammals: The role of plant secondary compounds. *American Naturalist,* **108**, 269–289.

Frye, G.G., Connelly, J.W., Musil, D.D. & Forbey, J.S. (2013) Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. *Ecology,* **94**, 308–314.

Geervliet, J.B.F., Ariëns, S., Dicke, M. & Vet, L.E.M. (1998) Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Biological Control,* **11**, 113–121.

Geist, V. (1974) On the relationship of social evolution and ecology in ungulates. *American Zoologist,* **14**, 205–220.

Glover, J. (1963) The elephant problem at Tsavo. *African Journal of Ecology,* **1**, 30–39.

GRSC (1989) *A Glossary of Terms Used in Range Management*. Denver, Colorado, USA.

Guy, P.R. (1976) The feeding behaviour of elephant (*Loxodonta africana*) in the Sengwa area, Rhodesia. *South African Journal of Wildlife Research,* **6**, 55–63.

Harborne, J.B. (1991) The chemical basis of plant defence. *Plant defenses against mammalian herbivory* (eds R.T. Palo & C.T. Robbins), pp. 45. CRC Press, Boca Raton, Florida, USA.

Hart, B.L., Hart, L.A. & Pinter-Wollman, N. (2008) Large brains and cognition: Where do elephants fit in? *Neuroscience and Biobehavioral Reviews,* **32**, 86–98.

Heady, H.F. (1964) Palatability of herbage and animal preference. *Journal of Range Management,* **17**, 76–82.

Hobbs, T.N. & Swift, D.M. (1985) Estimates of habitat carrying capacity incorporating explicit nutritional constraints. *Journal of Wildlife Management,* **49**, 814–822.

Houston, A.I. & McNamara, J.M. (2014) Foraging currencies, metabolism and behavioural routines. *Journal of Animal Ecology,* **83**, 30–40.

Illius, A.W. & Gordon, I.J. (1993) Diet selection in mammalian herbivores: Constraints and tactics. *An Interdisciplinary Approach To Foraging Behavior.* (ed. R. Hughes), pp. 157–181. Blackwell Scientific Publishing, Oxford, United Kingdom.

Jarman, P.J. (1974) The social organisation of antelope in relation to their ecology. *Behaviour,* **48**, 215–267.

Johnson, S.D., Burgoyne, P.M., Harder, L.D. & Dötterl, S. (2011) Mammal pollinators lured by the scent of a parasitic plant. *Proceedings of the Royal Society of London B: Biological Sciences,* **278**, 2303–2310.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Ecosystem Management* (eds F.B. Samson & F.L. Knopf), pp. 130–147. Springer, New York, USA.

Juntheikki, M.R. (1996) Comparison of tannin-binding proteins in saliva of Scandinavian and North American moose (*Alces alces*). *Biochemical Systematics and Ecology,* **24**, 595–601.

Kerley, G.I.H. & Landman, M. (2006) The impacts of elephants on biodiversity in the Eastern Cape subtropical thickets: Elephant conservation. *South African Journal of Science,* **102**, 395–402.

Kerley, G.I.H. & Shrader, A.M. (2007) Elephant contraception: Silver bullet or a potentially bitter pill?: Commentary. *South African Journal of Science,* **103**, 181–182.

Klaassen, R.H.G., Nolet, B.A., van Gils, J.A. & Bauer, S. (2006) Optimal movement between patches under incomplete information about the spatial distribution of food items. *Theoretical Population Biology,* **70**, 452–463.

Kleiber, M. (1947) Body size and metabolic rate. *Physiological Reviews,* **27**, 511–541.

Kluever, B.M., Howery, L.D., Breck, S.W. & Bergman, D.L. (2009) Predator and heterospecific stimuli alter behaviour in cattle. *Behavioural Processes,* **81**, 85–91.

Kohl, K.D., Pitman, E., Robb, B.C., Connelly, J.W., Dearing, M.D. & Forbey, J.S. (2015) Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. *Journal of Comparative Physiology B: Biochemistry & Molecular Biology,* **185**, 425–434.

Krueger, W.C., Laycock, W.A. & Price, D.A. (1974) Relationships of taste, smell, sight, and touch to forage selection. *Journal of Range Management,* **27**, 258–262.

Kuzyk, G.W., Cool, N.L., Bork, E.W., Bampflde, C., Franke, A. & Hudson, R.J. (2009) Estimating economic carrying capacity for an ungulate guild in western Canada. *Open Conservation Biology Journal,* **3**, 24–35.

Launchbaugh, K.L., Provenza, F.D. & Pfister, J.A. (2001) Herbivore response to anti-quality factors in forages. *Journal of Range Management,* **54**, 431–440.

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.

Leopold, A. (1933) *Game Management*. Scribner, New York, USA.

Mattson, W. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics,* **11**, 119–161.

McArt, S.H., Spalinger, D.E., Collins, W.B., Schoen, E.R., Stevenson, T. & Bucho, M. (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology,* **90**, 1400-1411.

McArthur, C., Hagerman, A.E. & Robbins, C.T. (1991) Physiological strategies of mammalian herbivores against plant defences. *Plant defenses against mammalian herbivory* (eds R.T. Palo & C.T. Robbins), pp. 103–114. CRC Press, Boca Raton, Florida, USA.

McArthur, C., Sanson, G.D. & Beal, M.A. (1995) Salivary proline-rich proteins in mammals: Roles in oral homeostasis and counteracting dietary tannin. *Journal of Chemical Ecology,* **21**, 663–691.

Mehansho, H., Ann, D.K., Butler, L.G., Rogler, J. & Carlson, D.M. (1987a) Induction of proline-rich proteins in hamster salivary glands by isoproterenol treatment and an unusual growth inhibition by tannins. *Journal of Biological Chemistry,* **262**, 12344–12350.

Mehansho, H., Butler, L.G. & Carlson, D.M. (1987b) Dietary tannins and salivary proline-rich proteins: Interactions, induction, and defense mechanisms. *Annual review of nutrition,* **7**, 423–440.

Mehansho, H., Clements, S., Sheares, B.T., Smith, S. & Carlson, D.M. (1985) Induction of proline-rich glycoprotein synthesis in mouse salivary glands by isoproterenol and by tannins. *Journal of Biological Chemistry,* **260**, 4418–4423.

Mentis, M.T. & Tainton, N.M. (1981) *Acceptability and Palatability*. University of Natal Press, Pietermartizburg, South Africa.

Miller, A.K., Hensman, M.C., Hensman, S., Schultz, K., Reid, P., Shore, M., Brown, J., Furton, K.G. & Lee, S. (2015) African elephants (*Loxodonta africana*) can detect TNT using olfaction: Implications for biosensor application. *Applied Animal Behaviour Science,* **171**, 177–183.

Mirza, S.N. & Provenza, F.D. (1990) Preference of the mother affects selection and avoidance of foods by lambs differing in age. *Applied Animal Behaviour Science,* **28**, 255–263.

Mitko, L., Weber, M.G., Ramirez, S.R., Hedenström, E., Wcislo, W.T. & Eltz, T. (2016) Olfactory specialization for perfume collection in male orchid bees. *Journal of Experimental Biology,* **219**, 1467–1475.

Mole, S., Butler, L.G. & Iason, G. (1990) Defense against dietary tannin in herbivores: A survey for proline rich salivary proteins in mammals. *Biochemical Systematics and Ecology,* **18**, 287–293.

Morellet, N., Gaillard, J.-M., Hewison, A.J., Ballon, P., Boscardin, Y., Duncan, P., Klein, F. & Maillard, D. (2007) Indicators of ecological change: New tools for managing populations of large herbivores. *Journal of Applied Ecology,* **44**, 634–643.

Muller, K. (2013) What drives the seasonal movements of African elephants (*Loxodonta africana)* in Ithala Game Reserve. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Niinemets, Ü., Loreto, F. & Reichstein, M. (2004) Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science,* **9**, 180–186.

Nobler, J.D. (2016) Risky business: Tradeoffs between nutrition, toxicity, and predation by a specialist mammalian herbivore. Unpublished MSc thesis, Boise State University, Boise, Idaho, USA.

Oppenheim, F.G., Offner, G.D. & Troxler, R.F. (1985) Amino acid sequence of a proline-rich phosphoglycoprotein from parotid secretion of the subhuman primate *Macaca fascicularis*. *Journal of Biological Chemistry,* **260**, 10671–10679.

Owen-Smith, N. (1988) *Megaherbivores: The Influence of Very Large Body Size on Ecology*. Cambridge University Press, Cambridge, United Kingdom.

Owen-Smith, N. & Chafota, J. (2012) Selective feeding by a megaherbivore, the African elephant (*Loxodonta africana*). *Journal of Mammalogy,* **93**, 698–705.

Owen-Smith, N. & Cooper, S.M. (1987) Palatability of woody plants to browsing ruminants in a South African savanna. *Ecology,* **68**, 319–331.

Owen-Smith, N., Kerley, G., Page, B., Slotow, R. & Van Aarde, R. (2006) A scientific perspective on the management of elephants in the Kruger National Park and elsewhere. *South African Journal of Science,* **102**, 389-394.

Pellew, R.A. (1984) The feeding ecology of a selective browser, the giraffe (*Giraffa camelopardalis tippelskirchi*). *Journal of Zoology,* **202**, 57–81.

Peñuelas, J. & Llusià, J. (2004) Plant VOC emissions: Making use of the unavoidable. *Trends in Ecology and Evolution,* **19**, 402–404.

Pichersky, E. & Gershenzon, J. (2002) The formation and function of plant volatiles: Perfumes for pollinator attraction and defense. *Current opinion in plant biology,* **5**, 237–243.

Pietrzykowski, E., McArthur, C., Fitzgerald, H. & Goodwin, A. (2003) Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. *Journal of Applied Ecology,* **40**, 458–469.

Pimm, S.L. & van Aarde, R.J. (2001) African elephants and contraception. *Nature,* **411**, 766–766.

Plotnik, J.M., Shaw, R.C., Brubaker, D.L., Tiller, L.N. & Clayton, N.S. (2014) Thinking with their trunks: Elephants use smell but not sound to locate food and exclude nonrewarding alternatives. *Animal Behaviour,* **88**, 91–98.

Provenza, F.D. (1994) Ontogeny and social transmission of food selection in domesticated ruminants. *Behavioral Aspects of Feeding* (eds B.G. Galef Jr., M. Mainardi & P. Valsecchi), pp. 147-164. Harwood Academic Publishers, Pennsylvania.

Provenza, F.D. (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management,* **48**, 2–17.

Provenza, F.D. & Balph, D. (1987) Diet learning by domestic ruminants: Theory, evidence and practical implications. *Applied Animal Behaviour Science,* **18**, 211–232.

Provenza, F.D., Burritt, E.A., Clausen, T., Bryant, J., Reichardt, P. & Distel, R.A. (1990) Conditioned flavor aversion: A mechanism for goats to avoid condensed tannins in blackbrush. *American Naturalist,* **136**, 810–828.

Provenza, F.D., Villalba, J.J., Haskell, J., MacAdam, J.W., Griggs, T.C. & Wiedmeier, R.D. (2007) The value to herbivores of plant physical and chemical diversity in time and space. *Crop Science,* **47**, 382–398.

Pyke, G.H., Pulliam, R.H. & Charnov, E.L. (1977) Optimal foraging: A selective review of theory and tests. *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.

Rhoades, D.F. & Cates, R.G. (1976) Toward a general theory of plant antiherbivore chemistry. *Biochemical Interaction Between Plants and Insects* (eds J. Wallace & R. Mansell), pp. 168–213. Springer, New York, USA.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C. & Mautz, W.W. (1987) Role of tannins in defending plants against ruminants: Reduction in protein availability. *Ecology,* **68**, 98–107.

Rohloff, J. (2015) Analysis of phenolic and cyclic compounds in plants using derivatization techniques in combination with GC-MS-based metabolite profiling. *Molecules,* **20**, 3431-3462.

Salminen, J.-P. & Karonen, M. (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology,* **25**, 325-338.

Schmitt, M.H., Ward, D. & Shrader, A.M. (2016) Salivary tannin-binding proteins: An ecological and evolutionary advantage for goats? *Journal of Mammology,* **In review**.

Scholes, R. & Biggs, R. (2005) A biodiversity intactness index. *Nature,* **434**, 45–49.

Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. (2005) *Insect-Plant Biology*. Oxford University Press, Oxford, United Kingdom.

Seigler, D.S. (1998) Monoterpenes. *Plant Secondary Metabolism*, pp. 324-352. Springer US, Boston, MA.

Shimada, T. (2006) Salivary proteins as a defense against dietary tannins. *Journal of Chemical Ecology,* **32**, 1149–1163.

Shimada, T. & Saitoh, T. (2003) Negative effects of acorns on the wood mouse *Apodemus speciosus*. *Population Ecology,* **45**, 7-17.

Shipley, L.A. (2007) The influence of bite size on foraging at larger spatial and temporal scales by mammalian herbivores. *Oikos,* **116**, 1964–1974.

Shipley, L.A., Davis, E.M., Felicetti, L.A., McLean, S. & Forbey, J.S. (2012) Mechanisms for eliminating monoterpenes of sagebrush by specialist and generalist rabbits. *Journal of Chemical Ecology,* **38**, 1178–1189.

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.

Shuttleworth, A. & Johnson, S.D. (2009) The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology,* **23**, 931–940.

Skinner, J.D. & Chimimba, C.T. (2005) *The Mammals of the Southern African Sub-Region.* Cambridge University Press, Cape Town, South Africa.

Slotow, R., Garaï, M.E., Reilly, B., Page, B. & Carr, R.D. (2005) Population dynamics of elephants re-introduced to small fenced reserves in South Africa. *South African Journal of Wildlife Research,* **35**, 23–32.

Slotow, R., Whyte, I., Hofmeyr, M., Kerley, G., Conway, T.J. & Scholes, R.J. (2008) Lethal management of elephants. *Elephant Management: A Scientific Assessment of South Africa* (eds R.J. Scholes & K.G. Mennell). Witwatersrand University Press, Johannesburg, South Africa.

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.

Spielman, A.I. & Bennick, A. (1989) Isolation and characterization of six proteins from rabbit parotid saliva belonging to a unique family of proline-rich proteins. *Archives of Oral Biology,* **34**, 117–130.

Stephens, D.W. & Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton.

Sterner, R.W. & Elser, J.J. (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press.

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

Stutz, R.S., Banks, P.B., Proschogo, N. & McArthur, C. (2016a) Follow your nose: Leaf odour as an important foraging cue for mammalian herbivores. *Oecologia,* **182**, 643-651.

Stutz, R.S., Croak, B.M., Proschogo, N., Banks, P.B. & McArthur, C. (2016b) Olfactory and visual plant cues as drivers of selective herbivory. *Oikos*, doi:10.1111/oik.03422.

Tainton, N. (1999) *Veld Management in South Africa*. University of Natal Press, Pietermaritzburg, South Africa.

Tholl, D., Boland, W., Hansel, A., Loreto, F., Röse, U.S. & Schnitzler, J.P. (2006) Practical approaches to plant volatile analysis. *The Plant Journal,* **45**, 540–560.

Thomson, R. (2003) *A Game Warden's Report: The State of Wildlife in Africa at the Start of the Third Millennium*. Magron, Hartebeespoort, South Africa.

Thorhallsdottir, A.G., Provenza, F.D. & Balph, D.F. (1990) Ability of lambs to learn about novel foods while observing or participating with social models. *Applied Animal Behaviour Science,* **25**, 25–33.

Trollope, W.S.W., Trollope, L.A. & Bosch, O.J.H. (1990) Veld and pasture management terminology in southern Africa. *Journal of the Grassland Society of Southern Africa,* **7**, 52–61.

Ulappa, A.C., Kelsey, R.G., Frye, G.G., Rachlow, J.L., Shipley, L.A., Bond, L., Pu, X. & Forbey, J.S. (2014) Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. *Journal of Mammalogy,* **95**, 834-842.

Valeix, M., Fritz, H., Sabatier, R., Murindagomo, F., Cumming, D. & Duncan, P. (2011) Elephant-induced structural changes in the vegetation and habitat selection by large herbivores in an African savanna. *Biological Conservation,* **144**, 902–912.

van der Merwe, N.J., Thorp, J.A. & Bell, R.H.V. (1988) Carbon isotopes as indicators of elephant diets and African environments. *African Journal of Ecology,* **26**, 163–172.

Van Soest, P.J. (1994) *Nutritional Ecology of the Ruminant,* 2nd edn. Cornell University Press, Ithaca, New York, USA.

Villalba, J.J., Provenza, F.D. & Han, G.D. (2004) Experience influences diet mixing by herbivores: Implications for plant biochemical diversity. *Oikos,* **107**, 100–109.

Vourc'h, G., De Garine-Wichatitsky, M., Labbé, A., Rosolowski, D., Martin, J.-L. & Fritz, H. (2002) Monoterpene effect on feeding choice by deer. *Journal of Chemical Ecology,* **28**, 2411–2427.

Wallis, I.R., Nicolle, D. & Foley, W.J. (2010) Available and not total nitrogen in leaves explains key chemical differences between the eucalypt subgenera. *Forest Ecology and Management,* **260**, 814-821.

Ward, D., Muller, K. & Shrader, A.M. (2016) Soil fertility on granite and sedimentary soils is associated with seasonal differences in foraging by elephants. *Plant and Soil*, doi:10.1007/s11104-11016-13067-y.

Windels, S.K. & Hewitt, D.G. (2011) Effects of plant secondary compounds on nutritional carrying capacity estimates of a browsing ungulate. *Rangeland Ecology and Management,* **64**, 264–275.

Wrangham, R. & Waterman, P. (1981) Feeding behaviour of vervet monkeys on *Acacia tortilis* and *Acacia xanthophloea:* With special reference to reproductive strategies and tannin production. *J Anim Ecol*, 715–731.

Wyatt, J.R. & Eltringham, S.K. (1974) The daily activity of the elephant in the Rwenzori National Park, Uganda. *African Journal of Ecology,* **12**, 273–289.

Chapter Two

African elephants use olfactory cues emitted from plants when making foraging decisions

Abstract

There are many decisions that mammalian herbivores need to make when foraging, including what plants to eat and which patches to visit. The mechanisms that they could employ include visual examination, direct sampling of the vegetation, and/or olfactory (odour) cues emitted from the vegetation. However, the importance of olfactory cues for foraging mammals remains poorly understood and the aim of our study was thus to identify whether elephants use olfactory cues to make feeding decisions. Moreover, if they did, were these cues (i.e. odour) only obtained when the elephants were next to the plant, or could they be detected prior to arriving at the plant (i.e. to make patch choice decisions)? To do this, we used scent-based choice experiments between various preferred and non-preferred plants across two spatial scales. We also used coupled gas chromatography-mass spectrometry (GC-MS) analysis of headspace extracts to explore the variation in volatile organic compounds (VOCs) that were emitted by the different plant species. At the smaller, feeding-station scale, the elephants could make a selection by only moving their trunks, while at the larger between-patch scale, we used a Y-maze to allow the elephants to move towards the patch they selected. We found that elephants select their preferred plant species across both spatial scales. Moreover, VOC combinations of any two preferred plant species were typically similar in odour, while the odours of the preferred and avoided combinations, and avoided and avoided combinations were usually very different. The ability to differentiate between plant odours allows elephants to reduce their search time by allowing them to target preferred plant species both within a feeding station and between patches. Our results show that olfactory cues likely play an important role in driving herbivore foraging decisions across multiple spatial scales.

Key words: Diet selection, foraging, odour, search time, spatial scale, volatile organic compounds, Y-maze

Introduction

Mammalian herbivores make a vast number of foraging decisions across a broad range of spatial scales (Senft *et al.* 1987). At a small scale, these herbivores can take thousands of bites per day (Illius & Gordon 1990). At larger scales, they can move across a number of plant communities on a daily basis (Senft *et al.* 1987), and strategically move around their environment on a seasonal basis (Shrader *et al.* 2012). Thus, herbivores are faced with a dynamic foraging environment, which they need to navigate effectively. Ultimately, both small and large-scale movements across the landscape are driven by foraging decisions, with the final goal of maximising nutritional intake rates (Senft *et al.* 1987; Shipley 2007; Owen-Smith *et al.* 2010; Morgan *et al.* 2016). However, a key question that remains unanswered is, what cues do they use to make foraging decisions at these different scales?

A challenge that herbivores face is that they rarely, if ever, know the distribution or quality of available resources, particularly in changing environments (Ward 1992; Wilmshurst *et al.* 1995; Klaassen *et al.* 2006; Ward 2010). For example, at both small and large scales, the abundance and distribution of resources varies spatially and temporally. Plant species and individuals within a species can vary in nutritional composition and defence investment. Nutritional composition can be beneficial (e.g. crude protein, digestibility) and detrimental (e.g. fibre, lignin), while investment in defences can be chemical (e.g. secondary metabolites, such as tannins, terpenes and alkaloids) (Freeland & Janzen 1974; Rhoades 1979; Belovsky & Schmitz 1994; Bell 2012; Bennett *et al.* 2015) or physical (Young 1987; Ward *et al.* 2012; Barton 2015; Kariñho-Betancourt *et al.* 2015). These differences are frequently correlated with the dietary preference of a plant species (Cooper & Owen-Smith 1985; Barton & Koricheva 2010; Shrader *et al.* 2012). Thus, preference can be linked with quality (Shrader *et al.* 2012).

Herbivores have to locate preferred resources, which can be costly. Moving from patch to patch at random would likely increase search time and energy loss associated with travelling between patches (Charnov 1976; Owen-Smith *et al.* 2010). Thus, herbivores need to make informed decisions about how and where to feed. Moreover, they should forage in a manner that maximises their nutritional intake, and minimizes travel costs (Pyke *et al.* 1977; Owen-Smith *et al.* 2010; Houston & McNamara 2014). However, when faced with imperfect knowledge about the abundance and distribution of resources, what mechanisms do herbivores use to reduce search time and thus improve foraging choices and ultimately energy gain?

One way they could do this is by continuously sampling forage to update information on nutritional quality (Krebs & McCleery 1984; Ruedenauer *et al.* 2016). However, to obtain adequate information on a wide range of plant species, herbivores would need to sample large portions of the landscape throughout the year, which could result in increased travel costs. A second option, would be to use visual cues. However, there are several issues associated with this, including variation in visual acuity and colour detection among herbivore species (Entsu *et al.* 1992; Piggins & Phillips 1996; Jacobs *et al.* 1998) that may limit success in making dietary selections (Rutter *et al.* 2004). An additional issue is that visual cues can be easily obstructed by objects in the landscape, such as a preferred plant growing among a number of less preferred plants (Stutz *et al.* 2015).

Another option is for herbivores to use odours (volatile organic compounds) emitted by plants (Illius & Gordon 1993; Baluska & Ninkovic 2010). Insects use olfactory cues to forage and to find locations to oviposit (Geervliet *et al.* 1998; Raguso 2008; Mitko *et al.* 2016). However, the degree to which mammalian herbivores use odours when foraging is largely unknown (Provenza & Balph 1987; Pietrzykowski *et al.* 2003; Bedoya-Pérez *et al.* 2014). Green leaves produce a variety of different volatiles including various aliphatics

(especially green leaf volatiles) and terpenoids (including both monoterpenes and sesquiterpenes) (Peñuelas & Llusià 2004). These are known to play various roles in plant signalling and plant defence but their importance for interactions with mammalian herbivores are not well explored (Bedoya-Pérez *et al.* 2014). Furthermore, plant odours could be linked to preference for a particular item as a result of a conditioned response to past post-ingestive consequences (Villalba *et al.* 2015). For example, several studies have found that mammalian herbivores have learned to avoid certain plants due to negative postingestive feedback stemming from plant secondary metabolites (PSMs) (Provenza & Balph 1987; Provenza *et al.* 1990; Kyriazakis *et al.* 1998; Bedoya-Pérez *et al.* 2014).

Due to the nature of VOCs that comprise odour profiles, plant odour likely can be detected from much greater distances than visual cues and can pass through visually obstructing barriers (Bell 2012; Stutz *et al.* 2015). While odour has the potential to be directed by the wind, and can be affected by temperature and light (Niinemets *et al.* 2004), it can still be a useful tool for herbivores to detect preferred plant species across multiple spatial scales (Bell 2012). For example, herbivores could use odours to make informed foraging choices about the location and availability of preferred food items. Because these odours can emanate from distant patches, use of plant odours by herbivores could reduce search time and energy expenditure while foraging.

To explore the degree to which herbivores may use plant odours to make foraging decisions across different spatial scales, we focused on the foraging of African elephants (*Loxodonta africana)*. Due to their large body size, elephants have very high absolute nutritional requirements, necessitating a large number of foraging decisions within a day. Although they can tolerate a certain degree of low-quality vegetation, studies have indicated that they are extremely selective foragers (Owen-Smith & Chafota 2012; Pretorius *et al.* 2012). Elephants, like many other herbivores, forage in an environment where resources are

often clustered in patches (Cohen *et al.* 1999; De Knegt *et al.* 2008; Crane *et al.* 2016). As a result, they have to search and move through areas of low food availability, expending energy without gaining energy, to reach areas of higher resource availability. To forage in a nutritionally maximising and energetically efficient manner, elephants would need to make foraging decisions that reduce search time for preferred food items within and between these clusters. Due to their keen sense of smell (Miller *et al.* 2015), we predicted that elephants are able to use plant odours to make foraging decisions. Furthermore, we predicted that the combination of plant species presented to elephants, as well as the difference in preference rank between plant species, would influence the elephant's foraging choice. We tested these predictions in a choice experiment across a fine spatial scale, mimicking foraging decisions within a feeding station. In addition, we tested the use of plant odour by elephants at a larger spatial scale. To do this, we used a Y-maze, which allowed us to determine if an elephant could make between patch foraging decisions using plant odours at a distance beyond their body length.

Methods

All aspects of this experiment were approved by the institutional animal ethics committee (Reference number: AREC/106/015). To explore the role that odour plays in the foraging decisions of African elephants, we conducted two experiments. The first tested whether elephants used odour to make foraging decisions at the feeding-station scale (<0.5 m), and the second tested whether they use it at a larger spatial scale (5 m), which we considered to be equivalent to decisions made between two patches. Both experiments eliminated eyesight and touch as variables driving elephant foraging decisions and focused solely on scent.

All research was completed during August 2015 at the *Adventures with Elephants* facility near Bela Bela, Limpopo Province, South Africa. We used five semi-tame sub-adult individuals between 15-20 years old (three females, two males) for the feeding-station

experiment, and three of these same individuals (one female, two males) for the between-patches experiment. We only used three individuals for the between-patch experiment because two of the elephants were unwilling to walk into the Y-maze. For both of these experiments, we were only interested in whether elephants used odour to make foraging decisions. Thus, we did not include sex as a variable in our analyses.

Plant species

We used 12 woody plant species in our scent-based experiments (see below). These species comprised 75% of the elephants' diets at our study site and were categorized as preferred or avoided using an acceptability index (see below) (Owen-Smith & Cooper 1987). To form the acceptability index, we followed the semi-tame, free-ranging elephants in their native habitats, ~50 m behind along feeding paths, while they foraged. Along each path, we used a belt transect that was 50 m long x 10 m wide (i.e. 5 m on either side of the feeding path, n=100). We chose a width of 5 m because this is the reach of a foraging elephant's trunk (Shrader *et al.* 2012). Along each path, we recorded the abundance of every plant species present as well as the number of times a given plant species was consumed by an elephant along the transect.

To generate the acceptability index (AI) of a species, the number of times a particular plant species was eaten was divided by the proportional abundance of that plant species in the same area (Owen-Smith & Cooper 1987). After compiling an index for every plant species present, we then identified the plant species that were preferred (i.e. most selected proportional to its abundance), principal (selected overall most frequently – a function of acceptability and abundance), or avoided (rarely eaten in relation to its abundance). Preferred plant species had AIs of >0.5 and avoided plant species had AIs below 0.3. Principal plant species were eaten the most frequently of all species encountered and had AIs ranging between 0.28-0.5. For our study, we focused on the six most-eaten plant species that

comprised ~75% of the elephants' diets (preferred and principal) (*Pappea capensis* Sapindaceae, *Dombeya rotundifolia* Malvaceae, *Terminalia sericea* Combretaceae, *Combretum zeyheri* Combretaceae, *Grewia monticola* Malvaceae, and *Euclea crispa* Ebenaceae), and the five most avoided (*Vitex rehmannii* Lamiaceae, *Searsia pyroides* Anacardiaceae, *Searsia lancea* Anacardiaceae, *Euclea undulata* Ebenaceae, and *Olea europaea* Oleaceae), as well as a novel favourite, the combretum mistletoe (*Viscum combreticola* Santalaceae). This mistletoe was often out of reach for the elephants, and thus difficult to access. However, it was the most favoured species present at the study site (Appendix I, Table Ai.1).

Plant odour

To verify that the odour profiles of the plants were different, we collected odour samples from vegetative parts (leaves and stems only) of each species used in the experiments (n = 8 individual plants sampled per species). Volatile organic compounds (VOCs), were collected from each plant species using dynamic-headspace extraction methods (Tholl 2006). This was done by enclosing a branch in a polyacetate bag (NaloPhan®, Kalle, Germany) and sucking air from the bag for 3 h through a small cartridge filled with 1 mg each of Tenax® TA (60/80) (SupelcoTM; Bellefonte, PA, USA) and Carbotrap® B (20-40 mesh) (Sigma-Aldrich Co.; St Louis, MO, USA) using a PAS500 Personal Air Sampler (Spectrex, Redwood City, California). Control samples were collected for the same duration from empty polyacetate bags and used to identify environmental contaminants.

Volatiles were analysed by gas chromatography-mass spectrometry (GC-MS) using a Varian (Palo Alto, CA) CP3800 gas chromatograph (fitted with a Varian 1079 injector with a ChromatoProbe thermal desorption device) coupled to a Varian 1200 quadrupole mass spectrometer. A polar (Bruker BR-Swax) capillary column was used. A detailed description of these methods is presented in Shuttleworth & Johnson (2009). Compounds were identified

using the NIST 2011 mass spectral library. In most cases, identifications were confirmed by comparison of retention times with published retention indices (Kovats) and/or injection of synthetic standards (for a complete table of VOCs identified, see Appendix II, Table Aii.1). Absolute amounts of volatiles emitted were estimated by comparison of peak areas from samples with peak areas obtained from injection of a known amount of methyl benzoate (injected and run under identical conditions to samples) (Shuttleworth 2016). We have previously established that, for our analytical apparatus, methyl benzoate yields a peak area: nanogram (ng) relationship that is close to the average obtained from 200 compounds from various compound classes.

Feeding-station experiment

In the feeding-station experiment, we determined whether the elephants preferred or avoided plant species in the rank order that they selected them in the field. We conducted a scent-based choice experiment using two identical ~120 L plastic bins placed side-by-side (Appendix I, Figure Ai:1A). Each bin contained a branch from a single tree species. To ensure that only olfactory cues were available to the elephants, we inserted a PVC board into the side of each bin ~10 cm from the top rim. This prevented the elephants from touching and seeing what was in each bin. The PVC board slid across the opening of the bin and fitted tightly around the edges of the interior of each bin (see Appendix I, Figure Ai:1). The board could be slid open once the elephants made their selection to allow the elephant to consume the item. To allow odour to waft out from inside the bin, we drilled ~200 small holes (1 cm diameter) through the PVC board.

To provide odours for the elephants to select between, we concealed a clipping of a favoured and/or avoided plant species harvested from the surrounding savanna inside each bin. We clipped branches to the equivalent size of an elephant's "small" trunkful (~35 g, see Schmitt et al. 2016) to standardize size across all trials. The clipped end of the plant was

coated with Vaseline® to prevent emission of excess damage volatiles from the cut (Finnerty & McArthur unpublished data). We tested 11 species of plants (six preferred and five avoided) in a full factorial design with all 11 plant species being tested against each other, but not against themselves. This resulted in 55 combinations. Furthermore, we also included the combretum mistletoe (*Viscum combreticola*), which we tested against the least (*Olea europaea*) and second-least (*Euclea undulata*) preferred plant species. This resulted in 57 combinations in total.

To ensure that the elephant did not observe the experimental set-up, a professional handler instructed the elephants to face away (180°) from the testing arena. Once we had placed the plant clippings inside each bin, we placed the bins side-by-side with the opening to the PVC grid facing away from where the elephant was standing. Once established, the elephants were then instructed to turn, face forward, and to "smell" the bins. At this point, the elephant would step up to the bins and place their trunks on each PVC board and inhale the odours from each patch (Appendix I, FigureAi:1B). After sniffing both bins, the elephants were instructed to remove their trunks. We then instructed the elephants to "choose", at which point they placed their trunk in the bin they preferred. To reinforce the choice, we gave the elephants the clipping to eat from inside of the bin they selected. The bin that was not chosen was removed and the elephant was not allowed to see or eat the clipping within. This procedure was repeated five times consecutively for every elephant for each combination (we accounted for this serial correlation in our statistical analyses, see below). The position of each plant species, as well as the handler holding each bin, was randomized throughout the experiment by use of a random number generator. In addition, we cleaned the bins using water and a clean cloth prior to changing the plant species hidden inside to remove any residual odour. For photographic representation of the experiment see Appendix I, Figure Ai:1A.

Between-patch experiment

We focused our between-patch selection experiment on only the preferred versus avoided combinations. From this experiment we aimed to determine: (1) whether the elephants showed significant selection for the more preferred option across all combinations, and (2) to determine whether difference in rank between the two plant species influenced selection.

To do this, we used a Y-maze approach where elephants had to make a choice between two plant species over a 5 m distance. To further explore the preferred versus avoided category we tested the following combinations: (1) the most preferred species (*Pappea capensis*) versus the most avoided (*Olea europaea*), (2) the most preferred species (*Pappea capensis*) versus the second-most avoided (*Euclea undulata*), (3) the novel most-preferred species (*Viscum combreticola*) versus the most avoided (*Olea europaea*), (4) the novel most-preferred species (*Viscum combreticola*) versus the second-most avoided (*Euclea undulata*), (5) the lowest ranked of the preferred species (*Euclea crispa*) versus the most avoided species (*Olea europaea*), and (6) the lowest ranked of the preferred species (*Euclea crispa*) versus the highest ranked of the avoided species (*Vitex rehmannii*).

For this experiment, we built a Y-maze large enough for a bull elephant to walk through (for schematic, see Appendix I, Figure Ai:2). The height of the maze was 2.5 m, and the walkways were 2.5 m wide, which was > 1 m wider than the elephants used in our study. The entrance into the Y was 1.5 m long, and each arm was 4 m in length. Because the elephants had to be able to get out of the Y-maze, we left the end of each arm open, but included a small chamber that housed the small trunkful of the food item (which the elephant could not see) off the side end of each arm. To ensure the elephants were able to smell the plant samples from the start of the maze, we placed a fan in each of the chambers behind the plants, which blew the plant odours down each arm of the Y-maze.

At the start of the experiment, we instructed each elephant to stand at the start of the

maze and smell down each arm (Appendix I, Figure Ai:2). After the elephants smelled each

arm of the Y-maze for ~10 s, the animals were instructed by their handlers to "choose". At

that point, they walked down one arm of the Y-maze and were able to consume the plant

sample in the small chamber at the end of the arm. To avoid bias and odour contamination, all

observers and handlers stood directly behind the elephant, and no person walked through the

arms of the Y-maze, or stood at the end of the Y-maze. We repeated this experiment 10 times

per individual elephant per combination. We used a random number generator to randomize

the side we placed each plant species. To ensure that there was no failure due to the

dissipation of the plant odours, we only conducted Y-maze trials on windless mornings.

Statistical methods
Plant odour

We used a pairwise one-way ANOSIM randomization test (Anderson 2001) to examine

differences in odour between the 57 combinations of the preferred and avoided plant species.

ANOSIM calculates the test statistic R, which is a relative measure of the separation between

previously defined groups (e.g. preferred vs. avoided plant species), based on differences of

mean rank similarities between and within groups. R can range between 0 and 1, with 0

indicating completely random groupings (i.e. preferred and avoided plants do not exhibit

different odours) and 1 indicating that samples within groups (e.g. preferred vs. avoided

species) are more different from each other than to any sample from a different group (i.e.

preferred and avoided plants exhibit different odours) (Clark *et al.* 2007). We used 10,000

random permutations of the grouping vector (preferred vs. avoided) based on Euclidean

distances to obtain an empirical distribution of R under the null hypothesis to establish

significance using Primer v. 6 (Anderson 2001).

Behavioural choice

Both the species-choice and the between-patch selection experiments involved a series of binary choices (i.e. two bins, or each arm of the Y-maze). Because we used the same elephants within each of our experiments, we treated individuals as the subjects for repeated measures in generalized estimating equations (GEEs). We used GEEs because of potential non-independence of our data, which could stem from an individual possibly remembering previous trials. The model incorporated an exchangeable correlation matrix, and binomial error distribution with a logit link function. To analyze significance, we used score statistics because it is better suited for smaller sample sizes (Self & Mauritsen 1988). We then back-transformed data from the logit-scale for graphical representation. This back-transformation resulted in asymmetrical confidence intervals (CIs).

To determine if the elephants differentiated between the plant species at the feeding-station scale (i.e. bin experiment) based on plant odour, we analysed the proportion of choices made by the elephants for the more preferred plant species as described in our acceptability index. We used means and their 95% CIs to establish whether the elephants' preference between the plant species differed from the expected 50% distribution under random selection for each plant available. For the feeding-station scale (bin experiment), we used GEEs to determine (1) whether the elephants showed significant preference for the more preferred option across all combinations of the different plants that elephants encountered, (2) to assess the role that combination type (i.e. two preferred species, two avoided species, or one preferred and one avoided species) played in diet choice, and (3) to determine whether difference in rank between the two plant species (calculated from the acceptability indices) influenced diet choice.

We used elephant choice as the Boolean response variable. When species were from the same category (preferred vs. preferred, or avoided vs. avoided), we established preference

based on the acceptability index as outlined above. In separate GEEs, we tested the factors of combination (i.e. which species comprise a combination), combination type (i.e. two preferred species tested against each other, two avoided species tested against each other, or one preferred and one avoided species tested against each other), and difference in rank as independent variables with choice as the response variable. We could not run an interaction effect between combination type and difference in rank because not all combinations used all possible differences in rank (e.g. two preferred/avoided options can never be a rank difference of ≥ 7, whereas preferred vs. avoided can range from 1–13).

To explore whether elephants use scent to make foraging decisions between patches (i.e. using the Y-maze), we used GEEs to determine (1) whether the elephants showed significant preference for the more preferred plant species across all combinations, and (2) to determine whether difference in rank between to the two plant species influenced diet choice. All combinations in this experiment comprised one preferred species and one avoided species, so we did not explore the influence of combination type for this experiment. We used elephant choice as the Boolean response variable. In separate models, we tested the factors of combination (i.e. which plant species comprise a combination), and difference in rank.

Results

Plant odour

When we examined the composition of the volatile organic compounds (i.e. odour profiles) from each plant species, we were able to establish that each species had a detectable and unique odour. The actual scent data (specific VOCs) are not presented here, only a measure of the similarity between plant species' odour profiles. Results from our pairwise ANOSIM indicate that all pairs of plant species had significantly different odour profiles, although some pairs exhibited low R values, suggesting that differences were less clear in these combinations (Appendix I, Table Ai.2). Thus, elephants could potentially differentiate between plant

species by odour alone. Interestingly, pairwise comparisons between the odours of preferred plant species yielded low R values, while comparisons of the odour of preferred species with that of avoided species and comparisons between avoided species yielded higher R values (Appendix I, Table Ai.2). This suggests greater similarity between the odours of preferred species greater differences between preferred and avoided and between avoided species (Appendix I, Table Ai.2).

Feeding-station experiment

In the choice between bins, we found that the combination of plant species presented to the elephants significantly influenced their choice (GEE: $\chi^2 = 789.957$, P<0.0001). Across all 57 combinations, elephants selected for their preferred species ~63% of the time, and showed no preference between the two options ~37% of the time. The elephants never consistently selected for the less preferred plant species.

In the above model, we did not differentiate the 57 combinations between combination types (i.e. two preferred species tested against each other, two avoided species tested against each other, or one preferred and one avoided species tested against each other), nor did we include the difference in rank between plant species within a combination. Thus, to potentially explain the frequency where the elephants showed no preference (37%), we ran two additional GEEs. When we tested for combination type, we found that this significantly influenced elephant preference (GEE: $\chi^2 = 46.444$, P<0.0001; Figure 2.1). Specifically, when given the choice between two preferred species or two avoided species, they showed no significant preference. However, when the elephants were given the choice between a preferred and an avoided species, they significantly selected for the preferred species (Figure 2.1).
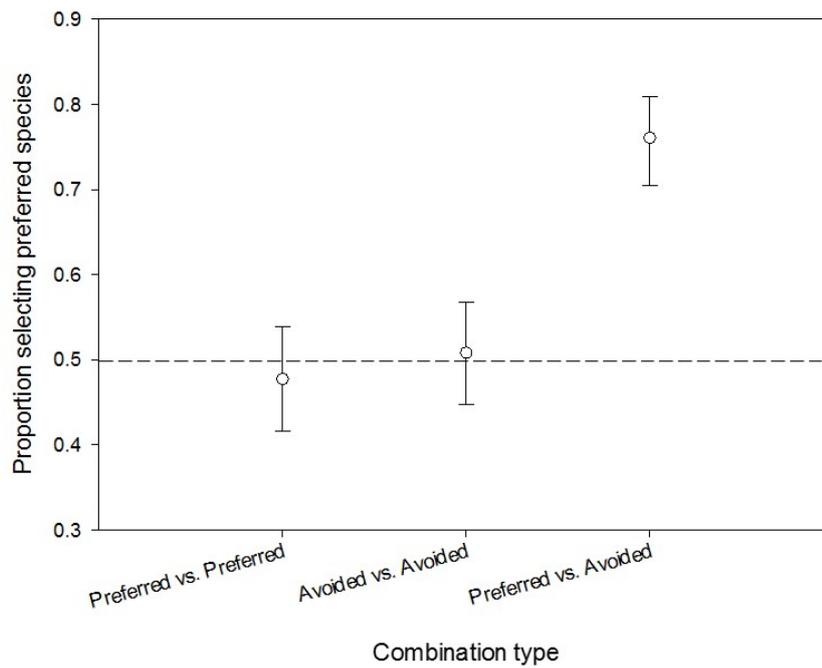
Figure 2.1: Proportion of elephants selecting the more preferred species as a function of combination type. Marginal means (+95% Confidence Intervals) of the proportion of selection of a given option are plotted. For the preferred vs. preferred and avoided vs. avoided combination types, there is overlap with the 0.5 expectation (i.e. random selection), indicating no preference for the more preferred species. For the preferred vs. avoided combination there is no overlap with the 0.5 expectation, indicating preference for the more preferred species.

In addition to combination type, the difference in rank between species also influenced selection (GEE: χ^2= 509.154, P<0.0001). Specifically, as the difference in rank increased, the proportion of elephants selecting the preferred option increased (Figure 2.2). Interestingly, we found that a difference of rank of 1 or 2 places resulted in the elephants showing no significant differences in selection (i.e. random - 0.5).
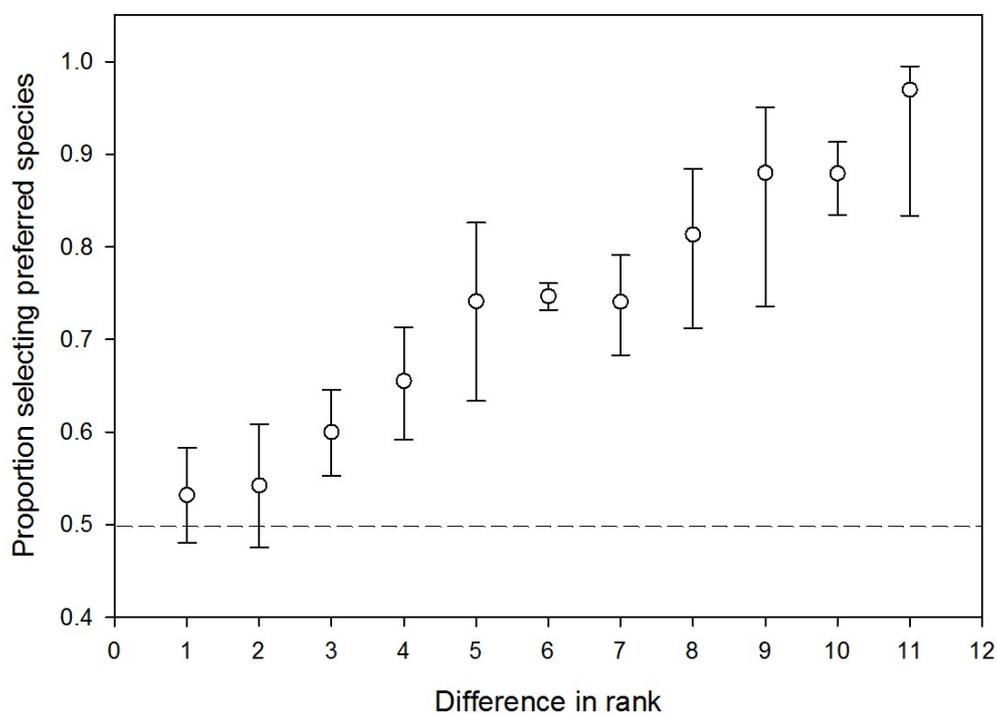
Figure 2.2: Proportion of elephants selecting the more preferred species as a function of difference in rank between options. Marginal means ($\pm$95% Confidence Intervals) of the proportion of selection of a given option are plotted. For the difference in rank of 1 & 2, there is overlap with the 0.5 expectation (i.e. random selection), indicating no preference for the more preferred species. For the remaining differences in rank categories, there is no overlap with the 0.5 expectation under random selection, indicating preference for the more preferred species.

Between-patch experiment

In the Y-maze experiment, we found that elephants were able to make scent-based decisions over a large spatial scale. Across all 6 combinations that we tested, elephants always selected for the most preferred plant species (GEE: χ^2= 10.372, P=0.006). Furthermore, elephants were able to differentiate between differently ranked plants and always selected the more preferred plant species (as determined by the acceptability index), regardless of difference in rank (GEE: χ^2= 10.372, P=0.006; Figure 2.3).
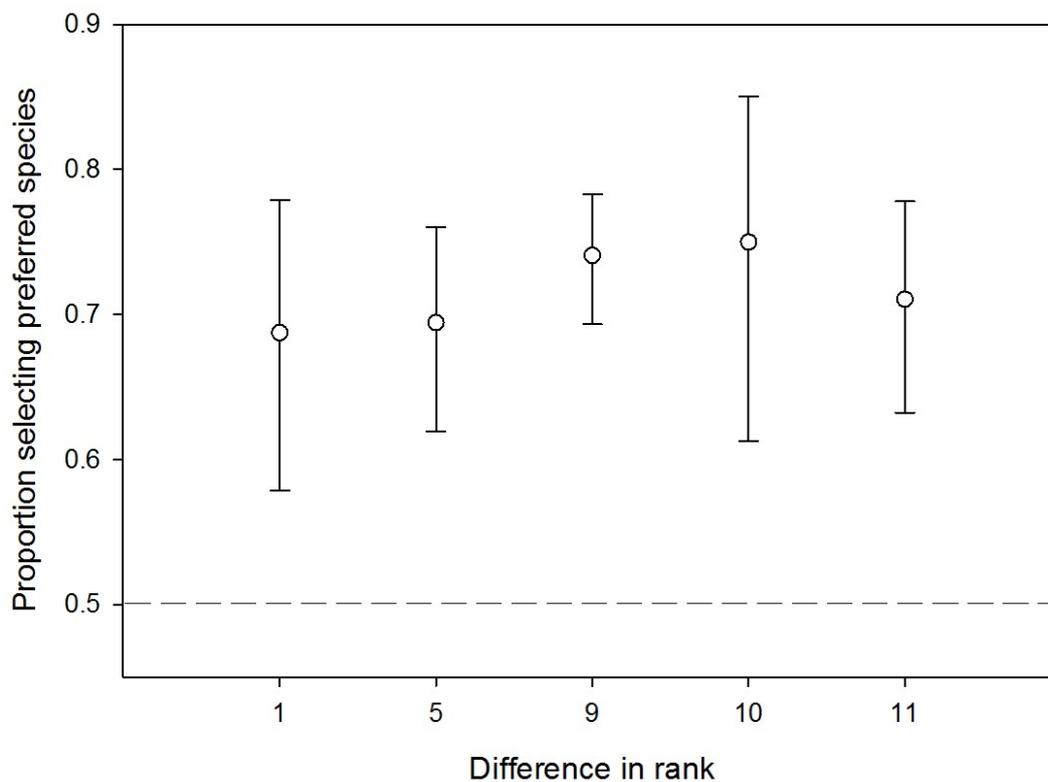
Figure 2.3: Proportion of elephants selecting the more preferred species as a function of difference in rank between options. Marginal means (+95% Confidence Intervals) of the proportion of selection of a given option are plotted. For all difference in rank categories, there is no overlap of the 95% CI with the 0.5 expectation (i.e. random selection), indicating preference for the more preferred species.

Discussion

There are a number of ways in which animals are able to assess vegetation in terms of biomass, availability, and nutritional quality when making foraging decisions. The mechanisms that mammalian herbivores could use include direct sampling of vegetation, visual examination of available resources, and assessment of olfactory cues emitted from the vegetation (Krueger *et al.* 1974; Krebs & McCleery 1984; Fortin 2003; Klaassen *et al.* 2006; Stutz *et al.* 2015; Stutz *et al.* 2016a; Stutz *et al.* 2016b). By exploring the use of olfactory cues, we found that African elephants were able to detect, distinguish, and use plant odour to make foraging decisions across different spatial scales. As a result, this suggests that odour is a key component that elephants use when making diet selections.

The goal of a forager is to maximise nutritional intake. However, the energy used to move between patches diminishes the energy gained from feeding within patches (Schoener 1971; Bergman *et al.* 2001). Our findings suggest that elephants are using olfactory signals to identify good patches and assess what tree species are contained within patches. By identifying what species are available in a feeding station (e.g. within a tree cluster) and between patches (e.g. between tree-clumps) via olfaction, free-ranging elephants could then use this information to determine the location of target high-quality/preferred species within a patch as well as to decide which patches to visit, and potentially how long to stay in a given patch.

In our study, elephants selected for their more preferred plant species more frequently across two spatial scales based on the odour emitted by the plants. Interestingly, when we examined selection at the feeding-station scale, we found that the elephants only showed significant preference for any option when choices were between a preferred and an avoided species. This is unexpected because within each of the preferred and avoided categories, there was a range of preference in AIs (Appendix I, Table Ai.1). For the preferred species, the AIs

have a very broad range from 0.28-0.87 of preference, while for the avoided plant species, the AIs have a smaller range (<0.2) of preference. Although dietary preference varies, the index we formed suggests that even within the preferred and avoided categories, the elephants should theoretically still show preference for one of the options.

The lack of preference between two preferred or two avoided species at the feeding-station scale occurred despite the ability of the elephants to differentiate plant species based on odour. The sense of smell of African elephants is superior to that of domestic dogs (Miller *et al.* 2015), which is better than the olfactory detection of many current GC-MS instruments (Harper *et al.* 2005). One reason for lack of differentiation could be that plants within each preference category (i.e. preferred and avoided) are all so similar nutritionally that when faced with the choice between two items from the same preference category, the elephants show no preference due to these similarities. However, this is unlikely because I found that plant species within the same preference category are not nutritionally similar (Chapter 3). The elephants' lack of preference could be explained by the potential presence of deleterious compounds, such as tannins, alkaloids, or monoterpenes. The elephants may be using a dietary mixing strategy (Freeland & Janzen 1974; Westoby 1974) where they limit the intake of any deleterious plant secondary metabolites by mixing the avoided plant species they consume during the trials. Dietary mixing to avoid ingesting an avoided plant species has been identified in mammalian herbivores (Rogosic *et al.* 2006; Papachristou *et al.* 2007; Copani *et al.* 2013).

An alternative reason for the lack of selection within categories could stem from the difference in scales at which our diet-preference data were collected and selection trials were run. Because the scale at which we collected diet-preference data was much larger than the feeding-station scale, our rankings may not perfectly match the elephants' preferences at smaller spatial scales. The elephants may change their tactics to feed less selectively at larger

spatial scales when the energetic costs to travel to the next food item outweigh the nutritional benefits (Murray 1991). Selectivity might increase at a smaller spatial scale, reflecting the lower energetic costs involved in diet selections at that scale. Consistent with this, we found that as the difference in rank between two plant species increased at the feeding-station scale, the overall selection for the more favoured option increased. This suggests that the elephants made diet selections based on the increasing profit gained by selecting the higher ranking item. However, this selection was significant only when the rank difference was two or more.

At a small scale, odour is a key information source that herbivores can use to identify target species, even when the odour or sight of the favoured species is masked by less preferred options (Stutz *et al.* 2015). A recent study showed that swamp wallabies (*Wallabia bicolor*) are able to find a preferred plant species even when it is hidden inside a stand of less desirable options (Stutz *et al.* 2015). The wallabies actively sniffed the air around the stand of plants, and eventually located the target species hidden inside the stand. Similarly, reindeer (*Rangifer tarandus*) were able to distinguish good and poor lichen sources via olfactory cues below 90 cm of snow (Helle 1984).

It is unlikely that foragers would be omniscient about the location and availability of resources in their environment (Bazely 1988; Wilmshurst *et al.* 1995; Ward 2010). Therefore, using odour cues, elephants are able to locate potential food items across multiple spatial scales. Our findings are consistent with the idea that elephants are capable of using odour cues to make foraging decisions at distances beyond their body length to select a patch. Additionally, elephants also use odour cues to make feeding decisions between plants at the feeding station scale. These foraging decisions based on plant odours could be a result of a conditioned response to past post-ingestive consequences (Villalba *et al.* 2015). Several past studies have found that mammalian herbivores have learned to avoid certain plants due to negative postingestive feedback (Provenza & Balph 1987; Provenza *et al.* 1990; Kyriazakis *et*

al. 1998; Bedoya-Pérez *et al.* 2014). Thus, it is possible that foragers can identify these plants by their smell and make the link between the smell of these plants and the negative consequences of eating them. Our experimental design did not test for the role of conditioned flavour aversions in elephant diet choice directly. However, it is likely that the elephants in our study learned to associate odours with postingestive costs and incorporated this into their diet selections. Given our results, odour cues could be a tool that numerous other browsing species use, however, further studies should be conducted to establish this.

References

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* **26**, 32–46.

Baluska, F. & Ninkovic, V. (2010) *Plant Communication from an Ecological Perspective.* Springer Berlin, Germany.

Barton, K.E. (2015) Tougher and thornier: general patterns in the induction of physical defence traits. *Functional Ecology,* **30**, 181–187.

Barton, K.E. & Koricheva, J. (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *American Naturalist,* **175**, 481–493.

Bazely, D. (1988) Foraging behaviour of sheep (*Ovis aries* L.) grazing on swards of perennial ryegrass (*Lolium perenne* L.). Unpublished PhD thesis, University of Oxford, Oxford, United Kingdom.

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.

Bell, W.J. (2012) *Searching Behaviour: The Behavioural Ecology of Finding Resources.* Springer Science & Business Media, Dordrecht, Netherlands.

Belovsky, G.E. & Schmitz, O.J. (1994) Plant defenses and optimal foraging by mammalian herbivores. *Journal of Mammalogy,* **75**, 816–832.

Bennett, A.E., Grussu, D., Kam, J., Caul, S. & Halpin, C. (2015) Plant lignin content altered by soil microbial community. *New Phytologist,* **206**, 166–174.

Bergman, C.M., Fryxell, J.M., Gates, C.C. & Fortin, D. (2001) Ungulate foraging strategies: Energy maximizing or time minimizing? *Journal of Animal Ecology,* **70**, 289–300.

Charnov, E.L. (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology,* **9**, 129–136.

Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., Gross, K.L., Pennings, S.C., Suding, K.N. & Grace, J.B. (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters,* **10**, 596–607.

Cohen, Y., Pastor, J. & Moen, R. (1999) Bite, chew, and swallow. *Ecological Modelling,* **116**, 1–14.

Cooper, S.M. & Owen-Smith, N. (1985) Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia,* **67**, 142–146.

Copani, G., Hall, J.O., Miller, J., Priolo, A. & Villalba, J.J. (2013) Plant secondary compounds as complementary resources: Are they always complementary? *Oecologia,* **172**, 1041–1049.

Crane, K.K., Mosley, J.C., Mosley, T.K., Frost, R.A., Smith, M.A., Fuller, W.L. & Tess, M.W. (2016) Elk foraging site selection on foothill and mountain rangeland in spring. *Rangeland Ecology & Management,* **69**, 319–325.

De Knegt, H.J., Groen, T.A., Van De Vijver, C.A.D.M., Prins, H.H.T. & Van Langevelde, F. (2008) Herbivores as architects of savannas: Inducing and modifying spatial vegetation patterning. *Oikos,* **117**, 543–554.

Entsu, S., Dohi, H. & Yamada, A. (1992) Visual acuity of cattle determined by the method of discrimination learning. *Applied Animal Behaviour Science,* **34**, 1–10.

Fortin, D. (2003) Searching behavior and use of sampling information by free-ranging bison (*Bos bison*). *Behavioral Ecology and Sociobiology,* **54**, 194–203.

Freeland, W.J. & Janzen, D.H. (1974) Strategies in herbivory by mammals: The role of plant secondary compounds. *American Naturalist,* **108**, 269–289.

Geervliet, J.B.F., Ariëns, S., Dicke, M. & Vet, L.E.M. (1998) Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids C*otesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae). *Biological Control,* **11**, 113–121.

Harper, R.J., Almirall, J.R. & Furton, K.G. (2005) Identification of dominant odor chemicals emanating from explosives for use in developing optimal training aid combinations and mimics for canine detection. *Talanta,* **67**, 313–327.

Helle, T. (1984) Foraging behaviour of the semi-domestic reindeer (*Rangifer tarandus* L.) in relation to snow in Finnish Lapland. *Report from Kevo Subarctic Research Station*, pp. 35-47. Kevo Subarctic Research Station, Utsjoki, Finland.

Houston, A.I. & McNamara, J.M. (2014) Foraging currencies, metabolism and behavioural routines. *Journal of Animal Ecology,* **83**, 30–40.

Illius, A.W. & Gordon, I.J. (1990) Constraints on diet selection and foraging behaviour in mammalian herbivores. *Behavioural Mechanisms of Food Selection* (ed. R.N. Hughes), pp. 369–393. Springer, Berlin, Germany.

Illius, A.W. & Gordon, I.J. (1993) Diet selection in mammalian herbivores: Constraints and tactics. *An Interdisciplinary Approach To Foraging Behavior.* (ed. R. Hughes), pp. 157–181. Blackwell Scientific Publishing, Oxford, United Kingdom.

Jacobs, G.H., Deegan, J.F. & Neitz, J. (1998) Photopigment basis for dichromatic color vision in cows, goats, and sheep. *Visual neuroscience,* **15**, 581–584.

Kariñho-Betancourt, E., Agrawal, A.A., Halitschke, R. & Núñez-Farfán, J. (2015) Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist,* **206**, 796–806.

Klaassen, R.H.G., Nolet, B.A., van Gils, J.A. & Bauer, S. (2006) Optimal movement between patches under incomplete information about the spatial distribution of food items. *Theoretical Population Biology,* **70**, 452–463.

Krebs, J.R. & McCleery, R.H. (1984) Individual optimization in behavioural ecology. *Behavioural Ecology: An Evolutionary Approach* (eds J.R. Krebs & N.B. Davies). Blackwell Scientific, Oxford, United Kingdom.

Krueger, W.C., Laycock, W.A. & Price, D.A. (1974) Relationships of taste, smell, sight, and touch to forage selection. *Journal of Range Management,* **27**, 258–262.

Kyriazakis, I., Anderson, D. & Duncan, A. (1998) Conditioned flavour aversions in sheep: the relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. *British Journal of Nutrition,* **79**, 55-62.

Miller, A.K., Hensman, M.C., Hensman, S., Schultz, K., Reid, P., Shore, M., Brown, J., Furton, K.G. & Lee, S. (2015) African elephants (*Loxodonta africana*) can detect TNT

using olfaction: Implications for biosensor application. *Applied Animal Behaviour Science,* **171**, 177–183.

Mitko, L., Weber, M.G., Ramirez, S.R., Hedenström, E., Wcislo, W.T. & Eltz, T. (2016) Olfactory specialization for perfume collection in male orchid bees. *Journal of Experimental Biology,* **219**, 1467–1475.

Morgan, K.V., Hurly, T.A., Martin, L. & Healy, S.D. (2016) Presentation order affects decisions made by foraging hummingbirds. *Behavioral Ecology and Sociobiology,* **70**, 21–26.

Murray, M.G. (1991) Maximizing energy retention in grazing ruminants. *Journal of Animal Ecology,* **60**, 1029–1045.

Niinemets, Ü., Loreto, F. & Reichstein, M. (2004) Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science,* **9**, 180–186.

Owen-Smith, N. & Chafota, J. (2012) Selective feeding by a megaherbivore, the African elephant (*Loxodonta africana*). *Journal of Mammalogy,* **93**, 698–705.

Owen-Smith, N. & Cooper, S.M. (1987) Assessing food preferences of ungulates by acceptability indices. *Journal of Wildlife Management,* **51**, 372–378.

Owen-Smith, N., Fryxell, J.M. & Merrill, E.H. (2010) Foraging theory upscaled: the behavioural ecology of herbivore movement. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* **365**, 2267–2278.

Papachristou, T.G., Dziba, L.E., Villalba, J.J. & Provenza, F.D. (2007) Patterns of diet mixing by sheep offered foods varying in nutrients and plant secondary compounds. *Applied Animal Behaviour Science,* **108**, 68–80.

Peñuelas, J. & Llusià, J. (2004) Plant VOC emissions: Making use of the unavoidable. *Trends in Ecology and Evolution,* **19**, 402–404.

Pietrzykowski, E., McArthur, C., Fitzgerald, H. & Goodwin, A. (2003) Influence of patch characteristics on browsing of tree seedlings by mammalian herbivores. *Journal of Applied Ecology,* **40**, 458–469.

Piggins, D. & Phillips, C. (1996) The eye of the domesticated sheep with implications for vision. *Animal Science,* **62**, 301–308.

Pretorius, Y., Stigter, J.D., de Boer, W.F., van Wieren, S.E., de Jong, C.B., de Knegt, H.J., Grant, C.C., Heitkönig, I., Knox, N., Kohi, E., Mwakiwa, E., Peel, M.J.S., Skidmore, A.K., Slotow, R., van der Waal, C., van Langevelde, F. & Prins, H.H.T. (2012) Diet selection of African elephant over time shows changing optimization currency. *Oikos,* **121**, 2110–2120.

Provenza, F.D. & Balph, D. (1987) Diet learning by domestic ruminants: Theory, evidence and practical implications. *Applied Animal Behaviour Science,* **18**, 211–232.

Provenza, F.D., Burritt, E.A., Clausen, T., Bryant, J., Reichardt, P. & Distel, R.A. (1990) Conditioned flavor aversion: A mechanism for goats to avoid condensed tannins in blackbrush. *American Naturalist,* **136**, 810–828.

Pyke, G.H., Pulliam, R.H. & Charnov, E.L. (1977) Optimal foraging: A selective review of theory and tests. *Quarterly Review of Biology,* **52**, 137–154.

Raguso, R.A. (2008) Wake up and smell the roses: The ecology and evolution of floral scent. *Annual Review of Ecology, Evolution, and Systematics*, 549–569.

Rhoades, D.F. (1979) Evolution of plant chemical defense against herbivores. *Herbivores: Their Interaction With Secondary Plant Metabolites*, pp. 3–54. Academic Press, New York, USA.

Rogosic, J., Estell, R.E., Skobic, D., Martinovic, A. & Maric, S. (2006) Role of species diversity and secondary compound complementarity on diet selection of Mediterranean shrubs by goats. *Journal of Chemical Ecology,* **32**, 1279–1287.

Ruedenauer, F.A., Spaethe, J. & Leonhardt, S.D. (2016) Hungry for quality—individual bumblebees forage flexibly to collect high-quality pollen. *Behavioral Ecology and Sociobiology,* **70**, 1–9.

Rutter, S.M., Orr, R.J., Yarrow, N.H. & Champion, R.A. (2004) Dietary preference of dairy cows grazing ryegrass and white clover. *Journal of Dairy Science,* **87**, 1317–1324.

Schoener, T.W. (1971) Theory of feeding strategies. *Annual Review of Ecology and Systematics,* **11**, 369–404.

Self, S.G. & Mauritsen, R.H. (1988) Power/sample size calculations for generalized linear models. *Biometrics,* **44**, 79–86.

Senft, R., Coughenour, M., Bailey, D., Rittenhouse, L., Sala, O. & Swift, D. (1987) Large herbivore foraging and ecological hierarchies. *BioScience,* **37**, 789–799.

Shipley, L.A. (2007) The influence of bite size on foraging at larger spatial and temporal scales by mammalian herbivores. *Oikos,* **116**, 1964–1974.

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.

Shuttleworth, A. (2016) Smells like debauchery: The chemical composition of semen-like, sweat-like and faintly foetid floral odours in Xysmalobium (Apocynaceae: Asclepiadoideae). *Biochemical Systematics and Ecology,* **66**, 63-75.

Shuttleworth, A. & Johnson, S.D. (2009) A key role for floral scent in a wasp-pollination system in *Eucomis* (Hyacinthaceae). *Annals of Botany,* **103**, 715–725.

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

Stutz, R.S., Banks, P.B., Proschogo, N. & McArthur, C. (2016a) Follow your nose: Leaf odour as an important foraging cue for mammalian herbivores. *Oecologia,* **182**, 643-651.

Stutz, R.S., Croak, B.M., Proschogo, N., Banks, P.B. & McArthur, C. (2016b) Olfactory and visual plant cues as drivers of selective herbivory. *Oikos*, doi:10.1111/oik.03422.

Tholl, D. (2006) Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current opinion in plant biology,* **9**, 297–304.

Villalba, J., Provenza, F., Catanese, F. & Distel, R. (2015) Understanding and manipulating diet choice in grazing animals. *Animal Production Science,* **55**, 261-271.

Ward, D. (1992) The role of satisficing in foraging theory. *Oikos,* **63**, 312–317.

Ward, D. (2010) A parsimonious optimal foraging model explaining mortality patterns in Serengeti wildebeest. *Ecological Modelling,* **221**, 2406–2408.

Ward, D., Shrestha, M.K. & Golan-Goldhirsh, A. (2012) Evolution and ecology meet molecular genetics: Adaptive phenotypic plasticity in two isolated Negev desert populations of *Acacia raddiana* at either end of a rainfall gradient. *Annals of Botany,* **109**, 247–255.

Westoby, M. (1974) An analysis of diet selection by large generalist herbivores. *American Naturalist,* **108**, 290-304.

Wilmshurst, J.F., Fryxell, J.M. & Hudson, R.J. (1995) Forage quality and patch choice by wapiti (*Cervus elaphus*). *Behavioral Ecology,* **6**, 209–217.

Young, T.P. (1987) Increased thorn length in *Acacia depranolobium*—an induced response to browsing. *Oecologia,* **71**, 436–438.

Chapter Three

To what extent do elephants use pre-ingestive cues and/or postingestive feedback when making diet choices?

Abstract

To date, the factors that drive elephant diet selection are not well understood. A number of studies have focused on postingestive feedback measures to describe diet selection, which include aspects of vegetation that can only be assessed by a herbivore after they commit to eating a particular item. However, these postingestive measures including crude protein, digestibility, and tannin content, have not explained elephant diet choice in other studies. Instead, many herbivorous species, including elephants, may rely on pre-ingestive cues (aspects of plants that can be assessed prior to ingestion, i.e. swallowing) to select particular plants when foraging. These could include a range of attributes such as the appearance, smell, or taste of a plant. As all plants emit odours composed of various volatile organic compounds (VOCs), elephants could obtain information about a plant prior to ingestion. However, the extent to which they use odour cues when making foraging decisions is unknown. Thus, the aim of our study was to assess the extent to which elephants use postingestive feedback measures and/or pre-ingestive cues (specifically odour) when making foraging decisions. Ultimately, we found that terpenes play a stronger role in elephant diet choice than phenolics (such as tannins) and other postingestive feedback measures. This is likely because terpenes are a pre-ingestive cue that also incur postingestive costs, which the elephants can use prior to making foraging decisions, unlike other postingestive feedback measures. Postingestive feedback measures indicated that preferred plant species would actually be less favourable than the avoided species because they were less digestible and had higher PSMs (tannins) and lower ratio of palatable (crude protein, digestibility) to unpalatable (tannins) categories than the avoided plant species. Pre-ingestive cues indicated that avoided plant species contained both high levels and a wide diversity of PSM odour cues, particularly monoterpenes, known antiherbivore compounds. As a result, these pre-ingestive odour cues are likely an important factor that elephants use to make diet selections.

Key words: crude protein, digestibility, foraging decisions, monoterpenes, tannins

Introduction

Woody plant species can differ in their nutritional composition as well as their chemical and physical defences (Freeland 1991; Harborne 1991; Rohner & Ward 1997; Metlen *et al.* 2009; Kariñho-Betancourt *et al.* 2015). Variation in nutritional (e.g. crude protein, energy) and anti-nutritional factors (e.g. tannins, terpenes) can even be found between individuals within a single plant species (Metlen *et al.* 2009; Ward *et al.* 2012). For mammalian herbivores, such variation is important because plants are frequently low in overall quality (McNab 1980; Damuth 1981; Ley *et al.* 2008). A key trait of many of these factors is that they are detected after ingestion via postingestive feedback. However, some features of plants, such as the presence or concentration of specific plant secondary metabolites (PSMs), can be detected prior to consumption.

Many herbivorous species may rely on these pre-ingestive cues (aspects of plants that can be assessed prior to ingestion, i.e. swallowing) to select particular plants when foraging (McCrickerd & Forde 2016). These cues could include a range of attributes such as the appearance, smell, or taste of a plant. Visual cues could include an index of red colour in parts of leaves that are high in anti-herbivore compounds called anthocyanins (Manetas 2006). While visual cues are still a possible way that herbivores could gain pre-consumptive knowledge about a plant, there are several issues associated with visual cues, including variation in visual acuity and colour detection among herbivore species (Entsu *et al.* 1992; Piggins & Phillips 1996; Jacobs *et al.* 1998), which may limit success in making dietary selections (Rutter *et al.* 2004). Alternatively, several studies have suggested that plant odour could be a useful cue for herbivores (Stutz *et al.* 2015; Stutz *et al.* 2016a; Stutz *et al.* 2016b), although the role of odour in this respect remains poorly explored.

All plants emit odours composed of various Volatile Organic Compounds (VOCs) – low molecular weight (typically less than 350 Unified Atomic Mass Units (amu)) compounds

with a sufficiently high vapour pressure to evaporate at ambient temperatures (Baluska & Ninkovic 2010). Leaves produce a variety of different VOCs, some of which are PSMs known to play a role in plant defence against herbivory (Peñuelas & Llusià 2004). However, the degree to which they drive mammalian foraging decisions is not well known (Bedoya-Pérez *et al.* 2014).VOCs could thus represent useful cues from which herbivores could obtain information about a plant prior to ingestion (Illius & Gordon 1993; Bedoya-Pérez *et al.* 2014).

Pre-ingestive cues could act as a direct or indirect indicator of specific PSM content. Thus, these cues could act as a guide for herbivores to predict potential postingestive costs associated with PSMs in forage items. For example, terpenes, which can be detected prior to consumption because they are volatile, also have postingestive costs. In contrast, although tannins are a family of PSMs that have commonly been the focus of many studies (e.g. Cooper & Owen-Smith 1985; Robbins *et al.* 1987; Provenza *et al.* 1990; Gilboa 1995; Mkhize *et al.* 2015), they cannot be detected directly via scent because their molecules are too large to evaporate at normal temperatures (Schoonhoven *et al.* 2005). It is possible, however, that herbivores either remember the particular odour of tannin-rich plants, or they use pre-ingestive cues, such as specific VOCs, that are correlated with tannin concentration (postingestive costs) to avoid tannin-rich browse items. As a result, conditioned flavour aversion may be associated with tannins as a possible mechanism that herbivores use to make foraging decisions (Provenza *et al.* 1990; Lawler *et al.* 1999).

Understanding herbivore diet selection is important, especially when a herbivore has a large impact on their environment due to their foraging behaviour. One such species is the African elephant (*Loxondonta africana*), which is the largest extant terrestrial animal. Paired with this large body size is the need to consume large amounts of food to maintain their metabolic requirements, with individuals elephants ingesting 100–200 kg of vegetation per day (Wyatt & Eltringham 1974; Guy 1976). During their foraging bouts, elephants frequently

uproot, knock down, or otherwise modify plants in their environment (Ben-Shahar 1993; Pringle 2008). As a result, they are considered to be ecosystem engineers because they alter their environment through their foraging behaviour (Bond 1994; Jones *et al.* 1994). These elephant-induced alterations have been linked to modification of habitat use of other animal species as well as changes in vegetation structure and community composition (Ben-Shahar 1993; Chamaillé-Jammes *et al.* 2007; Pringle 2008; Valeix *et al.* 2011).

Elephants are mixed-feeders, yet remain selective with respect to which plant species they consume (Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013). To understand factors that influence the foraging decisions of elephants, a number of studies have focused on postingestive feedback measures (Plumptre 1995; Foguekem *et al.* 2011; Shrader *et al.* 2012; Muller 2013). These measures include aspects of vegetation that can only be assessed by a herbivore after they commit to eating a particular item (Illius & Gordon 1993; Provenza 1995), such as crude protein, digestibility, and total polyphenols (Plumptre 1995; Shrader *et al.* 2012; Muller 2013). However, elephant diet selection is not very well explained by assessment of postingestive feedback measures, possibly because the aspects studied to date, which have been useful in identifying grazers' diets (e.g. crude protein and digestibility: Wilmshurst & Fryxell 1995; Stears 2014), do not appear to drive elephant feeding choices (Ward *et al.* 2016).

From a previous study, we know that African elephants recognize odour emitted from plants and use it to make foraging decisions (Chapter 2). However, the extent to which elephants use odour cues when making foraging decisions is unknown. To address this, we assessed the extent to which elephants used postingestive feedback measures and pre-ingestive cues (specifically odour) when making foraging decisions. Specifically, we explored whether certain pre-ingestive cues may also indicate postingestive costs. If elephants did use pre-ingestive cues, we wanted to identify what aspects of the plant-odour profiles influenced

dietary selection. For postingestive feedback measures, we explored whether four key food quality measures (crude protein, digestibility, total polyphenols and tannin content) and the ratios of the nutritional factors to antinutritional factors *sensu* Makkar *et al.* (2007) (i.e. crude protein: tannins, crude protein: total polyphenols, digestibility: tannins, digestibility: total polyphenols) could explain patterns of diet selection. If these indices play an important role, we predicted that the elephants' preferred plant species would be higher in crude protein, be more digestible, and have lower tannin and total polyphenol content compared to the avoided plant species. To test if pre-ingestive cues explained elephant diet selection, we explored relative amounts of key compound classes (e.g. monoterpenes, sesquiterpenes, and aliphatic acids) and specific VOCs (e.g. linalool, (E)-ocimene, and limonene) that are known to affect herbivore intake (Vourc'h *et al.* 2002; Bedoya-Pérez *et al.* 2014), as well as total emissions of VOCs from the odour profiles of preferred and avoided plants. We then used this information to identify which compound(s) influenced diet selection. If pre-ingestive cues explain diet selection, we predicted that anti-herbivory PSMs would be lower in both quantity and emissions in the preferred plant species compared to the avoided plant species.

Methods

All methods were approved by the University of KwaZulu-Natal's Animal Ethics committee (permit number: AREC/106/015).

Acceptability index

To determine the dry-season diet of elephants, we monitored a small population (N = 6) of elephants near Bela Bela, Limpopo province in South Africa (S 24° 46' 53.43" E 27° 57' 5.03"). To explore plant preference, we formulated an acceptability index of the woody plant species preferred and avoided by the elephants. To do this, we located semi-tame, free-ranging elephants and followed their feeding paths, walking about 50 m behind them so as not to disturb their feeding. Along each path, we recorded data within a belt transect (50 m long x

10 m wide (i.e. 5 m on either side of the feeding path), n=100 transects). A width of 5 m encompasses the reach of a foraging elephant's trunk (Shrader *et al.* 2012). Along each path, the abundance of every woody plant species that was present as well as the number of times each was eaten by an elephant were recorded. To generate the acceptability index (AI) of a species, the number of times a particular plant species was eaten was divided by the proportional abundance of that plant species in the sampled area (Owen-Smith & Cooper 1987). After compiling an index for every plant species present, plant species were categorized as preferred (i.e. most selected proportional to its abundance), principal (selected overall most frequently – a function of acceptability and abundance), or avoided (rarely eaten in relation to its abundance). Preferred plant species had AIs of >0.5 and avoided plant species had AIs below 0.3. Principal plant species were eaten the most frequently of all species encountered and had AIs ranging between 0.28-0.5. For our study, we focused on the six most-eaten plant species that comprised ~75% of the elephants' diets (preferred and principal) (*Pappea capensis* Sapindaceae*, Dombeya rotundifolia* Malvaceae*, Terminalia sericea* Combretaceae*, Combretum zeyheri* Combretaceae*, Grewia monticola* Malvaceae*,* and *Euclea crispa* Ebenaceae), and the five most avoided (*Vitex rehmannii* Lamiaceae*, Searsia pyroides* Anacardiaceae*, Searsia lancea* Anacardiaceae*, Euclea undulata* Ebenaceae, and *Olea europaea* Oleaceae).

Plant collection

We collected leaf samples from individual plants from each of the six most eaten plant species as well as the five most avoided plant species for chemical analysis. From each tree, leaves were removed by hand-plucking from all parts of the canopy. This ensured that the collected leaves were an accurate representation of the entire tree. Once plucked, leaves were placed in paper bags and dried in a drying oven at 55 ℃ for 48 h. After drying, we milled the leaves through a 1 mm sieve to prepare them for chemical analysis.

Postingestive feedback: wet chemistry

All plant samples were analysed for nitrogen, digestibility, total polyphenols, and tannin content. Samples were analysed for nitrogen content (g nitrogen per g dry matter) using the Kjeldahl method (Bradstreet 2015) at the Soil Fertility and Analytical Services at Cedara Agricultural College, KwaZulu-Natal, South Africa. Nitrogen was multiplied by 6.25 to estimate % crude protein (Van Soest 1994). We determined dry matter digestibility by cellulase digestion (presented as "proportion digested") (Zacharias 1986). Total polyphenols were measured using Hagerman's (2011) modified Prussian Blue protocol. Units from the spectrophotometer are given in gallic acid equivalents (GAE) because there is no absolute value of polyphenols (Hagerman 2011). We used the radial diffusion assay to measure the amount of tannins (Hagerman 2011).

Pre-ingestive cues: Volatile organic compound collection and analysis

We collected scent samples from vegetative parts (leaves and stems only) of the six most preferred and five most avoided plant species (n = 8 individuals per species). Volatile organic compounds (VOCs) were collected using dynamic-headspace extraction methods. This was done by enclosing a branchlet in a polyacetate bag (NaloPhan, Kalle, Germany) and sucking air from the bag for 3 h through a small cartridge filled with 1 mg each of Tenax® TA (60/80) (SupelcoTM; Bellefonte, PA, USA) and Carbotrap® B (20-40 mesh) (Sigma-Aldrich Co.; St Louis, MO, USA) using a PAS500 Personal Air Sampler (Spectrex, Redwood City, California). Control samples were also collected for 3 h from empty polyacetate bags and used to identify environmental contaminants. Because each plant species has varying densities and sizes of leaves, we counted the number of leaves enclosed in the polyacetate bag for every sample so we could later calculate the total volatile emissions per mm^2 leaf surface area. For this, we collected >20 leaf samples from each plant species and estimated the average surface

area of a leaf from each species by taking length and width measurements and using the formula for an ellipse (surface area = π*a*b).

Volatiles were analysed by gas chromatography-mass spectrometry (GC-MS) using a Varian (Palo Alto, CA) CP3800 gas chromatograph (fitted with a Varian 1079 injector with a ChromatoProbe thermal desorption device) coupled to a Varian 1200 quadrupole mass spectrometer. A polar (Bruker BR-Swax) capillary column was used. A detailed description of these methods is presented in Shuttleworth & Johnson (2009b). Compounds were identified using the NIST 2011 mass spectral library. Where possible, identifications were confirmed by comparison of retention times with published retention indices (Kovats) and/or injection of synthetic standards (for a complete table of VOCs identified, see Appendix II, Table Aii.1). Absolute amounts of volatiles emitted were estimated by comparison of peak areas from samples with peak areas obtained from injection of a known amount of methyl benzoate (injected and run under identical conditions to samples). We have previously established that, for our analytical apparatus, methyl benzoate yields a peak area: nanogram (ng) relationship that is close to the average obtained from 200 compounds from various compound classes (Chapter 2).

Compound classes were assigned following (Knudsen *et al.* 2006) and reflect assumed biosynthetic pathways through which volatiles are produced. We divided aliphatics into functional groups (e.g. alcohols, aldehydes, etc.) and split terpenes into monoterpenes, irregular terpenes, and sesquiterpenes. Thus, the compound classes we identified were: aliphatic acids, aliphatic alcohols, aliphatic aldehydes, aliphatic alkanes, aliphatic esters, aliphatic ketones, benzenoids compounds, C-5 branched compounds, irregular terpenes, miscellaneous cyclic compound, monoterpene, nitrogen-containing compound, and sesquiterpenes. Any compound that could not be identified was placed in the "unknown" category (47 compounds). Unknown compounds ranged in frequency from 0-15 compounds

per plant species. All compounds were included in the analyses. If a compound was listed as 'trace amount', we used 0.001% for the statistical analysis (as per: Shuttleworth & Johnson 2009a).

Statistical analyses of postingestive feedback measures

We explored differences in chemical composition between preferred and avoided plant species using several statistical approaches. We had multiple dependent variables and thus may have an exaggerated Type I error. Consequently, we used multivariate analyses (ANOSIM and SIMPER) where appropriate. We used an ANOSIM randomization test (Anderson 2001) to examine differences in postingestive feedback measures between the two preference categories (i.e. preferred vs. avoided). ANOSIM calculates the test statistic R, which is a relative measure of the separation between previously defined groups (e.g. preferred vs. avoided plant species), based on differences of mean ranks between and within groups. R can range between 0 and 1, with 0 indicating completely random groupings and 1 indicating that samples within groups (e.g. preferred vs. avoided species) are more different from each other than any sample from a different group (Clark *et al.* 2007). We used 10,000 random permutations of the grouping vector to obtain an empirical distribution of R to establish significance (Anderson 2001).

Upon finding significant differences between the preference groups (see *Results*), we explored the direction of the differences using nested generalized linear models with a Tweedie distribution (Jørgensen 1987) and $\log_{10}$ link-function for each of the four factors (crude protein, digestibility, total polyphenols, and tannin content) in addition to ratios of the positive factors (crude protein, digestibility) to the negative factors (total polyphenols, tannins). The Tweedie family of distributions is appropriate for variables that can be represented by Poisson mixtures of gamma distributions and combines properties of continuous and discrete distributions (Jørgensen, 1987). Tweedie distributions are useful

when modelling responses with a mixture of zeros and positive values (Jørgensen, 1987). We used "species" nested within "preference category" (preferred vs. avoided) and ran separate models for each of the eight above-mentioned dependent variables. To avoid possible Type 1 error, we used Holm's (1979) correction of α for sequential analyses of the same null hypothesis.

Statistical analyses of leaf volatiles

Statistical comparison of leaf odours was conducted at the level of compound class and individual compounds (see above). To compare the overall volatile emissions for the preferred and avoided plant species, we used a nested generalized linear model with a Tweedie distribution and a log_{10} link-function with "species" nested within "preference category" (preferred vs. avoided). We used volatile emissions (ng) per mm^2 leaf surface area as the dependent variable. We then used a series of analyses to explore differences in (1) the relative contribution to dissimilarity of compound classes in preferred and avoided species, (2) number of volatile organic compounds within each class of compounds (e.g. monoterpenes, aliphatic acids, and benzenoid/phenylpropanoid compounds), and (3) the relative contribution of individual volatile organic compounds in the preferred and avoided species. Differences in VOC profiles between preferred and avoided plant species were tested using a one-way ANOSIM (Clarke & Gorley 2006). Volatiles characterizing the preferred and avoided categories were further explored using the similarity percentages (SIMPER) function in Primer (Clarke & Gorley 2006). SIMPER identifies compounds that contribute most to the mean similarity within a particular group and mean dissimilarity between groups (i.e. preferred and avoided species). Data were square-root transformed prior to analyses to downweight the influence of dominant compounds on the analysis. To explore the number of individual volatile organic compounds in the preferred and avoided plant species, we used a nested generalized linear model with a Tweedie distribution (Jørgensen 1987) and log_{10} link-

function with "species" nested within "preference category" (preferred vs. avoided). We used the number of compounds as the dependent variable.

We ran an ANOSIM to test for differences in relative contribution of all monoterpenes, a known antiherbivore defence in plants (Vourc'h *et al.* 2002), between the two preference categories (preferred vs. avoided plant species). Furthermore, we used a SIMPER analysis to identify which monoterpenes contributed to these differences. The SIMPER analysis identifies the mean dissimilarity of the monoterpenes between the plant species in the preferred vs. avoided plant species. We used volatile monoterpene emissions (ng) per mm^2 leaf surface area as the dependent variable.

Results

Postingestive feedback measures

The ANOSIM multivariate analysis for total polyphenols, tannins, digestibility, and crude protein revealed a significant difference between the preferred and avoided plant species with regard to their postingestive feedback measures (ANOSIM: R=0.108; p< 0.0001). After applying the Holm's correction to α, preferred and avoided plant species differed significantly in digestibility, tannins, crude protein: tannins, and digestibility: tannins (Table 3.1). Preferred plants had significantly higher levels of tannins than avoided plant species (Table 3.1). Contrary to our prediction, we found that avoided plant species were more digestible and had more favourable crude protein: tannins and digestibility: tannins ratios than preferred species (Table 3.1). The two preference categories did not significantly differ in the amount of total polyphenols, crude protein, and the ratio of crude protein: total polyphenols and digestibility: total polyphenols.

Table 3.1: Generalized Linear Model results showing how various postingestive feedback measures differed between preferred and avoided plant species. Bold values indicate the preference category (i.e. preferred or avoided) that was significantly larger.

Factor	Units	χ^2	df	p	Mean preferred $\pm$ SE	Mean avoided $\pm$ SE
Total polyphenols	GAE	5.077	1	0.024	2.03 $\pm$ 0.148	1.584 $\pm$ 0.13
Tannins	mm	23.7	1	0.0001	**7.654 $\pm$ 0.77**	3.426 $\pm$ 0.447
Digestibility	Proportion	28.409	1	0.0001	0.2 $\pm$ 0.003	**0.227 $\pm$ 0.004**
Crude protein	CP.g^{-1} DM	5.217	1	0.022	7.805 $\pm$ 0.151	7.31 $\pm$ 0.156
Crude protein: Tannins		17.645	1	0.0001	1.814 $\pm$ 0.237	**3.859 $\pm$ 0.445**
Crude protein: Total polyphenols		1.113	1	0.292	6.045 $\pm$ 0.533	6.92 $\pm$ 0.643
Digestibility: Tannins		33.825	1	0.0001	0.117 $\pm$ 0.013	**0.447 $\pm$ 0.006**
Digestibility: Total polyphenols		4.544	1	0.033	0.16 $\pm$ 0.143	0.21 $\pm$ 0.195

Pre-ingestive cues
Volatile emissions

Volatile emissions for preferred and avoided plant species differed significantly ($\chi^2 = 16.202$, df=1, p<0.0001), with preferred species (mean $\pm$ SE: 0.0010 $\pm$ 0.00014 ng/mm^2/min) having significantly lower volatile emissions than avoided plant species (mean $\pm$ SE: 0.0021 $\pm$ 0. 00027 ng/mm^2/min).

Compound classes

Compounds representing 14 compound classes were detected from headspace samples of *preferred and avoided plant species.* The multivariate analysis revealed a significant difference in the preferred versus avoided plant species with regard to the relative amounts of different compound classes (ANOSIM: R= 0.116; p= 0.005). The SIMPER analysis showed that plant species in the preferred and avoided categories had an average overall dissimilarity of 49.12% with respect to the relative amounts of the various compound classes to the overall odour (Table 3.2). Monoterpenes, aliphatic esters, benzenoids/phenylpropanoids, and sesquiterpenes contributed ~50% of the dissimilarity between the preference categories. However, of these categories, only monoterpene levels were significantly lower in the preferred plant species. Additionally, aliphatic acids, aliphatic alcohols, and aliphatic ketones were also lower in the preferred species, but they contributed much less (<7% each) to the overall percent dissimilarity between preferred and avoided plant species.

Table 3.2: Compound classes and their relative contributions to the first 90 % of average Bray–Curtis similarity between headspace scent samples collected from *preferred and avoided plant species with respect to their relative contributions to the overall odour profile* (SIMPER analysis). Dissimilarity/Standard Deviation (SD) is an indicator that discriminates between preference groups. Larger Dissimilarity/SD values indicate a larger percent dissimilarity between groups. Contribution % indicates the percentage of dissimilarity that each group contributes to the overall dissimilarity. Bold values indicate the preference category (i.e. preferred or avoided) that was larger.

Compound Class	Mean abundance in preferred plant species	Mean abundance in avoided plant species	Dissimilarity/SD	Contribution %
Monoterpene	3.73	**5.82**	1.32	20.06
Aliphatic ester	**5.22**	3.59	1.3	16.36
Sesquiterpene	**2.08**	1.9	1.14	10.79
Benzenoid/phenylpropanoid compounds	**3.04**	2.88	1.23	9.77
Miscellaneous cyclic compounds	**1.88**	1.24	1.26	8.05
Unknown	**1.86**	1.75	1.2	7.73
Aliphatic alcohol	0.85	**1.2**	0.76	6.81
Aliphatic acid	0.25	**1.34**	0.84	6.17
Aliphatic ketone	0.32	**1.02**	0.72	5.16

Our results indicated that there were significantly fewer compounds in the preferred plant species compared to the avoided plant species (ANOSIM: R=0.125; p= 0.001). The compound classes contributing to these differences in number of volatile organic compounds are identified in Table 3.3. Plant species in the preferred and avoided categories had an overall dissimilarity of 33.6 % with regards to the *number* of volatile organic compounds within each compound class as calculated by the SIMPER analysis. The classes that contributed ~ 50% of the dissimilarity between the groups were sesquiterpenes, aliphatic acids, miscellaneous

cyclic compounds, monoterpenes, and aliphatic ketones. However, only sesquiterpenes, aliphatic acids, and monoterpenes were lower in the preferred plant species.

Table 3.3: Compound classes and their relative contributions to the first ~90 % of average Bray–Curtis similarity between headspace scent samples collected from *preferred and avoided plant species* with respect to the number of compounds within each group (SIMPER analysis). These data represent the number of compounds within each compound class. Dissimilarity/Standard deviation (SD) indicates the differences between preference groups. Larger Dissimilarity/SD values indicate a larger percent dissimilarity between groups. Contribution % indicates the overall percentage of dissimilarity each group contributes to the overall dissimilarity. Bold values indicate the preference category (i.e. preferred or avoided) that was larger.

Compound Class	Preferred mean abundance	Avoided mean abundance	Dissimilarity/SD	Contribution %
Sesquiterpene	2.73	**2.93**	1.55	12.83
Miscellaneous cyclic compound	**3.03**	2.31	1.28	10.62
Aliphatic acid	0.63	**1.85**	1.14	10.27
Monoterpene	3.75	**4.15**	1.3	9.5
Unknown	3.34	**3.67**	1.11	8.46
Aliphatic alcohol	1.44	**2.24**	1.34	8.36
Aliphatic ketone	0.79	**0.99**	0.92	7.42
Aliphatic aldehyde	**1.1**	0.95	1.07	7.07
Aliphatic ester	**3.15**	2.4	1.1	6.47
Benzenoid compound	**4.68**	4.37	1.12	6.43
Irregular terpene	**1.04**	0	0.81	5.84

Volatile organic compounds

A total of 182 volatile organic compounds from various compound classes were detected from headspace samples of *preferred and avoided plant species.* Overall, we found a significant difference in the relative contribution of each of the volatile organic compounds in the preferred and avoided plant species (ANOSIM: R=0.248; p= 0.0001). The volatile organic compounds that contribute most to these differences are listed in Appendix III, Table Aiii.1. Interestingly, seven of the top 14 volatile organic compounds comprising ~30% of the differences were monoterpenes: (E)-ocimene, (Z)-ocimene, limonene, β-pinene, α-pinene, linalool, and *p*-cymenene. We found that the plant species in the preferred and avoided categories had an overall dissimilarity of about 72% with regard to the relative contribution of particular volatile organic compounds. Furthermore, when we evaluated the number of different volatile organic compounds present in the preferred and avoided plant species, we found that preferred plants produced significantly fewer compounds ($\chi2$= 97, df=1, p<0.0001) than avoided species (20.5 $\pm$ 1.18 compounds versus 34.9 $\pm$ 1.94 compounds; means $\pm$ SD).

Monoterpenes

We evaluated the differences in relative contributions of the monoterpenes in preferred and avoided plant species. We found a significant difference in the relative contribution of the various monoterpenes to the odour profiles of preferred and avoided plant species (ANOSIM: R=0.082; p= 0.0002). The SIMPER analysis indicated that 11 monoterpenes contributed most to these differences in relative contribution (Table 3.4). Plant species in the preferred and avoided plant categories had a dissimilarity of ~81%, and nine of the 11 monoterpenes contributing to ~92% of the relative contribution odour profiles were higher in the avoided plant species (Table 3.4). Furthermore, the number of monoterpenes (mean $\pm$ SD) among categories was significantly different, with preferred plant species having significantly fewer monoterpenes (2.4 $\pm$ 0.23) than the avoided species (6.4 $\pm$ 0.81; $\chi2$= 16, df=1, p<0.0001).

Avoided plant species had much higher volatile emissions of monoterpenes (mean$\pm$ SE: 0.0012 $\pm$ 0. 00019 ng/mm^2/min) than preferred plant species (mean $\pm$ SE: 0.0003 $\pm$0.00006 ng/mm^2/min; χ^2= 25.857, df=1, p<0.0001).

Table 3.4: Monoterpenes contributing to the first ~90 % of average Bray–Curtis similarity between headspace scent samples collected from *preferred and avoided plant species* (SIMPER analysis). Dissimilarity/Standard deviation (SD) indicates differences between preference groups. Larger Dissimilarity/SD values indicate a larger percent dissimilarity across groups. Contribution % indicates the overall percentage of dissimilarity each group contributes to the overall dissimilarity. Names in bold indicate higher relative contribution to odour profile in avoided plant species.

Monoterpenes	Preferred mean abundance	Avoided mean abundance	Dissimilarity/SD	Contribution%
(E)-Ocimene	1.95	**2.46**	0.96	22.27
(Z)-Ocimene	0.82	**1.45**	0.88	12.02
Limonene	0.49	**1.47**	0.79	9.93
Linalool	0.88	0.63	0.66	9.85
γ-Terpinene	0.38	0.34	0.41	8.38
β-Pinene	0.48	**1.29**	0.6	8.23
α-Pinene	0.27	**1.13**	0.94	7.14
Sabinene	0.45	**0.63**	0.46	5.07
p-Cymene	0	**0.8**	0.48	3.72
Terpinolene	0.03	**0.64**	0.48	3.29
p-Cymen-8-ol	0	**0.45**	0.7	2.49

Discussion

Because the African elephant may play such a large role shaping their environment as an ecosystem engineer through their selective feeding behaviour, it is important to understand what drives their diet selection. To date, studies have been largely unsuccessful at determining what features of the plants in their environment influence diet selection by elephants.

Theoretically, herbivores should select species that yield the highest energy gains with the lowest anti-nutritional costs within a time constraint (Pyke *et al.* 1977; Houston & McNamara 2014). For browsers and mixed-feeders, such as elephants, diet selection is complicated due to the presence of PSMs, many of which can have deleterious effects (Freeland & Janzen 1974; Peñuelas & Llusià 2004; Sorensen *et al.* 2005). A key question has remained: to what extent do large mammalian browsers use postingestive feedback measures and/or pre-ingestive cues to make diet selections? Elephants, despite their large body size, have been shown to be selective when making foraging decisions (Codron *et al.* 2006; Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013). Our study found that terpenes play a stronger role in elephant diet choice than phenolics. This is likely because terpenes are a pre-ingestive cue that also have postingestive costs, which the elephants can use prior to making foraging decisions, unlike other postingestive feedback measures.

Comparisons among postingestive feedback measures showed that preferred plant species would actually be less favourable than the avoided species for several reasons such as differences in palatable:unpalatable factors as well as tannin concentration. Elephants did not use crude protein to make their foraging choices. There was no significant difference in the crude protein concentrations between preferred and avoided plant species. Additionally, the lower digestibility in the preferred species, higher PSMs (tannins) and the lower ratio of palatable (crude protein, digestibility) to unpalatable (tannins) categories in the preferred species than in the avoided species further suggests that elephants did not use these postingestive measures to make their diet selections. This is in contrast to the results of Shrader *et al.* (2012) and Muller (2013). In both of these studies, elephants selected for options that had the most favourable palatable: unpalatable ratios. There are, however, differences in the crude protein and total polyphenol concentrations in the plants between our study and that of Shrader *et al.* (2012) and Muller (2013). These differences may be attributed

to the following: First, our study and the two aforementioned studies were conducted in different parts of South Africa. Ithala Game Reserve, where Shrader *et al.* (2012) and Muller (2013) conducted their studies, is in a more nutrient-rich area and has a higher mean annual rainfall than Bela Bela where our study was conducted (du Preez *et al.* 2011; Climate-Data 2017). In addition, the plant species eaten by the elephants in the different studies did not overlap, which also likely contributed to the nutritional differences observed.

In our study, elephants preferred plant species that contained significantly higher levels of tannins, which is surprising given their antinutritional properties (Shimada 2006). This is especially peculiar because there was no significant difference in the crude protein concentrations between preferred and avoided plant species. Thus, the elephants were selecting for plants despite their tannin concentrations, with no obvious nutritional benefit. Tannins are a key PSM that have been found to negatively influence herbivore diet selection (Cooper & Owen-Smith 1985; Owen-Smith & Chafota 2012; Mkhize *et al.* 2015). However, unlike many other PSMs, tannins cannot be detected directly via smell because they are large and heavy compounds (500- 20,000 amu) and are too heavy to become volatile (compounds easily volatilize between 100-200 amu) (Schoonhoven *et al.* 2005).

Although tannins are not volatile, they are astringent, which herbivores may associate with their deleterious effects (Provenza 1995). Furthermore, there could also be other cues correlated with tannin content of plants that herbivores could rely on to make foraging decisions. For example, diformylphloroglucinol compounds (DFPCs) are not volatile, but they correlate with volatile terpenes in *Eucalyptus* species. These volatile terpenes are used as a cue for the DFPCs by brushtail possums (*Trichosurus vulpecula*) (Lawler *et al.* 1999). Thus, elephants may use reliable correlations of tannins with volatile compounds in what is known as conditioned flavour aversion to minimize tannin intake, where they associate the flavour of a certain plant with postingestive costs (Provenza *et al.* 1990). Alternatively, they may use

learning from others in the herd to avoid the plant (Bryant *et al.* 1991) to minimize tannin intake. However, conditioned flavour aversion does not explain why the elephants consumed plant species with high tannin concentrations. Thus, it is likely that preference for these species occurred despite their high tannin concentrations. The question that remains is how the elephants deal with the ingestion of large amounts of tannin. One potential explanation could be that African elephants are able to neutralise tannins via salivary tannin-binding proteins (Chapter 4). Nonetheless, one would still expect that, when given the choice, elephants should still select for the plant species with lower tannin levels to maximize their gains (Owen-Smith & Chafota 2012).

Elephants are unlikely to rely solely on postingestive cues when making foraging decisions. This is because, even though elephants are selective, they cover large areas to obtain sufficient nutritious forage and sometimes may be forced to consume a number of different plant species (Shrader *et al.* 2012). Browsing herbivores may have to rely on previously learned information regarding which plants to eat or avoid or they may use pre-ingestive cues, such as plant odours, which can be used to assess the vegetation quality prior to consumption. Moreover, using pre-ingestive cues could help maximise energy gain, by minimising the intake of heavily defended and/or toxic plants, and reduce travel time between plants and/or patches by directing the herbivores to higher quality plants (Chapter 2). Our results suggest that elephants use olfactory cues, likely of terpenes, (especially at larger spatial scales) to make diet selections rather than relying solely on postingestive feedback measures. It is, however, possible that the elephants had linked olfactory cues of the different plants to postingestive feedback via conditioned learning prior to our study.

The elephants in our study avoided plant species whose scent profiles contained both high levels and a wide diversity of PSM odour cues, particularly monoterpenes. Monoterpenes have been found to negatively influence the intake of certain plant species

across a wide range of herbivore species (Vourc'h *et al.* 2002; Kirmani *et al.* 2010; Shipley *et al.* 2012; Frye *et al.* 2013; Utz *et al.* 2016). Moreover, a recent study has suggested that monoterpenes inhibit digestive enzymes (Kohl *et al.* 2015). Previous studies have established that herbivores can tolerate monoterpenes until a threshold is reached and then shift away from consuming forage items with elevated monoterpene content (Elliott & Loudon 1987; Camp *et al.* 2015; Nobler 2016). For example, Elliott and Loudon (1987) tested the response of red deer calves (*Cervus elaphus*) to monoterpenes commonly found in Sitka spruce (*Picea sitchensis*) and lodgepole pine (*Pinus contorta*) needles and found that the deer significantly avoided treatments that had high concentrations (i.e. high emission) of the monoterpenes. In another study on Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), European roe deer (*Capreolus capreolus*) and rusa deer (*Cervus timorensis russa*), all three species showed negative responses to high concentrations of monoterpenes in food items during feeding trials (Vourc'h *et al.* 2002). Duncan *et al.* (1994) found that the total monoterpene content of a plant negatively influenced the amount of biomass a deer takes from a given plant. These studies suggest that monoterpenes may play an important deterrent role with respect to mammalian herbivores which is well-supported by our findings.

We found that both preferred and avoided plant species have monoterpenes. However, avoided plants have more than double the number of different monoterpenes and the total monoterpene emissions were an order of magnitude greater than the emissions from preferred plant species. In all analyses of the preferred and avoided plant species odour profiles, monoterpenes were the main compound class that were consistently lower both in emissions as well as number of compounds present for preferred plants than avoided plants. Additionally, the overall total VOC emissions of preferred plant species were dramatically lower than avoided plant species.

Monoterpenes, like many PSMs, are an inducible plant defence (i.e. concentrations change in response to browsing pressure; Holopainen 2004). This leads to another possible reason why browsing herbivores may rely more heavily on pre-ingestive cues. Because there is a large amount of variation across and within plant species with respect to nutritional and chemical content, sometimes as a result of phenotypic response to herbivory or to increased soil nutrients (fertiliser) (Holopainen 2004; O'Reilly-Wapstra *et al.* 2004; Loney *et al.* 2006; Burghardt 2016), not all plants are consistently the best dietary options. Numerous studies have found that plants have differing levels of PSMs based on their life history, and these can often be reflected in their VOC profiles (Holopainen 2004; Metlen *et al.* 2009; Moore *et al.* 2014), which herbivores could detect via olfaction. As a result, these pre-ingestive odour cues would be an important and potentially more relevant indicator of an individual plant's nutritional and chemical composition.

Overall, our findings suggest that with regard to plant species encountered by the elephants in our study, terpenes play a stronger role in elephant diet choice than phenolics (such as tannins) and other postingestive feedback measures. This is likely because terpenes are a pre-ingestive cue that also incur postingestive costs, which the elephants may have learned from previous foraging activity prior to this study. The terpene odours could be used prior to making foraging decisions, unlike other postingestive feedback measures. Postingestive feedback measures of crude protein and digestibility likely work better for determining diet selection by grazers because of the reduced number and type of PSMs in grasses (Bryant *et al.* 1992). It is possible that in less chemically diverse systems such as in the Arctic tundra where there are few plant species, browsers may also use postingestive feedback measures such as crude protein, digestibility, and tannins as reliable cues (Bryant *et al.* 1991). However, in species-rich, chemically diverse environments such as African savannas (du Toit 1995), it is apparent that these factors are not reliable predictors of diet

choices by browsers. This may help explain why previous studies have had limited success in determining what drives browser diets based solely on postingestive feedback measures. Thus, it is important to explore pre-ingestive cues through the analysis of plant VOCs that those browsers encounter to determine what drives their foraging decisions. Future studies could also compare VOC terpenes with terpenes detected via wet chemistry of vegetation to determine if VOC terpenes are a good indicator of terpenes within the leaf. Additional studies of elephants could manipulate monoterpenes and test the influence of the concentration of specific monoterpene(s) or monoterpene combinations on elephant diet selection to gain a more precise understanding of the ability of elephants to recognize and react to their chemical environment. Having a better understanding of what drives the foraging behaviour of African elephants ultimately sheds light on not just the underlying drivers of their dietary selection, but also enhances our knowledge of what drives their feeding impacts in the ecosystems in which they live.

References

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* **26**, 32–46.

Baluska, F. & Ninkovic, V. (2010) *Plant Communication from an Ecological Perspective.* Springer Berlin, Germany.

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.

Ben-Shahar, R. (1993) Patterns of elephant damage to vegetation in northern Botswana. *Biological Conservation,* **65**, 249–256.

Bond, W.J. (1994) Keystone Species. *Biodiversity and Ecosystem Function* (eds E.-D. Schulze & H.A. Mooney), pp. 237–253. Springer Berlin, Germany.

Bradstreet, R.B. (2015) *The Kjeldahl Method for Organic Nitrogen.* Elsevier, New York, USA.

Bryant, J.P., Provenza, F.D., Pastor, J., Reichardt, P.B., Clausen, T.P. & du Toit, J.T. (1991) Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics,* **22**, 431–446.

Bryant, J.P., Reichardt, P.B. & Clausen, T. (1992) Chemically mediated interactions between woody plants and browsing mammals. *Journal of Range Management,* **45**, 18–24.

Burghardt, K.T. (2016) Nutrient supply alters goldenrod's induced response to herbivory. *Functional Ecology,* **30**, 1769-1778.

Camp, M.J., Shipley, L.A., Johnson, T.R., Forbey, J.S., Rachlow, J.L. & Crowell, M.M. (2015) Modeling trade-offs between plant fiber and toxins: A framework for quantifying risks perceived by foraging herbivores. *Ecology,* **96**, 3292–3302.

Chamaillé-Jammes, S., Valeix, M. & Fritz, H. (2007) Managing heterogeneity in elephant distribution: Interactions between elephant population density and surface-water availability. *Journal of Applied Ecology,* **44**, 625–633.

Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., Gross, K.L., Pennings, S.C., Suding, K.N. & Grace, J.B. (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters,* **10**, 596–607.

Clarke, K. & Gorley, R. (2006) Primer-E user manual and tutorial. *Primer-E Ltd.* Plymouth, United Kingdom.

Climate-Data (2017) Climate Data. pp. Global climate data sourced from climate models and openstreet mapping. https://en.climate-data.org/continent/africa/.

Codron, J., Lee-Thorp, J.A., Sponheimer, M., Codron, D., Grant, R.C. & de Ruiter, D.J. (2006) Elephant (*Loxodonta africana*) diets in Kruger National Park, South Africa: Spatial and landscape differences. *Journal of Mammalogy,* **87**, 27–34.

Cooper, S.M. & Owen-Smith, N. (1985) Condensed tannins deter feeding by browsing ruminants in a South African savanna. *Oecologia,* **67**, 142–146.

Damuth, J. (1981) Population density and body size in mammals. *Nature,* **290**, 699–700.

du Preez, C.C., van Huyssteen, C.W. & Mnkeni, P.N.S. (2011) Land use and soil organic matter in South Africa 1: a review on spatial variability and the influence of rangeland stock production. *South African Journal of Science,* **107**, 27-34.

du Toit, J.T. (1995) Determinants of the composition and distribution of wildlife communities in southern Africa. *Ambio,* **24**, 2–6.

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.

Elliott, S. & Loudon, A. (1987) Effects of monoterpene odors on food selection by red deer calves (*Cervus elaphus*). *Journal of Chemical Ecology,* **13**, 1343–1349.

Entsu, S., Dohi, H. & Yamada, A. (1992) Visual acuity of cattle determined by the method of discrimination learning. *Applied Animal Behaviour Science,* **34**, 1–10.

Foguekem, D., Tchamba, M.N., Gonwouo, L.N., Ngassam, P. & Loomis, M. (2011) Nutritional status of forage plants and their use by elephant in Waza National Park, Cameroon. *Scientific Research and Essays,* **6**, 3577–3583.

Freeland, W. (1991) *Plant Secondary Metabolites: Biochemical Coevolution with Herbivores.* CRC Press, Boca Raton, Florida, USA.

Freeland, W.J. & Janzen, D.H. (1974) Strategies in herbivory by mammals: The role of plant secondary compounds. *American Naturalist,* **108**, 269–289.

Frye, G.G., Connelly, J.W., Musil, D.D. & Forbey, J.S. (2013) Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. *Ecology,* **94**, 308–314.

Gilboa, N. (1995) Negative effects of tannins on livestock and their neutralization. Unpublished Ph.D thesis, Hebrew University of Jerusalem, Jerusalem, Israel.

Guy, P.R. (1976) The feeding behaviour of elephant (*Loxodonta africana*) in the Sengwa area, Rhodesia. *South African Journal of Wildlife Research,* **6**, 55–63.

Hagerman, A.E. (2011) Tannin chemistry. [http://www.users.muohio.edu/hagermae/]

Harborne, J.B. (1991) The chemical basis of plant defence. *Plant defenses against mammalian herbivory* (eds R.T. Palo & C.T. Robbins), pp. 45. CRC Press, Boca Raton, Florida, USA.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian journal of statistics,* **6**, 65–70.

Holopainen, J.K. (2004) Multiple functions of inducible plant volatiles. *Trends in Plant Science,* **9**, 529–533.

Houston, A.I. & McNamara, J.M. (2014) Foraging currencies, metabolism and behavioural routines. *Journal of Animal Ecology,* **83**, 30–40.

Illius, A.W. & Gordon, I.J. (1993) Diet selection in mammalian herbivores: Constraints and tactics. *An Interdisciplinary Approach To Foraging Behavior.* (ed. R. Hughes), pp. 157–181. Blackwell Scientific Publishing, Oxford, United Kingdom.

Jacobs, G.H., Deegan, J.F. & Neitz, J. (1998) Photopigment basis for dichromatic color vision in cows, goats, and sheep. *Visual neuroscience,* **15**, 581–584.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Ecosystem Management* (eds F.B. Samson & F.L. Knopf), pp. 130–147. Springer, New York, USA.

Jørgensen, B. (1987) Exponential dispersion models. *Journal of the Royal Statistical Society. Series B (Methodological),* **49**, 127–162.

Kariñho-Betancourt, E., Agrawal, A.A., Halitschke, R. & Núñez-Farfán, J. (2015) Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist,* **206**, 796–806.

Kirmani, S.N., Banks, P.B. & McArthur, C. (2010) Integrating the costs of plant toxins and predation risk in foraging decisions of a mammalian herbivore. *Oecologia,* **164**, 349–356.

Knudsen, J.T., Eriksson, R., Gershenzon, J. & Ståhl, B. (2006) Diversity and distribution of floral scent. *Botanical Review,* **72**, 1–120.

Kohl, K.D., Pitman, E., Robb, B.C., Connelly, J.W., Dearing, M.D. & Forbey, J.S. (2015) Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. *Journal of Comparative Physiology B: Biochemistry & Molecular Biology,* **185**, 425–434.

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.

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D. & Knight, R. (2008) Evolution of mammals and their gut microbes. *Science,* **320**, 1647–1651.

Loney, P.E., McArthur, C., Sanson, G.D., Davies, N.W., Close, D.C. & Jordan, G.J. (2006) How do soil nutrients affect within-plant patterns of herbivory in seedlings of *Eucalyptus nitens*? *Oecologia,* **150**, 409–420.

Makkar, H.P.S., Sidhuraiu, P. & Becker, K. (2007) *Plant Secondary Metabolites.* Springer, New York, USA.

Manetas, Y. (2006) Why some leaves are anthocyanic and why most anthocyanic leaves are red? *Flora,* **201**, 163–177.

McCrickerd, K. & Forde, C.G. (2016) Sensory influences on food intake control: Moving beyond palatability. *Obesity Reviews,* **17**, 18–29.

McNab, B.K. (1980) Food habits, energetics, and the population biology of mammals. *American Naturalist*, 106–124.

Metlen, K.L., Aschehoug, E.T. & Callaway, R.M. (2009) Plant behavioural ecology: Dynamic plasticity in secondary metabolites. *Plant, Cell & Environment,* **32**, 641–653.

Mkhize, N.R., Heitkönig, I.M., Scogings, P.F., Dziba, L.E., Prins, H.H. & de Boer, W.F. (2015) Condensed tannins reduce browsing and increase grazing time of free-ranging goats in semi-arid savannas. *Applied Animal Behaviour Science,* **169**, 33–37.

Moore, B.D., Andrew, R.L., Külheim, C. & Foley, W.J. (2014) Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytologist,* **201**, 733–750.

Muller, K. (2013) What drives the seasonal movements of African elephants (*Loxodonta africana)* in Ithala Game Reserve. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Nobler, J.D. (2016) Risky business: Tradeoffs between nutrition, toxicity, and predation by a specialist mammalian herbivore. Unpublished MSc thesis, Boise State University, Boise, Idaho, USA.

O'Reilly-Wapstra, J., McArthur, C. & Potts, B. (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Functional Ecology,* **18**, 677–684.

Owen-Smith, N. & Chafota, J. (2012) Selective feeding by a megaherbivore, the African elephant (*Loxodonta africana*). *Journal of Mammalogy,* **93**, 698–705.

Owen-Smith, N. & Cooper, S.M. (1987) Assessing food preferences of ungulates by acceptability indices. *Journal of Wildlife Management,* **51**, 372–378.

Peñuelas, J. & Llusià, J. (2004) Plant VOC emissions: Making use of the unavoidable. *Trends in Ecology and Evolution,* **19**, 402–404.

Piggins, D. & Phillips, C. (1996) The eye of the domesticated sheep with implications for vision. *Animal Science,* **62**, 301–308.

Plumptre, A. (1995) The chemical composition of montane plants and its influence on the diet of the large mammalian herbivores in the Pare National des Volcans, Rwanda. *Journal of Zoology,* **235**, 323–337.

Pringle, R.M. (2008) Elephants as agents of habitat creation for small vertebrates at the patch scale. *Ecology,* **89**, 26–33.

Provenza, F.D. (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management,* **48**, 2–17.

Provenza, F.D., Burritt, E.A., Clausen, T., Bryant, J., Reichardt, P. & Distel, R.A. (1990) Conditioned flavor aversion: A mechanism for goats to avoid condensed tannins in blackbrush. *American Naturalist,* **136**, 810–828.

Pyke, G.H., Pulliam, R.H. & Charnov, E.L. (1977) Optimal foraging: A selective review of theory and tests. *Quarterly Review of Biology,* **52**, 137–154.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C. & Mautz, W.W. (1987) Role of tannins in defending plants against ruminants: Reduction in protein availability. *Ecology,* **68**, 98–107.

Rohner, C. & Ward, D. (1997) Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia*. *Journal of Vegetation Science,* **8**, 717–726.

Rutter, S.M., Orr, R.J., Yarrow, N.H. & Champion, R.A. (2004) Dietary preference of dairy cows grazing ryegrass and white clover. *Journal of Dairy Science,* **87**, 1317–1324.

Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. (2005) *Insect-Plant Biology*. Oxford University Press, Oxford, United Kingdom.

Shimada, T. (2006) Salivary proteins as a defense against dietary tannins. *Journal of Chemical Ecology,* **32**, 1149–1163.

Shipley, L.A., Davis, E.M., Felicetti, L.A., McLean, S. & Forbey, J.S. (2012) Mechanisms for eliminating monoterpenes of sagebrush by specialist and generalist rabbits. *Journal of Chemical Ecology,* **38**, 1178–1189.

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.

Shuttleworth, A. & Johnson, S.D. (2009a) The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology,* **23**, 931–940.

Shuttleworth, A. & Johnson, S.D. (2009b) A key role for floral scent in a wasp-pollination system in *Eucomis* (Hyacinthaceae). *Annals of Botany,* **103**, 715–725.

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.

Stears, K. (2014) Key factors driving the foraging ecology of Oribi : fear, cattle and the quality and quantity of food. Unpublished PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

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

Stutz, R.S., Banks, P.B., Proschogo, N. & McArthur, C. (2016a) Follow your nose: Leaf odour as an important foraging cue for mammalian herbivores. *Oecologia,* **182**, 643-651.

Stutz, R.S., Croak, B.M., Proschogo, N., Banks, P.B. & McArthur, C. (2016b) Olfactory and visual plant cues as drivers of selective herbivory. *Oikos*, doi:10.1111/oik.03422.

Utz, J.L., Shipley, L.A., Rachlow, J.L., Johnstone-Yellin, T., Camp, M. & Forbey, J.S. (2016) Understanding tradeoffs between food and predation risks in a specialist mammalian herbivore. *Wildlife Biology,* **22**, 167–173.

Valeix, M., Fritz, H., Sabatier, R., Murindagomo, F., Cumming, D. & Duncan, P. (2011) Elephant-induced structural changes in the vegetation and habitat selection by large herbivores in an African savanna. *Biological Conservation,* **144**, 902–912.

Van Soest, P.J. (1994) *Nutritional Ecology of the Ruminant,* 2nd edn. Cornell University Press, Ithaca, New York, USA.

Vourc'h, G., De Garine-Wichatitsky, M., Labbé, A., Rosolowski, D., Martin, J.-L. & Fritz, H. (2002) Monoterpene effect on feeding choice by deer. *Journal of Chemical Ecology,* **28**, 2411–2427.

Ward, D., Muller, K. & Shrader, A.M. (2016) Soil fertility on granite and sedimentary soils is associated with seasonal differences in foraging by elephants. *Plant and Soil*, doi:10.1007/s11104-11016-13067-y.

Ward, D., Shrestha, M.K. & Golan-Goldhirsh, A. (2012) Evolution and ecology meet molecular genetics: Adaptive phenotypic plasticity in two isolated Negev desert populations of *Acacia raddiana* at either end of a rainfall gradient. *Annals of Botany,* **109**, 247–255.

Wilmshurst, J.F. & Fryxell, J.M. (1995) Patch selection by red deer in relation to energy and protein intake: a re-evaluation of Langvatn and Hanley's (1993) results. *Oecologia,* **104**, 297–300.

Wyatt, J.R. & Eltringham, S.K. (1974) The daily activity of the elephant in the Rwenzori National Park, Uganda. *African Journal of Ecology,* **12**, 273–289.

Zacharias, P. (1986) The use of the cellulase digestion procedure for indexing the dry matter digestibility of forages. *Journal of the Grassland Society of Southern Africa,* **3**, 117–121.

Chapter Four

Have African elephants evolved physiological mechanisms to deal with plant secondary metabolites?: Large body size and tannin-binding proteins in a non-ruminant

Abstract

Two important factors drive the foraging decisions of mammalian herbivores: body size and digestive physiology. The Jarman-Bell principle suggests that large animals have lower mass-specific metabolic requirements than smaller animals, but that they have larger overall nutrient requirements. Ultimately, this means that large animals like African elephants (*Loxodonta africana*) can ingest lower-quality food and still meet their metabolic requirements. An added benefit for elephants is that they are hindgut fermenters. This forces them to ingest greater amounts of low quality, high-fibre food compared to ruminants, because fibre does not limit passage rate and thus daily intake of hindgut fermenters. However, one unexplored factor that may also affect the foraging of these large herbivores is plant secondary metabolites. Recent studies have reported that elephants tend to avoid items high in these metabolites. This selectivity is contrary to the predictions of Jarman-Bell principle, suggesting that plant secondary metabolites may lower food quality to a level to where even megaherbivores (>1000 kg) like elephants are affected. If true, then elephants may have evolved physiological adaptations to help them tolerate these low-quality foods. To explore this, we tested elephant saliva for salivary tannin-binding proteins. We used an inhibition assay based on the radial diffusion assay for tannins. In addition, we determined if elephants had proline-rich proteins, a specific type of salivary tannin-binding proteins. Consistent with our prediction, we found that elephants had salivary tannin-binding proteins that reduce the activity of tannins by ~75%. However, we found that elephants lack proline-rich proteins. These results indicate that despite some of the advantages of being large (e.g. having lower mass-specific metabolic requirements), elephants are greatly affected by plant secondary metabolites.

Key words: Body size, Jarman-Bell principle, *Loxodonta africana*, salivary tannin-binding proteins

Introduction

Two key factors that influence foraging decisions of mammalian herbivores are body size and digestive physiology (Owen-Smith 1988). Body size influences mass-specific metabolic requirements (Kleiber 1947; Owen-Smith 1988). Kleiber (1947) found that metabolic requirements scale at $M^{0.75}$ (where M = body mass), which results in larger animals requiring less energy per unit body mass than smaller animals. Ultimately, this means that larger animals can tolerate lower quality foods than smaller animals (Jarman 1974). As a result, larger animals are able to feed on a wider range of food items. Although larger animals have a lower mass-specific metabolic rate, they do require more nutrients per day than small animals (Clauss *et al.* 2003; Speakman 2005). Independent observations of African ungulates by Jarman (1968; 1974) and Bell (1969; 1971) determined that large herbivores can tolerate foodstuffs of lower quality than small herbivores due to several factors including a higher gut fill to energy requirement ratio (Hackmann & Spain 2010) and a faster ingesta passage rate (Clauss *et al.* 2003). Geist (1974) named this the Jarman-Bell principle.

Another factor that influences foraging ecology is digestive physiology. Mammalian herbivores are either foregut fermenters or hindgut fermenters. Ruminants, a subcategory of foregut fermenters, have a four-chambered stomach, with each stomach playing a particular role in the digestion of plant material (Hume 1989). In contrast, most hindgut fermenters, with the exception of some marsupials and equids, have only a single-chambered stomach, and all plant material is fermented in the caecum, which is located at the join of the large and small intestines (Stevens & Hume 2004). A major advantage of being a hindgut fermenter is the ability to survive on lower quality (higher fibre) forage than ruminants (Bell 1971). This is due to these species having a faster digestive passage rate compared to ruminants, which allows for an increased intake rate of lower quality foods (Bell 1971). The slower rate of passage in ruminants is a result of the small particle size that is required for food to pass

through the ruminoreticulum (Bell 1971; Janis 1976). This results in a more efficient extraction of nutrients per unit intake than non-ruminants (Demment & Van Soest 1985), but this slower passage rate is costly if these herbivores feed on high-fibre plants, for example during the dry season. Thus, hindgut fermenters are better suited for eating a wider range of food varying in quality/fibre content than ruminants because they can effectively handle larger particle sizes than ruminants, which allows hindgut fermenters to have a faster passage rate (Owen-Smith 1988).

The largest extant herbivore (also known as a megaherbivore; i.e. > 1000 kg) is the African elephant (*Loxodonta africana*), which weighs between 3000 kg (females) to 7000 kg (males) (Skinner & Chimimba 2005). As predicted by the Jarman-Bell principle, African elephants should be able to maintain their fitness by being non-selective and eating low quality foods. This is a result of both their large body size and their digestive physiology (i.e. they are hind-gut fermenters). Owen-Smith and Chafota (2012) found that elephants have a very wide dietary breadth that incorporates grasses and leaves from trees and shrubs as well as high fibre forage such as twigs, bark, and roots. One major advantage that allows these megaherbivores to eat such low quality food is a decreased digestion time (i.e. faster passage rate of food). The African elephant's digestive system has a shorter gastro-intestinal tract for faster throughput of plant matter, a reduced caecum, and a wider small intestine and colon than theoretically expected (Clauss *et al.* 2003). Although this results in a faster passage rate but yields a lower nutritional gain, it allows these herbivores to eat more food per day than ruminants. Thus, their daily intake is much higher than a ruminant of a similar size (Demment & Van Soest 1985; Clauss *et al.* 2003).

According to the theory associated with body size and digestive physiology, African elephants should not be selective feeders. However, recent findings do not support this prediction. For example, a number of studies have shown that elephants forage selectively and

favour certain plant species over others (Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013). In particular, elephants have been shown to minimize their intake of plant secondary metabolites (PSMs), particularly monoterpenes, which are known to have anti-nutritional effects (Kohl *et al.* 2015). Interestingly, although elephants avoid certain PSMs, elephants still consume a diet that has elevated concentrations of total polyphenols and particularly, tannins, which are a group of polyphenols that are non-nitrogenous plant secondary defence compounds (Robbins *et al.* 1987; McArthur *et al.* 1991). Tannins have a number of negative side effects with the most predominant being the reduction of protein intake and digestibility (Robbins *et al.* 1987; Gordon & Illius 1996; Shimada 2006) which reduces the overall quality of food (Schmidt *et al.* 1998). Although elephants eat plant species that have elevated tannin concentrations, several studies have indicated that after a threshold level of tannin concentration, elephants shift away from consuming certain tannin-rich browse items (Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013).

According to Foose (1982), African elephants can only digest about 40% of ingested cellular matter. Due to the low amount of cellular matter digested and the fairly rapid passage rate reported for elephants (Clauss *et al.* 2003), it is unclear how the effects of secondary metabolites physiologically affect them. Given that elephants avoid plant species that have high monoterpene concentrations (Chapter 3), it is likely that PSMs do influence them physiologically. In the case of tannins, their presence in available browse material could possibly reduce the amount of protein that elephants are able to obtain (Robbins *et al.* 1987; Shimada 2006). If this is the case, this could explain why elephants may shift away from consuming tannin-rich items under certain circumstances. What still remains unanswered is whether the African elephant has additional mechanisms of coping with tannins beyond lowered nutritional demands associated with their body size and digestive physiology as well as behavioural avoidance.

One strategy for reducing the impact of tannins is the production of salivary tannin-binding proteins (McArthur et al. 1991; Shimada 2006). Tannin-binding proteins bind to tannins released from foraged material during mastication in the oral cavity (McArthur et al. 1991; Bennick 2002; Shimada 2006). These proteins can increase the net amount of protein available for digestion (i.e. trade the loss of large amounts of dietary protein for a little salivary tannin-binding protein). Tannin-binding proteins are physiologically costly to make, so most species only produce these proteins if the benefit they provide outweighs the cost of making them (McArthur *et al.* 1991; Skopec *et al.* 2004; Shimada 2006). Because elephants occasionally avoid eating tannin-rich species (e.g. Owen-Smith & Chafota 2012), this suggests that the costs of being large may force elephants to be more selective foragers than initially thought. We aimed to determine whether elephants also produce tannin-binding proteins to further reduce the consequences of ingesting a tannin-rich diet. Although, according to some body-size theories, elephants should not produce tannin-binding proteins as they can tolerate a low quality diet. However, as they may in fact be greatly affected by tannins (Freeland 1991), they may use them to reduce the deleterious effects.

To our knowledge, the two major types of tannin-binding salivary proteins, proline-rich-proteins and histatins (Clauss *et al.* 2003; Shimada 2006), have never been tested for or identified in African elephant saliva. We note that numerous herbivore species have proline-rich proteins in their saliva (mule deer, *Odocoileus emionus*: Robbins et al. 1991; e.g. moose, *Alces alces*: Juntheikki 1996). Humans also have proline-rich proteins in their saliva (Bennick & Connell 1971; Bacon & Rhodes 1998). Many browsers and mixed-feeders, but not grazers, have been found to possess proline-rich proteins. However, the domestic goat (*Capra hircus*), was not found to have these compounds despite being a mixed-feeder (eats both grass and shrubs) (Austin et al. 1989; Distel & Provenza 1991; Makkar 2003). We tested elephant saliva against a negative control (goat saliva) because studies have noted the absence of proline-rich

proteins in goat saliva (Austin *et al.* 1989; Distel & Provenza 1991; Makkar 2003) and a positive control (human saliva) because prior studies noted the presence of proline-rich proteins in human saliva (Mandel *et al.* 1965; Maeda 1985). It is possible that the rapid passage rate and low level of digestion of African elephants require them to maximise the nutritional gain per bite. If that is the case, then there would be a selective pressure to produce tannin-binding proteins. Given that elephants behaviourally avoid foodstuffs that are high in other PSMs and items that are very tannin-rich, we predicted that elephants may use salivary tannin-binding proteins as another mechanism to reduce the negative impact that tannins have on nutritional uptake.

In addition to exploring tannin-binding proteins in elephant saliva, we also aimed to quantify the reduction of tannin levels prior to ingestion to average tannin output in faecal matter. Tannins that are detected in the faecal matter could be either the residual tannins that are locked in undigested plant materials, or tannins that passed through the gut without binding to proteins (Gedir *et al.* 2005). If there is no residual tannin found in the faecal matter, this could be attributed to one of two reasons. First, elephants are able to metabolize the tannins internally, either through tannin-binding proteins in their saliva or with another binding mechanism in the gut. Second, tannins form bonds to any available proteins (tannin-binding or nutritional proteins) and are neutralized (McArthur 1988).

Methods

All aspects of this study were approved by the University of KwaZulu-Natal's Animal Ethics Committee (095/13/Animal). Moreover, the elephants were not harmed or put under any additional stress during this study.

Sample collection

We acquired saliva from six (Male: *N*=3, Female: *N*=3) semi-tame African elephants near Bela Bela, Limpopo Province, South Africa. To induce salivation, we showed each elephant an orange slice, which caused saliva to flow freely. To collect the saliva, we used cotton swabs and sampled from the entire mouth, each swab touching each part of the oral cavity (tongue, cheeks, and sublingual region).We immediately sealed the swabs in plastic Eppendorf vials and froze the samples until laboratory analysis. We also acquired saliva from five goats (Male: *N*=2, Female: *N*=3) from the University of KwaZulu-Natal's research farm, Ukulinga, to use as a negative control (i.e. known not to possess proline in their saliva) for salivary proline-rich proteins in our SDS-PAGE analysis. In addition, five voluntary saliva samples were taken from human candidates (Male: *N*=3, Female: *N*=2) to use as a positive control (i.e. known to possess proline in their saliva) in our SDS-PAGE analysis (see below).

Proline-rich proteins

To test for the presence of proline-rich proteins, we used two different approaches. The first was a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970). We used a 12.5% running gel and 4% stacking gel to separate the various proteins present in elephant saliva. To prepare the saliva samples, we first separated the saliva from the cotton swabs by spinning them at 800 rpm in a centrifuge for 5 min. We used a Bradford's analysis (Bradford 1976) to assess the amount of protein in each sample. Thereafter, we diluted each saliva sample by 50% with phosphate-buffered saline (PBS) to reduce the potential negative effects of salts in our gels. We ran reducing gels, so we added β-mercaptoethanol, a reducing agent that separates proteins into their most basic forms or subunits, in a 1:10 ratio, to the sample buffer prior to addition to the saliva samples (as per Laemmli 1970). We loaded each gel with 25 μg/μL of protein and ran them at 18 mA per gel until the dye-front reached 0.5 cm from the edge of the gel. For each gel, we tested elephant

saliva against a positive control (human saliva) and a negative control (goat saliva) because prior studies noted the presence of proline-rich proteins in human saliva (Mandel *et al.* 1965; Maeda 1985). We also ran a negative control (goat saliva) because studies have noted the absence of proline-rich proteins in goat saliva (Austin *et al.* 1989; Distel & Provenza 1991; Makkar 2003).

The second approach we used was to run a comparative SDS-PAGE gels first using the staining and destaining method as *per* Laemmli (1970) as well as Beeley *et al.*'s (1991) method for staining and destaining to probe for proline-rich proteins. For Laemmli's method, we mixed 45% (v/v) methanol, 10% (v/v) acetic acid and 0.25% (w/v) Coomassie brilliant blue R-250. We left gels to stain overnight. They were then destained with 50% (v/v) methanol and 10% (v/v) acetic acid. Gels in this treatment were compared to gels that were stained in 0.1% w/v CBB R-250 mixed with 40% v/v ethanol and 10% v/v acetic acid for 3 hours and then destained for 4 days in 10% v/v acetic acid (Beeley *et al.* 1991).

Tannin-binding capacities

To test for the tannin-binding capabilities of elephant saliva, we used Hagerman's (1987) radial diffusion assay to test for tannin protein precipitation. Extracts from six of the most commonly foraged plant species (i.e. *Euclea crispa, Pappea capensis, Combretum zeyheri, Terminalia sericea, Grewia monticola, Dombeya rotundifolia*) of elephants were used (see Chapter 3). To extract total polyphenols from these plant species, we used 1 g of dried and milled leaf powder combined with 10 ml of 70% acetone. We sonicated the mixture for 30 min and then centrifuged for 10 min at 2500 x g. We poured off the supernatant and repeated the process. We combined both supernatants, which comprise roughly 75% of the available polyphenols in the plant material.

To make the radial diffusion plates, we followed Hagerman's (1987) procedure that precipitates tannin using bovine serum albumin (BSA). We made a series of 6 μL deep wells

in an Ouchterlony double immunodiffusion pattern (Ouchterlony 1953) instead of the traditional 4 wells per plate pattern (Hagerman 1987). This enabled us to replicate our experiment 5 times.

To test for the tannin-binding capabilities of elephant saliva, we tested 4 μL of plant extract alone and 4 μL of plant extract combined with 2 μL of elephant saliva. For each of the six plant species, we used five replicates of each treatment. We then tested these extracts against saliva from all six elephant individuals. We did not use goats as a negative control for the radial diffusion assay because of the potential that they could have other salivary tannin-binding proteins and did not want this to be a confounding result. Prior to the addition of the plant extract and saliva mixture, we mixed the two components in Eppendorf vials, vortexed them for five s, and allowed the mixture to react for 30 min. After reacting for 30 min, we pipetted the solution into the wells. We sealed each petri dish with parafilm and placed them into an incubator at 30 ºC for four days. On day four, we measured the perpendicular diameters of the tannin-binding ring that had formed around each well (Hagerman 2011).

Elephant dung and residual tannin concentrations

To gain better insight into the ability of African elephants to neutralize tannins, we tested elephant dung for residual tannin concentrations. We collected three dung samples from five of the individuals from which we collected saliva samples. Dung was collected during the dry season by following the elephant herd while the members foraged. After an individual defecated, an entire sample, uncontaminated by urine, was collected and placed in a sealed plastic bag for < 1 hr at which point the dung was left to air dry partially. After 24 h of air drying, the dung was then bagged for transportation (< 10 h) and dried in an oven at 60 ºC. We then milled the dry dung and followed the same tannin extraction protocol that we used to extract tannins from plant material. After extraction of the remaining tannins left in the dung, we inoculated radial diffusion gels with the extracts. As with the tannin-binding affinity

assay, we sealed each petridish with parafilm and placed them into an incubator at 30 $^{\circ}$C for four days. On day four, we measured the perpendicular diameters of the tannin-binding ring that had formed around each well.

Statistical analyses

To determine if elephant saliva could significantly decrease the amount of tannin-binding to the BSA when introduced to an array of wild forage species, we used a Wilcoxon test because our data were not normally distributed. We used mean ring diameter (mean of the perpendicular measurements of each tannin-binding ring) for all six plant species as our dependent factor and treatment (extract alone and extract with elephant saliva) as the independent factor. We initially incorporated amount of protein as a covariate, but found this to be non-significant so we removed it from the model.

For each individual plant species, we also ran a Wilcoxon test using mean ring diameter (average of the perpendicular measurements of each tannin-binding ring) as our dependent factor and treatment (extract alone and extract with elephant saliva) as our independent factor.

Results

Proline-rich proteins

Using the assay of Beeley *et al.* (1991), we found that the elephant saliva did not contain proline-rich proteins. However, saliva samples of all six elephant individuals yielded a single, non-pink/violet protein band at ~50 Dka. Our positive control (human saliva) yielded several pink/violet bands indicating the presence of proline-rich proteins (Beeley *et al.* 1991), while our negative control (goat saliva) had numerous protein bands but lacked proline-rich proteins indicated by the absence of a pink/violet band(s).

Overall tannin-binding capabilities

We found that when elephant saliva was mixed with the extracts of the most commonly

consumed plant species of elephants in Bela Bela, the saliva significantly reduced tannin-

binding capacity of the plant extracts (Wilcoxon test: Z= 11.19, P <0.0001). Overall, elephant

saliva reduced the diameter of the protein-precipitate ring by an average of 75%. In addition

to an overall analysis of the tannin-binding capabilities of elephant saliva, we also tested

elephants with each plant species individually using a Wilcoxon test (Table 4.1, Figure 4.1).

When we tested elephant dung for residual tannin levels, we found no detectable levels of

tannin.

Table 4.1: For all plant species, elephant saliva significantly reduced tannin-binding capacity

of the extract alone. P values were calculated as the difference between tannin levels in the

control (plant extract alone) solution and the combination of elephant saliva and plant extract

solution.

Species	*Z*	*P*	Mean Reduction (%)
Euclea crispa	4.2	<0.0001	87
Pappea capensis	4.4	<0.0001	79
Combretum zeyheri	3.9	<0.0007	66
Terminalia sericea	4.3	<0.0001	79
Grewia monticola	3.9	<0.0005	67
Dombeya rotundifolia	4.7	<0.0001	82

Tannin Binding Affinity
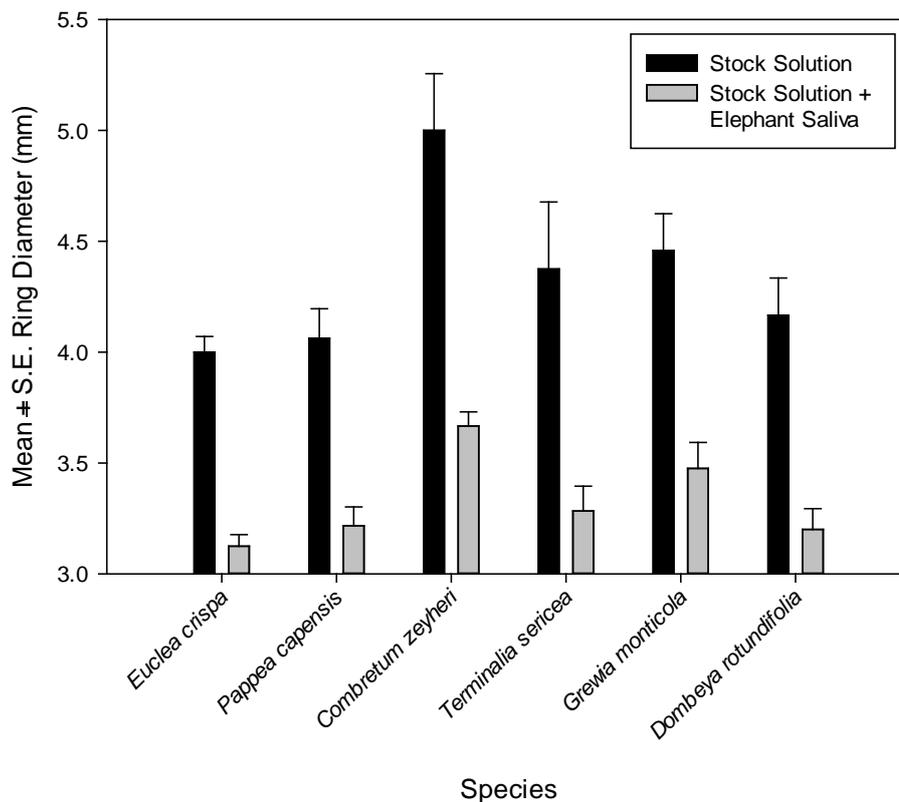
Figure 4.1: The mean (± S.E.) tannin-binding capacities of the six most commonly foraged plant species of elephants 1) 4 µL of plant extract alone and 2) combined with 2 µL of elephant saliva. Elephant saliva significantly decreased the amount of tannin that was left to bind with the bovine serum albumin in the petri dishes.

Discussion

We suggest that African elephants have protein(s) in their saliva that bind to tannins. Moreover, the tannin-binding affinity of these proteins is greater than we expected (i.e. reduces the mean diameter of the protein-precipitate ring by ~75%). Even though elephant saliva shows no trace of proline-rich-proteins using Beeley *et al.*'s (1991) assay, it is likely that the saliva may have other tannin-binding compounds such as histatin or another unnamed salivary tannin-binding protein (Shimada 2006). Another species that we also tested, the

domestic goat, consumes large quantities of tannin-rich leaves and yet its saliva also lacks proline (Distel & Provenza 1991; McArthur *et al.* 1991). Thus, we assume that the African elephant, which is capable of binding tannins, has another protein that binds these compounds. We found only a single protein band in all six of the elephant individuals' saliva, suggesting that whatever protein binds to tannin is held in this protein band. Furthermore, we found no detectable traces of tannins in elephant dung, suggesting that the vast majority of the tannins ingested are either metabolized or absorbed during digestion, or otherwise bound to available protein or salivary tannin-binding proteins (McArthur 1988). In some mammal species, tannic acid is not excreted in faeces because it is metabolized or degraded by symbiotic microflora in the gut (McArthur et al. 1991). However, teasing apart these mechanisms was beyond the scope of this study.

The presence of tannin-binding proteins in elephant saliva suggests that a large body size and a hindgut digestive physiology alone do not permit elephants to meet their metabolic requirements, contrary to the Jarman-Bell principle. This suggests that the relationships between body size, type of digestive system, and the quality of food (as determined by plant secondary metabolites) required by large herbivores should be re-examined.

The large body size of the African elephant, coupled with its hindgut digestive system, should result in tolerance of a wide range of plant types. The African elephant's digestive system is structured to increase the rate of passage of ingested food (Clauss *et al.* 2003). However, their large body size might actually not be as advantageous as once thought. Theoretically, their digestive passage time should be around 72 h, given their mass, to gain maximum nutritional yield from ingested food items (Foose 1982). However, Foose (1982) reported an empirically-determined ingesta passage rate of only 52 h. It appears that, evolutionarily speaking, the African elephant's digestive passage rate has been sped up to avoid holding onto digesta beyond total digestion to the point of total fermentation of plant

material (i.e. 72 h passage rate) (Clauss *et al.* 2003). With the empirically-determined

digestive passage rate of 52 h, elephants only breakdown 40% of cellular matter ingested

(Foose 1982). Considering an elephant's total nitrogen requirements, ca. 40% cellular

digestion is a low degree of digestion (Foose 1982), especially if nitrogen digestibility is also

low. Nitrogen limitation as a result of tannins in browse items has been found to be a key

factor influencing mammalian herbivore fitness (McArt *et al.* 2009; DeGabriel *et al.* 2014).

For example, the population dynamics of moose in wilderness areas in Alaska, United States

of America, are linked to summer nitrogen limitations (McArt *et al.* 2009). In one area during

summer, there was 23% more digestible protein available as a result of tannin concentrations

than another wilderness area, which significantly influenced the reproductive fitness of moose

in these two populations (McArt *et al.* 2009). Thus, the McArt *et al.* (2009) concluded that

summer dietary nitrogen availability may act as a nutritional constraint on moose. Given

McArt *et al.*'s (2009) findings, it is apparent that nitrogen limitation as a result of tannin

content plays a larger role in mammalian herbivore population success than previous thought.

Therefore, it is possible that, given their large body size, this rapid passage rate might be

insufficient for them to obtain an adequate nutritional intake with the negative effects of

tannins (on nitrogen digestion).

The addition of tannin-binding proteins in elephant saliva may allow a broader diet

than would be possible without tannin-binding proteins. In addition, elephants may further

maximize their energy uptake by neutralizing the negative impacts of tannins. African

elephants consume plant species that contain elevated levels of secondary metabolites

(Shrader *et al.* 2012; Muller 2013; Ward *et al.* 2016). However, they may avoid eating plant

species, or particular plant parts, that have extremely high concentrations of tannins or other

polyphenols, but can tolerate a lower amount in their diet, which is neutralized through a

combination of salivary tannin-binding proteins and gut flora. This could explain why Owen-

Smith and Chafota (2012) found that elephants avoided parts of *Combretum* spp. that contained high concentrations of phenols. Additionally, in a study done exploring factors that influence elephant movement in Ithala Game Reserve in South Africa, elephants shifted their foraging habitats, as well as foraging preferences, away from *Acacia nilotica* (which has high polyphenol concentrations) as the amount of total polyphenols increased seasonally, causing the amount of digestible protein to decline (Muller 2013). The presence of salivary tannin-binding proteins in addition to a behavioural avoidance of high levels of tannins suggest that tannins might actually pose a greater cost to megaherbivores than previously expected.

Elephants are not the first megaherbivore to display a combination of a behavioural aversion of browse material that has very high levels of secondary metabolites as well as salivary tannin-binding proteins. Ndlovu and Mundy (2008) found that in a cafeteria trial, black rhinoceros, *Diceros bicornis,* selected against plant species that have high levels of both soluble and insoluble tannins. In addition to this behavioural avoidance of high levels of tannins, black rhinoceros saliva also contains tannin-binding proteins (Clauss *et al.* 2005). Similar to elephants, several studies have found that black rhinoceros eat numerous browse species that contain elevated tannin levels (e.g. Dierenfeld *et al.* 1995; Shaw 2011). Thus, like elephants, black rhinoceros might also able to cope with a threshold amount of tannins (Clauss *et al.* 2005).

Salivary tannin-binding proteins are costly to produce. One study found that rats cease production of proline-rich proteins when they are not needed (Haghighat *et al.* 1996), and another found that black rhinoceros produce more tannin-binding proteins when foraging on browse containing higher concentration of tannins (Clauss *et al.* 2005). It remains unclear whether elephants have tannin-binding compounds that are inducible. Nonetheless, the current binding-affinity of the proteins we tested, coupled with behavioural avoidance, are likely to be key evolutionary adaptations to help maintain nutritional intake.

The Jarman-Bell principle does not take plant secondary metabolites, such as tannins, into account. However, our results highlight that, despite their large body size, and thus their ability to tolerate low-quality food, elephants have evolved tannin-binding proteins as a way of dealing with the negative effects of tannins. This suggests that these proteins are necessary for elephants to maximise their protein intake. Thus, consistent with other studies (Clauss *et al.* 2005; Owen-Smith & Chafota 2012; Muller 2013), our results indicate that plant secondary metabolites play a greater role in the evolutionary foraging strategies of megaherbivores than previously thought.

References

Austin, P.J., Suchar, L.A., Robbins, C.T. & Hagerman, A.E. (1989) Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *Journal of Chemical Ecology,* **15**, 1335–1347.

Bacon, J.R. & Rhodes, M.J.C. (1998) Development of a competition assay for the evaluation of the binding of human parotid salivary proteins to dietary complex phenols and tannins using a peroxidase-labeled tannin. *Journal of Agricultural and Food Chemistry,* **46**, 5083–5088.

Beeley, J.A., Khoo, K.S. & Lamey, P.J. (1991) Two-dimensional electrophoresis of human parotid salivary proteins from normal and connective tissue disorder subjects using immobilised pH gradients. *Electrophresis,* **12**, 493–499.

Bell, R.H.V. (1969) The use of the herb layer by grazing ungulates in the Serengeti. *Animal Populations in Relation to their Food Resources* (ed. A. Watson), pp. 111–124. Blackwell, Oxford, United Kingdom.

Bell, R.H.V. (1971) A grazing ecosystem in the Serengeti. *Scientific American,* **224**, 86–93.

Bennick, A. (2002) Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology & Medicine,* **13**, 184–196.

Bennick, A. & Connell, G.E. (1971) Purification and partial characterization of four proteins from human parotid saliva. *Biochemical Journal,* **123**, 455–464.

Bradford, M.M. (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilization principle of protein-dye binding. *Analytical Biochemistry,* **72**, 248–254.

Clauss, M., Frey, R., Kiefer, B., Lechner-Doll, M., Loehlein, W., Polster, C., Rössner, G.E. & Streich, J.W. (2003) The maximum attainable body size of herbivorous mammals: Morphophysiological constraints on foregut, and adaptations of hindgut fermenters. *Oecologia,* **136**, 14–27.

Clauss, M., Gehrke, J., Hatt, J.-M., Dierenfeld, E.S., Flach, E.J., Hermes, R., Castell, J., Streich, J.W. & Fickel, J. (2005) Tannin-binding salivary proteins in three captive rhinoceros species. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* **140**, 67–72.

DeGabriel, J.L., Moore, B.D., Felton, A.M., Ganzhorn, J.U., Stolter, C., Wallis, I.R., Johnson, C.N. & Foley, W.J. (2014) Translating nutritional ecology from the laboratory to the

field: milestones in linking plant chemistry to population regulation in mammalian browsers. *Oikos,* **123**, 298-308.

Demment, M.W. & Van Soest, P.J. (1985) A nutritional explanation for body-size patterns of ruminant and nonruminant herbivores. *American Naturalist,* **125**, 641–672.

Dierenfeld, E.S., du Toit, R. & Braselton, E.W. (1995) Nutrient composition of selected browses consumed by black rhinoceros (*Diceros bicornis*) in the Zambezi Valley, Zimbabwe. *Journal of Zoo and Wildlife Medicine,* **26**, 220–230.

Distel, R.A. & Provenza, F.D. (1991) Experience early in life affects voluntary intake of blackbrush by goats. *Journal of Chemical Ecology,* **17**, 431–450.

Foose, T.J. (1982) Trophic strategies of ruminant versus nonruminant ungulates. Unpublished PhD thesis, University of Chicago, Chicago, USA.

Freeland, W. (1991) *Plant Secondary Metabolites: Biochemical Coevolution with Herbivores.* CRC Press, Boca Raton, Florida, USA.

Gedir, J.V., Sporns, P. & Hudson, R.J. (2005) Extraction of condensed tannins from cervid feed and feces and quantification using a radial diffusion assay. *Journal of Chemical Ecology,* **31**, 2761–2773.

Geist, V. (1974) On the relationship of social evolution and ecology in ungulates. *American Zoologist,* **14**, 205–220.

Gordon, I.J. & Illius, A.W. (1996) The nutritional ecology of African ruminants: A reinterpretation. *Journal of Animal Ecology,* **65**, 18–28.

Hackmann, T.J. & Spain, J.N. (2010) Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. *Journal of Dairy Science,* **93**, 1320–1334.

Hagerman, A.E. (1987) Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology,* **13**, 437–449.

Hagerman, A.E. (2011) Tannin chemistry. [http://www.users.muohio.edu/hagermae/]

Haghighat, M., Moetamed, A., Vaseghi, T. & Aminlari, M. (1996) Isoprenaline induces biosynthesis of proline-rich proteins in the salivary glands of rat but not in sheep. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology,* **115**, 165–168.

Hume, I.D. (1989) Optimal Digestive Strategies in Mammalian Herbivores. *Physiological Zoology,* **62**, 1145-1163.

Janis, C. (1976) The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. *Evolution,* **30**, 757–774.

Jarman, P.J. (1968) The effect of the creation of Lake Kariba upon the terrestrial ecology of the middle Zambezi valley. Unpublished PhD thesis, University of Manchester, Manchester, United Kingdom.

Jarman, P.J. (1974) The social organisation of antelope in relation to their ecology. *Behaviour,* **48**, 215–267.

Juntheikki, M.R. (1996) Comparison of tannin-binding proteins in saliva of Scandinavian and North American moose (*Alces alces*). *Biochemical Systematics and Ecology,* **24**, 595–601.

Kleiber, M. (1947) Body size and metabolic rate. *Physiological Reviews,* **27**, 511–541.

Kohl, K.D., Pitman, E., Robb, B.C., Connelly, J.W., Dearing, M.D. & Forbey, J.S. (2015) Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. *Journal of Comparative Physiology B: Biochemistry & Molecular Biology,* **185**, 425–434.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**, 680–685.

Maeda, N. (1985) Inheritance of the human salivary proline-rich proteins: A reinterpretation in terms of six loci forming two subfamilies. *Biochemical Genetics,* **23**, 455–464.

Makkar, H.P.S. (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research,* **49**, 241–256.

Mandel, I.D., Thompson Jr, R.H. & Ellison, S.A. (1965) Studies on the mucoproteins of human parotid saliva. *Archives of Oral Biology,* **10**, 499–507.

McArt, S.H., Spalinger, D.E., Collins, W.B., Schoen, E.R., Stevenson, T. & Bucho, M. (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology,* **90**, 1400-1411.

McArthur, C. (1988) Variation in Neutral Detergent Fiber Analysis of Tannin-Rich Foliage. *The Journal of Wildlife Management,* **52**, 374-378.

McArthur, C., Hagerman, A.E. & Robbins, C.T. (1991) Physiological strategies of mammalian herbivores against plant defences. *Plant defenses against mammalian herbivory* (eds R.T. Palo & C.T. Robbins), pp. 103–114. CRC Press, Boca Raton, Florida, USA.

Muller, K. (2013) What drives the seasonal movements of African elephants (*Loxodonta africana)* in Ithala Game Reserve. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Ndlovu, M. & Mundy, P.J. (2008) Browse preference of captive black rhinos at Chipangali Wildlife Orphanage, Zimbabwe. *Pachyderm*, 41–46.

Ouchterlony, Ö. (1953) Antigen-antibody reactions in gels. *Acta Pathologica Microbiologica Scandinavica,* **32**, 231–240.

Owen-Smith, N. (1988) *Megaherbivores: The Influence of Very Large Body Size on Ecology*. Cambridge University Press, Cambridge, United Kingdom.

Owen-Smith, N. & Chafota, J. (2012) Selective feeding by a megaherbivore, the African elephant (*Loxodonta africana*). *Journal of Mammalogy,* **93**, 698–705.

Robbins, C.T., Hagerman, A.E., Austin, P.J., McArthur, C. & Hanley, T.A. (1991) Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *Journal of Mammalogy,* **72**, 480–486.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C. & Mautz, W.W. (1987) Role of tannins in defending plants against ruminants: Reduction in protein availability. *Ecology,* **68**, 98–107.

Schmidt, K.A., Brown, J.S. & Morgan, R.A. (1998) Plant defenses as complementary resources: a test with squirrels. *Oikos,* **81**, 130-142.

Shaw, J.A. (2011) Adaptive resource use in a re-introduced black rhinoceros population. Unpublished PhD thesis, University of the Witwatersrand, Johannesburg, South Africa.

Shimada, T. (2006) Salivary proteins as a defense against dietary tannins. *Journal of Chemical Ecology,* **32**, 1149–1163.

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.

Skinner, J.D. & Chimimba, C.T. (2005) *The Mammals of the Southern African Sub-Region*. Cambridge University Press, Cape Town, South Africa.

Skopec, M.M., Hagerman, A.E. & Karasov, W.H. (2004) Do salivary proline-rich proteins counteract dietary hydrolyzable tannin in laboratory rats? *Journal of Chemical Ecology,* **30**, 1679–1692.

Speakman, J.R. (2005) Body size, energy metabolism and lifespan. *Journal of Experimental Biology,* **208**, 1717–1730.

Stevens, E.C. & Hume, I.D. (2004) *Comparative Physiology of the Vertebrate Digestive System,* 2nd edn. Cambridge University Press, Cambridge, UK.

Ward, D., Muller, K. & Shrader, A.M. (2016) Soil fertility on granite and sedimentary soils is associated with seasonal differences in foraging by elephants. *Plant and Soil*, doi:10.1007/s11104-11016-13067-y.

Chapter Five

Incorporating secondary metabolites, tannin-binding proteins, and diet breadth into carrying-capacity models for African elephants

This chapter has been published in *Ecological Modelling*.

Contents lists available at ScienceDirect

Ecological Modelling

Incorporating secondary metabolites, tannin-binding proteins, and diet breadth into carrying-capacity models for African elephants

Melissa H. Schmitt*, David Ward[1], Adrian M. Shrader

School of Life Sciences, University of KwaZulu-Natal, South Africa

Article info

Abstract

Elephant populations have been historically difficult to manage and have sometimes been subjected to drastic management procedures. One way to monitor elephant populations to estimate a sustainable carrying capacity is through the use of standing-crop carrying-capacity models. Previous carrying-capacity models designed for grazers have posed issues when applied to browsers such as elephants, predicting much higher carrying-capacity estimates than are realized in nature. This might be attributed to the level of plant secondary metabolites, which limit the available nitrogen in browse material that browsers and mixed feeders encounter while foraging. Many browsers and mixed-feeders, including elephants, have a physiological mechanism to tolerate a portion of encountered tannins in the form of salivary tannin-binding proteins. We constructed an optimal-foraging model for carrying capacity for elephants that incorporates the negative effects of plant secondary metabolites and the partial neutralization of these chemicals by the tannin-binding affinity of elephant saliva. In addition, our model includes diet breadth and dietary contribution of browsed species, browse quality, and the available standing crop of browsed species. Ultimately, our model produced more plausible estimates of elephant carrying capacity when compared with estimates that use 100% nitrogen availability and use. Moreover, as the key variables needed for the model can be obtained easily, our model is not site-specific or limited to elephants, but rather can be applied to a wide range of browsing herbivores across a number of reserves of different sizes.

Introduction

African elephant (*Loxodonta africana*) populations present many management challenges (Kerley and Shrader, 2007; Midgley et al., 2005; Whyte et al., 1998). In southern Africa, elephant numbers are estimated to be about 20,000 individuals and are increasing by about 4% per year (Blanc, 2007, 2008; Blanc et al., 2005). Through their foraging, elephant populations can have negative impacts on the landscape (Conybeare, 2004; Guldemond and van Aarde, 2008; Kerley and Landman, 2006; Valeix et al., 2011). The primary reason for their negative effects on vegetation is that elephants require 100–200 kg of vegetation per day to meet their ener- getic needs and protein requirements (Guy, 1976; Osborn, 2004; Wyatt and Eltringham, 1974). In addition to the large quantities of

* Corresponding author. Current address: School of Life Sciences, Uni- versity of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa. Tel.: +27 33 260 5143; fax: +27 33 260 5105.
E-mail address: missyschmitt@gmail.com (M.H. Schmitt).
[1] Present address: Biological Sciences, Kent State University, Kent, OH 44242, USA.

vegetation that they consume, they can also be destructive foragers, often breaking, pushing over, or otherwise damaging trees (Midgley et al., 2005). While this destructive behaviour does create impor- tant habitat features for a host of other species, it can limit resources for other herbivores (Pringle, 2008).

Due to their impacts on the landscape, elephant popula- tions have been subjected to drastic and invasive management techniques to limit local populations, including contraception, relo- cation, and culling (Kerley and Shrader, 2007; van Aarde et al., 1999; Whyte et al., 1998). Within Africa, protected areas are challenged to establish a balance between number of elephants and available resources. One approach to monitor and manage this issue is the use of carrying-capacity models for mammalian herbivores based on the available standing crop (biomass) in an area to predict the num- ber of individuals that could be supported (e.g. Jones and Sandland, 1974; Hobbs and Swift, 1985). This has been done for a number of species, primarily grazers (Harlan, 1958; Jones and Sandland, 1974; Mott, 1960; Riewe, 1961; Sandland and Jones, 1975).

Many of the early models assume a simple linear relation- ship between live weight gain per animal or live weight gain per hectare and stocking rate (number of mammalian herbivores in an

area) (Cowlishaw, 1969; Jones and Sandland, 1974; Riewe, 1961; Sandland and Jones, 1975). However, others suggest a more curvi-linear relationship between live weight gain per animal or hectare and stocking rate (Owen and Ridgman, 1968). Most of these early models use live weight gain (per animal or hectare), a known relation of animal production, to predict optimum carrying capacity (Jones and Sandland, 1974). There are differing opinions as to which of these early theoretical models reflect the most accurate relationship between carrying capacity and animal gain (Jones and Sandland, 1974; Sandland and Jones, 1975).

Until the 1980s, there were very few carrying-capacity models that could be applied to wild herbivores. This was mainly because a corresponding number of Livestock Units (LU), an equivalent measure for all livestock based on their metabolic requirements, was never defined (Meissner, 1982). Meissner (1982) made one of the first attempts to form equivalent LUs for wild animals such as blesbok (*Damaliscus pygargus phillips*), buffalo (*Syncerus caffer*), zebra (*Equus quagga*), giraffe (*Giraffa camelopardalis*), and elephants in an effort to more accurately predict carrying capacity based on metabolic requirements for each species and age-classes within each species. While this model was one of the first to include explicit values for wild animals, one key factor remained constant: all models assumed that foraging areas are homogenous in nutrient availability as well as nutrient utilization by animals. However, Hobbs and Swift (1985) incorporated explicit forage-quality values (e.g. nitrogen and metabolizable energy) into an optimal foraging model of carrying-capacity. The Hobbs and Swift (1985) model yielded a more precise representation of realized carrying capacity as a function of animal foraging choices and available nutrients. Forage quality and availability can vary both spatially and temporally, which can influence foraging animals' intake rates (Ungar and Noy-Meir, 1988) and selectivity (Sinclair and Gwynne, 1972). By incorporating site- and species-specific values of forage to a carrying-capacity model, Hobbs and Swift (1985) allowed for a more realistic estimate of what optimal carrying capacity should be for an area.

A problem that existed for the implementation of carrying-capacity models was that they were appropriate for grazers (eat grasses) only, which has posed issues when applied to browsers (eat woody plants such as trees and shrubs). In general, browsers encounter plants that have higher concentrations of protein than do grazers, which is beneficial for mammalian herbivores (Mattson, 1980; Van Soest, 1994). However, the woody plants that browsers feed on are generally quite well defended by secondary metabolites (i.e. not used for primary metabolism of the plant – Herms and Mattson, 1992). Grazers, on the other hand, usually encounter plants (grasses) with lower protein concentrations than woody plants but these plants are less well-defended by secondary metabolites than are woody plants (Chesselet et al., 1992). This often results in the failure of applying carrying-capacity models designed for grazers such as cattle and sheep to browsing mammals (i.e. because browsers encounter higher levels of secondary metabolites in their forage than do grazers). The reason for this problem with carrying-capacity models is that plant secondary metabolites (PSMs), especially tannins, typically reduce the amount of available nitrogen/crude protein in the forage (Robbins et al., 1991). As a result, grazing models applied to browsers and/or mixed-feeders typically overestimate carrying capacity because they assume that all nitrogen is available in browse material and thus generate substantially higher carrying-capacity estimates than can be realized in nature (Windels and Hewitt, 2011).

Windels and Hewitt (2011) developed a model based on the Hobbs and Swift (1985) model to predict the carrying capacity of browsers and mixed-feeders. In doing so, they took into account the amount of secondary metabolites in available browse species, which should improve carrying-capacity estimates. They found

that by including the effects of plant secondary metabolites on protein and energy, their model yielded lower, and more realistic estimates of carrying capacity. While Windels and Hewitt's (2011) model produced plausible estimates of carrying capacity for white-tailed deer (*Odocoileus virginianus*) in Mexico, there is a challenge to their approach. This approach includes the need to assess the amount of non-protein nitrogen. The % crude protein is conventionally calculated by weighting nitrogen by 6.25 for each plant species. However, not all nitrogen is proteinaceous (Mattson, 1980). This is a fairly complex process and would most likely not be feasible for resource managers to conduct for all plant species eaten by specific herbivores.

A factor that has not been extensively explored in previous carrying-capacity models is the tannin-binding affinity of a browser's saliva. Many mammalian species have adapted to browsing on tannin-rich plant species via the production of salivary tannin-binding proteins (McArthur et al., 1991; Shimada, 2006). These salivary proteins bind to released tannins before the tannins can bind to nitrogen released from the digesting plant material, neutralizing some of the negative impacts of tannins (McArthur et al., 1991; Shimada, 2006). The tannin-binding affinity of saliva can vary among species (Clauss et al., 2005). Each animal species that frequently encounters tannins can neutralize different levels of tannins (Clauss et al., 2005). Thus, access to different proportions of nitrogen by browsers is species-dependent. This adjusted available nitrogen value would then influence the estimated carrying capacity of an area. As a result, we aim to explore the addition of this factor into a carrying-capacity model to examine if this yields a more precise representation of carrying capacity.

To generate an improved carrying-capacity model for elephants, we conducted an in-depth study of elephant foraging behaviour during the dry season. This is the most food-limiting season for mammalian herbivores due to low nutrient quality and lower food availability. Moreover, it is the critical period that determines how many animals an area can support sustainably (Coe et al., 1976; Owen-Smith, 2002b). Employing Hobbs and Swift's (1985) concept of incorporating explicit nutritional values, we used their model as a framework for our carrying-capacity model. We also focused on the negative effects of plant secondary metabolites (which Hobbs and Swift (1985) did not address) and the neutralization of these chemicals by the tannin-binding affinity of elephant saliva. In addition, our model includes diet breadth and dietary contribution of browsed species, browse quality, and the available standing crop of browsed species. We aimed to develop a tool that protected areas and conservation/management bodies would be able to implement. Due to the robustness of the model, it is not intended to be site-specific and potentially could be generalized for other large mammalian browsers.

Methods

Carrying-capacity models require a priori knowledge about foraging behaviour, including information about intake rate (g/day), plant species preference (proportion contribution), available nitrogen in each plant species eaten (forage quality) (g nitrogen/g dry matter), the available biomass of each plant species (g/ha), and the tannin-binding affinity of the saliva of the model's target species. To gather these data, we studied a population of semi-tame elephants near Bela Bela in the Limpopo province, South Africa. These six elephants (male: $N = 3$, female: $N = 3$) were between 10 and 20 years old during the duration of our study and ranged from ~2.2 to 3.5 tonnes in weight. None of the female elephants were pregnant or nursing nor were the males in musth. All observations were made during the winter dry season (June–August 2013), when the animals fed exclusively on browse, and did not graze.

Determining which plant species comprise the majority of elephant diets

We determined the diet of elephants during the dry season when food availability was at its lowest and food quality was at its worst. To do this, we located foraging elephants and followed them at a distance of about 50 m along their feeding paths. Along each path, we used a belt transect (50 m long × 10 m wide (i.e. 5 m on either side of the feeding path), $n = 100$) to establish an acceptability index that described the species of plants elephants preferred or avoided. To formulate an acceptability index, we recorded the abundance of every plant species present along the 50 m × 10 m transect. The width of the transect was chosen because that is the reach of a foraging elephant's trunk (Shrader et al., 2012). In addition, we recorded the number of times a given plant species was eaten by an elephant along the transect. This number was then divided by the proportional abundance of that plant species in the same area to generate an acceptability index (Owen-Smith and Cooper, 1987). After compiling this index, we then identified the number of plant species that comprised 75% of the elephants' diets. From these species, we then determined which ones were preferred (i.e. most selected proportional to its abundance) or principal (selected overall most frequently – a function of acceptability and abundance). Due to unfavourable conditions or density dependence, it is possible that the proportional contribution of the different plant species could change both spatially and/or temporally.

Forage quality

Similar to Hobbs and Swift (1985), we assessed the quality (i.e. nitrogen) of the utilized plant species. We selected nitrogen as an index for forage quality because we wanted to explore the relationship between salivary tannin-binding proteins and the semi-neutralization of tannins, resulting in protein (=nitrogen* 6.25) availability (Shimada, 2006; Shimada and Saitoh, 2003). We focused on protein because it is widely known that this is the most limiting factor (Bernays and Chapman, 1994; Hobbs and Swift, 1985; Krasov and Martínez del Rio, 2007; White, 1993; Windels and Hewitt, 2011) and that its intake rate is affected by the response of the target herbivore to tannin, a known reducer of effective protein intake (e.g. Cooper and Owen-Smith, 1985; Robbins et al., 1987; Windels and Hewitt, 2011). We collected samples of the most favoured woody plant species, and focused specifically on the part of the plant that the elephants ate. For example, elephants typically ate only the leaves from the majority of their most foraged plant species (*Euclea crispa, Pappea capensis, Terminalia sericea*) while avoiding the woody matter and roots from these same species. However, elephants ate both the leaves and bark of other favoured species (*Dombeya rotundifolia, Combretum zeyheri*), and the leaves and woody matter of another (*Grewia monticola*). Overall, elephants consumed leaves as the vast majority of their diet from all preferred or principal species. Thus, we focused on the leaf biomass ingested from these plants as so little of their diet was comprised of stems and branches. We dried all samples in an oven at 60 °C for 48 h and then milled them through a 1 mm screen. All samples were then sent to an analytical laboratory (Cedara Agricultural College, KZN) for determination of g nitrogen per g dry matter (i.e. food quality) (Kjeldahl method). We then estimated % crude protein by multiplying total nitrogen by 6.25 (Van Soest, 1994).

Available biomass

To obtain a biomass estimate for available standing crop forage for each principal and preferred plant species, we first collected samples from 10 individual plants from each species and in each case, estimated what fraction of the canopy the sample represented. Each sample was dried in an oven at 60 °C for 48 h. Thereafter, the leaves were separated from the woody matter and these components were dried separately. Given that the elephants selected leaves mostly, we used biomass (g) of leaves as the measure of available standing crop.

For each of the principal and preferred sample trees from which we took biomass clippings, we also took measurements to calculate their volume, including canopy depth (CD) (distance from lowest productive branch to tallest living twig), canopy radius (r) (average of two perpendicular diameters), and tree height (h) (distance from ground to tallest living twig). In addition, we also noted the canopy shape (e.g., conical, half sphere, or cylindrical). These measurements were then used to estimate volume using the appropriate formula according to the canopy shape (i.e. for a cylinder: $V = \pi r^2 h$ and for a half-sphere: $V = 2/3 \pi r^3$).

To calculate the total available standing crop biomass of the principal and preferred species in the reserve, we located individuals of these plant species along additional vegetation belt transects that were 10 m wide (Shrader et al., 2012) ($n = 5$) and 1.2 km long. The volume of each tree within these transects was measured according to the same procedure listed above. For each tree species, we then used a linear regression using the paired values of leaves per canopy (in g) against the estimated volume of the canopy. The formula produced from each species-specific analysis was then used to estimate the available biomass (g) of leaves according to the volume of each individual's canopy. Once we estimated the available biomass (g) for each plant species in each transect, we then averaged the available biomass for each species across each transect. We multiplied available biomass by the number of transects that comprise the total reserve size ($N = 250$ transects). We then divided these numbers by the number of hectares in the reserve (300 ha) to obtain an estimated value for biomass (g) of standing crop available for each top species per hectare.

Bite mass

Because elephant diets vary so much across different populations (e.g. proportion of grass vs. woody browse contributing to overall diet; Codron et al., 2006), we were unable to use prior estimates of bite masses due to differences in food sources. As a result, we estimated bite masses of all foraged plant species in terms of the categories "extra-small," "small," "medium," "large," and "extra-large" *trunkfuls* because elephants use their trunks to put food into their mouths. While a single trunkful could yield numerous bites for the elephant, we only estimated ingested matter from a single trunkful, regardless of the number of bites. Trunkful-size category samples were representations of exactly what was eaten. For example, extra-small trunkfuls comprised leaves only, small trunkfuls consisted of a majority of leaves and some woody matter, and medium trunkfuls had a much larger proportion made up of woody matter. Often the woody matter was rejected and leaves were primarily ingested. Large and extra-large trunkful categories were seldom observed but were made up of a majority of woody matter but the elephants focused mainly on the leaves in these categories. Any rejected matter from all size categories was also estimated which was then subtracted when estimating actual bite mass.

We estimated trunkful size by collecting foraged material directly from individual elephants prior to ingestion and also by approximating trunkful mass by clipping similar sized branches. For each trunkful size category, we collected 10 sample bites. These trunkfuls were dried for 48 h in a drying oven at 60 °C, then weighed to determine average bite mass (g dry matter) for each size category.

Bite rate

Bite rates were gathered by observing a focal individual's foraging bouts throughout the course of a day. We took scan samples every 15 s. We converted the scan sampling into bite rate by calculating the proportion of bites per unit time (Martin and Bateson, 2007). We noted the foraging action each elephant was making (e.g., searching for a new patch, handling time (chewing or processing a trunkful), or actively taking a trunkful).

Intake rate

We calculated dry-season intake rates. Quantifying elephant intake (Ir) in g day^{-1} is described in Eq. (1):

$$Bm * Br = Ir \tag{1}$$

where Bm is the bite mass (g), Br is the bite rate (bites per day) and Ir is the intake rate (g per day).

For elephants actively taking in a foraged trunkful, we noted the size category of the bite to get an estimate of bite mass. We also determined the species and part of the plant selected. The size of any rejected plant matter that was either spat out or dropped was estimated using our "trunkful-size" scale to estimate mass and noted as a proportion of the trunkful that was rejected. We then subtracted the rejected mass from the estimated bite mass category to generate an estimate of ingested bite mass.

Because we used a discrete model for estimating intake rate, we then calculated the average intake rate by substituting the average trunkful mass for a given trunkful size, relative to the bite rate for each observation. We then calculated the mean of all scan-sample observations ($N = 11,209$). In addition to our own collected data on intake rate, we also used intake-rate data from Owen-Smith (1988). Furthermore, we also included two overestimates to test the sensitivity of the model (see Section 4).

Tannin-binding proteins

In our model, we also included the partial neutralization of tannins in browse material resulting from the tannin-binding affinity of elephant saliva. The results of a previous study (Schmitt, 2014) suggest that the tannin-binding proteins in elephant saliva likely reduce the impacts of tannins by ±75%. Information is lacking about the relationship between total tannin concentration (as measured by the bovine serum albumin assay – Hagerman 2014) and its negative impacts on digestible nitrogen (AE Hagerman, 2014, *pers. comm.*[2]). Thus, for the sake of this model, we assumed a 1:1 relationship, with 25% of available nitrogen being lost to tannin-binding prior to digestion, while the rest is preserved via the action of salivary tannin-binding proteins, resulting in a 75% availability of nitrogen for nutrient uptake (Schmitt, 2014). Due to the tannin-binding affinity of elephant saliva, we assumed that they can access 75% of nitrogen in their forage material.

Additionally, we also incorporated another source of variation in available crude protein. The nitrogen*6.25 = crude protein estimate works well for ruminants because the microbiome can convert non-protein nitrogen to microbial protein (Leng and Nolan, 1984). However, this may not be true for non-ruminant herbivores, such as elephants, rhinos and horses. On average only 61% of the crude protein in legumes and grasses is available to horses (Council, 2007). Because there is no available crude protein value available for elephants, we tested our model using the value estimated for horses. In a browser, tannin binding should theoretically act on that 61%. If

tannin-binding protein protects 75% of the available protein, then 45% crude protein would be available ($0.75 \times 61\%$). As a result, we also ran our model with 45% crude protein availability.

Principal and preferred species model

We created a model based on the theoretical framework of Hobbs and Swift's (1985) carrying-capacity model. In addition to several of Hobbs and Swift's (1985) factors (calculations for concentration of nitrogen in eaten plants, animal days per hectare, and the inclusion of herbivore intake rate and crude protein requirement) our model included three new factors:

1) tannin-binding affinity of saliva and adjusted nutritional values incorporating the anti-nutritional effects of tannin as the inverse of tannin-binding affinity of elephant saliva (75%) (see "Herbivore's Traits": Figure 5.1),
2) dietary breadth, including both preferred and principal tree species to better reflect what elephants actually ate (see "Diet Choice" under "Herbivore's Requirements": Figure 5.1), and
3) proportional contribution of each tree species to an elephant's diet which accounts for the preferential mixing that animals do when foraging (Provenza et al., 2003) (see "Diet Choice" under "Herbivore's Requirements": Figure 5.1). We included these new variables because they should provide an improved estimate of carrying capacity.

Model structure

First, to estimate animal days per hectare (number of days an individual can survive in an area with the given resources), it is necessary to calculate all available total nitrogen in the available forage items according to forage dietary contribution (proportion that each forage item contributed to the herbivore's diet in the area, taking into account dietary mixing) per hectare (Hobbs and Swift, 1985):

$$\sum_{1-n} \left((B) * (Nl) * (Pc) \right) = (Nta) \tag{2}$$

where B is the biomass (grams available per hectare), Nl is the nutrient level (gram nitrogen per gram dry matter), Pc is the proportion contribution of a species (0.01–0.99) and Nta is the available total nitrogen per hectare (grams nitrogen available per hectare).

Then, to adjust the total nitrogen to a more realistic level, we incorporated the negative influences of secondary metabolites and the partial neutralization of these due to the tannin-binding proteins (Schmitt, 2014). Because elephant saliva reduces the negative impacts of tannin to 75%, we assumed that only 75% of the total nitrogen is available for nutrient absorption. Additionally, we explored how this semi-neutralization of tannins would influence a 61% nitrogen availability (in the case of horses: Council, 2007), which ultimately results in only 45% nitrogen available, instead of 75%. In place of the tannin-binding affinity variable of 75%, the available crude protein estimate of 45% was substituted.

$$Nta * Tba = Na \tag{3}$$

where Nta is the available total nitrogen per hectare (grams nitrogen per hectare); Tba is the tannin-binding affinity (0.75) and Na is the adjusted available nitrogen (grams nitrogen per hectare).

To calculate animal days from the adjusted amount of N available per hectare, we calculated the amount of crude protein g^{-1} needed to sustain one elephant for one day. Elephants require a diet that is made up of 10% crude protein per day (i.e. 100 g CP kg^{-1}) (KR

[2] Dr. AE Hagerman, Professor of Biochemistry at Miami University, Oxford, Ohio, hagermae@miamioh.edu.
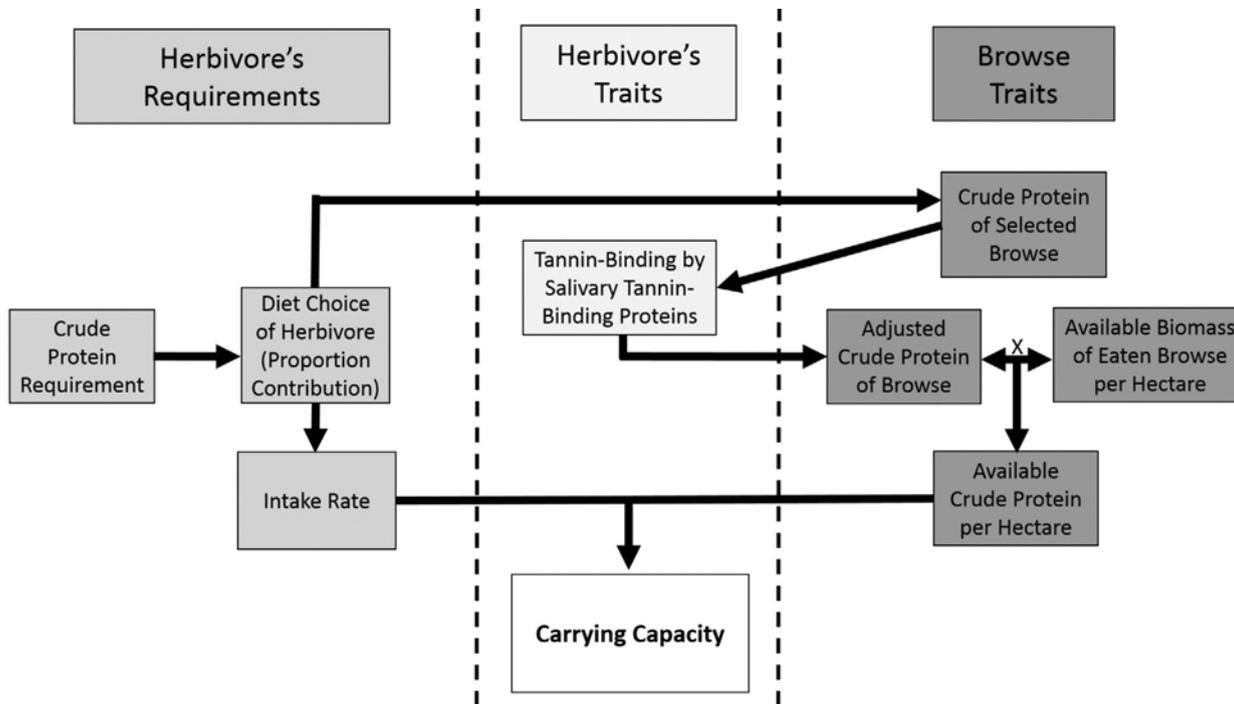
Figure 5.1: Conceptual framework of our principal and preferred species carrying-capacity model.

Mbatha, 2012, *pers. comm.*[3]). Thus, we calculated the concentration of crude protein that each forage item contained and expressed it in grams nitrogen per gram dry matter. To obtain the amount of crude protein (CP) (expressed in g N) that is necessary in a day we first calculated:

$$\frac{Cp/6.25}{100} = Nt \tag{4}$$

where Cp is the percent crude protein required per day (percent), 6.25 is the conversion factor between crude protein and nitro- gen and Nt is the amount of nitrogen required per gram of dry matter.

Then to estimate the grams nitrogen required for elephants to meet their daily nutritional requirement (10% crude protein per day), we then incorporated daily intake rate in grams per day as follows:

$$Ir * Nt = Nd \tag{5}$$

where Ir is the intake rate (grams per day), Nt is the amount of nitrogen required per gram of dry matter and Nd is the amount of nitrogen required per day (g).

Finally, to calculate animal days ha^{-1}, we divided our estimated adjusted N per hectare, which reflects the negative impacts of tannins, by grams of nitrogen required by an elephant per day as follows:

$$\frac{Na}{Nd} = \frac{Animal\,Days}{ha} \tag{6}$$

where Na is the adjusted available nitrogen (grams nitrogen per hectare) and Nd is the amount of nitrogen required per day (g).

To explore the sensitivity of this model, we tested it with several different intake rates (60 kg day^{-1}, 75 kg day^{-1}, 100 kg day^{-1}, 150 kg day^{-1}) that are both within the range of dry-matter intake for African elephants as well as beyond the range of daily dry-matter intake (see Section 4). Although zoo elephants have been estimated to require 10% crude protein per day (KR Mbatha, 2012, *pers. comm*[3]), we also tested a range of percent crude protein required in a day (6–14% crude protein per day) to incorporate reasonable uncertainty in crude protein estimations. For clarification, a table of the terms used in this model, along with their definition and units, is available in Appendix A. To see a sample calculation, see Appendix B.

Results

Important dietary species

We observed elephants consuming 27 different plant species of the total 36 species present on the reserve. Trees and shrubs comprised the majority of the plant species present on the reserve but there were also many grasses present (e.g. *Aristida congesta*, *Sporobolus pyramidalis*, *Hyparrhenia hirta*, *Eragrostis curvula*). We note that, although elephants may be considered mixed feeders (see e.g. Codron et al., 2006), elephants only ate trees and shrubs during our study (see also Shrader et al., 2012). We established that the most preferred species (eaten in a larger proportion to its abundance) were *Pappea capensis*, *Dombeya rotundifolia*, and *Terminalia sericea* (Table 1). *Combretum zeyheri* was both a preferred and principal species while the species that were principal species only were *Grewia monticola* and *Euclea crispa* (Table 1).

Forage quality and available biomass

Nutritional concentration varied among the principal and preferred species, ranging from 0.009 to 0.016 g N g^{-1} dry matter (DM) (Table 2). Our field observations revealed that available biomass

Table 5.1: Principal and preferred plant species consumed. Preferred species are listed in bold.

Species	# times consumed	# times encountered	Proportion of times consumed
Pappea capensis	71	82	0.87
Dombeya rotundifolia	45	61	0.74
Terminalia sericea	92	132	0.70
Combretum zeyheri	134	205	0.65
Grewia monticola	171	339	0.50
Euclea crispa	155	559	0.28

Table 5.2: The nitrogen concentration per unit dry mass ($g\,N\,g^{-1}$ dry matter (DM)) and the available biomass for the principal and preferred plant species consumed.

| Species	$g\,N\,g^{-1}$ DM	Standard deviation	$g\,DM\,ha^{-1}$
Dombeya rotundifolia	0.016	0.0025	29,389
Grewia monticola	0.015	0.0017	2755
Pappea capensis	0.013	0.0008	26,111
Combretum zeyheri	0.012	0.0013	86,858
Euclea crispa	0.010	0.0008	10,674
Terminalia sericea	0.009	0.0035	44,449

Figure 5.2: The sensitivity of our principal and preferred species' model as estimated in animal days per hectare as a function of nine discrete levels of crude protein requirement (% per day) and four discrete intake rate (g per day) levels for the 75% nitrogen concentration.

also varied greatly among preferred and principal species. *C. zeyheri* was the most available and *G. monticola* was the least available species (Table 5.2).

Intake rate

We calculated the average intake rate for the six elephant individuals and found that they ingested a mean of 75 kg dry matter per day (standard deviation = ±15.07). This means intake rate is larger than another estimate of intake rates for African elephants ($60\,kg\,day^{-1}$) (Owen-Smith, 1988). However, the lower end of our intake rates (incorporating individual variance) overlaps with the estimate of Owen-Smith (1988). Our higher intake rate may be a result of energy demands of the individual elephants due to differences in environmental conditions and/or stress levels (Levitsky, 1970; Swennen et al., 1989).

Principal and preferred species model

We simulated the effects of different combinations of four discrete levels of intake rates and nine discrete levels of daily crude protein requirements on carrying capacity (animal days per hectare) at the 100%, 75%, 45% available nitrogen concentration (Table 5.3). Overall, we found that variation in intake rate had a relatively larger impact on carrying capacity than changes in daily crude protein requirements (Figure 5.2). A potential result of this non-linear relationship is that during periods of decreased food availability, when individuals may have to eat more (increase intake rates) to maintain their nutritional requirements, the overall carrying capacity of an area will drop more rapidly as a result. However, it is unlikely that elephants will ever increase their intake rates into the range of our overestimated intake rates. This is improbable because when the mass of the heaviest possible elephant is substituted into an equation estimating daily food intake (Owen-Smith, 1988), the resulting intake rate does not come into range of our over-estimates.

Discussion

A key way to estimate an optimal carrying capacity for an area is through the use of standing-crop carrying-capacity models (Hobbs and Swift, 1985; Windels and Hewitt, 2011). Standing-crop models assume that there is no replenishment of resources (i.e., no growth of existing plant individuals or recruitment). Many plant species are
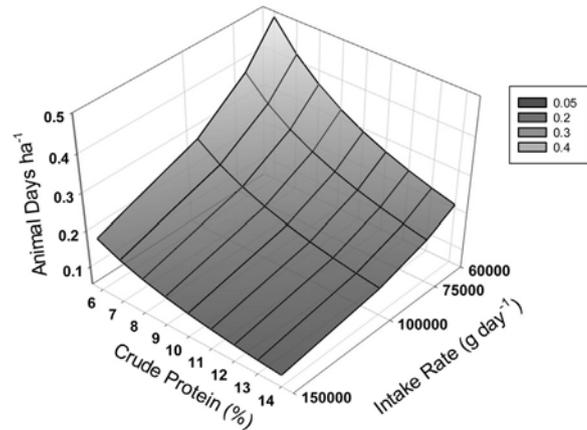
deciduous and are not productive during the dry season, which is a reflection of the environment found in almost all of southern Africa, which experiences summer rainfall (Tyson, 1986). Thus, applying a standing crop carrying-capacity model to the available dry-season biomass gives an approximation of the highest carrying capacity an area can sustain during the least productive season. This effectively estimates the carrying capacity for the entire year because the dry season limits the overall carrying capacity in large herbivore species with a low reproductive output such as the elephant (Coe et al., 1976).

Numerous standing-crop models have been developed to estimate carrying capacity (Fritz and Duncan, 1994; Hobbs and Swift, 1985; Windels and Hewitt, 2011). While such models have proven to be quite successful for grazers, these models have been problematic when applied to browsers. This is most likely because browsers encounter plant chemical defences in their forage items, such as tannins, which can reduce the amount of available nitrogen for nutrient absorption (Robbins et al., 1991). In contrast, grazers seldom encounter tannins and only a few grass species have detectable levels of polyphenols (Chesselet et al., 1992). The result is that the predicted carrying capacity is greater than what can actually be realized in nature. In an effort to develop a tool to manage and monitor the carrying capacity of elephants, we integrated several factors, including characteristics of the diet, and the ability of the forager to combat secondary metabolites (in this case, tannins) that can reduce the quality of their food, that have not been considered in previous standing-crop carrying-capacity models (e.g. Hobbs and Swift, 1985).

Principal and preferred species model

The first novel factor that we considered was the negative effects of plant secondary metabolites, and the partial neutralization of these chemicals by the tannin-binding affinity of saliva. Elephants

Table 5.3: Principal and preferred species' model predictions of animal days ha^{-1} for two levels of nitrogen concentration, viz. 100%, reflecting what conventional carrying-capacity models use, and 75%, reflecting the partial neutralization of secondary metabolites from tannin-binding proteins in elephant saliva and the reduction in available nitrogen due to residual tannins, and 45% reflecting what a horse (hindgut fermenter) can access incorporating the partial neutralization of secondary metabolites from tannin-binding proteins. Animal days ha^{-1} was represented as a function of four discrete levels of intake rate (IR) and nine discrete levels of daily requirement of % crude protein (CP).

IR (g day^{-1})	CP (% day^{-1})	100% nitrogen concentration	75% nitrogen concentration	45% nitrogen concentration
60,000	14	0.28	0.21	0.13
75,000	14	0.22	0.17	0.10
100,000	14	0.17	0.13	0.08
150,000	14	0.11	0.08	0.05
200,000	14	0.08	0.06	0.04
60,000	13	0.30	0.22	0.14
75,000	13	0.24	0.18	0.11
100,000	13	0.18	0.13	0.08
150,000	13	0.12	0.09	0.05
200,000	13	0.09	0.07	0.04
60,000	12	0.32	0.24	0.15
75,000	12	0.26	0.19	0.12
100,000	12	0.19	0.15	0.09
150,000	12	0.13	0.10	0.06
200,000	12	0.10	0.07	0.04
60,000	11	0.35	0.27	0.16
75,000	11	0.28	0.21	0.13
100,000	11	0.21	0.16	0.10
150,000	11	0.14	0.11	0.06
200,000	11	0.11	0.08	0.05
60,000	10	0.39	0.29	0.18
75,000	10	0.31	0.23	0.14
100,000	10	0.23	0.18	0.11
150,000	10	0.16	0.12	0.07
200,000	10	0.12	0.09	0.05
60,000	9	0.43	0.32	0.19
75,000	9	0.35	0.26	0.16
100,000	9	0.26	0.19	0.12
150,000	9	0.17	0.13	0.08
200,000	9	0.13	0.10	0.06
60,000	8	0.49	0.36	0.22
75,000	8	0.39	0.29	0.18
100,000	8	0.29	0.22	0.13
150,000	8	0.19	0.15	0.09
200,000	8	0.15	0.11	0.07
60,000	7	0.56	0.42	0.25
75,000	7	0.44	0.33	0.20
100,000	7	0.33	0.25	0.15
150,000	7	0.22	0.17	0.10
200,000	7	0.17	0.13	0.08
60,000	6	0.65	0.49	0.29
75,000	6	0.52	0.39	0.23
100,000	6	0.39	0.29	0.18
150,000	6	0.26	0.19	0.12
200,000	6	0.19	0.15	0.09

are able to neutralize about 75% of the tannins found in their browse material (assuming that there is a 1:1 relationship between total tannin concentration and its negative impacts on digestible nitro- gen), thus allowing them access to most of the available nitrogen in the browse materials. The addition of this parameter reduces the available nitrogen for nutrient uptake by 25%. We also mod- elled a 45% crude protein availability to reflect a 61% availability of total crude protein (in the case of horses, another hindgut fer- menter: Council, 2007) interacting with the 75% tannin-binding affinity of elephant saliva. We found that 100% nitrogen availabil- ity yielded more animal days per hectare than 75% or 45%, but that there was only a 0.11 animal days ha^{-1} difference between 75% (0.29 animal days ha^{-1}) and 45% (0.18 animal days ha^{-1}) availability.

The other new factors that we incorporated into our model describe the manner in which browsers forage. A major assump- tion of the Hobbs and Swift (1985) model is that animals will forage optimally such that they will eat the forage item that is most nutrient-rich first before moving on to less nutrient-rich items.

Although a forage species may be lower in nutrient level than the required amount (g N g^{-1} DM), an animal could achieve the nutritional target value by ingesting *more* of that forage species (Hobbs and Swift, 1985). Using this concept as a framework for our model, we constructed a model that included diet breadth (principal and preferred species) and the dietary contribution of each browsed species. By incorporating diet breadth and dietary contribution, we could control for various factors that influence foraging decisions (e.g. digestibility, assorted toxins that might be tolerated through dilution or diet mixing) without measur- ing them, which allows for a more streamlined, less complex, model.

Many other models assume that herbivores choose to eat 100% of all palatable species. Rather, our model restricts the elephants' intake such that it matches the dietary contribution of the different plant species. Another difference about our model is the addition of both principal and preferred species. With the addition of principal species into the model, our model assumed that elephants utilize more species than their preferred species only. Other models, (e.g.

Hobbs and Swift's (1985) model) only incorporated plant species that fall above the threshold daily nutrient requirement, and usually only one species below the threshold nutrient requirement. In reality, many more plant species than the limited number used in other models are actually eaten (e.g., principal species). We argue that these species are important when predicting carrying capacity of an area because these are the plant species that animals primarily rely upon for survival (Owen-Smith, 2002a).

A number of studies have shown that animals do not forage optimally (Janetos and Cole, 1981; Wilmshurst et al., 1995). We argue that our new model is a more precise reflection of carrying capacity than other standing-crop models because our model does not assume optimal foraging. Instead, our model uses the animal's diet breadth and the dietary contribution of each forage species to estimate carrying capacity. This is a more realistic method for estimating carrying capacity because it takes into account the ways that animals forage rather than making the assumption that animals forage optimally.

This is not the first study to attempt to use Hobbs and Swift's (1985) carrying-capacity model as a basis for a new standing-crop carrying-capacity model. Windels and Hewitt (2011) altered Hobbs and Swift's (1985) model to incorporate the negative impacts of tannins for mixed feeders. They also included a further reduction in the amount of available crude protein by subtracting the amount of non-protein nitrogen in each foraged species. Although their model produced plausible carrying-capacity estimates, the level of chemical information required about each forage species makes the model challenging for resource managers to implement.

Model assumptions

Our model makes assumptions about standing crop, including the fact that plants would not coppice or re-sprout during the dry season. Moreover, our model does not incorporate diet shift as a result of reduced availability or palatability of plant species. Rather, it uses the diet breath recorded from the elephants. Additionally, it does not take inter-annual differences (e.g., drought and/or burning can reduce the amount of available biomass, and dry-season rainfall can increase the amount of available biomass) into account. These inter-annual differences could influence available standing crop biomass or species selection by the browser. While data from a single dry season could produce a carrying-capacity estimate for the elements that were influenced by that particular season's carrying-capacity factors, the application of our model over several dry seasons could better predict a sustainable carrying capacity. Our current model could be applied to both theoretical and empirical estimates of reduced/increased biomass availability to simulate certain natural events such as fire or rainfall. Furthermore, the estimates of intake rate and preference of the animals that we studied could be influenced by their environment or by density-dependence of elephants. This could ultimately result in changes in intake rate and preference, which should be considered when our model is applied to other populations.

Another question that has been explored in similar standing-crop carrying-capacity models is the time period the carrying capacity estimate reflects (i.e. a 90-day dry season or a 365-day period). The dry season is the limiting time for herbivores in southern Africa, as well as other parts of the world due to low food availability as well as reduced nutrient levels (Coe et al.,

1976). Hobbs and Swift's (1985) model assumes that the carrying capacity estimate is for a 365-day period while Windels and Hewitt's model (2011) makes seasonal estimates. Because elephants have a long gestation period, they do not respond rapidly to favourable conditions by means of rapid population growth (Calef, 1988; Moss, 2001; Owen-Smith, 1988). Thus, for such species, our model's carrying capacity estimates for the dry season (i.e. the most food-limiting time) would reflect a 365-day period.

Another aspect where assumptions were made in our model is the crude-protein intake of African elephants. While we used a value calculated for the zoological gardens, there are other methods one could use to estimate elephant nutritional requirements. For example, the average size of an elephant in our study ($\sim$3950 kg) would require 4977 g crude protein per day, assuming that the daily requirement for maintenance of body mass of African elephants is the same as a domestic horse (1.26 g kg^{-1}). Assuming that food intake is 1.2% of body mass for African elephants (Owen-Smith, 1988), an elephant weighing 3950 kg should eat 47.4 kg dry matter per day. Thus, this elephant could theoretically eat a diet of 10.5% crude protein (4977 g crude protein*100/47,400 g dry matter). Barboza et al. (2009) have also noted that most large herbivores can subsist on lower intake rates if their amino acid intake is sufficient. However, it is unlikely that such intakes would be adequate because many herbivores eat to fill their gut and are digestion-limited feeders (Jeschke et al., 2002).

Implementation of principal and preferred species model

Although our calculations predict carrying capacity for a specific site, we developed a model that is not site-specific. Moreover, our approach should be straightforward for management staff to implement in protected areas with no further research than is already currently being executed. Some of the information needed to run this model is site-specific, but it is information that management bodies would require to calculate for any forage-based standing-crop model. For example, elephant diet selection varies spatially as does the available biomass of foraged species. However, the majority of the variables needed to use this model are constants, such as elephant intake rate, tannin-binding affinity of elephant saliva, and nutrient concentration of foraged plant species. This means that the estimated values for these parameters from previous studies could be applied by conservation/management bodies to estimate carrying capacity for elephants for any specific reserve, assuming that crude protein values are available for those plant species. For example, numerous plant species in South Africa have been analyzed for nitrogen/crude protein content, particularly articles outlining mammalian diets (e.g. Ganqa et al., 2005; Kos et al., 2012). Additionally, with very few modifications, this model could be applied to other browsing and mixed-feeding species. These modifications would include the tannin-binding affinity of a range of browser and mixed-feeder saliva, as well as adjusted intake rate and plant species data.

Acknowledgments

Appendix A.

See Table A1.

Table A1: List of terms and their definitions and units used in the preferred and principal species model.

Parameter	Definition	Unit
Biomass	Amount of forage item available per hectare	Grams dry matter of a given species per hectare
Nutrient level	Concentration of nitrogen in a forage item	Grams total nitrogen per gram dry matter
DM	Dry matter	Grams
Proportion contribution	Proportional contribution of a single forage item to an elephant's diet	Proportion ranging between 0.01 and 0.90
Available total nitrogen per hectare	Amount of total nitrogen per species in a single hectare	Grams total nitrogen per hectare
Tannin-binding affinity	The tannin binding level of saliva	% of tannins bound
Adjusted N per hectare	An adjusted estimate of concentration nitrogen available for absorption after the negative impacts of tannins has been incorporated per hectare	Grams nitrogen per gram dry matter per hectare
Crude protein (%)	Nitrogen*6.25	Crude protein per gram dry matter
Intake rate	Amount of food eaten by an elephant per day	Grams dry matter per day
Nitrogen required per day	Amount of nitrogen (concentration) that an elephant requires in its diet per day	Grams nitrogen per day
Animal days per hectare	A unit that expresses the number of days that a single animal can survive on a hectare given the animal's requirements and the available resources in a hectare	Animal days per hectare

Appendix B. Application of the algorithm and sample calculations to estimate carrying capacity

We used the values from Table B1 to estimate carrying capacity for the reserve (measured per hectare). Ultimately, all values will be used to estimate carrying capacity by summing the available nitrogen of the six woody plant species. However, for the sake of brevity, in the early portions of the example where individual species nitrogen values must be estimated, we only use *D. rotundifolia* to illustrate our calculation. This sample calculation is an example of 75% crude protein availability, 12% crude protein required per day with an intake rate of 7500 g crude protein per day.

To estimate animal days per hectare we calculated all available nitrogen according to forage dietary contribution per hectare:

$$\sum_{1-n} \big((B) * (Nl) * (Pc)\big) = (Nta) \qquad (A.1)$$

where B is the biomass (grams available per hectare), Nl is the nutrient level (gram nitrogen per gram dry matter), Pc is the proportion contribution of a species (0.01–0.99) and Nta is the available total nitrogen per hectare (grams nitrogen available per hectare).

Using the values for the six plant species (see Table B1), we obtain:

$$\sum_{1-n} \big((29389) * (0.016) * (0.07)\big) + \big((2755) * (0.015) * (0.26)\big)$$
$$+ \big((26111) * (0.013) * (0.11)\big)$$
$$+ \big((86858) * (0.012) * (0.20)\big)$$
$$+ \big((10674) * (0.010) * (0.23)\big)$$
$$+ \big((44449) * (0.009) * (0.14)\big) = \left(\frac{373.57 \text{ g N}}{\text{ha}}\right)$$

Table B2: The crude protein percent (g nitrogen) per gram dry matter equivalent.

Crude protein (% day^{-1})	g nitrogen g^{-1} DM
14%	0.022
13%	0.020
12%	0.019
11%	0.018
10%	0.016
9%	0.014
8%	0.013
7%	0.011
6%	0.009

To adjust the available nitrogen values to a more realistic level, we estimated that only 75% of nitrogen is available for nutrient absorption because of the partial neutralization of tannins by elephant's salivary tannin-binding proteins.

$$((Nta) * (Tba)) = Na \qquad (A.2)$$

where Nta is the available total nitrogen per hectare (grams nitrogen per hectare), Tba is the tannin-binding affinity (0.75) and Na is the adjusted available nitrogen (grams nitrogen per hectare) (Tables B2 and B3).

Thus, the adjusted available nitrogen (g) from *D. rotundifolia* is:

$$= \frac{280.16 \text{ g N}}{\text{ha}}$$

To calculate animal days from the adjusted amount of N available per hectare, we calculated the amount of crude protein per gram needed to sustain one elephant for one day. To calculate

Table B1: Key plant species, including both principal and preferred, comprising ~75% of the study population's total diet as well as the available biomass, nutritional information (% crude protein (CP) was estimated by multiplying g N by 6.25), and the proportional contribution to the 75% of the total diet of each plant species.

Species	kg DM ha^{-1}	g DM ha^{-1}	% CP g^{-1} DM	g N g^{-1} DM	g N avail ha^{-1}	Proportion contribution	g N avail*proportion diet ha^{-1}
Dombeya rotundifolia	29.39	29,389	10.10	0.016	474.93	0.07	33.25
Grewia monticola	2.76	2755	9.65	0.015	42.54	0.26	11.06
Pappea capensis	26.11	26,111	8.19	0.013	342.17	0.11	37.64
Combretum zeyheri	86.86	86,858	7.50	0.012	1042.30	0.20	208.46
Euclea crispa	10.67	10,674	6.42	0.010	109.65	0.23	25.22
Terminalia sericea	44.45	44,449	5.82	0.009	413.91	0.14	57.95

Table B3: Mass (g) of crude protein obtained per day as a function of intake rate (IR) and percent crude protein (CP) requirement per day expressed as concentration in terms of grams of nitrogen per unit dry mass (DM).

IR level (g day^{-1})	CP (g N g^{-1} DM) 14%	CP (g N g^{-1} DM) 13%	CP (g N g^{-1} DM) 12%	CP (g N g^{-1} DM) 11%	CP (g N g^{-1} DM) 10%	CP (g N g^{-1} DM) 9%	CP (g N g^{-1} DM) 8%	CP (g N g^{-1} DM) 7%	CP (g N g^{-1} DM) 6%
60,000	1344	1248	1152	1056	960	864	768	672	576
75,000	1680	1560	1440	1320	1200	1080	960	840	720
100,000	2240	2080	1920	1760	1600	1440	1280	1120	960
150,000	3360	3120	2880	2640	2400	2160	1920	1680	1440
200,000	4480	4160	3840	3520	3200	2880	2560	2240	1920

the *concentration* (g) of crude protein (CP) that is necessary in a day:

$$\left(\frac{\frac{(Cp)}{(6.25)}}{(100)} \right) = (Nt) \tag{A.3}$$

where Cp is the percent crude protein required per day (%), 6.25 is the conversion factor between crude protein and nitrogen, and Nt is the amount of nitrogen required per gram of dry matter.

$$= \left(\frac{\frac{\left(\% \frac{12}{Day} \right)}{(6.25)}}{(100)} \right) = 0.019$$

Second, to incorporate daily intake of a subadult animal (from our results):

$$((Ir) * (Nt)) = (Nd) \tag{A.4}$$

where IR is the intake rate (grams per day), Nt is the amount of nitrogen required per gram of dry matter and Nd is the amount of nitrogen required per day (g).

$$= \left((7500) * (0.019) \right) = \frac{1440 \text{ g N}}{Day}$$

Finally, to calculate animal days ha^{-1}:

$$\left(\frac{(Na)}{(Nd)} \right) = \frac{Animal \ Days}{ha} \tag{A.5}$$

where Na is the adjusted available nitrogen (grams nitrogen per hectare) and Nd is the amount of nitrogen required per day (g)

$$= \frac{280.16}{1440} = 0.19$$

References

Barboza, P.S., Parker, K.L., Hume, I.D., 2009. Integrative Wildlife Nutrition. Heidelberg, Springer - Verlag.

Bernays, E.A., Chapman, R.F., 1994. *Host-Plant Selection by Phytophagous Insects*. Chapman and Hall, New York, NY.

Blanc, J.J., 2007. African elephant status report 2007: an update from the African elephant database. 2831709709.

Blanc, J.J., 2008. *Loxodonta africana*, IUCN ed. IUCN Red List.

Blanc, J.J., Barnes, R.F.W., Craig, C.G., Douglas-Hamilton, I., Dublin, H.T., Hart, J.A., Thouless, C.R., 2005. Changes in Elephant Numbers in Major Savanna Populations in Eastern and Southern Africa. IUCN, pp. 18.

Calef, G.W., 1988. Maximum rate of increase in the African elephant. Afr. J. Ecol. 26, 323–327.

Chesselet, P., Wolfson, M.M., Ellis, R.P., 1992. A comparative histochemical study of plant polyphenols in southern African grasses. J. Grassl. Soc. South. Afr. 9, 119–125.

Clauss, M., Gehrke, J., Hatt, J.-M., Dierenfeld, E.S., Flach, E.J., Hermes, R., Castell, J., Streich, J.W., Fickel, J., 2005. Tannin-binding salivary proteins in three captive rhinoceros species. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 140, 67–72.

Codron, J., Lee-Thorp, J.A., Sponheimer, M., Codron, D., Grant, R.C., de Ruiter, D.J., 2006. Elephant (*Loxodonta africana*) diets in Kruger National Park, South Africa: spatial and landscape differences. J. Mammal. 87, 27–34.

Coe, M.J., Cumming, D.H., Phillipson, J., 1976. Biomass and production of large African herbivores in relation to rainfall and primary production. Oecologia 22, 341–354. Conybeare, A.M., 2004. Elephant impacts on vegetation and other biodiversity in the broadleaved woodlands of S-Central Africa. In: Timberlake, J.R., Childes, S.L. (Eds.), Biodiversity of the Four Corners Area: Technical Reviews, vol. 2. Biodi- versity Foundation for Africa/Zambezi Society, Harare, Zimbabwe/Bulawayo, pp. 477–508.

Cooper, S.M., Owen-Smith, N., 1985. Condensed tannins deter feeding by browsing ruminants in a South African savanna. Oecologia 67, 142–146.

Council, N.R., 2007. *Nutrient Requirements of Horses*, 6th ed. National Academy Press, Washington, DC.

Cowlishaw, S.J., 1969. The carrying capacity of pastures. Grass Forage Sci. 24, 207–214.

Fritz, H., Duncan, P., 1994. On the carrying capacity for large ungulates of African savanna ecosystems. Proc. R. Soc. Lond. Ser. B: Biol. Sci. 256, 77–82.

Ganqa, N.M., Scogings, P.F., Raats, J.G., 2005. Diet selection and forage quality factors affecting woody plant selection by black rhinoceros in the Great Fish River Reserve, South Africa. S. Afr. J. Wildl. Res. 35, 77–83.

Guldemond, R., van Aarde, R., 2008. A meta-analysis of the impact of African elephants on savanna vegetation. J. Wildl. Manage. 72, 892–899.

Guy, P.R., 1976. The feeding behaviour of elephant (*Loxodonta africana*) in the Sengwa area, Rhodesia. S. Afr. J. Wildl. Res. 6, 55–63.

Harlan, J.R., 1958. Generalized curves for gain per head and gain per acre in rates of grazing studies. J. Range Manage. 11, 140–147.

Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol., 283–335.

Hobbs, T.N., Swift, D.M., 1985. Estimates of habitat carrying capacity incorporating explicit nutritional constraints. J. Wildl. Manage. 49, 814–822.

Janetos, A.C., Cole, B.J., 1981. Imperfectly optimal animals. Behav. Ecol. Sociobiol. 9, 203–209.

Jeschke, J.M., Kopp, M., Tollrian, R., 2002. Predator functional responses: discriminating between handling and digesting prey. Ecol. Monogr. 72, 95–112.

Jones, R.J., Sandland, R.L., 1974. The relation between animal gain and stocking rate: derivation of the relation from the results of grazing trials. J. Agric. Sci. 83, 335–342.

Kerley, G.I.H., Landman, M., 2006. The impacts of elephants on biodiversity in the Eastern Cape Subtropical Thickets: elephant conservation. S. Afr. J. Sci. 102, 395–402.

Kerley, G.I.H., Shrader, A.M., 2007. Elephant contraception: silver bullet or a potentially bitter pill? Commentary. S. Afr. J. Sci. 103, 181–182.

Kos, M., Hoetmer, A.J., Pretorius, Y., de Boer, W.F., de Knegt, H., Grant, C., Kohi, E., Page, B., Peel, M., Slotow, R., 2012. Seasonal diet changes in elephant and impala in mopane woodland. Eur. J. Wildl. Res. 58, 279–287.

Krasov, W.H., Martínez del Rio, C., 2007. *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton University Press, Princeton, NJ.

Leng, R., Nolan, J., 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67, 1072–1089.

Levitsky, D.A., 1970. Feeding patterns of rats in response to fasts and changes in environmental conditions. Physiol. Behav. 5, 291–300.

Martin, P., Bateson, P.P.G., 2007. *Measuring Behaviour: An Introductory Guide*, 3rd ed. Cambridge University Press, New York.

Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11, 119–161.

McArthur, C., Hagerman, A.E., Robbins, C.T., 1991. Physiological strategies of mammalian herbivores against plant defences. In: Palo, R.T., Robbins, C.T. (Eds.), Plant Defenses Against Mammalian Herbivory. CRC Press Inc., Boca Raton, FL, pp. 103–114.

Meissner, H.H., 1982. Theory and application of a method to calculate forage intake of wild southern African ungulates for purposes of estimating carrying capacity. S. Afr. J. Wildl. Res. 12, 41–47.

Midgley, J.J., Balfour, D., Kerley, G.I.H., 2005. Why do elephants damage savanna trees? S. Afr. J. Sci. 101, 213–215.

Moss, C.J., 2001. The demography of an African elephant (*Loxodonta africana*) population in Amboseli, Kenya. J. Zool. 255, 145–156.

Mott, G.O., 1960. Grazing pressure and the measurement of pasture production. In: Proceedings 8th international Grassland Congress.

Osborn, F.V., 2004. Seasonal variation of feeding patterns and food selection by crop-raiding elephants in Zimbabwe. Afr. J. Ecol. 42, 322–327.

Owen, J.B., Ridgman, W.J., 1968. The design and interpretation of experiments to study animal production from grazed pasture. J. Agric. Sci. 71, 327–335.

Owen-Smith, N., 1988. *Megaherbivores: The Influence of Very Large Body Size on Ecol-ogy*. Cambridge University Press, Cambridge, UK.

Owen-Smith, N., 2002a. *Adaptive Herbivore Ecology: From Resources to Populations in Variable Environments*. Cambridge University Press.

Owen-Smith, N., 2002b. A metaphysiological modelling approach to stability in herbivore–vegetation systems. Ecol. Model. 149, 153–178.

Owen-Smith, N., Cooper, S.M., 1987. Assessing food preferences of ungulates by acceptability indices. J. Wildl. Manage., 372–378.

Pringle, R.M., 2008. Elephants as agents of habitat creation for small vertebrates at the patch scale. Ecology 89, 26–33.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Rumin. Res. 49, 257–274.

Riewe, M.E., 1961. Use of the relationship of stocking rate to gain of cattle in an experimental design for grazing trials. Agron. J. 53, 309–313.

Robbins, C.T., Hanley, T.A., Hagerman, A.E., Hjeljord, O., Baker, D.L., Schwartz, C.C., Mautz, W.W., 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68, 98–107.

Robbins, C.T., Hagerman, A.E., Austin, P.J., McArthur, C., Hanley, T.A., 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. J. Mammal. 72, 480–486.

Sandland, R.L., Jones, R.J., 1975. The relation between animal gain and stocking rate in grazing trials: an examination of published theoretical models. J. Agric. Sci. 85, 123–128.

Schmitt, M.H., 2014. *The Influences of Plant Secondary Metabolites on Calculating Carrying Capacities for the African Elephant, Loxodonta africana, School of Life Sciences*. University of KwaZulu-Natal.

Shimada, T., 2006. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 32, 1149–1163.

Shimada, T., Saitoh, T., 2003. Negative effects of acorns on the wood mouse *Apodemus speciosus*. Popul. Ecol. 45, 7–17.

Shrader, A.M., Bell, C., Bertolli, L., Ward, D., 2012. Forest or trees: at what scale do elephants make foraging decisions? Acta Oecol. 42, 3–10.

Sinclair, A.R.E., Gwynne, M.D., 1972. Food selection and competition in the East African buffalo (*Syncerus caffer Sparrman*). Afr. J. Ecol. 10, 77–89.

Swennen, C., Leopold, M.F., De Bruijn, L.M., 1989. Time-stressed oystercatchers, *Haematopus ostralegus*, can increase their intake rate. Anim. Behav. 38, 8–22.

Tyson, P.D., 1986. *Climatic Change and Variability in Southern Africa*. Oxford University Press, USA.

Ungar, E.D., Noy-Meir, I., 1988. Herbage intake in relation to availability and sward structure: grazing processes and optimal foraging. J. Appl. Ecol. 25, 1045–1062.

Valeix, M., Fritz, H., Sabatier, R., Murindagomo, F., Cumming, D., Duncan, P., 2011. Elephant-induced structural changes in the vegetation and habitat selection by large herbivores in an African savanna. Biol. Conserv. 144, 902–912.

van Aarde, R.J., Whyte, I., Pimm, S., 1999. Culling and the dynamics of the Kruger National Park African elephant population. Anim. Conserv. 2, 287–294.

Van Soest, P.J., 1994. *Nutritional Ecology of the Ruminant*, 2nd ed. Cornell University Press, Ithaca, New York.

White, T.C., 1993. *The Inadequate Environment: Nitrogen and the Abundance of Animals*. Springer Science & Business Media.

Whyte, I., van Aarde, R.J., Pimm, S.L., 1998. Managing the elephants of Kruger National Park. Anim. Conserv. 1, 77–83.

Wilmshurst, J.F., Fryxell, J.M., Hudson, R.J., 1995. Forage quality and patch choice by wapiti (*Cervus elaphus*). Behav. Ecol. 6, 209–217.

Windels, S.K., Hewitt, D.G., 2011. Effects of plant secondary compounds on nutritional carrying capacity estimates of a browsing ungulate. Rangel. Ecol. Manage. 64, 264–275.

Wyatt, J.R., Eltringham, S.K., 1974. The daily activity of the elephant in the Rwenzori National Park, Uganda. Afr. J. Ecol. 12, 273–289.

Chapter Six

Conclusions

The broad aim of my PhD was to explore how volatile organic compounds (VOCs), plant secondary metabolites (PSMs), and salivary tannin-binding proteins influenced the foraging behaviour of African elephants, and how these factors affected their carrying capacities. To achieve this aim, I used a wide range of techniques from a variety of fields in an attempt to gain a holistic understanding of elephant foraging behaviour. I conducted three experiments focussing on 1) the role of odour cues in elephant foraging decisions (Chapter 2), 2) the volatile organic compounds (VOCs) the elephants focussed on to make these decisions (Chapter 3), and 3) whether elephants had tannin-binding proteins in their saliva to help counteract the effects of tannins (Chapter 4). I then combined the results of these experiments into a single carrying-capacity model for elephants (Chapter 5).

The results of these experiments suggest that elephants use pre-ingestive olfactory cues to make informed foraging decisions within feeding stations and between patches (Chapters 2 and 3). This suggests that elephants likely know more about the distribution, and potentially the quality, of resources across the landscape, than previously thought. For example, I found that elephants are able to make foraging decisions beyond their body lengths (Chapter 2), suggesting that the movement patterns of African elephants may be driven by olfactory cues of food resources. Similar to findings in my study, both swamp wallabies (*Wallabia bicolor*) and greater sage-grouse (*Centrocercus urophasianus*) use olfactory cues emitted from preferred plant species to locate food items across a landscape (Frye *et al.* 2013; Stutz *et al.* 2015; Stutz *et al.* 2016). However, the question that still remains is what information is contained in these chemical signals?

To answer this question, I explored the chemicals that are emitted as VOCs from both preferred and avoided plant species (Chapter 3). The elephants avoided plant species whose scent profiles contained both high levels and a wide diversity of PSM odour cues, particularly monoterpenes. Similar to findings of many other studies, monoterpenes have been found to negatively influence the preference of certain plant species for a wide range of herbivore species including several species of deer (*Odocoileus hemionus sitkensis*, *Capreolus capreolus*, and *Cervus timorensis russa*), cottontail rabbits (*Sylvilagus nuttalli*), brushtail possums (*Trichosurus vulpecula*) and Greater Sage-Grouse (*Centrocercus urophasianus*) (Vourc'h *et al.* 2002; Kirmani *et al.* 2010; Shipley *et al.* 2012; Frye *et al.* 2013; Utz *et al.* 2016).

However, by using these pre-ingestive cues, elephants may target plants with high levels of secondary compounds that cannot be detected via olfaction (e.g. tannins). This is something that I (Chapter 3) and others have found (e.g. Owen-Smith & Chafota 2012; Shrader *et al.* 2012; Muller 2013). Yet, the fact that elephants are able to tolerate tannins up to a point, suggested that they had one or more mechanisms for coping with the negative effects of tannins. I found this to be the use of salivary tannin-binding proteins (Chapter 4). However, the presence of these tannin-binding proteins contradicts theoretical expectations regarding body-size and forage tolerance (Bell 1971; Geist 1974; Jarman 1974). It appears that the African elephant is under sufficient nutritional selective pressure to have evolved these proteins to cope with plant defences. Thus, my findings about the presence of tannin-binding proteins in elephants echo those of Clauss *et al.* (2005) for black rhinoceros. The occurrence of tannin-binding proteins also raises questions about our understanding of the relationship between body size, digestive physiology, and PSMs for other browsers and mixed-feeders. Ultimately, these tannin-binding proteins increase the amount of available crude protein for the elephants, which can greatly affect the carrying capacity for this species.

Overall, these findings have implications for understanding landscape-scale movements, habitat use, and foraging patterns of elephants. To gain a better understanding of how this might influence carrying capacities, I drew from the findings from my first three data chapters, and developed a carrying-capacity model for elephants based on Hobbs and Swift's (1985) carrying-capacity model. Older methods of estimating carrying capacity included estimates of available biomass and intake rate of the stocked animals, and as models improved, new factors such as rainfall and vegetation availability, protein content, and diet preferences were included (Coe *et al.* 1976; Hobbs & Swift 1985; Schmidt *et al.* 1995). These different models worked with varying degrees of success, however, and they were most successful with grazers (Cowlishaw 1969; Meissner 1982; Meissner *et al.* 1990; Schmidt *et al.* 1995). Although these models were an advancement from their predecessors, they were missing information regarding aspects of the foraging behaviour of their target species. Consequently, I used results from my study to incorporate diet preference, dietary contribution of preferred plant species, and the negative influences of tannins and the semi-neutralization of them by salivary tannin-binding proteins, which no model had incorporated to date.

Ultimately, I found that incorporating information regarding the foraging habitats of the target species yielded a more accurate reflection of the carrying capacity of an area. My model was able to take into account the preference for certain plant species, which I found was likely driven by the VOCs emitted from these plants, as well as the ability of elephants to tolerate a certain level of tannins. Importantly, I have highlighted the importance of PSMs for a megaherbivore, and have shown how a carrying-capacity model that requires relatively few input parameters can be used to estimate the size of a sustainable elephant population within a fenced reserve. However, although a model might predict a sustainable population size, it may not be feasible to maintain the population without intensive management (e.g. culling,

contraception, translocation). With minor adjustments, my carrying-capacity model could be applied to any terrestrial herbivore (or guild of herbivores) in any habitat.

Future Research

The results from my four data chapters (Chapters 2, 3, 4, and 5) provide a framework for future investigations to explore the role of plant-animal interactions in foraging behaviour of large mammalian herbivores. Potential future projects could expand on aspects of diet selection with regard to occurrence and functioning of PSMs as cues, their implications for browsers and mixed-feeders, and investigation of how tannin-binding proteins influence a range of browsers or mixed feeders. Potential projects could include the following:

Do monoterpenes drive elephant diet choice?

To follow up from my first two data chapters (Chapters 2 and 3), I suggest using an experimental approach to determine whether volatile monoterpenes dictate elephant diet choice, and if so, which one(s) have the largest influence. Furthermore, it might also be beneficial to incorporate manipulations where odours are paired with varying post-ingestive consequences to test the extent to which odour drives conditioned preferences or whether it acts as a signal in itself.

To do this, I would recommend using an experimental design similar to the one I used for my odour experiment at the feeding-station scale (Chapter 2). Instead of different woody plant species, one could use the non-chemically defended species (e.g. bana grass *Pennisetum purpureum* or oats *Avena sterilis*, both grasses with no known PSMs), in separate bins and add synthetic monoterpenes to each item. The feeding trials could use the monoterpenes that we identified in the plants in the elephants' habitat including: (E)-ocimene, (Z)-ocimene, limonene, linalool, γ-terpinene, β-pinene, α-pinene, sabinene, o-cymene, terpinolene, and p-

cymen-8-ol (Chapter 3). Monoterpenes could be tested individually against a control as well as against each other to determine what, if any, influence they have on diet selection. Furthermore, one could also use a similar approach to Nobler (2016), and determine whether individual monoterpene compounds are a stronger deterrent than a combination of monoterpenes. By using a combination of monoterpenes, this would reflect a more natural situation where elephants would encounter more than a single monoterpene in a given plant species.

Because monoterpenes potentially have varying post-ingestive consequences, it would be interesting to present elephants with novel monoterpenes to establish their preference/avoidance prior to ingesting them and then compare their preference after they have had a sufficient amount of time to experience any post-ingestive consequences. This could then by followed by trials with high-quality forage coated with the novel monoterpene(s) to determine if the elephants continually avoid eating specific monoterpenes based on their odour, linked with the post-ingestive feedback. Experiments like this would be able to determine the extent to which odour drives conditioned preferences or whether it acts as a signal in itself.

Detection of forage quality by browsers prior to feeding

Another question relevant to mechanisms of diet selection is the use of olfactory cues by browsers to gather information about the chemical composition (PSMs) and nutritional quality of items prior to making foraging decisions. Answering this question could involve a two-part experiment:

1) A single species of plant could be manipulated using several treatments that mimic various levels of browsing- and fire-management schemes that could affect browse quality (e.g. combinations of clipping and burning treatments and appropriate controls – Hean &

Ward 2012). When the plants have matured and all manipulations are complete, the first sampling phase would involve collection of volatiles from each individual plant in each treatment. I would use the headspace extraction method to entrap VOCs emitted from each plant (e.g. Johnson & Jürgens 2010). Each sample would be run through a gas chromatograph and the chemical signatures from each species identified. The chemical signature for each treatment within a species could then be compared to the chemical signatures of the other treatments.

2) A behavioural choice test could then be employed with one plant from each treatment lined up in a testing arena. Using semi-tame elephants such as I did would allow the researcher to introduce the animal to each plant in the arena for a short time so that each elephant can have ample time to smell each option. After all options have been smelled, the elephants would then be guided to a position equidistant from all browse options. The elephant would then be allowed to make a foraging decision. The final sampling step would be collection of plant material for nutritional analysis. This would occur directly after the behaviour experiment. Each plant would be tested for crude protein, digestibility, and PSMs. The wet chemistry values would then be compared to the VOC values to determine whether there was a link between scent and chemical composition. In addition, the elephants' preferences could be compared to the wet and VOC chemical results to determine if the elephants could discern a difference between the nutritional quality of treatments.

Does age, sex, or sexual status influence diet choice of browsers with respect to PSM content?

A number of studies have indicated that the age, sex, and reproductive status of individuals influence diet selection with respect to nutritional quality (Mirza & Provenza 1990; Provenza *et al.* 1990; Thorhallsdottir *et al.* 1990; Stronge *et al.* 1997; Parker *et al.* 2009; Gaytán *et al.*

2015; Mellado 2016). For example, age plays an important role in diet selection for goats (*Capra hircus*); younger goats select for forage items that are higher in crude protein and more digestible than adult goats (Gaytán *et al.* 2015). Furthermore, differences in diet selection have been noted for goats and Nubian ibex (*Capra nubiana*) depending on their sex (Gross *et al.* 1996; Stronge *et al.* 1997; Mellado 2016). For example, shrubs account for a significantly larger proportion of the diets of male goats (Gross *et al.* 1996; Stronge *et al.* 1997) than females, likely because males are larger than females and have higher energy demands and shrubs may fulfil that need despite their lower overall quality. In addition, pregnant and lactating female herbivores have higher nutritional demands than non-pregnant females (Parker *et al.* 2009). Behavioural interactions and competition for mates in male herbivores (e.g. bighorn sheep *Ovis canadensis* during rutting season) also cause an elevation in nutritional demands (Ruckstuhl 1998). All of these factors could contribute to differences in nutritional demands between the sexes, manifesting in different diet choices. Moreover, pregnant female ungulates (and their foetuses) are often more sensitive to PSMs and thus may avoid consuming potentially deleterious plants (Panter *et al.* 1992; Pfister 1999).

Young animals are generally naïve when it comes to making foraging decisions and often have to learn about which PSMs to avoid (Thorhallsdottir *et al.* 1990). Their foraging decisions, and the rate at which they learn can depend on the foraging decisions of their mother, or whether they feed on their own (Thorhallsdottir *et al.* 1990). This could potentially explain why herbivores in the same herd, feeding in the same areas, have different diet selection. Furthermore, these differences in plant selection with regard to nutrient and PSM content could be a reflection of the differing nutritional demands across the age, sex, and reproductive-status classes (Parker *et al.* 2009; Mellado 2016). For example, one study found that female elephants with young were more selective feeders than large males (Stokke

1999). To meet such demands, individuals would need to reduce the intake of any anti-nutritional factors (i.e. PSMs).

I would recommend exploring diet selection of different ages and sexes of browsers/mixed feeders (e.g. goats, impala) with respect to volatile PSMs found in available plants. One could use the giving-up density (GUD) approach (Brown 1988; Kotler *et al.* 1994; Bedoya-Pérez *et al.* 2014). This approach uses an artificial patch filled with a measured amount food mixed into an inedible medium to reflect a natural foraging patch. This inedible medium constitutes a realistic cost to foraging in this patch. The GUD is the amount of food left in the patch after a forager abandons it, with smaller amounts of food indicating greater preference for the variables being tested in that patch (Brown 1988). The artificial patches can be filled with pellets that are laced with different PSMs and presented to individuals of different ages, sexes, and reproductive status (Shrader *et al.* 2008). GUDs can be used to quantify how herbivores balance foraging in a chemically defended landscape with mating, territorial defence, and growth.

An alternative approach could be to use a similar method to the one I used to determine diet choice in Chapters 2 and 3. For each age and sex class, one would establish what volatile PSMs occur in avoided plant species for each age and sex class, and then use odour trials to determine if any PSM(s) are driving differences in selection. For both experimental designs, I would predict that the browsers with the highest pressure to meet their nutritional demands (e.g. pregnant females, juveniles, rutting males) would be the most selective with regard to reducing volatile PSMs in their diets.

Elephant utilization of high-quality forage with a high handling cost
Another study concerning the diet selection of elephants and energetic costs of foraging that could be conducted addresses the question of why elephants select mistletoes when there are

other more easily accessed forage items available. In my foraging observations, I noticed that a species of mistletoe, *Viscum combreticola* was heavily sought after by the elephants near Bela Bela. *V. combreticola* is a hemiparasite on *Combretum* spp. and is primarily found parasitizing the large-fruited bushwillow (*Combretum zeyheri*) and the red bushwillow (*Combretum apiculatum*). Elephants expend large amounts of energy and considerable effort (e.g. knocking down large adult trees) to acquire mistletoes, often barely foraging on the host plant, despite the fact that *Combretum* is a favoured species (Chapter 2). One hypothesis is that elephants seek out mistletoes either because they lack high concentrations of PSMs or are richer in other nutrients than the host species (such as nitrogen) (Ehleringer *et al.* 1986). However, Okubamichael *et al.* (2011) found that some host trees had higher nitrogen concentrations than their mistletoe parasites. If this is the case, the apparently large energetic costs of foraging for mistletoes must presumably be outweighed by their benefits. Moreover, the understanding of those benefits would shed light on the foraging strategies of elephants in low-nutrient, high-PSM ecosystems. A potential approach would be to use an optimality model including the costs and benefits of obtaining mistletoes in nutrient-poor, chemically-rich landscapes.

The tannin-binding affinity of herbivore species' saliva across a range of body sizes and digestive physiologies

I suggest using a phylogenetic comparative method to examine the relationships of foraging niche (i.e. browser, grazer, and mixed-feeder), body size, and type of digestion (e.g. ruminants vs hind-gut fermenters) to examine tannin-binding affinity of saliva (Chapter 4) from a range of mammalian herbivore species. The results of (Clauss *et al.* 2005) found that three species of rhinoceroses had salivary tannin-binding proteins despite the fact that white rhinoceroses are grazers and tannins are seldom encountered by grazers. Given the

phylogenetic link between these three rhino species and the presence of salivary tannin-binding proteins, I predict that more closely related species will have more similar salivary tannin-binding affinities than less related species. Furthermore, I predict that browsers will have higher salivary tannin-binding affinity than that of grazers, because tannins are primarily found in browse items and not grasses. Additionally, as body size decreases, I predict that the tannin-binding affinity of browsers' saliva will increase because tannins reduce the overall quality of browse and smaller-bodied herbivores may be more negatively affected by PSMs (due to their higher energy demands) than larger animals (Geist 1974). Alternatively, it is possible that large-bodied herbivores may be more effected by PSMs, because larger herbivores process PSMs more slowly than smaller animals (Freeland 1991). Finally, I predict that the tannin-binding affinity should be greater for ruminants than hind-gut fermenters because ingesta passage rate of ruminants is longer than for hindgut fermenters, giving tannins a greater probability of being absorbed during digestion.

To test for the tannin-binding capabilities of the saliva, I would recommend using the same technique that I used for the elephant saliva in Chapter 4, except that one could test the saliva against different stock solutions of tannic acid instead of plant extracts. This would then allow exploration of the relationship between tannin-binding affinity of saliva and factors such as body size and digestive physiology.

Testing my carrying-capacity model on populations of wild elephants and incorporating competition between browsers and mixed-feeders

My carrying-capacity model could be used to predict the carrying capacity of wild elephants, such as those in Ithala Game Reserve (IGR) in KwaZulu-Natal, South Africa. Elephants in IGR pose a serious management issue because they frequently leave the reserve. The foraging behaviour of the elephants of IGR has been extensively studied (Shrader *et al.* 2012; Muller

2013; Ward *et al.* 2016). A researcher could apply these data to my model in an effort to predict IGR's elephant carrying capacity. A modelled carrying capacity that is smaller than the current population of elephants could help explain the tendency for individuals to forage outside the reserve boundaries. An approach such as this could be applied to the elephant population of any protected area.

The second part of this study would be to incorporate another important factor that could influence carrying capacities, which is competition between species of browsers and mixed-feeders. A possible approach would be to use my carrying-capacity model and alter the available biomass for each browser/mixed-feeder by calculating available biomass in different vegetation-height classes. Elephants directly compete with a range of browsing species such as blue duiker *Philantomba monticola*, common duiker *Sylvicapra grimmia*, and bushbuck *Tragelaphus sylvaticus* (Lessing 2006). However, elephants can access a wider range of food types that overlap with the feeding heights of impala *Aepyceros melampus*, kudu *Tragelaphus strepsiceros*, black rhino *Diceros bicornis*, and giraffe *Giraffa camelopardalis* (du Toit 1990; Wilson 2002; Lessing 2006). Many of these species have some dietary overlap in species preference and browse height, but have differing degrees of preference (Pellew 1983; Pellew 1984; Furstenburg & Van Hoven 1994; de Garine-Wichatitsky *et al.* 2004; Ndlovu & Mundy 2008; Kotze & Zacharias 2010; Shaw 2011).

Another adjustment to the model could include a measure of rarity of a particular herbivore species. For example, if a herbivore is endangered and in need of increasing its population size, one could set the numbers of browser A at a fixed number and then maximize the numbers of browser B (the rare species). As a result, one could incorporate these factors and constraints into my model to predict carrying capacity as a function of foraging competition using species-specific foraging preferences.

References

Bedoya-Pérez, M.A., Issa, D.D., Banks, P.B. & McArthur, C. (2014) Quantifying the response of free-ranging mammalian herbivores to the interplay between plant defense and nutrient concentrations. *Oecologia,* **175**, 1167–1177.

Bell, R.H.V. (1971) A grazing ecosystem in the Serengeti. *Scientific American,* **224**, 86–93.

Brown, J.S. (1988) Patch use as an indicator of habitat preference, predation risk, and competition. *Behavioral Ecology and Sociobiology,* **22**, 37–47.

Clauss, M., Gehrke, J., Hatt, J.-M., Dierenfeld, E.S., Flach, E.J., Hermes, R., Castell, J., Streich, J.W. & Fickel, J. (2005) Tannin-binding salivary proteins in three captive rhinoceros species. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,* **140**, 67–72.

Coe, M., Cumming, D. & Phillipson, J. (1976) Biomass and production of large African herbivores in relation to rainfall and primary production. *Oecologia,* **22**, 341–354.

Cowlishaw, S.J. (1969) The carrying capacity of pastures. *Grass and Forage Science,* **24**, 207–214.

de Garine-Wichatitsky, M., Fritz, H., Gordon, I.J. & Illius, A.W. (2004) Bush selection along foraging pathways by sympatric impala and greater kudu. *Oecologia,* **141**, 66–75.

du Toit, J.T. (1990) Feeding-height stratification among African browsing ruminants. *African Journal of Ecology,* **28**, 55–61.

Ehleringer, J.R., Cook, C.S. & Tieszen, L.L. (1986) Comparative water use and nitrogen relationships in a mistletoe and its host. *Oecologia,* **68**, 279–284.

Freeland, W. (1991) *Plant Secondary Metabolites: Biochemical Coevolution with Herbivores*. CRC Press, Boca Raton, Florida, USA.

Frye, G.G., Connelly, J.W., Musil, D.D. & Forbey, J.S. (2013) Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. *Ecology,* **94**, 308–314.

Furstenburg, D. & Van Hoven, W. (1994) Condensed tannin as anti-defoliate agent against browsing by giraffe (*Giraffa camelopardalis*) in the Kruger National Park. *Comparative Biochemistry and Physiology Part A: Physiology,* **107**, 425–431.

Gaytán, L., Salem, A.F.Z., Rodríguez, A., García, J.E., Arévalo, J.R. & Mellado, M. (2015) Age and season effects on quality of diets selected by Criollo crossbred goats on rangeland. *Animal Production Science,* **55**, 758–765.

Geist, V. (1974) On the relationship of social evolution and ecology in ungulates. *American Zoologist,* **14**, 205–220.

Gross, J.E., Alkon, P.U. & Demment, M.W. (1996) Nutritional ecology of dimorphic herbivores: Digestion and passage rates in Nubian ibex. *Oecologia,* **107**, 170–178.

Hean, J.W. & Ward, D. (2012) Fire and herbivory are not substitutable: Evidence from regrowth patterns and changes in physical and chemical defences in *Acacia* seedlings. *Journal of Vegetation Science,* **23**, 13–23.

Hobbs, T.N. & Swift, D.M. (1985) Estimates of habitat carrying capacity incorporating explicit nutritional constraints. *Journal of Wildlife Management,* **49**, 814–822.

Jarman, P.J. (1974) The social organisation of antelope in relation to their ecology. *Behaviour,* **48**, 215–267.

Johnson, S.D. & Jürgens, A. (2010) Convergent evolution of carrion and faecal scent mimicry in fly-pollinated angiosperm flowers and a stinkhorn fungus. *South African Journal of Botany,* **76**, 796–807.

Kirmani, S.N., Banks, P.B. & McArthur, C. (2010) Integrating the costs of plant toxins and predation risk in foraging decisions of a mammalian herbivore. *Oecologia,* **164**, 349–356.

Kotler, B.P., Gross, J.E. & Mitchell, W.A. (1994) Applying patch use to assess aspects of foraging behavior in Nubian ibex. *Journal of Wildlife Management,* **58**, 299–307.

Kotze, D.C. & Zacharias, P.J.K. (2010) Utilization of woody browse and habitat by the black rhino (*Diceros bicornis*) in western Itala Game Reserve. *African Journal of Range & Forage Science,* **10**, 36–40.

Lessing, J. (2006) Elephant feeding behaviour and forage offtake implications in the Addo Elephant National Park. Unpublished MSc thesis, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa.

Meissner, H., Spreeth, E., De Villiers, P., Pietersen, E., Hugo, T. & Terblanche, B. (1990) Quality of food and voluntary intake by elephant as measured by lignin index. *South African Journal of Wildlife Research,* **20**, 104–110.

Meissner, H.H. (1982) Theory and application of a method to calculate forage intake of wild southern African ungulates for purposes of estimating carrying capacity. *South African Journal of Wildlife Research,* **12**, 41–47.

Mellado, M. (2016) Dietary selection by goats and the implications for range management in the Chihuahuan Desert: A review. *Rangeland Journal,* **38**, 331–341.

Mirza, S.N. & Provenza, F.D. (1990) Preference of the mother affects selection and avoidance of foods by lambs differing in age. *Applied Animal Behaviour Science,* **28**, 255–263.

Muller, K. (2013) What drives the seasonal movements of African elephants (*Loxodonta africana)* in Ithala Game Reserve. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Ndlovu, M. & Mundy, P.J. (2008) Browse preference of captive black rhinos at Chipangali Wildlife Orphanage, Zimbabwe. *Pachyderm*, 41–46.

Nobler, J.D. (2016) Risky business: Tradeoffs between nutrition, toxicity, and predation by a specialist mammalian herbivore. Unpublished MSc thesis, Boise State University, Boise, Idaho, USA.

Okubamichael, D.Y., Griffiths, M.E. & Ward, D. (2011) Host specificity, nutrient and water dynamics of the mistletoe *Viscum rotundifolium* and its potential host species in the Kalahari of South Africa. *Journal of Arid Environments,* **75**, 898–902.

Owen-Smith, N. & Chafota, J. (2012) Selective feeding by a megaherbivore, the African elephant (*Loxodonta africana*). *Journal of Mammalogy,* **93**, 698–705.

Panter, K.E., Keeler, R.F., James, L.F. & Bunch, T.D. (1992) Impact of plant toxins on fetal and neonatal development: A review. *Journal of Range Management*, 52–57.

Parker, K.L., Barboza, P.S. & Gillingham, M.P. (2009) Nutrition integrates environmental responses of ungulates. *Functional Ecology,* **23**, 57–69.

Pellew, R.A. (1984) The feeding ecology of a selective browser, the giraffe (*Giraffa camelopardalis tippelskirchi*). *Journal of Zoology,* **202**, 57–81.

Pellew, R.A.P. (1983) The impacts of elephant, giraffe and fire upon the *Acacia tortilis* woodlands of the Serengeti. *African Journal of Ecology,* **21**, 41–74.

Pfister, J.A. (1999) Behavioral strategies for coping with poisonous plants. *Grazing behavior of livestock and wildlife* (ed. K. K. Launchbaugh, KD Sanders, and JC Mosley), pp. 45–59. Idaho Forest, Wildlife, and Range Experimental Station Bulletin, Moscow, Idaho, USA.

Provenza, F.D., Burritt, E.A., Clausen, T., Bryant, J., Reichardt, P. & Distel, R.A. (1990) Conditioned flavor aversion: A mechanism for goats to avoid condensed tannins in blackbrush. *American Naturalist,* **136**, 810–828.

Ruckstuhl, K.E. (1998) Foraging behaviour and sexual segregation in bighorn sheep. *Animal Behaviour,* **56**, 99–106.

Schmidt, A., Theron, G. & Van Hoven, W. (1995) A comparison of some methods used to estimate the grazing capacity of a game ranch in Northern Province, South Africa. *Koedoe,* **38**, 123–128.

Shaw, J.A. (2011) Adaptive resource use in a re-introduced black rhinoceros population. Unpublished PhD thesis, University of the Witwatersrand, Johannesburg, South Africa.

Shipley, L.A., Davis, E.M., Felicetti, L.A., McLean, S. & Forbey, J.S. (2012) Mechanisms for eliminating monoterpenes of sagebrush by specialist and generalist rabbits. *Journal of Chemical Ecology,* **38**, 1178–1189.

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.

Shrader, A.M., Kotler, B.P., Brown, J.S. & Kerley, G.I.H. (2008) Providing water for goats in arid landscapes: Effects on feeding effort with regard to time period, herd size and secondary compounds. *Oikos,* **117**, 466–472.

Stokke, S. (1999) Sex differences in feeding-patch choice in a megaherbivore: elephants in Chobe National Park, Botswana. *Canadian Journal of Zoology,* **77**, 1723–1732.

Stronge, D., Fordham, R. & Minot, E. (1997) The foraging ecology of feral goats *Capra hircus* in the Mahoenui giant weta reserve, southern King Country, New Zealand. *New Zealand Journal of Ecology,* **21**, 81–88.

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

Stutz, R.S., Banks, P.B., Proschogo, N. & McArthur, C. (2016) Follow your nose: Leaf odour as an important foraging cue for mammalian herbivores. *Oecologia,* **182**, 643-651.

Thorhallsdottir, A.G., Provenza, F.D. & Balph, D.F. (1990) Ability of lambs to learn about novel foods while observing or participating with social models. *Applied Animal Behaviour Science,* **25**, 25–33.

Utz, J.L., Shipley, L.A., Rachlow, J.L., Johnstone-Yellin, T., Camp, M. & Forbey, J.S. (2016) Understanding tradeoffs between food and predation risks in a specialist mammalian herbivore. *Wildlife Biology,* **22**, 167–173.

Vourc'h, G., De Garine-Wichatitsky, M., Labbé, A., Rosolowski, D., Martin, J.-L. & Fritz, H. (2002) Monoterpene effect on feeding choice by deer. *Journal of Chemical Ecology,* **28**, 2411–2427.

Ward, D., Muller, K. & Shrader, A.M. (2016) Soil fertility on granite and sedimentary soils is associated with seasonal differences in foraging by elephants. *Plant and Soil*, doi:10.1007/s11104-11016-13067-y.

Wilson, S.L. (2002) The influence of body size on the foraging behaviour of a Thicket Biome browser. Unpublished PhD thesis, University of Port Elizabeth, Port Elizabeth, South Africa.

Appendix I

Table Ai.1: Acceptability Index (AI) of preferred and avoided plant species.

Species	Acceptability	# Times Consumed	# Times Encountered	AI
Viscum combreticola	Novel Preferred	NA	NA	NA
Pappea capensis	Preferred	71	82	0.87
Dombeya rotundifolia	Preferred	45	61	0.74
Terminalia sericea	Preferred	92	132	0.70
Combretum zeyheri	Preferred	134	205	0.65
Grewia monticola	Principal	171	339	0.50
Euclea crispa	Principal	155	559	0.28
Vitex rehmannii	Avoided	15	78	0.19
Searsia pyroides	Avoided	18	98	0.18
Searsia lancea	Avoided	52	410	0.13
Euclea undulata	Avoided	3	83	0.04
Olea europaea	Avoided	0	222	0.00

Table Ai.2: Results from pairwise ANOSIM contrasts of odour profiles from each species in every combination presented to the elephants. P = preferred, A = avoided.

Combinations	R Statistic	Significance Level (%)
Pappea capensis (P), *Euclea crispa* (P)	0.446	0.0002
Pappea capensis (P), *Combretum zeyheri* (P)	0.485	0.0002
Pappea capensis (P), *Dombeya rotundifolia* (P)	0.299	0.004
Combretum zeyheri (P), *Euclea crispa* (P)	0.371	0.0002
Combretum zeyheri (P), *Dombeya rotundifolia* (P)	0.324	0.0006
Terminalia sericea (P), *Euclea crispa* (P)	0.493	0.0002
Terminalia sericea (P), *Pappea capensis* (P)	0.485	0.0002
Terminalia sericea (P), *Combretum zeyheri* (P)	0.435	0.0003
Terminalia sericea (P), *Dombeya rotundifolia* (P)	0.406	0.0003
Grewia monticola (P), *Euclea crispa* (P)	0.54	0.0002
Grewia monticola (P), *Pappea capensis* (P)	0.605	0.0002
Grewia monticola (P), *Combretum zeyheri* (P)	0.565	0.0003
Grewia monticola (P), *Terminalia sericea* (P)	0.474	0.001
Grewia monticola (P), *Dombeya rotundifolia* (P)	0.472	0.0008
Dombeya rotundifolia (P), *Euclea crispa* (P)	0.499	0.0002
Euclea undulata (A), *Vitex rehmannii* (A)	0.999	0.0002
Euclea undulata (A), *Searsia pyroides* (A)	1	0.0002
Searsia lancea (A), *Euclea undulata* (A)	0.998	0.0002
Searsia lancea (A), *Vitex rehmannii* (A)	0.983	0.0002
Searsia lancea (A), *Searsia pyroides* (A)	0.735	0.0002
Searsia lancea (A), *Olea europaea* (A)	0.973	0.0002
Searsia pyroides (A), *Vitex rehmannii* (A)	0.994	0.0002
Olea europaea (A), *Euclea undulata* (A)	1	0.0002
Olea europaea (A), *Vitex rehmannii* (A)	1	0.0002
Olea europaea (A), *Searsia pyroides* (A)	0.988	0.0002
Euclea undulata (A), *Euclea crispa* (P)	0.79	0.0002
Searsia lancea (A), *Euclea crispa* (P)	0.608	0.0002
Searsia lancea (A), *Dombeya rotundifolia* (P)	0.793	0.0002
Pappea capensis (P), *Euclea undulata* (A)	0.826	0.0002
Pappea capensis (P), *Searsia lancea* (A)	0.756	0.0002
Pappea capensis (P), *Vitex rehmannii* (A)	0.928	0.0002
Pappea capensis (P), *Searsia pyroides* (A)	0.813	0.0002
Pappea capensis (P), *Olea europaea* (A)	0.511	0.0005
Combretum zeyheri (P), *Euclea undulata* (A)	0.907	0.0002
Combretum zeyheri (P), *Searsia lancea* (A)	0.71	0.0002
Combretum zeyheri (P), *Vitex rehmannii* (A)	0.941	0.0002
Combretum zeyheri (P), *Searsia pyroides* (A)	0.628	0.0002

Combretum zeyheri (P), *Olea europaea* (A)	0.459	0.0005
Viscum combreticola (P), *Euclea undulata* (A)	0.859	0.0002
Viscum combreticola (P), *Olea europaea* (A)	0.74	0.0002
Vitex rehmannii (A), *Euclea crispa* (P)	0.789	0.0002
Terminalia sericea (P), *Euclea undulata* (A)	0.978	0.0002
Terminalia sericea (P), *Searsia lancea* (A)	0.85	0.0002
Terminalia sericea (P), *Vitex rehmannii* (A)	1	0.0002
Terminalia sericea (P), *Searsia pyroides* (A)	0.839	0.0002
Terminalia sericea (P), *Olea europaea* (A)	0.575	0.0003
Grewia monticola (P), *Euclea undulata* (A)	0.912	0.0002
Grewia monticola (P), *Searsia lancea* (A)	0.763	0.0002
Grewia monticola (P), *Vitex rehmannii* (A)	0.934	0.0002
Grewia monticola (P), *Searsia pyroides* (A)	0.773	0.0002
Grewia monticola (P), *Olea europaea* (A)	0.68	0.0003
Searsia pyroides (A), *Euclea crispa* (P)	0.633	0.0002
Olea europaea (A), *Euclea crispa* (P)	0.63	0.0003
Olea europaea (A), *Dombeya rotundifolia* (P)	0.411	0.0006
Dombeya rotundifolia (P), *Euclea undulata* (A)	0.819	0.0002
Dombeya rotundifolia (P), *Vitex rehmannii* (A)	0.822	0.0002
Dombeya rotundifolia (P), *Searsia pyroides* (A)	0.758	0.0002

Figure Ai.1: A representation of both the feeding-station and between-patch experiments. Panels A&B illustrate the feeding stations composed of two bins (i.e. species choice), while C&D show the Y-maze used in the between-patch selection experiment. Slots in panel A indicate where the PVC boards were inserted.
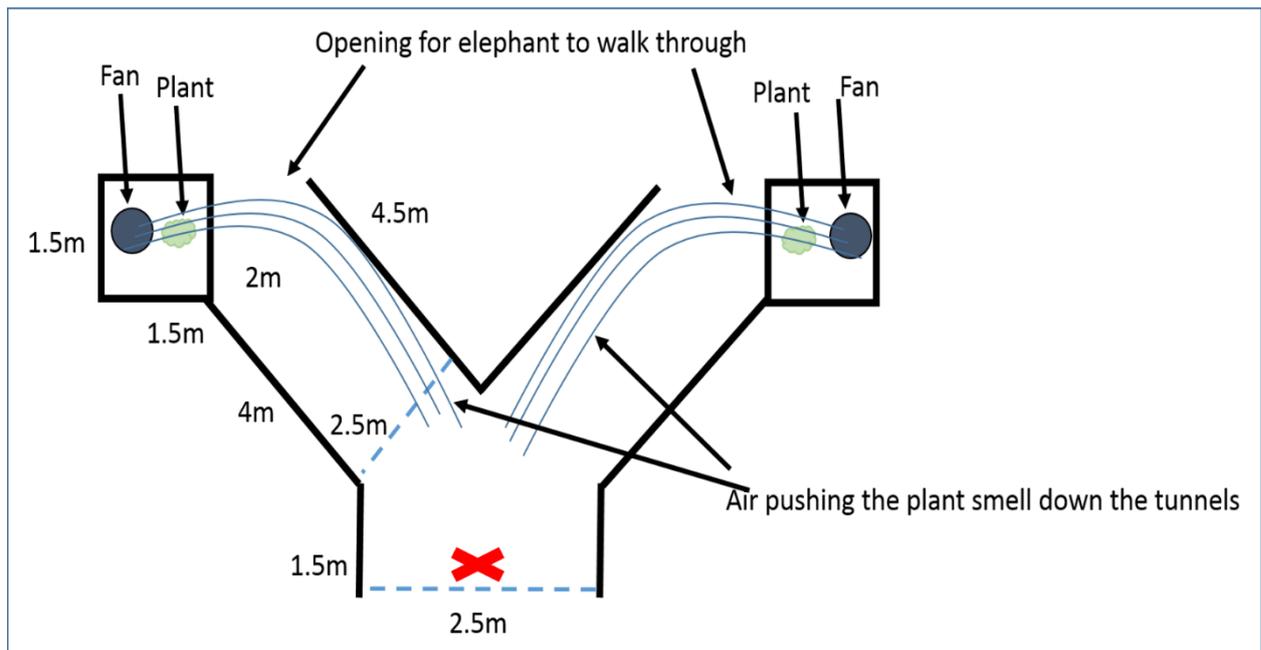
Figure Ai.2: Schematic of the Y-maze. Red "X" indicates the location where the elephants were instructed to stop and smell the air from each arm of the maze and then make their foraging decision.

Appendix II

Table Aii.1: Volatile organic compounds identified in all plant odour samples used for these studies.

Compound	Compound Class	Kovats	Varian GC 2013 RT 1
Aliphatics			
Alkanes			
Decane	Aliphatic alkane	1159	6.553
Undecane	Aliphatic alkane	1187	7.522
Dodecane	Aliphatic alkane	1225	8.422
Alcohols			
Hexan-1-ol	Aliphatic alcohol	1340	10.435
(Z)-Hex-3-en-1-ol	Aliphatic alcohol	1380	10.995
(E)-Hex-2-en-1-ol	Aliphatic alcohol	1383	11.04
Octan-2-ol	Aliphatic alcohol	1395	11.204
1-Heptanol	Aliphatic alcohol	1431	11.672
(E)-Oct-2-en-1-ol	Aliphatic alcohol	1602	13.664
Nonanol	Aliphatic alcohol	1653	14.21
Decanol	Aliphatic alcohol	1761	15.31
Hexadecan-1-ol	Aliphatic alcohol	2409	21.335
Aldehydes			
(E)-Hex-2-enal	Aliphatic aldehyde	1248	8.898
(E)-Oct-2-enal	Aliphatic aldehyde	1414	11.456
(E,E)-Hepta-2,4-dienal	Aliphatic aldehyde	1483	12.319
Decanal	Aliphatic aldehyde	1487	12.367
(E)-Non-2-enal	Aliphatic aldehyde	1522	12.771
(Z)-Dec-2-enal	Alpihatic aldehyde	1648	14.157
Dodecanal	Aliphatic aldehyde	1709	14.783
Ketones			
Heptan-2-one	Aliphatic ketone	1219	8.307
6-Methylheptan-2-one	Aliphatic ketone	1253	9
Octan-2-one	Aliphatic ketone	1286	9.586
3,5-Dimethyloctan-2-one	Aliphatic ketone	1342	10.462
Nonan-2-one	Aliphatic ketone	1376	10.947
Decan-2-one	Aliphatic ketone	1473	12.198
Hexane-2,5-dione	Aliphatic ketone	1489	12.386
Undecan-2-one	Aliphatic ketone	1587	13.5
Dodecan-2-one	Aliphatic ketone	1704	14.735
Esters			
Amyl acetate	Aliphatic ester	1213	8.17
Methyl caproate	Aliphatic ester	1221	8.334

Hexyl acetate	Aliphatic ester	1279	9.458
(Z)-Hex-3-en-1-yl acetate	Aliphatic ester	1312	10.001
(E)-Hex-2-en-1-yl acetate	Aliphatic ester	1321	10.145
Heptyl acetate	Aliphatic ester	1359	10.709
(Z)-Hex-3-en-1-yl propanoate	Aliphatic ester	1368	10.832
Ethyl octanoate	Aliphatic ester	1415	11.469
(Z)-Hex-en-1-yl butyrate	Aliphatic ester	1440	11.785
(Z)-Hex-3-en-1-yl isovalerate	Aliphatic ester	1451	11.932

Acids

2-Methyl propanoic acid	Aliphatic acid	1560	13.205
Butanoic acid	Aliphatic acid	1623	13.895
Pentanoic acid	Aliphatic acid	1744	15.144
4-Methylpentanoic acid	Aliphatic acid	1811	15.8
Hexanoic acid	Aliphatic acid	1866	16.329
Hex-5-enoic acid	Aliphatic acid	1934	16.969
2-Ethylhexanoic acid	Aliphatic acid	1975	17.354
Hept-6-enoic acid	Aliphatic acid	2054	18.085
Octanoic acid	Aliphatic acid	2098	18.486
Oct-7-enoic acid	Aliphatic acid	2165	19.105
Nonanoic acid	Aliphatic acid	2209	19.501
Decanoic acid	Aliphatic acid	2315	20.474

Aromatics

Benzaldehyde	Benzenoid compound	1521	12.765
Methyl benzoate	Benzenoid compound	1619	13.855
Phenylacetaldehyde	Benzenoid compound	1644	14.112
p-Methoxystyrene	Benzenoid compound	1683	14.52
2-Hydroxybenzaldehyde	Benzenoid compound	1687	14.562
Benzyl acetate	Benzenoid compound	1733	15.028
Methyl salicylate	Benzenoid compound	1792	15.618
Phenylethyl acetate	Benzenoid compound	1830	15.98
Guaiacol	Benzenoid compound	1882	16.477
Benzyl alcohol	Benzenoid compound	1907	16.722
Benzyl 3-methylbutanoate	Benzenoid compound	1920	16.84
Phenylethyl alcohol	Benzenoid compound	1945	17.077
p-Cresol	Benzenoid compound	2124	18.723
m-Cresol	Benzenoid compound	2131	18.795
(Z)-Hex-3-en-1-yl benzoate	Benzenoid compound	2177	19.211
Eugenol	Benzenoid compound	2215	19.553
Ethyl 4-ethoxybenzoate	Benzenoid compound	2219	19.592
Benzoic acid	Benzenoid compound	2481	22.011

C-5 Branched compounds

Isovaleric acid	C-5 Branched compound	1668	14.362
2-Methylbutanoic acid	C-5 Branched compound	1670	14.383

Terpenoids

Monoterpenes

α-Pinene	Monoterpene	1158	6.528
Sabinene	Monoterpene	1188	7.54
β-Pinene	Monoterpene	1213	8.16
Eucalyptol	Monoterpene	1234	8.609
Limonene	Monoterpene	1240	8.731
(Z)-Ocimene	Monoterpene	1248	8.894
γ-Terpinene	Monoterpene	1261	9.141
(E)-Ocimene	Monoterpene	1262	9.162
o-Cymene (could be p-cymene)	Monoterpene	1272	9.344
Terpinolene	Monoterpene	1284	9.549
p-Cymenene	Monoterpene	1423	11.569
2,6-Dimethylocta-3,7-diene-2,6-diol	Monoterpene	1437	11.757
(Z)-Linalool oxide (furanoid)	Monoterpene	1449	11.903
Linalool	Monoterpene	1525	12.809
Pinocarvone	Monoterpene	1562	13.227
α-Terpineol	Monoterpene	1692	14.618
endo-Borneol	Monoterpene	1700	14.698
Carvone	Monoterpene	1744	15.136
Myrtenol	Monoterpene	1809	15.781
(E,E)-2,6-Dimethylocta-3,5,7-triene-2-ol	Monoterpene	1817	15.86
(Z)-p-Mentha-6,8-dien-2-ol	Monoterpene	1849	16.165
p-Cymen-8-ol	Monoterpene	1867	16.336
(Z)-p-mentha-1(7),8-dien-2-ol	Monoterpene	1910	16.744
2,6-Dimethylocta-1,7-diene-3,6-diol	Monoterpene	2166	19.11
Carvacrol	Monoterpene	2257	19.939
Sesquiterpenes			
α-Copaene	Sesquiterpene	1471	12.177
β-Bourbonene	Sesquiterpene	1506	12.592
α-Bergamotene	Sesquiterpene	1571	13.324
(Z)-β-Caryophyllene	Sesquiterpene	1571	13.333
Caryophyllene	Sesquiterpene	1587	13.5
Humulene	Sesquiterpene	1666	14.348
Germacrene D	Sesquiterpene	1710	14.798
α-Muurolene	Sesquiterpene	1723	14.925
β-Caryophyllene oxide	Sesquiterpene	2031	17.873
Spathulenol	Sesquiterpene	2172	19.167
m/z: 204*,105,119,95,91,67	Unknown sesquiterpene	1438	11.769
m/z: 204*, 105,43,91,53,55,72,79	Unknown sesquiterpene	1455	11.974
m/z: 204*,105,119,93,120,91,92	Unknown sesquiterpene	1466	12.114
m/z: 161, 43, 119, 41, 105	Unknown sesquiterpene	1479	12.266
m/z: 204*,120,105,91,161,79,93	Unknown sesquiterpene	1568	13.299
m/z: 204*,105,161,91,109,119,81	Unknown sesquiterpene	1588	13.519
m/z: 204*,91,105,93,107,79,119	Unknown sesquiterpene	1607	13.717
m/z: 204*,105,161,119,77,136	Unknown sesquiterpene	1632	13.991

m/z: 204*,91,93,105,107,79	Unknown sesquiterpene	1646	14.141
m/z: 204*,92,91,55,83,105,70	Unknown sesquiterpene	1654	14.224
m/z: 204*,91,93,105,79,119,107	Unknown sesquiterpene	1664	14.32
m/z: 204*,91,43,93,119,84,82	Unknown sesquiterpene	1683	14.52
m/z: 204*,105,91,93,119,121,122	Unknown sesquiterpene	1689	14.583
m/z: 204*,136,121,91,43,105,93	Unknown sesquiterpene	1701	14.705
m/z: 204*,91,119,55,105,41,161	Unknown sesquiterpene	1758	15.282
m/z: 204*,91,105,119,161,93,79	Unknown sesquiterpene	1772	15.415
m/z: 220*,91,109,93,79,107,159	Unknown Sesquiterpene	2198	19.403
Irregular terpenes			
6-Methyl-5-hepten-2-ol	Irregular terpene	1437	11.748
Sabina ketone	Irregular terpene	1634	14.007
Geranyl acetone	Irregular terpene	1873	16.397
Miscellaneous cyclic compounds			
2-Pentylfuran	Miscellaneous cyclic compound	1254	9.016
2-Furfural	Miscellaneous cyclic compound	1451	11.924
5,5-Dimethyl-2(5H)-furanone	Miscellaneous cyclic compound	1606	13.711
Dihydro-5-methyl-2(3H)-furanone	Miscellaneous cyclic compound	1614	13.801
Butyrolactone	Miscellaneous cyclic compound	1637	14.038
4-Hexanolide	Miscellaneous cyclic compound	1717	14.873
δ-Hexalactone	Miscellaneous cyclic compound	1824	15.921
γ-Heptalactone	Miscellaneous cyclic compound	1830	15.985
4-Octanolide	Miscellaneous cyclic compound	1955	17.167
γ-Nonalactone	Miscellaneous cyclic compound	2079	18.319
Indole	Nitrogen-containing compound	2486	22.058
Unknowns			
m/z: 106, 91, 43, 55, 71	Unknown	1192	7.653
m/z: 43,67,41,57,68,86	Unknown	1239	8.723
m/z: 150*,69,41,81,79,82,53	Unknown	1303	9.86
m/z: 128*,43,99,55,112,70,71	Unknown	1304	9.87
m/z: 56,55,70,57,69,43	Unknown	1353	10.617
m/z: 136*,121,91,105,79,119,55	Unknown	1359	10.701
m/z: 95,43,82,109,67,111	Unknown	1401	11.289
m/z: 43,57,98,55,58,71	Unknown	1406	11.352
m/z: 53, 47, 71, 41, 55, 81	Unknown	1413	11.439
m/z: 43,80,79,39,41,77,81	Unknown	1416	11.478

m/z: 56,57,55,70,41,69,43	Unknown	1431	11.671
m/z: 81,110,39,53,41,57	Unknown	1450	11.912
m/z: 79,110,95,77,94,67	Unknown	1450	11.919
m/z: 43, 71, 112, 41, 27	Unknown	1554	13.141
m/z: 123*,57,82,67,43,81,41	Unknown	1556	13.158
m/z: 41, 55, 43, 57, 82, 71	Unknown	1559	13.196
mz: 99,43,57,71,100,42,55	Unknown	1570	13.322
m/z: 83, 55, 81, 95, 41	Unknown	1577	13.391
m/z: 112*,83,55,57,84	Unknown	1592	13.559
m/z: 41, 43, 57, 55, 71	Unknown	1604	13.691
m/z: 97,43,69,42,45,41	Unknown	1616	13.817
m/z: 55,83,97,69,41,57,56	Unknown	1633	14.002
m/z: 42,41,56,86,39,43	Unknown	1635	14.026
m/z: 43,55,58,71,95,85,99	Unknown	1670	14.391
m/z: 43,95,58,71,55,85,41	Unknown	1674	14.424
m/z: 126*,111,55,67,43,98,71,83	Unknown	1675	14.443
m/z: 82,81,43,54,39,55,53	Unknown	1679	14.482
m/z: 117*,91,90,43,89,65,118	Unknown	1680	14.49
m/z: 98*,43,55,41,39,70	Unknown	1731	15.005
m/z: 96,43,95,67,68,81	Unknown	1757	15.265
m/z: 134*,119,91,65,120,92,98	Unknown	1796	15.654
m/z: 71,43,99,41,59,53	Unknown	1811	15.798
m/z: 119, 43, 109, 143, 91	Unknown	1815	15.842
m/z: 85, 57, 41, 29, 43	Unknown	1998	17.568
m/z: 57,85,39,41,43,55,31	Unknown	2000	17.584
m/z: 99, 43, 71, 87, 114	Unknown	2016	17.733
m/z: 55,81,82,41,67,79	Unknown	2045	18.002
m/z: 74, 87, 41, 43, 55	Unknown	2057	18.109
m/z: 43, 95, 67, 55, 41, 107	Unknown	2099	18.5
m/z: 126*,98,55,83,11,84,43	Unknown	2140	18.871
m/z: 55,43,41,57,82,96	Unknown	2163	19.084
m/z: 43, 58, 71, 57, 59, 41	Unknown	2167	19.116
m/z: 133,91,43,148,105,79	Unknown	2285	20.196
m/z: 136,91,79,93,41,69	Unknown	2350	20.789
m/z: 93,91,105,107,109,95	Unknown	2382	21.084
m/z: 83,97,57,55,69,43	Unknown	2594	23.09

Appendix III

Table Aiii.1: Volatile organic compounds contributing to the first ~90 % of average Bray–Curtis similarity between headspace scent samples collected from *preferred and avoided plant species* (from SIMPER analysis). Compounds are listed in decreasing order of % contribution. Dissimilarity/SD indicates differences between preference groups. Larger Dissimilarity/SD values indicate a larger percent dissimilarity across groups. Contribution % indicates the overall percentage of dissimilarity that each group contributes to the overall dissimilarity. Unknown VOCs are presented with the molecular mass first (if known) indicated by a * followed by the base peak and remaining fragments in decreasing order of abundance.

Volatile organic compounds	Preferred mean abundance	Avoided mean abundance	Dissimilarity/SD	Contribution %
(E)-Ocimene	1.08	1.27	1.16	2.91
(Z)-Hex-3-en-1-yl acetate	2.07	1.61	1.2	2.67
(Z)-Ocimene	0.55	0.76	0.98	2.43
Caryophyllene	0.94	0.76	1.14	2.43
Limonene	0.39	0.93	1.21	2.23
β-Pinene	0.17	0.82	1.11	2.19
α-Pinene	0.22	0.66	0.7	2
Benzaldehyde	1.25	0.95	1.1	1.98
(Z)-Hex-3-en-1-ol	0.55	0.44	0.86	1.9
Dihydro-5-methyl-2(3H)-furanone	0.65	0.51	1.09	1.84
Linalool	0.72	0.52	1.16	1.82
p-Cymenene	0	0.68	0.93	1.8
Phenylethyl alcohol	0.67	0.78	1.18	1.78
4-Hexanolide	0.68	0.54	1.07	1.76
Hexyl acetate	0.62	0.24	1	1.73
mz: 99,43,57,71,100,42,55	0.56	0.46	1.11	1.63
Benzyl alcohol	1.01	0.95	1.08	1.6
4-Octanolide	0.34	0.55	1.11	1.48
Hexanoic acid	0.12	0.57	0.92	1.46
Methyl salicylate	0.58	0.53	1.25	1.43

Sabinene	0.17	0.35	0.51	1.28
Octanoic acid	0.11	0.48	0.77	1.24
p-Cresol	0.31	0.44	1.2	1.23
m/z: 150*,69,41,81,79,82,53	0.36	0.25	0.83	1.17
p-Cymen-8-ol	0	0.45	0.84	1.15
Decan-2-one	0.21	0.31	0.74	1.09
γ-Terpinene	0.23	0.24	0.6	1.05
β-Bourbonene	0.37	0.15	0.87	1.04
γ-Heptalactone	0.43	0	0.73	1.03
Humulene	0.19	0.29	0.76	1.03
m/z: 126*,98,55,83,11,84,43	0	0.36	0.73	0.99
Terpinolene	0.04	0.35	0.53	0.98
γ-Nonalactone	0.28	0.26	0.89	0.97
Pentanoic acid	0.07	0.3	0.68	0.9
o-Cymene	0	0.4	0.49	0.89
Hexane-2,5-dione	0.12	0.26	0.58	0.88
Nonanol	0	0.33	0.61	0.81
Undecan-2-one	0	0.33	0.5	0.8
δ-Hexalactone	0.25	0.1	0.64	0.8
β-Caryophyllene oxide	0.09	0.27	0.82	0.77
2-Hydroxybenzaldehyde	0.14	0.16	0.55	0.75
(E)-Oct-2-enal	0.19	0.12	0.51	0.7
(Z)-Hex-en-1-yl butyrate	0.15	0.18	0.59	0.7
p-Methoxystyrene	0	0.22	0.47	0.7
(E)-Hex-2-enal	0.15	0.13	0.42	0.67
Nonanoic acid	0.08	0.22	0.52	0.66
m/z: 134*,119,91,65,120,92,98	0.08	0.2	0.69	0.66
1-Heptanol	0.03	0.19	0.46	0.65
Guaiacol	0.26	0	0.61	0.65
6-Methylheptan-2-one	0	0.26	0.5	0.64
Decanal	0.22	0.07	0.51	0.63
Myrtenol	0	0.28	0.75	0.63
5,5-Dimethyl-2(5H)-furanone	0.06	0.21	0.55	0.62
m/z: 204*,91,105,93,107,79,119	0	0.29	0.5	0.62
4-Methylpentanoic acid	0	0.25	0.5	0.62
α-Terpineol	0	0.28	0.72	0.62
Methyl caproate	0	0.27	0.49	0.6
m/z: 117*,91,90,43,89,65,118	0.09	0.19	0.62	0.6
Eucalyptol	0.02	0.22	0.41	0.6
α-Muurolene	0.07	0.21	0.69	0.59
m/z: 74, 87, 41, 43, 55	0	0.24	0.45	0.58
m/z: 204*,91,119,55,105,41,161	0	0.24	0.71	0.57
m/z: 204*,91,43,93,119,84,82	0	0.26	0.58	0.57
m/z: 43,80,79,39,41,77,81	0.19	0.04	0.55	0.56
m/z: 204*,91,93,105,107,79	0.02	0.24	0.51	0.54

m/z: 43,57,98,55,58,71	0	0.17	0.44	0.53
(Z)-Hex-3-en-1-yl isovalerate	0.07	0.17	0.58	0.53
m/z: 96,43,95,67,68,81	0.22	0	0.38	0.52
Pinocarvone	0	0.23	0.49	0.51
(Z)-Dec-2-enal	0.2	0	0.56	0.49
m/z: 204*,92,91,55,83,105,70	0	0.21	0.47	0.47
Decanol	0	0.19	0.5	0.47
m/z: 55,83,97,69,41,57,56	0.19	0	0.6	0.46
m/z: 82,81,43,54,39,55,53	0.19	0	0.42	0.46
m/z: 43,55,58,71,95,85,99	0.2	0	0.53	0.46
6-Methyl-5-hepten-2-ol	0.19	0	0.36	0.44
Ethyl 4-ethoxybenzoate	0.19	0	0.58	0.44
m/z: 43,67,41,57,68,86	0.17	0	0.31	0.43
Geranyl acetone	0.17	0	0.57	0.43
m/z: 204*,91,93,105,79,119,107	0	0.2	0.48	0.42
Decanoic acid	0	0.17	0.45	0.42
Amyl acetate	0.17	0	0.34	0.4
α-Bergamotene	0	0.13	0.42	0.39
Dodecan-2-one	0	0.16	0.46	0.38
m/z: 204*,105,119,93,120,91,92	0.03	0.15	0.5	0.38
m/z: 79,110,95,77,94,67	0	0.14	0.62	0.37
m-Cresol	0	0.12	0.42	0.37
Germacrene D	0.04	0.11	0.45	0.37
α-Copaene	0.06	0.11	0.37	0.36
m/z: 204*,105,161,91,109,119,81	0	0.16	0.5	0.36
(Z)-Hex-3-en-1-yl propanoate	0	0.13	0.42	0.35
endo-Borneol	0	0.16	0.49	0.34
Benzyl acetate	0.15	0	0.41	0.34
3,5-Dimethyloctan-2-one	0	0.14	0.38	0.34
Dodecane	0	0.14	0.37	0.33
Decane	0	0.14	0.36	0.32
m/z: 43,95,58,71,55,85,41	0.02	0.09	0.39	0.32
m/z: 41, 43, 57, 55, 71	0.13	0	0.29	0.31
m/z: 95,43,82,109,67,111	0.13	0	0.32	0.3
(Z)-β-Caryophyllene	0.05	0.06	0.43	0.29
Oct-7-enoic acid	0	0.12	0.42	0.29
Octan-2-ol	0	0.11	0.45	0.29
Undecane	0	0.12	0.36	0.29
m/z: 43, 95, 67, 55, 41, 107	0.12	0	0.37	0.29