

https://dspace.org/

Avian frugivory of Cinnamomum camphora in an urban environment: visiting bird species and the implications for seed dispersal and seedling emergence

McGowan, Lauren M.

2019
https://hdl.handle.net/10539/29439

Downloaded from a DSpace repository. https://dspace.org/

Avian frugivory of *Cinnamomum camphora* in an urban environment: visiting bird species and the implications for seed dispersal and seedling emergence

---

Lauren M. McGowan

682497

A Dissertation submitted to the Faculty of Science,

University of the Witwatersrand,

Johannesburg, South Africa

in fulfilment of the requirements for the degree of Master of Science

August 2019

Table of Contents

DECLARATION i
ABSTRACT ii
Keywords: iv
ACKNOWLEDGEMENTS v
GLOSSARY OF TERMS and ABBREVIATIONS vi
CHAPTER ONE 1
References 13
CHAPTER TWO 22
THE ROLE OF VISITING BIRD SPECIES, FRUIT PRODUCTION AND RIPENESS ON POTENTIAL SEED DISPERSAL OF *CINNAMOMUM CAMPHORA* IN AN URBAN ENVIRONMENT
Abstract 22
Keywords: 23
Introduction 23
Methods 27
Results 35
Discussion 45
Conclusion 50
References 50
Appendix 1. 59
CHAPTER THREE 60
ARE BIRDS NECESSARY? THE ROLE OF BIRDS IN SEEDLING EMERGENCE OF *CINNAMOMUM CAMPHORA* IN AN URBANISED ENVIRONMENT
Abstract 60
Keywords 61
Introduction 61
Methods 67
Results 72
Discussion 81
Conclusion 91
References 92
CHAPTER FOUR 100
References 108

DECLARATION

I declare that this Dissertation is my own work. It is being submitted for the requirements of a Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any other University or tertiary institution.



Lauren M. McGowan

14 August 2019

Supervisors:

Prof. Craig T. Symes (*University of the Witwatersand*)

Prof. Ed T. F. Witkowski (*University of the Witwatersand*)

ABSTRACT

Alien invasive plant species have become a major threat to biodiversity worldwide. Through mutualistic relationships with local frugivorous bird species, many fleshy-fruited invasive plant species have established populations that have become difficult to monitor and control.

Cinnamomum camphora (L.) Presl. (Laureceae) is an alien invasive plant species found across many countries including Australia and South Africa. *Cinnamomum camphora* has been classified as different invasive categories in only five of the nine provinces in South Africa. *Cinnamomum camphora* has been classified as Category 1b in four provinces (Eastern Cape, Limpopo, KwaZulu-Natal) and Category 3 in the Western Cape (with the exception of 'Champion trees'. However, *C. camphora* has not been categorised in the remaining four provinces (Gauteng, North West, Northern Cape and Mpumalanga), leading to the possibility of the species becoming highly invasive without correct monitoring and control of already established populations. *Cinnamomum camphora* is adapted to growth in disturbed habitats, meaning that dispersal is possible within urban environments. This study aimed to investigate how bird species responded to the production and ripeness of *C. camphora* fruit, as well as the implications for seed dispersal and seedling emergence.

Bird observations and fruit production and ripeness sampling showed that there was an increase in the number of visiting birds as fruit production increased during the fruiting season (February to June) and as a higher proportion of fruit became ripe. The primary bird visitor species were a combination of both seed predators and legitimate seed dispersers with 35.3% and 52.9% of bird observations respectively. The most common bird was the Rock Dove (*Columba livia*), a known seed predator, with 26.4%, while the most common seed

disperser was the Karoo Thrush (*Turdus smithi*) with 19.1 % of total bird observations. Primary activities consisted of birds consuming *C. camphora* fruit and birds found resting in *C. camphora* trees.

Seedling emergence testing in a greenhouse trial showed that *C. camphora* seeds that remained within intact fruit were still able to successfully emerge (and hence had germinated in the soil) even when previous studies suggested otherwise. Percentage seedling emergence over the 12 month greenhouse trial period for the four seed/fruit stages were 22.6%, 38.3%, 40.8% and 38.2% for the unripened fruit, ripened fruit, shed fruit and consumed fruit stages respectively. Seedlings from seeds that had been ingested and voided by birds took significantly longer to emerge compared to seeds that had been within intact fruit stages (ripened, shed and unripened). Unripened fruit took a significantly shorter time for seedlings to emerge than the other three stages. Furthermore, consumed seeds planted in both April and May of 2016 took a significantly longer time to emerge than the other three fruit stages.

Seed viability using tetrazolium on the remaining seeds in the soil showed that only 15.5% of all *C. camphora* seeds remained viable after 12 months of the seedling emergence trial. The majority of seeds were either unviable, 29%, or had decomposed due to factors such as fungal infections or predation by insect larvae. However, while the seed viability was not significantly different between the four fruit/seed stages, bird ingestion resulted in a slower rate of emergence, indicating that birds reduced the overall seed vigour of *C. camphora*. Overall it was found that birds served mainly as *C. camphora* dispersal agents and not as both dispersers and germination mediators, except that birds lowering the rate of seedling emergence, not percentage emergence. This makes *C. camphora* populations potentially difficult to control and monitor, especially within urban environments, and suggests that the populations are likely to spread further via bird dispersal.

Keywords: Avian frugivory, bird-plant interactions, disperser effectiveness, frugivory, fruiting phenology, germination, invasive plant species, seed dispersal effectiveness, seed predators, seed viability, seed vigour, seedling emergence.

ACKNOWLEDGEMENTS

This master's project has been a huge undertaking for me for the past few years and I know that I would have not been able to complete it if it was not for many people helping me every step of the way.

To my supervisors, Prof. Craig Symes and Prof. Ed Witkowski, thank you for helping me along this massive journey. Thank you Prof. Symes for your support and guidance for the majority of my degree, and thank you Prof. Witkowski for taking over as my supervisor towards the end of 2017. Thank you for supporting me in your own ways and for the suggestions and guidance you gave me through the course of my write up. Thank you for having to put up with my delays and for pushing me every time I began to procrastinate. This dissertation would have never seen completion without your input and support.

To my mother and father, thank you for all of your love and support throughout the past few years. Thank you for encouraging me to continue with my work and for always asking how everything is going. Thank you especially for assisting me in travelling to and from the campus over the weekends and holidays in order to capture both my observation and seedling emergence data. To Candice Neves, thank you for constantly checking in, and encouraging my progress without judgement, and ensuring that I was not falling behind on my due dates. To Tamryn Hamilton, thank you for being my travel buddy for early morning observation days and for being my assistant when it came to measuring the tree sizes of my sample set. Finally to Stephanie Payne, thank you for allowing me to read through your dissertation in order to get a sense of what would be required of me and how to best present my work, and thank you for your help way back towards the start of this study,

GLOSSARY OF TERMS and ABBREVIATIONS

Champion Trees – Alien tree species (individuals or groups) that are protected under Section 12 of the National Forestry Act of 1998 due to their aesthetic, cultural, historic or tourism value.

DBH – 'Stem Diameter at Breast Height', standard measurement when determining tree diameter, measured at 1.37m.

Endozoochory – Process in which seeds are dispersed by dispersal species after ingestion either through regurgitation or excretion.

Exozoochory – Dispersal method of seeds through adhesion to the feathers or fur of a dispersal agent.

IAPs – 'Invasive Alien Plants'

ISTA – 'International Seed Testing Association'

Legitimate Seed Disperser – Species that consume entire fruits followed by dispersal of viable seeds through endozoochory.

Polyphagy – the ability of an animal species to have a variety of foods as part of its diet.

SDE – 'Seed Dispersal Effectiveness'

Seed Predator – Species that destroy seeds or seed embryos through mandibulation or gizzard action.

CHAPTER ONE

GENERAL INTRODUCTION

Alien invasive species

Invasive alien plant (IAP) species make up a large, important component of global change (Jeschke *et al*. 2013; Amodeo *et al*. 2017). Invasive alien plant species invade new environments, sometimes prior to, more often after the loss of any indigenous plant species and often replace any remaining species as the primary food source to local dispersers (Buckley *et al.* 2006; Gosper and Vivian-Smith 2006; Jordaan *et al*. 2011). Disturbed habitats can result in the decline of herbivores, frugivores and pollinators (Chupp and Battaglia 2016). The mutualisms between native plants and their dispersers are interrupted with these declines and can lead to native species becoming locally extinct while invasive species can colonize and establish new relationships with the remaining indigenous species (Chupp and Battaglia 2016).

South Africa's Mediterranean-climate region is second only to Australia for the number of invasive alien plant species (Richardson and Rejmánek 2011; Mokotjomela *et al*. 2013a). South Africa has been largely invaded by fleshy-fruited invasive plant species that are dispersed by both native and non-native bird species (Jordaan *et al*. 2011; Wilson and Downs 2012; Mokotjomela *et al*. 2013a; Thabethe *et al*. 2015b). The management of fleshy-fruited invasive plant species has become one of the main challenges when it comes to biodiversity conservation (Buckley *et al*. 2006; Amodeo *et al*. 2017). Management measures that have been attempted in South Africa include preventing more of the species being imported into the country, regulation of the use of alien species, and eradication methods for species that have low populations over a limited number of areas, while species that have

become well-established have their impacts reduced (Zengeya *et al*. 2017).Understanding and predicting the invasiveness of alien species has become a high priority within invasion science (Pyšek *et al.* 2015; Gioria and Pyšek 2017).

Numerous invasive alien plant species are fleshy-fruited and rely on mutualistic relationships with local native and non-native disperser species for dispersal, resulting in increased invasiveness, hence and to overcoming barriers that may otherwise prevent them from colonising new habitats (Cronk and Fuller 1995; Richardson *et al.* 2000; Richardson and Pyšek 2006; Jordaan *et al*. 2011; Amodeo *et al*. 2017; Sebastián-González 2017), while the disperser species receives resources for nesting and energetic rewards in the form of fruit from the plant species (Thabethe *et al*. 2015b).

Seed dispersal is an important part of an invasive plant species' life cycle as it is the process in which seeds are successfully dispersed by abiotic vectors such as wind and water, or biotic vectors such as animals, either via endozoochory (within – via consumption) or ectozoochory (on the outside, e.g. attached to fur or feathers) dispersal (Wenny *et al*. 2016). Seed dispersal effectiveness (SDE) is the contribution that a disperser species makes towards the reproduction of a plant species and is made of two components, qualitative and quantitative (Schupp 1993; Roxburgh 2007; Schupp *et al.* 2010; Mokotjomela *et al*. 2016). The qualitative aspect of SDE refers to the treatment that a seed receives from both the disperser and the microhabitat into which the seed is dispersed (Roxburgh 2007; Schupp *et al.* 2010), while the quantitative component refers to the number of dispersers visiting a plant species and the number of seeds that species disperses (Schupp 1993; Roxburgh 2007; Schupp *et al.* 2010). Both of these components are influenced by both frugivore and fruit characteristics.

Fruit characteristics

Invasive plant species show an advantage over native plant species due to various fruit traits that influence a frugivores' fruit choice (Wenny *et al*. 2016). Avian frugivores have been shown to prefer different plant species depending on a variety of traits, such as fruit nutritional composition, fruit size, colour, availability and accessibility, and seed size and load (Thabethe *et al*. 2015a). These traits impact the selection of invasive plant species over native plant species by either attracting and encouraging bird species to consume the fruits of the invasive plants, or cause the birds to leave the fruiting plants shortly after consuming a few fruits (Gosper *et al*. 2005), thereby influencing the rates of spread for those species (Buckley *et al*. 2006; Westcott and Fletcher 2011; Mokotjomela *et al.* 2013a; Thabethe *et al*. 2015a). Fruit traits that increase either the probability and/or the quality of seed dispersal must be included when determining the likelihood, or potential extent of plant invasions (Gosper *et al*. 2005; Westcott and Fletcher 2011). Several studies have been conducted on the comparisons between fruit consumed and fruits available in a habitat (Izhaki 2002; Bradford *et al*. 2008; Kunz and Linsenmair 2010; Westcott and Fletcher 2011).

The most important fruit traits that influence frugivore fruit choice are those focused on fruit crop size and fruiting phenology of invasive plant species (Gosper 2004; Gosper and Vivan-Smith 2010; Westcott and Fletcher 2011). Fruit density, accessibility and the crop size of invasive plant species are often investigated to determine whether there is any variation or difference compared to native plant species (Gosper *et al*. 2005). Alien plant species that produce crops that have a large abundance of conspicuous fruits often attract a wide variety of frugivorous species and are the most heavily used species (Knight 1986; Westcott and Fletcher 2011; Mokotjomela *et al*. 2013a; Gioria and Pyšek 2017).

Furthermore, the timing of fruit production and period in which the fruit is available can determine the abundance of dispersers and their behaviour towards their fruit choices (Gosper *et al*. 2005). Invasive plant species have distinct phenology patterns that allows for specific frugivores to be attracted (Gosper *et al*. 2005), which often includes attractive fruiting displays over longer periods of time and also during lean times when fruit abundance is usually low (Gosper 2004; Gosper and Vivan-Smith 2010; Westcott and Fletcher 2011; Wenny *et al*. 2016), with some species even fruiting year round (Thabethe *et al*. 2015a).

Traits of the fruit itself are also important in terms of influencing fruit selection by birds (Westcott and Fletcher 2011). The size of the fruit is one of these important traits (Sallabanks 1993; Corlett 2005; Chupp and Battaglia 2016; Wenny *et al*. 2016), as plant species that have large fruits limits the number of frugivorous species, with large enough gapes to hold and swallow the fruit, that are attracted to the plant (Carnicer *et al.* 2008; Chamberlain and Holland 2009; Burns 2013; Wenny *et al*. 2016; Sebastián-González 2017). Studies found that plants, both native and invasive, that have fruit sizes which are compatible with a bird species gape size are able to attract a large variety of species (especially frugivorous species) for successful dispersal (Gosper *et al*. 2005; Burns 2013; Chupp and Battaglia 2016; Sebastián-González 2017), as fruit handling time is shortened therefore leading to more fruit and subsequently seeds being consumed and potentially dispersed (Chupp and Battaglia 2016). Native and invasive plant species with fruits smaller than 15mm were found to attract a larger diversity and number of bird species (Gosper *et al*. 2005). Smaller fruit also means smaller seed sizes, which helps with effective dispersal as the seeds of smaller invasive plant species are able to be readily consumed and dispersed by a wide variety of frugivorous bird species, instead of being discarded and dropped beneath the parent plant (Gosper *et al*. 2005; Wenny *et al*. 2016).

Bird species have brilliant colour vision (Knight 1986; Gosper *et al*. 2005), and so bird-dispersed plants have visual displays of small, brightly coloured propagules that possess a thin fruit skin and remain attached to the parent plant at maturity (Herrera 2002; Vander Wall and Beck 2012). Nearly 30% of invasive plant species that are mainly dispersed by birds have black, red or blue fruits (Chupp and Battaglia 2016). Invasive alien plant species within South Africa make use of these visual cues in order to attract more frugivores away from neighbouring native plants that are fruiting at the same time (Knight 1986; Mokotjomela *et al*. 2013a), as well as to increase the fruit removal rate and seed dispersal of the IAP species (Gosper *et al*. 2005).

Many alien plants which have colourful fruit displays that are high in numbers also have increased nutritional value compared to competing native species (Jordaan and Downs 2012; Mokotjomela *et al*. 2013a; Wenny *et al*. 2016). Birds prefer fruits that are associated with increased nutritional rewards (Chupp and Battaglia 2016).Some studies found that invasive plant species have higher energy, lipid or protein content (Gosper 2004; Kueffer *et al*. 2009; Gosper and Vivian-Smith 2010) and lower seed loads (Corlett 2005). However, there are also studies that contrast this, with smaller fruits and lower lipid content being reported (Gosper and Vivian-Smith 2010). Fruit traits such as the ratio of pulp to indigestible seeds can affect the choices that birds make (Gosper *et al*. 2005).

Disperser characteristics

Traits of visiting frugivores are also important in terms of the dispersal quality and quantity of invasive alien species (Buckley *et al.* 2006; Westcott and Fletcher 2011). Quantitative traits includes factors such as gape size (Chupp and Battaglia 2016; Wenny *et al*. 2016; Sebastián-González 2017), number of dispersers and dietary constraints placed on the

frugivore (Buckley *et al.* 2006), while qualitative traits include frugivore behaviour, seed passage time, digestive physiology and body size (Schupp *et al.* 2010; Amodeo *et al*. 2017).

Bird species can be divided into three forms of fruit handling behaviour, legitimate seed dispersers, seed discarders and seed predators. Legitimate seed dispersers are bird species that swallow entire fruits or a portion of the fruit that contains the seed (Herrera 1984; Levey 1987; Schupp 1993; Jordano and Schupp 2000; Gosper *et al*. 2005; Díaz Vélez *et al*. 2018). Fruits are handled very little before consumption by dispersers (Herrera 1984; Levey 1987; Schupp 1993; Díaz Vélez *et al*. 2018), and viable seeds remain in the disperser's gut for a time before being regurgitated or excreted (Herrera 1984; Díaz Vélez *et al*. 2018). This leads to a high seed dispersal for seeds that have been consumed (Gosper *et al*. 2005; Díaz Vélez *et al*. 2018).

Seed discarders separate the seed from fruit during fruit handling, either from pressing the fruit against a branch or by fruit remains staying attached to a feeding perch (Levey 1987; Schupp 1993; Jordano and Schupp 2000; Gosper *et al*. 2005; Díaz Vélez *et al*. 2018). Seed shadows formed by these bird species are relatively small as seeds are often discarded beneath the source plant, or in rare cases, close to the source plant as fruit is carried to nearby trees and fallen seeds are vulnerable to secondary predation (Jordano and Schupp 2000; Gosper *et al*. 2005; Díaz Vélez *et al*. 2018).

Seed predator species are detrimental towards effective seed dispersal, as seeds are destroyed during various processes (Herrera 1984; Jordano and Schupp 2000; Gosper *et al*. 2005; Thabethe *et al*. 2015b; Díaz Vélez *et al*. 2018). Seeds can be destroyed during handling in the bill while the fruit pulp is consumed (the case with some parrot species), through action within the gizzard (found with some pigeons, especially *Columba* species) or by the seed contents being consumed directly by the predator species (Jordano and Schupp 2000; Gosper

et al. 2005; Díaz Vélez *et al*. 2018). However, there are rare cases in which seeds can escape damage and be successfully dispersed by seed predators (Carrion-Tacuri *et al*. 2012; Mokotjomela *et al*. 2015; Thabethe *et al*. 2015b; Mokotjomela *et al*. 2016).

Bird movement after foraging (for species that deposit undamaged seeds) is important in terms of invasive plant spread (Gosper *et al*. 2005). Birds that remain in a parent plant after feeding would be less effective as a successful disperser than those that leave the parent plant shortly after foraging (Gosper *et al*. 2005). Smaller seed dispersers were found to spend less time in invasive plants than larger dispersers or seed predators in south-east Queensland, Australia (Stansbury and Vivian-Smith 2003).

Disperser effects on germination

Frugivorous dispersers can have a variety of behaviours which influence germination either from ingestion, processing or defecation (Amodeo *et al*. 2017). These processes may either increase, decrease or have no effect on germination (Traveset 1998; Traveset *et al*. 2007; Thabethe *et al*. 2015b; Mokotjomela *et al*. 2016; Amodeo *et al*. 2017).

Frugivores are able to change the physical properties of the seeds of some plant species' facilitates germination, such as the removal of the fruit pulp (Fricke *et al*. 2016). Numerous studies have shown that germination is more successful after ingestion has occurred (Jordaan *et al*. 2011a; Thabethe *et al*. 2015b; Mokotjomela *et al*. 2016). Dispersers are often thought to enhance the germination of various plant species through pulp removal (de-inhibition) or through seed coat modification (scarification) (Yagihashi *et al*. 1998, Thabethe *et al*. 2015b), as there are only a few instances in which the seeds of various plant species' failed to germinate without the assistance of being ingested by dispersers (Temple 1977; Yagihashi *et al*. 1998). However, some studies have found that ingested seeds can actually lose viability, leading to a reduced to no net effect on germination success (LaRosa

1985; Wilson and Downs 2012; Thabethe *et al*. 2015b). Frugivores which do not disperse seeds away from the parent plant but still remove the seed from the fruit pulp, increase the seed's survival compared to seeds remaining within the fruit pulp (Fedriani *et al.* 2012; Fricke *et al*. 2016).

Therefore, the role of bird dispersers in the process of invasion should not be underestimated when it comes to the amount or number of fruit eaten during a visit and the subsequent 'processed seeds' (Jordaan *et al*. 2011a). Understanding the mechanisms and pathways by which IAP species establish in foreign countries is important as this information can assist in either the species eradication or management to control their spread, as well as an aid in biological control efforts (D'Avila *et al*. 2010; Dlamini *et al*. 2018).

Cinnamomum camphora

Cinnamomum camphora (L.) is an evergreen, fleshy fruited tree species native to subtropcial forests of China, as well as Vietnam, Japan and Korea (Chen *et al*. 2004; Kameyama and Nakajima 2018). It has become invasive in parts of England, Australia, parts of the United States of America, such as Florida (Firth 1981; Chen *et al.* 2004), and in South Africa (Invasive Species Compendium 2015; Anonymous 2019).

Cinnamomum camphora can be found in abundance in disturbed habitats such as along roadsides and in developed areas; although large fruiting individuals have also been noted infrequently in undisturbed forested habitats (Chupp and Battaglia 2016). Bird species have preferences for *C. camphora* in Asia and Australia, but North American bird species were found to not have a preference for *C. camphora* (Chupp and Battaglia 2016). *Cinnamomum camphora* has round shaped fruits that can range between 5-10mm (diameter) in size and are dark-blue in colour (Chupp and Battaglia 2016). In North America, *C. camphora* was tested as a substitute resource for native frugivorous bird species as the

abundance of a similar native species, *Persea borbonia*, was decreasing due to laurel wilt disease within the same habitat (Chupp and Battaglia 2016). Seedlings of *C. camphora* were seldom found during transect surveys in certain forested habitats, indicating that there was a limit to long-distance seed dispersal (Chupp and Battaglia 2016).

Three frugivorous bird species in South Afirca, namely Red-winged Starlings (*Onychognathus morio*), Dark-capped Bulbuls (*Pycnonotus tricolor*), and Speckled Mousebirds (*Colius striatus*), were able to maintain their body masses and meet their daily energetic requirements (to varying degrees) from fruits of *C. camphora* (Jordaan *et al*. 2011), which have a relatively low sugar content and high lipid content (Thabethe *et al*. 2015a). These species of birds have overlapping ranges with invasive *C. camphora* and can be found across South Africa, leading to these species being potential top dispersers of *C. camphora* (Henderson 2001; Hockey *et al*. 2005; Jordaan *et al*. 2011). These overlapping ranges of dispersers and *C. camphora* also indicates an increase of both the spread and invasion of *C. camphora* across South Africa compared to an invasion without the asssistance of numerous native species.

Rationale for investigating frugivory and seed dispersal in *Cinnamomum camphora*

Invasion sciences have often had research done within natural and semi-natural ecosystems (Richardson and Pyšek 2006; Gaertner *et al*. 2017). However, this same science is limited when it comes to urban areas, such as cities, including information about the introduction, establishment and spread of alien species (Gaertner *et al*. 2017; Salomon Cavin and Kull 2017). Urban development, ever increasing as the human population does, results in transformed and fragmented natural landscapes and often leads to negative effects for native organisms, such as the introduction of alien species (Hulme 2009; Herrera *et al*. 2011; Westcott and Fletcher 2011; Coetzee *et al.* 2018). Not only has human activity influenced the

likelihood of an introduction of an exotic species, but an increased rate of disturbance of a recipient habitat also increases the chance of naturalisation of an exotic species (Pyšek *et al*. 2010; Herrera *et al*. 2011; Westcott and Fletcher 2011). These fragmented ecosystems present new challenges for the determination and management of biological invasions (Gaertner *et al*. 2017). However, due to the lack of ecological and conservational value they may bring, cities and other urban areas are often left out of high priority invasion management schemes (Gaertner *et al*. 2017).

Invasive alien species within South Africa can be classified according to whether their negative impacts on the environment outweigh their socio-economic benefits (Zengeya *et al*. 2017). A species is classified as a 'beneficial' species when the socio-economic benefits, such as tourism, are more prominent than negative environmental impacts (Zengeya *et al*. 2017). *Cinnamomum camphora* has been classified as one such species (Zengeya *et al*. 2017). *Cinnamomum camphora* was introduced to Cape Town, South Africa as early as the late 1600s (Anonymous 2019), where several individuals were planted within the Vergelegen Estate (DAFF 2016; Zengeya *et al*. 2017). These individuals have become known as national heritage trees or 'champion trees' and have therefore not been listed in the Alien and Invasive Species (A&IS) Regulations and are protected under Section 12 of the *National Forests Act* of 1998 (DAFF 2016; Zengeya *et al*. 2017).

Apart from these individual 'champion trees', *C. camphora* trees as a whole are being controlled and/or regulated in areas where there are no listed champions (Zengeya *et al*. 2017). This has lead to the species becoming classified differently depending on the province where populations have been registered, with the species classified as Category 1b in four of the five provinces in which it listed (Eastern Cape, Limpopo, KwaZulu-Natal and Mpumalanga) (Invasive Species Compendium 2015; Zengeya *et al*. 2017), and as Category 3

in the Western Cape, where the species can be utilised, but not sold or distributed (Invasive Species Compendium 2015; Zengeya *et al*. 2017).

However, *C. camphora* populations in the remaining four South African provinces (Gauteng, Free State, Northern Cape and the North West) are unlisted in the A&IS Regulations (Invasive Species Compendium 2015; Zengeya *et al*. 2017). This means that *C. camphora* within these four provinces is not being monitored or regulated, leading to the potential that the species could form uncontrollable populations, in which the benefits of having this species is likely to soon be outweighed by the negative impacts. This is reinforced by Jordaan *et al*. (2011) who determined that invasive alien plant species within South Africa (including *C. camphora*) are able to germinate readily and when combined with bird dispersal, especially when birds are not necessary for successful germination. Similarly, Mokotjomela (2013b) and Thabethe *et al*. (2015b) found that frugivorous avian dispersers make controlling IAP problematic as they are able to facilitate rapid spread of those alien species.

When *C. camphora* in unmonitored provinces also occur within urban environments that are not highly prioritised in terms of invasion sciences and management, there becomes a possibility that any established or emerging populations could be dispersed out to surrounding semi-natural and natural environments and form new, potentially uncontrollable populations that can have highly negative impacts on the environment.

Understanding the ecological principles that lead to invasive succession, including dispersal methods and rapid growth effects, is important in determining the development and implementation of management strategies (Thabethe *et al*. 2015b). Through the use of dispersal ecology, we can work on predicting *C. camphora*'s invasion behaviour within urban ecosystems by observing the movement patterns of dispersers, and then predicting potential

dispersal from these data (Westcott and Fletcher 2011). Using the results from seed germination and viability trials, seed shadows can be predicted for *C. camphora* by combining the seedling emergence data with data collected on which dispersers visit the trees, how long those dispersers retain the seeds, and the movement of those dispersers through the urban environment (Bartuszevige and Gorchov 2006). The gathering of these types of data will help in determining the relationship between *C. camphora* and dispersers (native and non-native) and the resulting seed dispersal effectiveness within an urban environment.

Aims and objectives

The aim of this study was to investigate the role of different frugivorous bird species on fruit consumption, and subsequent seed dispersal and potential seedling establishment of *C. camphora* within an urban environmnet.

The first objective of this study, which is the focus of Chapter Two, was to determine potential seed dispersal of *C. camphora* within an urban environment in relation to the specific visiting bird species, as well as fruit production and ripeness. This was done through calculation of the fruit production and ripeness of twelve *C. camphora* trees found within the University of Witwatersrand, Johannesburg for the duration of the 2016 fruiting season and the responses of the various visiting bird species. These twelve trees were within an environement that was heavily influenced by many anthropogenic structures and buildings, with sections of both invasive and native plants scattered throughout the campuses. Further observations were conducted to identify the primary visiting bird species and whether legitimate seed dispersers or seed predators were more abundant, and the interactions between these species and *C. camphora*.

The second objective, the focus of Chapter Three, investigated germination ecology of *C.* camphora, especially the effects of bird ingestion, on the rate and numbers of seedlings emerging in a greenhouse trial. Thereafter, seeds that had not emerged during the 12 month trial period were chemically tested for seed viability and these results compared between the four fruit/seed stages. The four fruit/seed stages are: i) Consumed seeds, ii) Ripened fruit, iii) Shed fruit and iv) Unripened fruit. The integration and synthesis of the combined results from chapters two and three will help towards predicting the future rate of invasive spread of *C. camphora* and whether the species will have enhanced seed dispersal and hence distribution within urban environments. The two data chapters, Chapter Two and Chapter Three, have been write in the format of journal papers.

References

Amodeo, M. R., Vázquez, M. B. and Zalba, S. M. 2017. Generalist dispersers promote germination of an alien fleshy-fruited tree invading natural grasslands. *PLoS ONE* 12(2): 1-17.

Anonymous. 2019. Cinnamomum camphora. *Howling Pixel.* (https://howlingpixel.com/i-en/Cinnamomum_camphora#cite_note-usgs-10) Accessed 24 June 2019.

Bartuszevige, A. M. and Gorchov, D. L. 2006. Avian seed dispersal of an invasive shrub. *Biological Invasions* 8: 1013-1022.

Bradford, M. G., Dennis, A. J. and Westcott, D. A. 2008.Diet and dietary preferences of the southern cassowary (*Casuarius casuarius*) in North Queensland, Australia. *Biotropica* 40: 338-343.

Buckley, Y. M., Anderson, S., Catterall, C. P., Corlett, R. T., Engel, T., Gosper, C. R., Nathan, R., Richardson, D. M., Setter, M., Spiegel, O., Vivian-Smith, G., Voigt, F. A., Weir, J. E. S. and Westcott, D. A. 2006. Management of plant invasions mediated by frugivore interactions. *Journal of Applied Ecology* 43: 848-857.

Burns, K. C. 2013. What causes size coupling in fruit-frugivore interaction webs? *Ecology* 94: 295-300.

Carnicer, J., Brotons, L., Sol, D. and De Cáceres, M. 2008. Random sampling, abundance extinction dynamics and niche-filtering immigration constraints explain the generation of species richness gradients. *Global Ecology and Biogeography* 17: 352 362.

Carrion-Tacuri, J., Berjano, R., Guerrero, G., Figueroa, E., Tye, A. and Castillo, J. M. 2012. Predation on seeds of invasive *Lantana camara* by Darwin's finches in the Galapagos. *Wilson Journal of Ornithology* 124: 338-344.

Chamberlain, S. A. and Holland, J. N. 2009. Body size predicts degree in ant-plant mutualistic networks. *Functional Ecology* 23: 196-202.

Chen, Z.-H., Wu, B., Li, J.-Y., Zhao, J.-G., Zhou, X.-Y. and Zhang, Y.-K. 2004. Germination of the seeds and growth of the seedlings of *Cinnamomum camphora* (L.) Presl. *Plant Species Biology* 19: 55-58.

Chupp, A. D. and Battaglia, L. L. 2016. Bird-plant interactions and vulnerability to biological invasions. *Journal of Plant Ecology* 9: 1-11.

Coetzee, A., Barnard, P. and Pauw, A. 2018. Urban nectarivorous bird communities in Cape Town, South Africa, are structured by ecological generalisation and resource distribution. *Journal of Avian Biology* 49(6): 1-11.

Corlett, R. 2005. Interactions between birds, fruit bats and exotic plants in urban Hong Kong, South China. *Urban Ecosystem* 8: 275-283.

Cronk, Q. C. B. and Fuller, J. L. 1995. *Plant Invaders.* London: Chapman and Hall.

D'Avila, G., Gomes-Jr, A., Canary, A. C. and Bugoni, L. 2010. The role of avian frugivores on germination and potential seed dispersal of the Brazilian pepper *Schinus terebinthifoloius*. *Biota Neotropica* 10: 45-51.

Department of Agriculture, Forestry and Fisheries (DAFF). 2016. *Champion trees*, (http://www.daff.gov.za/daffweb3/Branches/Forestry-Natural-Resources-Management/Forestry-Regulation-Oversight/Sustainable-Forestry/Champion-Trees)

Díaz Vélez, M. C., Sérsic, A. N., Traveset, A. and Paiaro, V. 2018. The role of frugivorous birds in fruit removal and seed germination of the invasive alien *Cotoneaster franchetii* in central Argentina. *Austral Ecology* 43(5): 1-9.

Dlamini, P., Zachariades, C. and Down, C. T. 2018. The effect of frugivorous birds on seed dispersal and germination of the invasive Brazilian pepper tree (*Schinus terebinthifolius*) and Indian laurel (*Litsea glutinosa*). *South African Journal of Botany* 114: 61-68.

Fedriani, J. M., Zywiec, M. and Delibes, M. 2012. Thieves or mutualists? Pulp feeders enhance endozoochore local recruitment. *Ecology* 93: 575-587.

Firth, D. J. 1981. Camphora laurel (*Cinnamomum camphora*): a new weed in north-eastern New South Wales. *Australian Weeds* 1: 26-28.

Fricke, E. C., Haak, D. C., Levey, D. J. and Tewksbury, J. J. 2016. Gut passage and secondary metabolites alter the source of post-dispersal predation for bird-dispersed chili seeds. *Oecologia* 181: 905-910.

Gaertner, M., Wilson, J. R. U., Cadotte, M. W., MacIvor, J. S., Zenni, R. D. and Richardson, D. M. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. *Biological Invasions* 19: 3461-3469.

Gioria, M. and Pyšek, P. 2017. Early bird catches the worm: germination as a critical step in plant invasion. *Biological Invasions* 19: 1055-1080.

Gosper, C. R. 2004. Fruit characteristics of invasive bitou bush, *Chrysanthemoides monilifera* (Asteraceae), and a comparison with co-occurring native plant species. *Australian Journal of Botany* 52: 223-230.

Gosper, C. R., Stansbury, C. D. and Vivian-Smith, G. 2005. Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. *Diversity and Distributions* 11: 549-558.

Gosper, C. R. and Vivian-Smith, G. 2006. Selecting replacements for invasive plants to support frugivores in highly modified sites: a case study focusing on *Lantana camara.* *Ecological Management and Restoration* 7: 197-203.

Gosper, C. R. and Vivian-Smith, G. 2010. Fruit traits of vertebrate-dispersed alien plants: smaller seeds and more pulp sugar than indigenous species. *Biological Invasions* 12: 2153-2163.

Henderson, L. 2001. *Alien Weeds and Invasive Plants*. Cape Town: Agricultural Research Council.

Herrera, C. M. 1984. A study of avian frugivores, bird-dispersed plants, and their interaction in Mediterranean scrublands. *Ecological Monographs* 54(1): 1-23.

Herrera, C. M. 2002. Seed dispersal by vertebrates, in: Herrera, C. M. and Pellmyr, O. (Eds.), *Plant-Animal Interactions: An Evolutionary Approach*. Blackwell Publishing, Oxford, United Kingdom, pp. 185-208.

Herrera, J. M., Morales, J. M. and García, D. 2011. Differential effects of fruit availability and habitat cover for frugivore-mediated seed dispersal in a heterogeneous landscape. *Journal of Ecology* 99: 1100-1107.

Hockey, P. A. R., Dean, W. R. J. and Ryan, P. G. 2005. *Roberts' Birds of southern Africa*. Seventh edition. The Trustees of the John Voelcker Bird Book Fund, Cape Town.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10-18.

Invasive Species Compendium. 2015. *Cinnamomum camphora* (camphor laurel). Cabi.org,http://www.cabi.org/isc/datasheet/13519

Izhaki, I. 2002. The role of fruit traits in determining fruit removal in east Mediterranean ecosystems, in: Levey, D. J., Silva, W. R. and Galetti, M. (Eds), *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*. CAB International Publishing, Wallingford, pp. 161-175.

Jeschke, J., Keesing, F. and Ostfeld, R. 2013. Novel organisms: comparing invasive species, GMOs, and emerging pathogens. *Ambio* 42: 541-548.

Jordaan, L. A., Johnson, S. D. and Downs, C. T. 2011. Digestion of fruit of invasive alien plants by three southern African avian frugivores. *Ibis* 153: 863-867.

Jordaan, L. A. and Downs, C. T. 2012. Nutritional and morphological traits of invasive and exotic fleshy-fruits in South Africa. *Biotropica* 44: 738-743.

Jordano, P. and Schupp, E. W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* 70(4): 591 615.

Kameyama, Y. and Nakajima, H. 2018. Environmental conditions for seed germination and seedling growth of *Cinnamomum camphora* (Lauraceae): the possibility of regeneration in an abandoned deciduous broad-leaved forest, eastern Japan. *Journal of Forest Research* 23(3): 190-194.

Knight, R. S. 1986. Fruit displays of indigenous and invasive alien plant in the south-western Cape. *South African Journal of Botany* 52: 249-255.

Kueffer, C., Kronauer, L. and Edwards, P. J. 2009. Wider spectrum of fruit traits in invasive than native floras may increase the vulnerability of oceanic islands to plant invasions. *Oikos* 118: 1327-1334.

Kunz, B. K. and Linsenmair, K. E. 2010. Fruit traits in baboon diet: a comparison with plant species characteristics in West Africa. *Biotropica* 42: 363-371.

LaRosa, A. M., Smith, C. W. and Gardener, D. E. 1985. Role of alien and native birds in the dissemination of firetree (*Myricafava Ait.-Myriacaceae*) and associated plants in Hawaii. *Pacific Science* 39: 372-378.

Levey, D. J. 1987. Seed size and fruit-handling techniques of avian frugivores. *The American Naturalist* 129 (4): 471-485.

Mokotjomela, T. M., Musil, C. F. and Esler, K. J. 2013a. Frugivorous birds visit fruits of emerging alien shrub species more frequently than those of native shrub species in the South African Mediterranean climate region. *South African Journal of Botany* 86: 73 78.

Mokotjomela, T. M., Downs, C. T., Esler, K. and Knight, J. 2016. Seed dispersal effectiveness: a comparison of four bird species feeding on seeds of invasive *Acacia cyclops* in South Africa. *South African Journal of Botany* 105: 259-263.

Pyšek, P., Chytrý, M. and Jarošík, V. 2010. Habitats and landuse as determinants of plant invasions in the temperate zone of Europe, in: Perrings, C., Mooney, H. and Williamson, M. (Eds), *Bioinvasions and Globalization: Ecology, Economics, Management and Policy*. Oxford University Pres, Oxford, pp. 66-81.

Pyšek, P., Manceur, A., Alba, C., McGregor, K., Pergl, J., Štajerová, K., Chytrý, M., Danihelka, J., Kartesz, J., Klimešová, J., Lučanová, M., Moravcová, L., Nishino, M., Sádlo, J., Suda, J., Tichý, L. and Kühn, I. 2015. Naturalization of central European plants in North America: species traits, habitats, propagule pressure, residence time. *Ecology* 96: 762-774.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. and Rejmanek, M. 2000. Plant invasions – the role of mutualisms. *Biological Review* 75: 65-93.

Richardson, D. M. and Pyšek, P. 2006. Plant invasions-merging the concepts of species invasiveness and community invisibility. *Progress in Physical Geography* 30: 409-431.

Richardson, D. M. and Rejmánek, M. 2011. Trees and shrubs as invasive alien species – a global review. *Diversity and Distributions* 17: 788-809.

Roxburgh, L. 2007. The effect of gut processing on the quality of mistletoe seed dispersal. *Journal of Tropical Ecology* 23: 377-380.

Sallabanks, R. 1993. Fruit defenders vs fruit thieves – winter foraging behaviour in American robins. *Journal of Field Ornithology* 64: 42-48.

Saloman Cavin, J. and Kull, C. A. 2017. Invasion ecology goes to town: from disdain to sympathy. *Biological Invasions* 19: 3471-3487.

Schupp, E. W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. *Vegetatio* 107/108: 15-29.

Schupp, E. W., Jordano, P. and Gomez, J. M. 2010. Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* 188: 333-353.

Sebastián-González, E. 2017. Drivers of species' role in avian seed-dispersal mutualistic networks. *Journal of Animal Ecology* 86: 878-887.

Stansbury, C. D. and Vivian-Smith, G. 2003. Interactions between frugivorous birds and weeds in Queensland as determined from a survey of birders. *Plant Protection Quarterly* 18: 157-165.

Temple, S. A. 1977. Plant-animal mutualism: coevolution with dodo leads to near extinction of plant. *Science* 197: 885-886.

Thabethe, V., Wilson, A-L., Hart, L. A. and Downs, C. T. 2015a. Digestive efficiency of indigenous and invasive avian species fed fruit of invasive alien plants in South Africa. *African Zoology* 50(4): 293-297.

Thabethe, V., Wilson, A-L., Hart, L. A. and Downs, C. T. 2015b. Ingestion by an invasive parakeet species reduces germination success of invasive alien plants relative to ingestion by indigenous turaco species in South Africa. *Biological Invasions* 17: 3029-3039.

Traveset, A. 1998. Effect of seed passage through vertebrate frugivores' gut on germination: a review. *Seed Science and Research* 22: 243-248.

Traveset, A., Robertson, A. W. and Rodríguez-Pérez, J. 2007. A review on the role of endozoochory in seed germination, in Dennis, A. J., Schupp, E. W., Green, R. J., Westcott, D. A. (Eds.). *Seed dispersal: theory and its application in a changing world.* Cambridge: CAB International, Columns Design Ltd., Reading. Pp. 78-103.

Vander Wall, S. B. and Beck, M. J. 2012. A comparison of frugivory and scatter-hoarding seed-dispersal syndromes. *Botanical Review* 78: 10-31.

Wenny, D. G., Şekercioğlu, Ç. H., Cordeiro, N. J., Rogers, H. S. and Kelly, D. 2016. Chapter 5: Seed Dispersal by Fruit-Eating Birds, in Şekercioğlu, Ç. H., Wenny, D. G. and Whelan, C. J. (Eds.). *Why Birds Matter: Avian Ecological Function and Ecosystem Services*. The University of Chicago Press, Chicago. Pp. 107-146.

Westcott, D. A. and Fletcher, C. S. 2011. Biological invasions and the study of vertebrate dispersal of plants: opportunities and integration. *Acta Oecologica* 37: 650-656.

Wilson, A. L. and Downs, C. T. 2012. Knysna turacos (*Tauraco corythaix*) do not improve seed germination of ingested fruit of some indigenous South African tree species. *South African Journal of Botany* 78: 55-62.

Yagihashi, T., Hayashida, M. and Miyamoto, T. 1998. Effects of bird ingestion on seed germination of *Sorbus commixta*. *Oecologia* 114: 209-212.

Zengeya, T., Ivey, P., Woodford, D. J., Weyl, O, Novoa, A., Shackleton, R., Richardson, D. and van Wilgen, B. 2017. Managing conflicts-generating invasive species in South Africa: challenges and trade-offs. *Bothalia* 42(2): 1-11.

CHAPTER TWO

THE ROLE OF VISITING BIRD SPECIES, FRUIT PRODUCTION AND RIPENESS ON POTENTIAL SEED DISPERSAL OF INVASIVE *CINNAMOMUM CAMPHORA* IN AN URBAN ENVIRONMENT

Abstract

The potential invasiveness of alien plant species needs to be understood in order to help prevent populations from growing out of control and becoming major threats to native plant communities. Invasive plant species are often associated with birds as primary dispersers in order to overcome physical barriers and spread into new areas through mutualistic relationships and interactions between various traits. *Cinnamomum camphora* (L.) J. Presl. (Lauraceae) is a tree species that has become invasive in parts of South Africa but has not yet been classified in Gauteng according to the National Environmental: Biodiversity Act 10/2004. The potential invasiveness of *C. camphora* was determined by observing how the different bird species responded to fruit production, by assessing the primary bird species visiting *C. camphora* and the interactions those species had with *C. camphora*. The results showed that the number of visiting birds positively responded to fruit production as the number of ripened fruit increased. However, out of the 24 visiting species, only six species were found to be major visitors with more than 100 observations recorded. The primary visiting bird species ranged from seed predators (Rock Doves, *Columba livia*) with 35.3% of total bird observations in *C. camphora* trees, to legitimate seed dispersers (such as Red-winged Starlings, *Onychognathus morio*) with 52.9% of total observations. Primary bird activities comprised of resting in the trees and consuming *C. camphora* fruit. In conclusion, *C. camphora* attracted a large number of potential dispersers (N=22) during the 2017 fruiting season (February to June), with six species being classified as major visitors. Of

these six major bird species, four species were potential seed dispersers (Dark-capped Bulbuls, Karoo Thrushes, Common Mynas and Red-winged Starlings), with Red-winged Starlings and Dark-capped Bulbuls being polyphagous frugivores, and two potential seed predators (Rock Dove and Red-eyed Doves).

Keywords: Bird-plant interactions, disperser effectiveness, frugivory, fruiting phenology, seed predators

Introduction

Seed dispersal effectiveness is the contribution that a disperser species makes towards the reproduction of a plant species (Schupp 1993; Schupp *et al.* 2010; Mokotjomela *et al*. 2016). Dispersal over long distances also increases the chance of seeds being placed in safe microsites away from seed predator species to allow for successful recruitment at the end of the reproductive cycle of the plant species (Nathan and Muller-Landau 2000; Calviño-Cancela *et al*. 2006; Ronce 2007; Gross-Camp and Kaplin 2011; Jordano *et al*. 2011; Mokotjomela *et al*. 2016; White and Midgley 2017).

The seed dispersal of zoochorous plants in a spatially heterogeneous landscape can be determined by the environmental features of forest cover, or tree cover in other biomes, and fruit availability (Herrera *et al*. 2011). Habitat fragmentation, often caused by anthropogenic habitat disturbances, promotes the discontinuities found in the spatial distribution of any remaining habitat cover and fruit resources (Herrera *et al*. 2011; Gaertner *et al*. 2017). Spatial and temporal changes in environmental features aside from habitat cover influence the habitat suitability for dispersal processes (Herrera *et al*. 2011; Gaertner *et al*. 2017). An increased rate of disturbance of a recipient habitat also increases the chance of naturalisation of an exotic species (Pyšek *et al*. 2010; Westcott and Fletcher 2011; Gaertner *et al*. 2017).

Landscape structure is an important factor to consider for both ecologists and conservationists as it can influence the interactions between fruit and frugivores (Lehouck *et al.* 2009). Fragmented landscapes can help manage invasive plant species spread by creating barriers against some dispersers, such as large mining areas or large urbanised areas between green belts (Gosper *et al*. 2005). However, fragmentation can also increase invasive rates by creating stepping stones for frugivorous birds as they pass through (Gosper *et al*. 2005; Buckley *et al*. 2006; Galetti *et al*. 2016) and cause rapid fruit removal as birds favour disturbed habitats as feeding sites. This leads to more seeds of invasive plants becoming dispersed (Gosper *et al*. 2005; Galetti *et al*. 2016). Fragmentation can also lead to invasive plant species spreading into the areas surrounding the dispersers' preferred habitat (Buckley *et al.* 2006).

Birds act as natural selection agents of the different fruit display traits (Sobral *et al*. 2010; Palacio and Ordano 2018). Two of the most important fruit traits invasive plant species have that attract and encourage frugivorous bird species to visit are the fruit crop size (number of fruit/tree) and the physical size of the individual fruit (Snow 1971; Sallabanks 1993; Gosper *et al*. 2005; Palacio and Ordano 2018).

Fruit crop size is considered to be the most important fruit trait influencing selection of invasive fruit by birds (Snow 1971; Palacio and Odano 2018). The size of an invasive plant species' fruit crop is important when compared with native, fruit-bearing plant species within the neighbouring area (Bach and Kelly 2004; Gosper *et al*. 2005; Chupp and Battaglia 2016). Knight (1986) found that invasive plant species in South Africa often had larger and more conspicuous fruit displays than the native species. Plant species that fruit over long periods of time or during lean times (Gosper 2004; Gosper and Vivian-Smith 2010; Westcott and Fletcher 2011), produce large crops and that produce large fruit displays are the most heavily used (Westcott and Fletcher 2011).

Animals rely primarily on their visual and olfactory senses to locate fruits but can also be drawn to large fruiting trees by the sounds of fruit falling to the ground (Schaefer 2011). It is traditionally thought that frugivorous bird species perceive fruit display as a signal that determines fruit choice and therefore fruit removal (Snow 1971; Richardson *et al*. 2000; Schaefer 2011; Palacio and Ordano 2018). The more conspicuous a fruit display is, the larger the area is from which dispersers can be attracted (Schaefer 2011). Fruit of trees can often be found on the crown making it easy for birds to see the overall fruit crop size (Noma and Yumoto 1997).

Frugivore traits are also important for both dispersal quality and quantity as well as dispersal distances of an invasive plant species (Buckley *et al.* 2006; Carnicer *et al.* 2008; Chamberlain and Holland 2009; Sebastián-González 2017). The quantity depends on traits such as availability, gape size, number of dispersers and dietary constraints placed on the frugivore (Buckley *et al.* 2006; Carnicer *et al.* 2008; Chamberlain and Holland 2009; Sebastián-González 2017). Gape size is one such important trait, if a fruit or seed is larger than the gape of a bird species, that species is unable to swallow the seed and become a potential disperser unless by other means such as by handling the fruit (Jordano 2000; Sebastián-González 2017).

Food handling by a disperser is also an important part of successful seed dispersal (Levey 1986; Symes and Downs 2001; Symes and Perrin 2003; Cousens *et al*. 2010). The primary function of fruit handling is to make fruit small enough to be swallowed by a frugivore that may originally be ‘gape-limited’ (Levey 1986; Symes and Downs 2001). Optimum fruit size results in reduced energy costs spent on handling (Symes and Downs 2001), which allows the disperser to have more time and energy to be spent on other daily activities (Levey 1986; Symes and Downs 2001).

Avian frugivores can be classified as ‘mashers’ or ‘gulpers’, with mashers spending more time processing food externally to receive small enough pieces to consume (Levey 1986; Symes and Downs 2001; Cousens *et al*. 2010), while gulpers are able to swallow more foods whole, and thus spending less time with fruit handling (Levey 1986; Symes and Downs 2001; Cousens *et al*. 2010).

Food handling can also include moving fruit away from the source plant before ingestion and discarding the seeds while ingesting the flesh (Symes and Perrin 2003; Cousens *et al*. 2010). Separation of seeds from fruit pulp by species before swallowing will limit dispersal; but will also reduce gut transit times for seeds that had been swallowed (Cousens *et al*. 2010). Grey-headed Parrots (*Poicephalus fuscicollis suahelicus*) often pick at a fruit to extract a kernel before dropping the rest of the fruit (Symes and Perrin 2003). Some individuals were recorded flying away from the source plant and either found perching in another tree to continue feeding, or found to drop the fruit while in flight (Symes and Perrin 2003).

The number of seeds that are dispersed for each visit is also determined by the length of time on which the disperser is visiting the plant (Levey 1986; Schupp 1993; Symes and Downs 2001). While staying in a plant for a longer period of time means that more fruit is handled and therefore more seeds dispersed, there is also a chance of the seeds being regurgitated or excreted below the plant instead of being dispersed away from the source plant, leading to the success of dispersal being bought into question (Levey 1986; Schupp 1993; Symes and Downs 2001; Symes and Perrin 2003).

Cinnamomum camphora (L.) J. Presl. (Lauraceae) was first introduced to South Africa as early as the late 1600s (Anonymous 2019). The species is categorised as an invasive plant species in five of the nine provinces; but is uncategorised for the remaining four

provinces in the National Environmental: Biodiversity Act (NEMBA) Act 10/2004 (Government Gazette 2016), making it a species that is at risk of becoming highly invasive and of concern to conservationists (Jordaan *et al.* 2011b). Gauteng is an uncategorised province with a high number of urbanised areas, mining sectors and farmlands. By knowing and understanding the dispersal effectiveness of *C. camphora* in an urbanised area, we can find ways of controlling current known level of infestation from producing highly invasive populations while preventing the establishment of new populations.

The aim of this study was to determine how the potential seed dispersal of *C. camphora* is affected by the different bird species found within an urban environment through observation of the fruit production over time and how the various bird species respond to fruit production over the fruiting season. Further observations and literature searches were made on whether the bird species were primarily seed predators or legitimate seed dispersers, the number of species that were predominantly frugivorous, and the type of major interactions between *C. camphora* and the major visiting bird species. It was predicted that the number of visiting birds would respond to the number of ripened *C. camphora* fruit on the trees, and that the predominant visiting species would be predominantly frugivores, spending the majority of their time eating ripened *C. camphora* fruit.

Methods

Study site and species

The study was conducted on the main campus of the University of the Witwatersrand, Braamfontein (26° 11' 34.8"S, 28° 01' 49.8"E; altitude: 1758 m above sea level) and took place from the middle of February until the start of July 2016. Situated in central Johannesburg, the university is in a highly urbanised area with only a few potential, fragmented sites for successful dispersal and establishment of plants, mostly in a northerly

direction such as the Johannesburg Botanical Garden, Zoo Lake, Melville Koppies Nature Reserve, Delta Park and a number of golf courses and small parks.

Twelve C. camphora individuals found in three areas across the campus were used for the study (Figure 2.1.). These trees were the most easily accessible (i.e. not behind fencing) and had fruit displays that were low enough to easily access fruiting phenology (Figure 2.1.).

Cinnamomum camphora (L.) J. Presl. (Lauraceae) grows best in areas where the mean annual rainfall is >1 000 mm per annum (Invasive Species Compendium 2015). A mature *C. camphora* tree is able to produce approximately 100 000 fruit in a single fruiting season (Firth 1981; Panetta 2001; Head and Muir 2004). The fruit is a drupe, with a diameter of 7.0 – 9.8 mm (Panetta 2001; Chen et al. 2004) and the single seed within each fruit measures 6.0 – 7.5 mm (Panetta 2001; Chen et al. 2004). The fruit coat is a shiny blue-black in colour and attracts birds (Panetta 2001; Jordaan et al. 2011a).



Figure 2.1. Locations of *Cinnamomum camphora* trees on the University of the Witwatersrand (26° 11' 34.8"S, 28° 01' 49.8"E; altitude: 1758m above sea level), Johannesburg campus located within a highly urbanised environment. Yellow markers with circles indicate *C. camphora* that were not used due to inaccessibility or lack of enough *C. camphora* fruit for the study (n=7). Markers with stars indicate *C. camphora* used for research data (n=12) with three colours indicating the different groups used for observation data collection; light blue - observation group A (n=4), pink - observation group B (n=4) and purple is observation group C (n=4). The two markers in the Northern area are trees that have relatively little human activity occurring around them, while the markers to the South and North-West areas encounter relatively heavy amounts of people walking underneath the trees throughout the day. Images taken during April 2016.

Tree size, fruit production over time and effects of weather during sampling

Tree height was determined through the use of a clinometer and with the assistance of a partner. Starting at a set distance of 10m away from the tree (measured using a 20m tape measure), the person holding the clinometer moved either further away or closer towards the tree until the shortest distance where the top of the tree canopy is visible. The distance from the base of the tree to the clinometer sighting point (D) was measured using the 20m tape measure (Figure 2.2.). The clinometer was used to obtain two angles, from eye level to the top of the tree (a) and from eye level to the base of the tree (b) (Figure 2.2.). The overall height (H) was calculated based on the angles and distances collected through the following basic trigonometric equations:

$C=90°-a$

$E=90°-b$

$A=\frac{\sin(a)*D}{\sin(C)}$

$B=\frac{\sin(b)*D}{\sin(E)}$

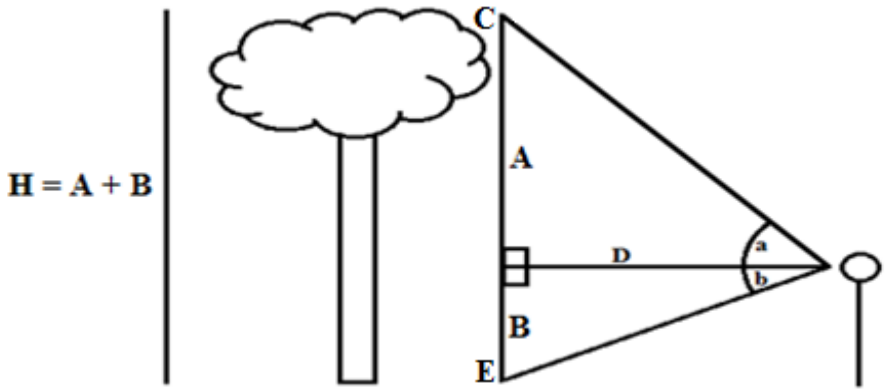


Figure 2.2. Diagram showing tree height measurement using a clinometer.

Stem diameter was measured at breast height (DBH: 1.37m above ground level) using a measuring tape and marked with a thumb tack. If the trunk had split into branches before the DBH point, then the smallest circumference below the divide was measured. String was wrapped tightly around the trunk at the thumb tack and the circumference marked on the string. The string was measured using the measuring tape in order to get the tree circumference. The diameter for each of the twelve trees was then calculated by dividing the tree circumference by Pi from the equation: Circumference=2(Pi*radius).

Measurements of tree canopy size followed the methods in Witkowski and Garner (2000). The longest canopy diameter (m) [d_1] and the diameter of the canopy perpendicular (m) [d_2] to the longest canopy diameter were measured for the twelve *C. camphora* trees using a measuring tape. These measurements were then used to calculate the canopy area (m^2) and canopy volume (m^3) (including tree height as well) for each tree by using the following equations:

Canopy area = Pi*(d_1/2)*(d_2/2)

Canopy volume = (4/3)*Pi*(d_1/2)*(d_2/2)*(height/2)

The fruiting phenology of *C. camphora* was recorded over the fruiting season, from mid-February to mid-July 2016. Each sampling session occurred once a week, on Wednesday, at around 7:00 am in the morning. If a bird observation session occurred on a Wednesday morning, the phenology sampling session would be moved to the Thursday to prevent disrupting the Wednesday bird observations.

The fruiting phenology of each of the 12 *C. camphora* trees was determined by using a similar method to the one performed by Noma and Yumoto (1997). An easy to reach and similarly sized fruit bearing branch (approximately 1 m length of branch covered in foliage and an approximate foliage cross-sectional area of $1m^2$), was selected and marked on each

tree. Lower branches were selected as *C. camphora* fruit was found to be consistent on both higher and lower branches, and a single branch selected so as to prevent conflict with other research on the tree (see Chapter 3). The fruit on each selected tree branch was counted three times per sampling session to get the total number of fruit (as counts remained consistent); and then another three times in order to get an absolute count of ripe fruit only. Fruit was considered ripe only if the entire surface was blue-black in colour. On each tree, the total number of similarly sized branches was counted in order to be able to scale up the data to the tree level to estimate fruit production/tree:

Total fruit/tree = number of fruit on selected branch*number of branches/tree.

Weather data, namely the mean monthly minimum and maximum temperatures (°C) and total monthly rainfall (mm) that occurred at the study site and its surrounding environment over the *C. camphora* fruiting season were downloaded during December 2017 from Time and Date (https://www.worldweatheronline.com). These data were used to assess whether there is a relationship between the number of ripe *C. camphora* fruit and mean minimum and maximum temperatures (°C) and total rainfall (mm) during the fruiting season months.

Bird observations

Bird observations were performed weekly throughout the 2016 fruiting season of *C. camphora* (mid-February until late-June). Observations were performed on any given day of the week (included public holidays) unless the university grounds were inaccessible. The 12 trees selected were divided into three groups for easier observation; and each group had trees that were not directly next to or opposite each other.

Each of the twelve trees was observed for five minutes three times each week, over twenty weeks. In order to ensure that all of the trees were observed equally throughout both

the day and the week, days were divided into three periods, i.e. morning (AM; 07:00-10:00), mid-day (MD; 10:30-13:30); and afternoon (PM; 14:00-17:00) (Fig. 2.3.). Each period was further divided into three, one hour long sessions (Figure 2.3.), in which one of the three groups of trees was observed (Figure 2.3.).

Observation recordings included factors such as: i) the individual tree being observed, ii) bird species observed, iii) starting time (observation start time if the bird was present, or else the time the bird arrived or changed activity) and departure time, iv) bird's position (in tree or beneath tree on ground), v) bird's activity which were divided into five categories (Table 2.1., Figure 2.4.), and vi) the food source/s being ingested (*Cinnamomum camphora* fruits/seeds; Scraps – leftover human food/trash; Insects; and Other – small, unidentifiable resources) (Appendix, Data Sheet 1). If a bird changed its activity, the new activity was only recorded if it occurred for longer than ten seconds.

Table 2.1. Descriptions of the five activities recorded during the bird observation trials.

Activity	Description
Chasing	Individuals chasing or being chased by another bird.
Feeding	Consumption of any food resources scavenged or harvested off/under tree canopy.
Provisioning	Putative parents feeding any fledglings.
Resting	Perching, sleeping or either self- or allo-preening.
Searching	Digging through foliage or scanning ground for resources, i.e. food and/or nesting materials.

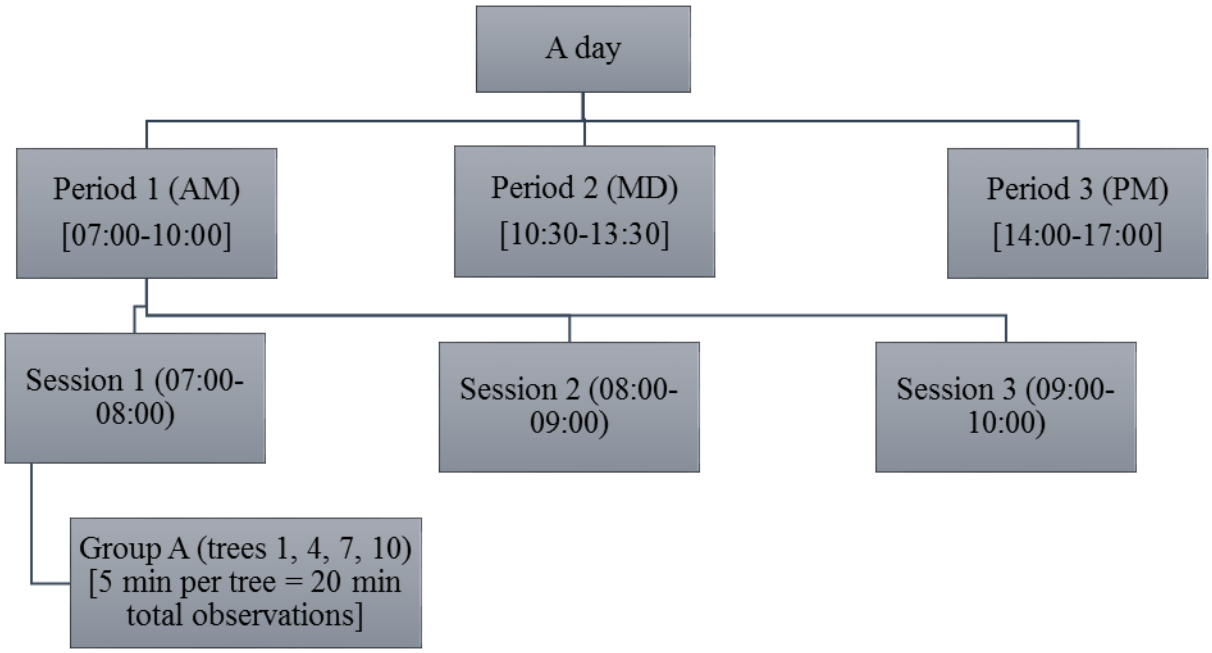


Figure 2.3. Breakdown of an observation day: divided into three periods (AM, MD (midday), PM), each with three sessions, and an example tree group to be observed during a session.



Figure 2.4. A) Red-winged Starling (*Onychognathus morio*), a seed disperser, feeding on *Cinnamomum camphora* fruit, B) Rock Dove (*Columba livia*), feeding on *C. camphora* seeds and/or fruit, and C) Karoo Thrush (*Turdus smithi*) perching in *C. camphora*. Photographs: LM McGowan

Data analysis

The relationship between the total ripened *C. camphora* fruit and the tree size measurements was assessed using both linear and non-linear regression analyses. Further linear and exponential regression analyses were used to assess the response of visiting birds and birds consuming *C. camphora* fruit to the overall number of fruit and number of ripened fruit per tree.

Several Chi-square tests for independence were used to determine the associations between: i) the number of ripened and unripened fruit/tree between the five months of the 2016 fruiting season, ii) seed predator and seed disperser species and the three predominant dietary components and, iii) the six major bird species and the four activities performed while visiting *C. camphora*.

Results

Tree size, fruit production over time and effects of whether during sampling

Cinnamomum camphora had a mean height of 12.30m and standard deviation of 5.01m (coefficient of variation (CV) = 41.4%) (Table 2.2.), and a stem diameter of 0.58m with a standard deviation of 0.23m (CV = 39.7%) (Table 2.2.). The mean canopy area and canopy volume for the twelve *C. camphora* trees were $131m^2.tree^{-1}$ (CV = 55.3%) and $190m^3.tree^{-1}$ (CV = 60.6%) respectively with standard deviations of $72.5m^2.tree^{-1}$ and $115.2m^3tree^{-1}$ respectively (Table 2.2.).

Table 2.2. Descriptive statistics of the four tree measurements of the twelve *Cinnamomum camphora* trees located on the University of the Witwatersrand Johannesburg campus.

Tree	Height (m)	Stem diameter at breast height (m)	Canopy area (m^2tree^{-1})	Canopy volume (m^3tree^{-1})
Mean	12.3	0.58	131.1	190.1
Standard deviation	5.01	0.23	72.5	115.2
Coefficient of variation (CV)	41.4	39.7	55.3	60.6

The fruiting period of *C. camphora* started at the end of summer and finished at the start of winter 2016 (Table 2.3.), namely from February to June 2016. The first three months of fruiting saw a high production of over 8000 mean fruit per *C. camphora* tree (Table 2.3.). This number halved by May to 4662 total fruit per tree before dramatically dropping down to a mean of only 162 fruit per tree at the end of the fruiting period (Table 2.3.).

During February and March 2016, the majority of fruit was unripe, with 99.4% and 92.9% respectively (Table 2.3.). The unripened fruit percentage dropped to 41.3% in April and fell further to 4.01% in May (Table 2.3.); but increased again in June to 20.4% (Table 2.3.). There was a significant association between the five months and numbers of ripened and unripened fruit ($5x2\chi^2 = 17066$, d.f. = 4, n = 29711, $P<0.00001$) (Table 2.3.).

Table. 2.3. The mean (± SE) and percentage number of overall fruit, ripened and unripened, per *Cinnamomum camphora* tree (n=12) over the five months of the 2016 fruiting season (χ^2 = 17066, d.f. = 4, n = 29711, P<0.00001).

	Ripened fruit		Unripened fruit		Total	
Month	Mean ± SE	%	Mean ± SE	%	Mean ± SE	%
February	48 ± 34.0	0.6	8138 ± 1385	99.4	8186 ± 1391	100
March	588 ± 143.4	7.2	7646 ± 906.7	92.9	8234 ± 959.7	100
April	4970 ± 560.6	58.7	3497 ± 419.7	41.3	8467 ± 847.5	100
May	4475 ± 577.3	96	187 ± 39.2	4.0	4662 ± 595.2	100
June	129 ± 27.9	79.6	33 ± 6.7	20.4	162 ± 33.2	100

The total number of fruit produced by the twelve *C. camphora* trees was weakly positively related to both the height of the trees (R^2 = 0.4128) (Figure 2.5.), and the stem diameter at breast height (DBH) (R^2 = 0.3791). Canopy area (R^2 = 0.3632) and canopy volume (R^2 = 0.3722) were weakly positively related to the total number of produced fruit per tree (Figure 2.5.).

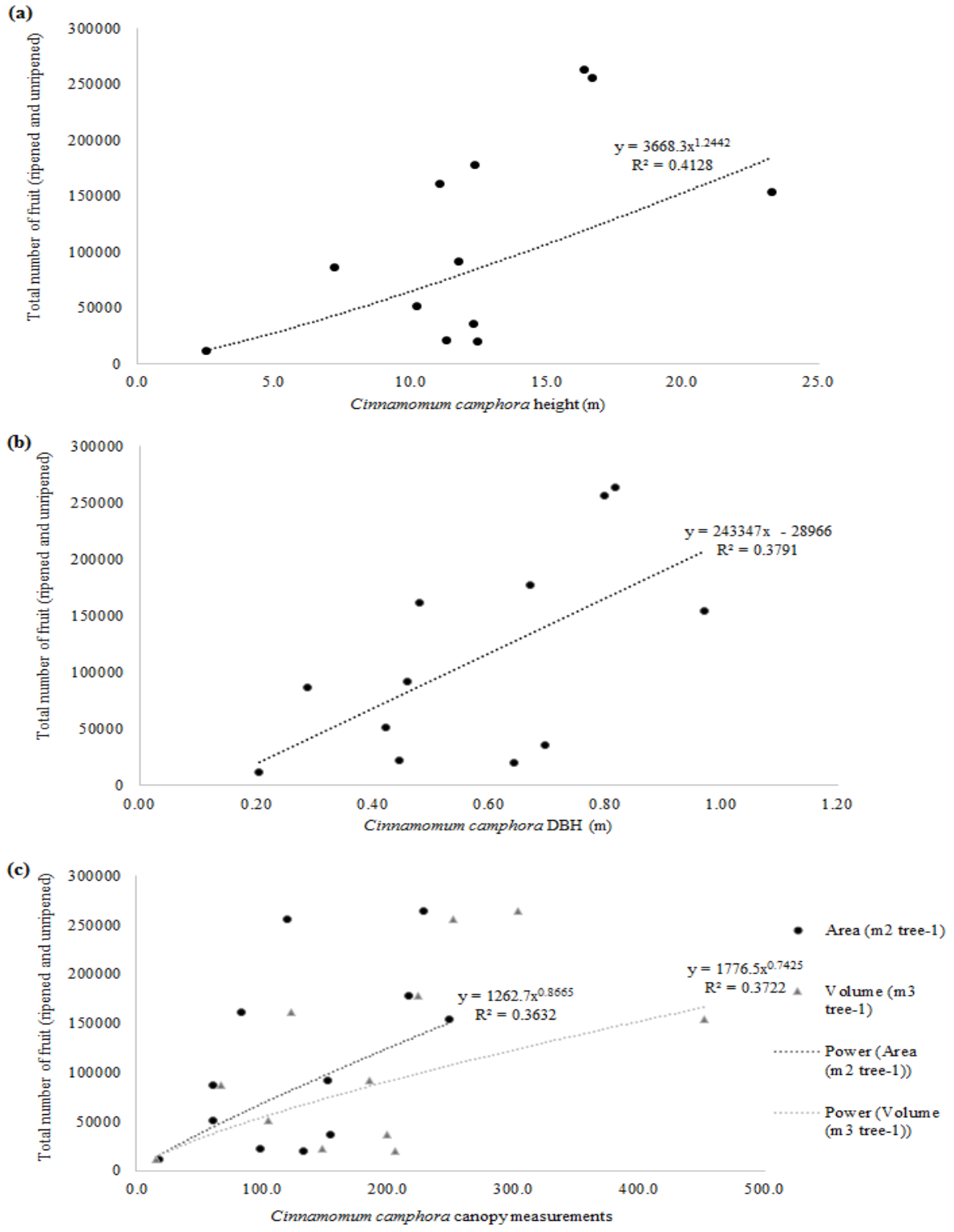


Figure 2.5. Fruit production of *Cinnamomum camphora* trees (n=12) for the 2016 fruiting season in relation to tree size, with corresponding regression analyses, as recorded by, (a) the height of *C. camphora* (m) (R^2 = 0.4145), (b) stem diameter at breast height (m) (R^2 = 0.3791), (c) canopy area ($m^2.tree^{-1}$) and volume ($m^3.tree^{-1}$) (Canopy area: R^2 = 0.3632; Canopy Volume: R^2 = 0.3722).

The number of ripened *C. camphora* fruit/tree increased with higher monthly rainfall (mm) in Johannesburg, i.e. when it was above 60mm (Figure 2.6.). After April, when the total monthly rainfall fell below 50mm, the mean number of ripened fruit started to decrease (Figure 2.6.).

The number of ripened fruit was high when the mean ambient temperature was higher than 25°C (Figure 2.6.). Towards the end of autumn and the beginning of winter (April to June 2016) as temperatures began to fall below 24°C, the number of *C. camphora* fruit began to decrease as well, going from 8467 fruit/tree in April to 162 in June 2016 (Figure 2.6.).

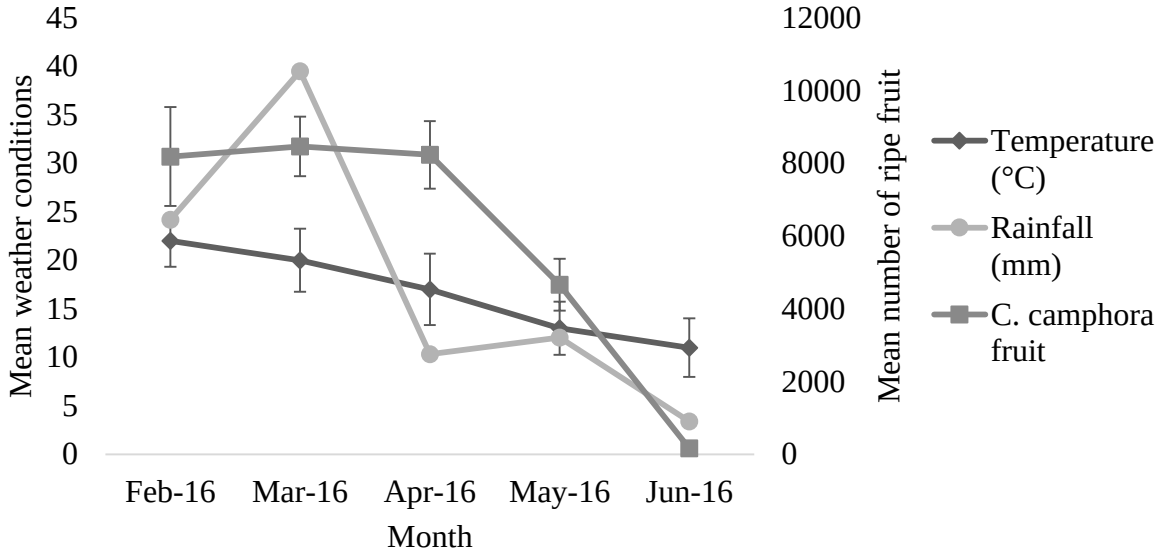


Figure 2.6. Total monthly rainfall (mm), and mean (±SD) monthly temperature (°C) in relation to the mean (±SE) total *Cinnamomum camphora* fruit/tree over the 2016 fruiting season.

Avian response to fruit production

The fruiting period of *C. camphora* occurred from early-February to late June 2016, with the ripened fruit appearing at the end of February (Figure 2.7.). When the mean number of ripened fruit/tree increased, so did the number of visiting birds (Figure 2.7.). There were two points during the fruiting period (week of 07 April and the weeks between 20 April and

11 May) in which the number of visiting birds decreased, with the week of 11 May being due to rainy weather conditions (Figure 2.7.). Birds visiting *C. camphora* to feed on ripened fruit followed the same pattern as the overall visitor number, but with a lower abundance (Figure 2.7.).

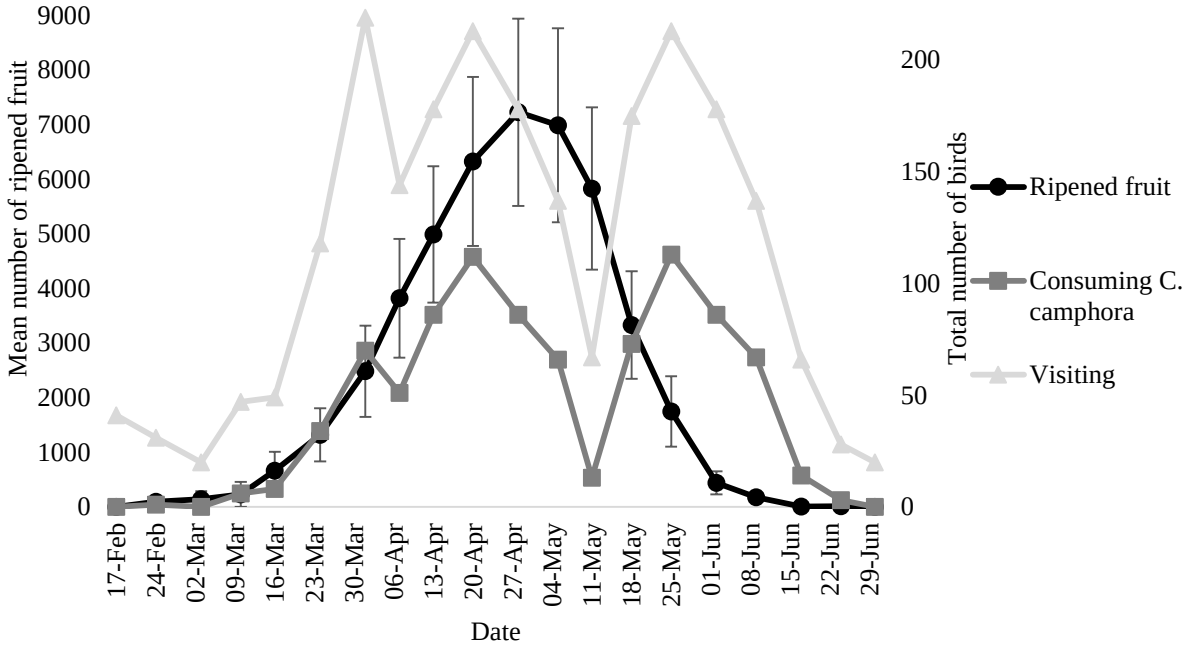


Figure 2.7. Total number of birds that visited each *Cinnamomum camphora* tree (n=12) and the total number of birds feeding on *C. camphora* fruit in relation to the mean number of ripened fruit per tree (±S.E.) over the fruiting phenology period of 2016.

Regression analysis showed that the relationship between the weekly mean of total *C. camphora* fruit (ripened and unripened) and the weekly mean number of birds visiting *C. camphora* was very weak and insignificant ($R^2 = 0.039$) (Figure 2.8.), as was the relationship between the weekly mean of total *C. camphora* fruit (ripened and unripened) and the weekly mean number of birds visiting *C. camphora* for the purpose of feeding on ripened fruit ($R^2 = 0.007$) (Figure 2.8.).

However, the regression analysis also showed that the relationship between both the weekly mean number of ripened *C. camphora* fruit and the mean number of visiting birds

weekly; and the mean number of visiting birds feeding on *C. camphora* fruit weekly were both weak but significant (Visiting birds: $R^2 = 0.3695$; Birds feeding on *C. camphora*: $R^2 = 0.3533$) (Figure 2.8.).

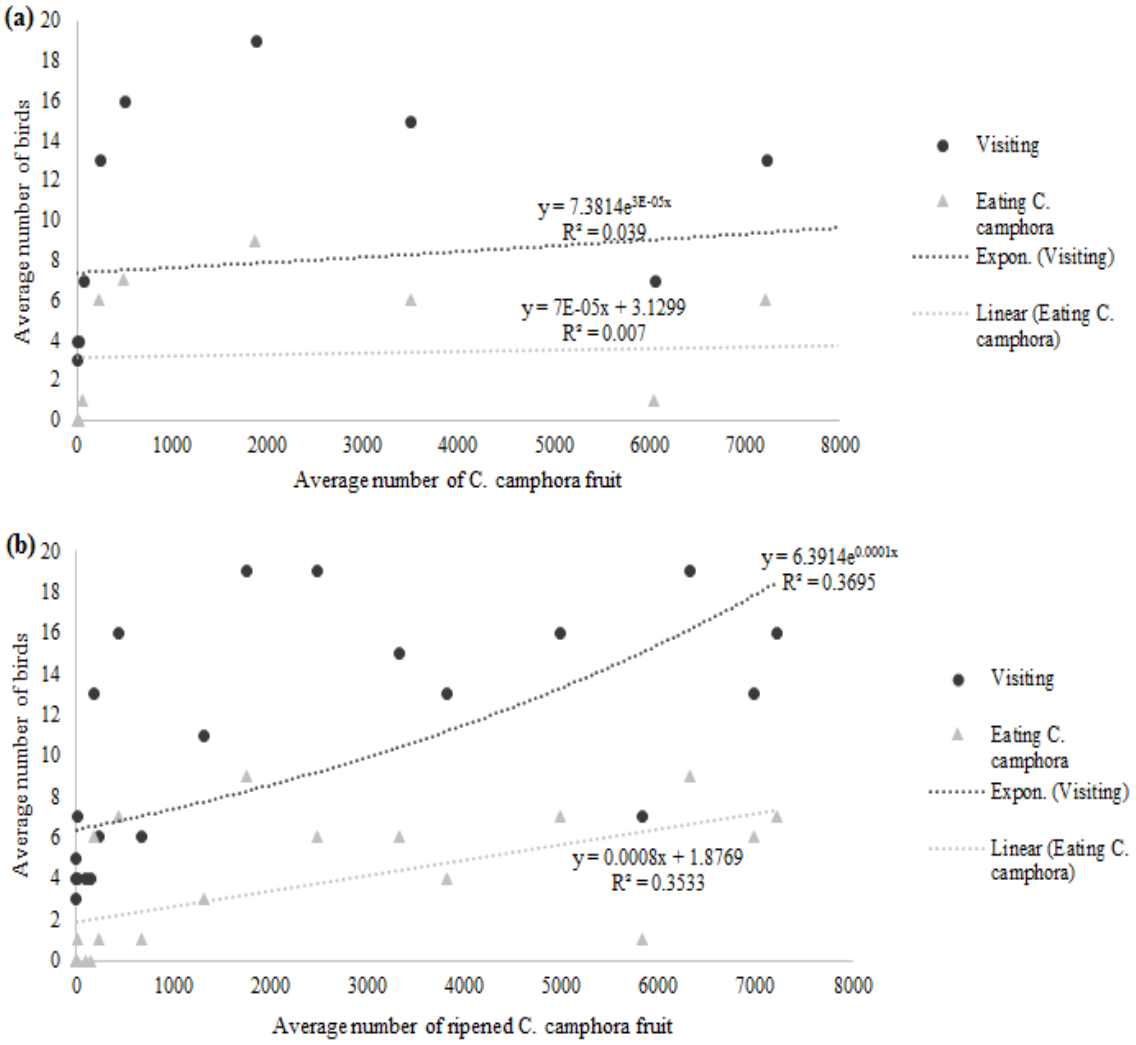


Figure 2.8. The relationship between the fruit availability of *Cinnamomum camphora* and visiting birds as measured by, (a) the weekly mean number of *C. camphora* fruit (ripened and unripened) per tree compared with the mean number of visiting birds per tree weekly ($R^2 = 0.039$), and the mean number of visiting birds for the purpose of feeding on fruit per tree weekly ($R^2 = 0.007$), (b) weekly mean number of ripened *C. camphora* fruit compared with the mean number of visiting birds weekly ($R^2 = 0.3695$); and the mean number of birds found feeding on *C. camphora* fruit weekly ($R^2 = 0.3533$).

Major avian species and degree of frugivory

A total of 2284 bird observations comprising 22 species visited the 12 *C. camphora* trees over the fruiting period (Table 2.4.). While 16 of the 22 species had fewer than 100 observations each and were therefore grouped together (Table 2.4.), the remaining six species had greater than 200 observations each and were considered to be the major visitors and contributors for potential dispersal of *C. camphora* (Table 2.4.). The most abundant visitor was the non-native Rock Dove (*Columba livia*) with 603 observations, accounted for 26.4% of the total visits over the fruiting period (Table 2.4.). This was follow by the native Karoo Thrush (*Turdus smithi*) and non-native Common Myna (*Acridotheres tristis*) with 436 (19.1%) and 327 (14.3%) observations respectively (Table 2.4.). The other three major species; Red-winged Starling (*Onychognathus morio*), Dark-capped Bulbul (*Pycnonotus tricolor*) and Red-eyed Dove (*Streptopelia semitorquata*), are all native species and had total observations of between 200 and 250 (Table 2.4.). The other 16 bird species only had 270 total observations and accounted for 11.8% of total visits (Table 2.4.).

Only two of the six main bird species were classified as frugivores, *O. morio* and *S. semitorquata*, the other four species were classified as omnivores, with *S. semiorquata* being classified as a granivorous omnivore (Table 2.4.). Observing which plant dietary components the species consumed showed that all six species consume the fruit, the seed, or both the fruit and seeds (Table 2.4.).

Table 2.4. The total abundance and percentage (%) of the different avian species visiting *Cinnamomum camphora* trees and whether they are native or non-native to South Africa, the abundant bird species' polyphagy classification, the main plant dietary component each species consumes and whether a seed disperser or seed predator.

Bird species	**Total observations**	**Percentage of total visits (%)**	**Native or non-native to South Africa**	**Polyphagy classification (Hockey *et al*. 2005)**	**Plant dietary component**
Rock Dove (*Columba livia*)	603	26.4	Non-native	Omnivorous	Plant matter and seeds
Karoo Thrush (*Turdus smithi*)	436	19.1	Native	Omnivorous	Fruit
Common Myna (*Acridotheres tristis*)	327	14.3	Non-native	Omnivorous	Fruit, nectar and seeds
Red-winged Starling (*Onychognathus morio*)	230	10.1	Native	Frugivorous	Fruit, nectar and seeds
Dark-capped Bulbul (*Pycnonotus tricolor*)	214	9.4	Native	Frugivorous	Fruit, nectar, petals and seeds
Red-eyed Dove (*Streptopelia semitorquata*)	204	8.9	Native	Granivorous	Flowers, fruit and seeds
Bird species <100 observations in total (n=16)	270	11.8	Mixed	Mixed	Mixed
Total	2284	100			

Avian behaviour when visiting Cinnamomum camphora *trees*

The top visiting species, Rock Dove, had the majority of observed individuals using *C. camphora* as a food source (51%), followed by 35% of individuals feeding on other food resources, such as human scraps, 9% using *C. camphora* as a resting spot; and only 6% performing other activities (Figure 2.9.).

The predominant frugivore species visiting *C. camphora*, Red-winged Starling, only had individuals performing two activities; 46% of which were feeding on *C. camphora* fruit; and the other 54% of individuals recorded using *C. camphora* as a resting spot (Figure 2.9.).

The remaining four major visitors had either 35 or 43% of individuals observed to be feeding on *C. camphora* fruit, between 41 and 54% of individuals found resting in *C. camphora*; and between three and six percent of individuals observed performing other minor activities (Figure 2.9.). Three of the four species, Common Myna, Karoo Thrush and Red-eyed Dove, had 8, 7 and 18% of observed individuals feeding on other food resources respectively (Figure 2.9), while the Dark-capped Bulbul was the same as the Red-winged Starling by feeding on no other food resources besides *C. camphora* (Figure 2.9). There was a significant association between the four activities performed among the six major visiting bird species (6×4 $\chi^2 = 493.5$, d.f. = 15, n= 203, $P<0.00001$) (Figure 2.9).

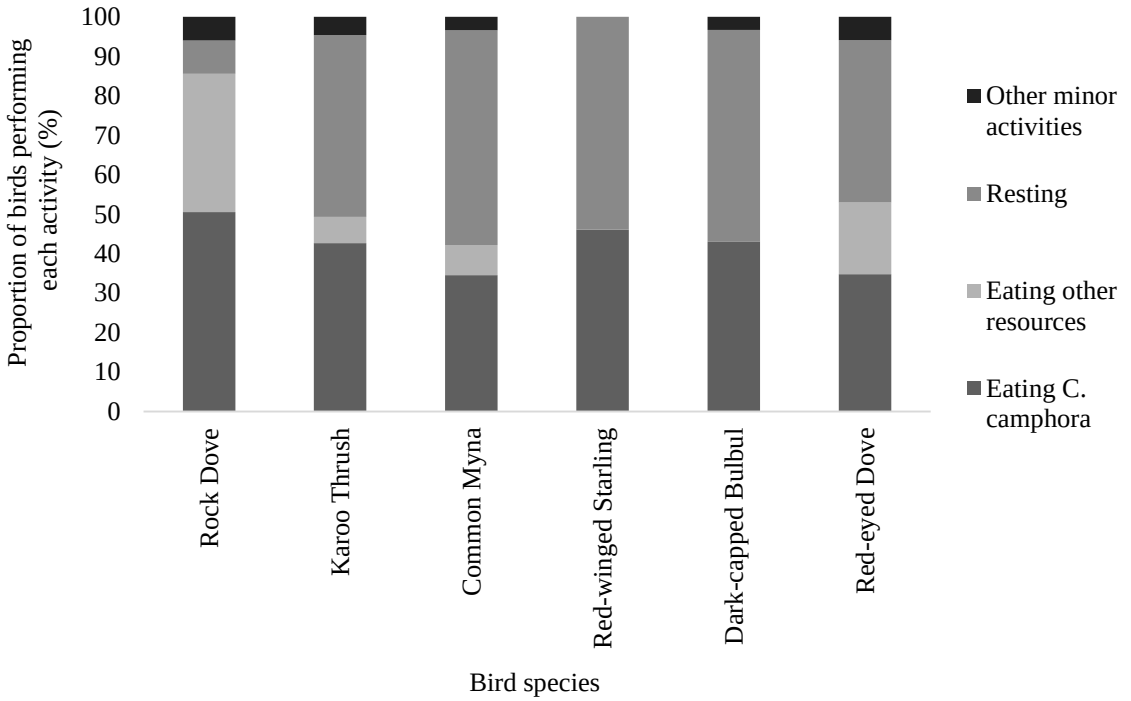


Figure 2.9. The proportion of birds from the six main bird species recorded visiting *Cinnamomum camphora* performing four different activities. ($\chi^2 = 493.5$, d.f. = 15, n= 203, P<0.00001).

Discussion

Cinnamomum camphora attracted a large number of potential dispersers during its 2016 fruiting season (Figure 2.9.). A reason for this could be because the fragmented landscape created by the buildings on campus prevent visiting bird species from being attracted to any smaller, neighbouring tree species fruiting at the same time as *C. camphora*. The crop sizes of invasive plant species fruiting seasons are often larger and fruiting continues for longer than the surrounding native species, allowing dispersers to persist in a habitat throughout the year when they might normally be unsupported without the invasive plant species being present (Buckley *et al*. 2006). Other possible reasons are that surrounding plants were observed to either not be fruiting at the same time as *C. camphora* or were not bird-dispersed species. A detailed list of all plant species found across the campus, including

fruiting phenology, dispersion methods and potential dispersal species (i.e. birds, mammals or insects).

Eight of the twelve observed trees had fruit crop sizes of around 8 000 fruit during the fruiting season (double counting could have occurred each month) (Figure 2.5.). These large fruit crop sizes combined with the visual appearance of *C. camphora* fruit (blue-black) made the trees more conspicuous to bird species, especially against the mainly neutral tones of the surrounding buildings, and therefore attracted large numbers of birds. Sallabanks (1993) found that the abundance of fruit was a primary factor in the selection of *Crataegus monogyna* Jacq. (Rosaceae) shrubs by American Robins (*Turdus migatorius*) in Western Oregon.

Another reason a large number of birds visited *C. camphora* could be due to the nutritional content of the fruit (Jordaan *et al*. 2011b). Jordaan *et al*. (2011b) observed that Dark-capped Bulbuls used both *C. camphora* as well as other plant species in order to meet its energetic requirements while the larger Red-winged Starlings were able to meet their daily energetic requirements on a diet of just *C. camphora* fruit. *Cinnamomum camphora* is known to be high in lipids and low in sugars compared with other invasive plant species, meaning that should birds require those levels in order to meet energetic requirements they can change their preferences accordingly (Jordaan *et al*. 2011b).

Major avian species and interactions with Cinnamomum camphora

The most common bird species found visiting *C. camphora* was the invasive Rock Dove (*C. livia*) (Table 2.5.), which is important as dove species are known to be seed predators (Gosper *et al.* 2005; Cousens *et al*. 2010). Seed predators are species that destroy seeds through either gizzard action or by directly consuming seed contents, with viable seeds surviving gut treatment only in rare cases (Gosper *et al.* 2005; Cousens *et al*. 2010; Thabethe

et al. 2015), and can have major impacts on the population dynamics, diversity and distribution of plants (Maron and Crone 2006; Bagchi *et al*. 2014; Fricke *et al*. 2016).

There are a few times in which a seed predator does disperse an invasive plant's seeds, such as when the predator carries the fruit (with seed still inside) to another plant for later consumption (Carrion-Tacuri *et al*. 2012; Thabethe *et al*. 2015). It was found that parrots and Darwin's Finches, seed predator species, acted as dispersers for both native and invasive plant species in Hawaii (Carrion-Tacuri *et al*. 2012). However, there are few studies that report on the overall effects of seed predators on invasive plant species (Carrion-Tacuri *et al*. 2012; Thabethe *et al*. 2015).

Red-eyed Doves had fewer seeds that survived gut transit with only 20% of seeds surviving (Mokotjomela *et al*. 2015; Mokotjomela *et al*. 2016). However, even with the low survival rate, *Streptopelia* species have large, ubiquitous ranges (Hockey *et al*. 2005), meaning that there is the possibility of long-distance seed dispersal services being provided to plant species that have seed traits that allow for a percentage to pass through intact, with or without germination influences (Mokotjomela *et al*. 2016). Individual Rock Doves during this study were noted at various times to ignore seedless *C. camphora* fruit, and instead consume seeds that had been regurgitated or excreted by other birds feeding in the tree.

Of the six major bird species found visiting *C. camphora*, four species were potential seed dispersers (Dark-capped Bulbuls, Karoo Thrushes, Common Mynas and Red-winged Starlings) (Table 2.5.), and of those four potential dispersers, Red-winged Starlings and Dark-capped Bulbuls are polyphagous frugivores. This meant that while a seed predator species had the highest number of observations, the majority of visitors were legitimate seed dispersers which increased the potential of *C. camphora* having successful seed dispersal.

All four of the legitimate seed disperser spent most of the time resting in *C. camphora* trees, with feeding on *C. camphora* fruit in second place (Figure 2.9.). However, these results could be due to the observational times which only accounted for 3780 min under the twelve trees for the duration of the fruiting season.

A study that observed the response of bird to three *C. camphora* trees during 2015 also found that the abundance of birds positively responded to the increase in ripening *C. camphora* fruit (McGowan 2015). However, while similar time spent on the major activities of resting and eating *C. camphora* fruit was observed (McGowan 2015), and the six major bird species observed during this study was observed during 2015 (McGowan 2015), Red-winged Starlings were found to be the top visiting species and potential dispersers during the 2015 fruiting season (McGowan 2015). Repeating this study over multiple consecutive years could help determine how the major bird visitors to *C. camphora* change over time, as well as if the bird abundances stay consistent in response to ripening *C. camphora* fruit.

Possibility of successful C. camphora *dispersal in an urban area*

Seed dispersal effectiveness depends on both the quality of dispersal and the quantity of seeds that are dispersed (Stoner and Henry 2009; Vander Wall and Beck 2012). While a seed may be consumed and successfully dispersed by animals, there is no guarantee that there will be successful germination (Stoner and Henry 2009). This is due to the distance that a disperser carries seeds away from the parent plant (Stoner and Henry 2009; García and Martínez 2012; Vander Wall and Beck 2012; White and Midgley 2017). Even if a disperser is effective in dispersing seeds far or well scattered, the seed might not fall into a suitable habitat for germination (Stoner and Henry 2009; Vander Wall and Beck 2012;White and Midgley 2017).

Seeds 4mm in diameter or larger, as with the case of *C. camphora* which has seed sizes ranging between 6-7.5mm in diameter (Panetta 2001; Chen *et al*. 2004), are known to be regurgitated by the bird species that consume them (Levey 1987). Jordaan *et al*. (2011a) however found that while, like most native frugivorous birds, Red-winged Starlings regurgitated *C. camphora* seeds; Dark-capped Bulbuls were found to mainly defecate *C. camphora* seeds. This is important as regurgitated seeds are not carried as far as seeds that are defecated (Levey 1987).

Jordaan *et al*. (2011a) found that Red-winged Starlings and Dark-capped Bulbuls had retention times of *C. camphora* seeds of 23-55min and 10-42min respectively. While the campus is located within a highly urbanised area (Figure 2.1.), there are still patches of green space spread throughout, indicating that should the two frugivorous species spend less time in *C. camphora* and fly out of campus grounds, there is a possibility of seeds being successfully dispersed. Further investigations can be made into observing the amount of time these specific species spend in *C. camphora* (by observing the trees for as long as possible), and observations into which direction individuals fly after leaving *C. camphora*. Should these species remain in the trees for the duration of their seed retention times, the *C. camphora* seeds dispersed will fall beneath the source plants and either be secondarily predated on by Rock Doves, trampled on by human traffic or, if the seeds manage to fall to the soil space beneath the tree, the seedlings removed by garden services.

One factor that was not explored in this chapter but can be further investigated is determining the parts of the day in which the primary activities (resting and feeding on *C. camphora*) took place. The spread of feeding and resting by all species throughout the day could be due to the species potentially becoming habituated to the disturbances on campus and the different numbers of people crossing under the trees throughout the day. Longer observations under specific individuals would help determine when the birds (with a focus on

the frugivores) spend the most time feeding on *C. camphora* in relation to other activities during the day.

Conclusion

Cinnamomum camphora is an invasive plant species within South Africa that can find potentially limited dispersal agents within highly urbanised areas. While bird species responded in high numbers as the proportion of ripened *C. camphora* fruit increased, a high number of these birds were seed predators. Seed predators disperse intact seeds in low numbers but their ubiquitous range means that, along with a frugivore's long seed retention time, seeds of *C. camphora* can potentially be dispersed across long distances. Furthermore, a highly urbanised area limits the distribution and area of safe microsite available for seedling establishment, as well as the impacts of secondary seed predators and gardening services. It will take further investigation into both how long major bird species visiting spend in *C. camphora* in relation to the species' seed retention times, as well observing where these species fly to after leaving *C. camphora* trees to start determining whether *C. camphora* can become an invasive species without monitoring in the future.

This research could also extend further into other landscape types, such as forests, abandoned farmlands and suburban gardens, to fully explore the dispersal agents and potential invasive spread of *C. camphora* in provinces where it is not currently being monitored.

References

Anonymous. 2019. Cinnamomum camphora. *Howling Pixel.* (https://howlingpixel.com/i-en/Cinnamomum_camphora#cite_note-usgs-10) Accessed 24 June 2019.

Bach, C. E. and Kelly, D. 2004. Effects of forest edges, fruit display size, and fruit colour on bird seed dispersal in a New Zealand mistletoe, *Alepis flavida*. *New Zealand Journal of Ecology* 28(1): 93-103.

Bagchi, R., Gallery, R. E., Gripenberg, S., Gurr, S. J., Narayan, L., Addis, C. E., Freckleton, R. P. and Lewis, O. T. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* 506: 85-88.

Buckley, Y. M., Anderson, S., Catterall, C. P., Corlett, R. T., Engel, T., Gosper, C. R., Nathan, R., Richardson, D. M., Setter, M., Spiegel, O., Vivian-Smith, G., Voigt, F. A., Weir, J. E. S. and Westcott, D. A. 2006. Management of plant invasions mediated by frugivore interactions. *Journal of Applied Ecology* 43: 848-857.

Calviño-Cancela, M., Dunn, R. R., van Etten, E. J. B. and Lamont, B. B. 2006. Emus as non-standard seed dispersers and their potential for long-distance dispersal. *Ecography* 29: 632-640.

Carnicer, J., Brotons, L., Sol, D. and De Cáceres, M. 2008. Random sampling, abundance extinction dynamics and niche-filtering immigration constraints explain the generation of species richness gradients. *Global Ecology and Biogeography* 17: 352 362.

Carrion-Tacuri, J., Berjano, R., Guerrero, G., Figueroa, E., Tye, A. and Castillo, J. M. 2012. Predation on seeds of invasive *Lantana camara* by Darwin's finches in the Galapagos. *Wilson Journal of Ornithology* 124: 338-344.

Chamberlain, S. A. and Holland, J. N. 2009. Body size predicts degree in ant-plant mutualistic networks. *Functional Ecology* 23: 196-202.

Chen, Z-H., Wu, B., Li, J-Y., Zhao, J-G., Zhou, X-Y. and Zhang, Y-K. 2004. Germination of the seeds and growth of the seedlings of *Cinnamomum camphora* (L.) Presl. *Plant Species Biology* 19: 55-58.

Chupp, A. D. and Battaglia, L. L. 2016. Bird-plant interactions and vulnerability to biological invasions. *Journal of Plant Ecology* 9: 1-11.

Cousens, R. D., Hill, J., French, K. and Bishop, I. D. 2010. Towards better prediction of seed dispersal by animals. *Functional Ecology* 24: 1163-1170.

Firth, D. J. 1981. Camphora laurel (*Cinnamomum camphora*): a new weed in north-eastern New South Wales. *Australian Weeds* 1: 26-28.

Fricke, E. C., Haak, D. C., Levey, D. J. and Tewksbury, J. J. 2016. Gut passage and secondary metabolites alter the source of post-dispersal predation for bird-dispersed chili seeds. *Oecologia* 181: 905-910.

Gaertner, M., Wilson, J. R. U., Cadotte, M. W., MacIvor, J. S., Zenni, R. D. and Richardson, D. M. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. *Biological Invasions* 19: 3461-3469.

Galetti, B. L., Piratelli, A. J. and Piña-Rodrigues, F. C. M. 2016. Fruit colour preference by birds and applications to ecological restoration. *Brazilian Journal of Biology* 76(4): 955-966.

García, D. and Martínez, D. 2012. Species richness matters for the quality of ecosystem services: a test using seed dispersal by frugivorous birds. *Proceedings of the Royal Society B* 279: 3106-3113.

Gosper, C. R. 2004. Fruit characteristics of invasive bitou bush, *Chrysanthemoides monilifera* (Asteraceae), and a comparison with co-occurring native plant species. *Australian Journal of Botany* 52: 223-230.

Gosper, C. R. and Vivian-Smith, G. 2010. Fruit traits of vertebrate-dispersed alien plants: smaller seeds and more pulp sugar than indigenous species. *Biological Invasions* 12: 2153-2163.

Gosper, C. R., Stansbury, C. D. and Vivian-Smith, G. 2005. Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. *Diversity and Distributions* 11: 549-558.

Government Gazette 2016. National Environmental Management: Biodiversity Act 10/2004. Alien and Invasive Species 2016.

Gross-Camp, N. D. and Kaplin, B. A. 2011. Differential seed handling by two African primates affects seed fate and establishment of large-seeded trees. *Acta Oecologica* 37: 578-586.

Head, L. and Muir, P. 2004. Nativeness, invasiness, and nation in Australian plants. *The Geographical Review* 94(2): 199-217.

Herrera, J. M., Morales, J. M. and García, D. 2011. Differential effects of fruit availability and habitat cover for frugivore-mediated seed dispersal in a heterogeneous landscape. *Journal of Ecology* 99: 1100-1107.

Hockey, P. A. R., Dean, W. R. J. and Ryan, P. G. 2005. *Roberts' Birds of southern Africa*. Seventh edition. The Trustees of the John Voelcker Bird Book Fund, Cape Town.

Invasive Species Compendium. 2015. *Cinnamomum camphora* (camphor laurel). Cabi.org,http://www.cabi.org/isc/datasheet/13519

Jordano, P. and Schupp, E. W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* 70(4): 591 615.

Jordaan, L. A., Johnson, S. D., Downs, C. T. 2011a. The role of avian frugivores in germination of seeds in fleshy-fruited invasive alien plants. *Biological Invasions* 13: 1917-1930.

Jordaan, L. A., Johnson, S. D. and Downs, C. T. 2011b. Digestion of fruit of invasive alien plants by three southern African avian frugivores. *Ibis* 153: 863-867.

Jordano, P., Forget, P-M., Lambert, J. E., Böhning-Gaese, K., Traveset, A. and Wright, S. J. 2011. Frugivores and seed dispersal: mechanisms and consequences for biodiversity of a key ecological interaction. *Biology Letters* 7: 321-323.

Knight, R. S. 1986. Fruit displays of indigenous and invasive alien plant in the south-western Cape. *South African Journal of Botany* 52: 249-255.

Lehouck, V., Spanhove, T., Vangestel, C., Cordeiro, N. J. and Lens, L. 2009. Does landscape structure affect resource tracking by avian frugivores in a fragmented Afrotropical forest? *Ecography* 32: 789-799.

Levey, D. J. 1986. Methods of seed processing by birds and seed deposition patterns, in: Estrada, A. and Fleming, T. H. (Eds.) *Frugivory and Seed Dispersal*. W. Junk, Dordrecht, pp. 147-158.

Levey, D. J. 1987. Seed size and fruit-handling techniques of avian frugivores. *The American Naturalist* 129 (4): 471-485.

Maron, J. L. and Crone, E. 2006. Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society B: Biological Sciences* 273: 2575-2584.

McGowan, L. 2015. Seed dispersal by birds in an urban environment. Unpublished Honours Research Project, University of the Witwatersrand, Gauteng, South Africa.

Mokotjomela, T. M., Hoffman, J. H. and Downs. C. T. 2015. The potential for birds to disperse the seeds of *Acacia cyclops*, an invasive alien plant species in South Africa. *Ibis* 157: 449-458.

Mokotjomela, T. M., Downs, C. T., Esler, K. and Knight, J. 2016. Seed dispersal effectiveness: a comparison of four bird species feeding on seeds of invasive *Acacia cyclops* in South Africa. *South African Journal of Botany* 105: 259-263.

Nathan, R. and Muller-Landau, H. C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15: 278-285.

Noma, N. and Yumoto, T. 1997. Fruiting phenology of animal-dispersed plants in response to winter migration of frugivores in a warm temperate forest on Yakushima Island, Japan. *Ecological Research* 12: 119-129.

Palacio, F. X. and Ordano, M. 2018. The strength and drivers of bird-mediated selection on fruit crop size: a meta-analysis. *Frontiers in Ecology and Evolution* 6(18): 1-14.

Panetta, F. D. 2001. Seedling emergence and seed longevity of the tree weeds *Celtis sinensis* and *Cinnamomum camphora*. *Weed Research* 41: 83-95.

Pyšek, P., Chytrý, M. and Jarošík, V. 2010. Habitats and landuse as determinants of plant invasions in the temperate zone of Europe, in: Perrings, C., Mooney, H. and

Williamson, M. (Eds), *Bioinvasions and Globalization: Ecology, Economics, Management and Policy*. Oxford University Pres, Oxford, pp. 66-81.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. and Rejmánek, M. 2000. Plant invasions – the role of mutualisms. *Biological Reviews* 75: 65-93.

Ronce, O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics* 38: 231-253.

Sallabanks, R. 1993. Hierarchical mechanisms of fruit selection by an avian frugivore. *Ecology* 74(5): 1326-1336.

Schaefer, H. M. 2011. Why fruits go to the dark side. *Acta Oecologica* 37: 604-610.

Schupp, E. W. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. *Vegetatio* 107/108: 15-29.

Schupp, E. W., Jordano, P. and Gomez, J. M. 2010. Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* 188: 333-353.

Sebastián-González, E. 2017. Drivers of species' role in avian seed-dispersal mutualistic networks. *Journal of Animal Ecology* 86: 878-887.

Sinclair, I., Hockey, P., Tarboton, W. and Ryan, P. 2011. *Sasol Birds of Southern Africa, Fourth Edition*. Struik Nature, Cape Town, South Africa.

Snow, D. W. 1971. Evolutionary aspects of fruit eating by birds. *Ibis* 113: 194-202.

Sobral, M. A. Larrinaga, A. R. and Guitián, J. 2010. Do seed-dispersing birds exert selection on optimal plant trait combinations? Correlated phenotypic selection on the fruit and seed size of hawthorn (*Crataegus monogyna*). *Evolutionary Ecology* 24: 1277-1290.

Stoner, K. E., and Henry, M. 2009 Seed dispersal and frugivory in tropical ecosystems. In *Tropical Biology and Conservation Management Volume* 5. (eds K. Del Claro, P. S. Oliveria, and V. Rico-Gray) pp. 176-193 . Eolss Publishers Co. Ltd., Oxford, United Kingdom.

Symes, C. T. and Downs, C. T. 2001. Feeding and energy intake in two avian frugivores, the Black-eyed Bulbul *Pycnonotus barbartus* (Passeriformes: Pycnonotidae) and Speckled Mousebird *Colius striatus* (Passeriformes: Coliidae). *Durban Museum Novitates* 26: 20-24.

Symes, C. T. and Perrin, M. R. 2003. Feeding biology of the Greyheaded Parrot, *Poicephalus fuscicollis suahelicus* (Reichenow), in Northern Province, South Africa. *Emu* 103: 49-58.

Thabethe, V., Wilson, A-L., Hart, L. A. and Downs, C. T. 2015. Ingestion by an invasive parakeet species reduces germination success of invasive alien plants relative to ingestion by indigenous turaco species in South Africa. *Biological Invasions* 17: 3029-3039.

Vander Wall, S. B. and Beck, M. J. 2012. A comparison of frugivory and scatter-hoarding seed-dispersal syndromes. *Botanical Reviews* 78: 10-31.

Westcott, D. A. and Fletcher, C. S. 2011. Biological invasions and the study of vertebrate dispersal of plants: opportunities and integration. *Acta Oecologica* 37: 650-656.

White, J. D. M. and Midgley, J. J. 2017. Dispersal of semi-fleshy fruits to rock crevices by a rock-restricted rodent. *South African Journal of Science* 113(11/12): 1-5.

World Weather Online. 31 October 2018.

https://www.worldweatheronline.com/johannesburg weather-averages/gauteng/za.aspx.

Appendix 1. Data collection sheet used bird observation sampling.

Week:_____ Day:_____ Date:_____
Period:_____ Session:_____
Group:____ P/A: Present/Arrival time

Tree no.	Time of obs. start	Bird species	Time (P/A)	Time depart	Position (Tree/Ground)	Activity	Food

CHAPTER THREE

ARE BIRDS NECESSARY? THE ROLE OF BIRDS IN SEEDLING EMERGENCE OF *CINNAMOMUM CAMPHORA* IN AN URBANISED ENVIRONMENT

Abstract

Generalist frugivores are known to be the primary dispersers of many invasive plant species, and for some plant species, also significantly enhance germination. Plant species that do not require birds as germination mediators are the most difficult to control as they will be able to germinate successfully without assistance. Although germination studies of *Cinnamomum camphora*, an evergreen tree species, have been conducted, few have focused on how seeds ingested by birds compare to seeds that remain inside the fruit in terms of seedling emergence for the species. Seedling emergence and seed viability of *C. camphora* across four fruit/seed stages (i) Consumed seeds, (ii) Ripened fruit, (iii) Shed fruit and (iv) Unripened fruit, were assessed to determine whether bird species significantly influence the (a) percentage and (b) rate of seedling emergence of *C. camphora* seeds tested in a greenhouse trial compared with seeds that were not ingested by birds. Overall birds did not significantly reduce percentage seed viability of *C. camphora*. However, while bird ingestion of seeds was not necessary for seeds to germinate, seedlings from ingested seeds took significantly longer to emerge (i.e. seed vigour was reduced), increasing the length in which seeds are vulnerable to seed predators in and on the ground. However, this longer period of time over which germination occurred due to bird ingestion may potentially increase the resilience of *C. camphora* to fluctuating weather patterns, potentially allowing for more seedling establishment opportunities. It was found that birds caused seeds to take longer for

seedlings to emerge. Hence the role of birds was primarily observed as *C. camphora* dispersal agents and not germination enhancers, except for reduced seed vigour.

Keywords: Avian frugivory, invasive plant species, seed dispersal effectiveness, seed viability, seedling emergence.

Introduction

Seed dispersal effectiveness

One of the key factors that enable an exotic plant species to become invasive is its ability to create mutualistic relationships with native frugivorous species which involves seed dispersal through exozoochorous or endozoochorous interactions (Richardson *et al.* 2000; Bartuszevige and Gorchov 2006; Twigg *et al*. 2009; Dlamini *et al*. 2018). The relationships formed allow dispersing species (often birds) to meet their daily energetic requirements through fruit pulp as a food resource; and the plant receives the means of effective seed dispersal away from the source plant (Krefting and Roe 1949; Schupp 1993; Robertson *et al*. 2006; Jordaan *et al*. 2011; Díaz Vélez *et al*. 2018), and an easier way of crossing over physical barriers compared to plants that are not animal-dispersed (Díaz Vélez *et al*. 2018; Traveset and Richardson 2014).

The qualitative component of effective seed dispersal refers to the contribution that the dispersing species makes towards the future reproduction of the plant species as well as the probability of seeds from that plant species which are dispersed remaining viable and successfully growing into a mature plant (Schupp 1993; Roxburgh 2007; Schupp *et al*. 2010; Díaz Vélez *et al*. 2018). Mokotjomela *et al*. (2016) determined seed dispersal effectiveness by the product of mean germination "rates" (%) (*quality*), and the average adult body masses of tested birds as potential seed load proxies (quantity per species). This component includes the likely scarification treatment during gut passage the dispersing species performs on the

seeds and the spatial distribution of the dispersed seeds (Krefting and Roe 1949; Bartuszevige and Gorchov 2006; Buckley *et al*. 2006; Roxburgh 2007; Traveset *et al.* 2008; Bradford and Westcott 2010; Díaz Vélez *et al*. 2018).

Effect of birds on seed germination and seedling emergence

Germination traits in invasive plant species are often examined through the measurement of germination success (final percentage germination), timing (germination time of the first seedling), and speed (rate of germination) (Gioria and Pyšek 2017). These traits are used as they are easy to measure and allow for the collection of data across multiple plant species over a relatively short time period (Gioria and Pyšek 2017). The process of seed dispersal by frugivorous birds involves the ingestion of fruit and seeds, which are mechanically and/or chemically treated in the digestive tract, followed by the seeds being voided from the body either through regurgitation or defecation (Barnea *et al*. 1991; Meisenburg and Fox 2002; Buckley *et al*. 2006; Jordaan *et al*. 2011; Dlamini *et al*. 2018). According to some authors, frugivores which are unable to disperse seeds away from the parent plant but still remove the seed from the fruit pulp increase the seed's survival compared to seeds remaining within the fruit pulp (Meyer and Witmer 1998; Fedriani *et al.* 2012; Fricke *et al*. 2016).

Once ingested, seeds undergo mechanical and/or chemical digestion (Barnea *et al*. 1991; Traveset *et al*. 2001; Dlamini *et al*. 2018). Mechanical digestion is when the gizzard either destroys seeds (therefore halting germination completely) or scarifies the seed coat, which can result in increased or reduced germination rates (Kleyheeg *et al*. 2017), while scarification is the chemical digestion seeds go through within the gut of a disperser species (Traveset *et al.* 2008; Kleyheeg *et al*. 2017; Díaz Vélez *et al*. 2018). Scarification is often referred to when comparing both the speed and percentage germination rates of ingested and

un-ingested seeds (Traveset 1998; Traveset *et al.* 2008). Some fleshy-fruited invasive alien plant (IAP) species have had the importance of scarification on germination emphasised (Díaz Vélez *et al*. 2018), while other studies have shown that only fruit pulp removal is necessary to increase seed germination (Díaz Vélez *et al*. 2018).

Seed ingestion by birds has varying effects on the germination of different plant species (Krefting and Roe 1949; Yagihashi *et al*. 1998; D'Avila *et al*. 2010; Dlamini *et al*. 2018). Seed coat abrasion and pulp removal are the principal ways in which germination rates can be affected by frugivory (Meisenburg and Fox 2002; Fricke *et al*. 2016; Díaz Vélez *et al*. 2018; Dlamini *et al*. 2018). Intact seeds that successfully pass through the digestive tract can have either an increased, decreased or unresponsive germination compared with seeds that are not passed (Barnea *et al.* 1991; Traveset 1998; Yagihashi *et al.* 1998; Figueroa and Castro 2002; Mokotjomela *et al*. 2015; Mokotjomela *et al*. 2016).

There are few examples of plant species in which the seeds were only slightly inhibited from germination after ingestion (Barnea *et al*. 1991). Sixteen of the 34 combinations tested by Barnea *et al*. (1991) had plant species that were not affected by bird ingestion, while the remaining 18 combinations were affected in some form, mostly with the percentage and speed of germination being increased. Traveset (1998) found that of the seeds of 200 plant species that went through gut passage, 50% experienced a change in germination rate. Of those 50%, one-third of the species had inhibited germination while the other two-thirds had the germination speed enhanced and percentage increased (Traveset 1998).

The majority of studies on the topic have shown that the passage of a seed through the digestive tract of a frugivore enhances the germination speed and percentage of the seed that germinate due to germination inhibitors in the fruit pulp (that would prevent germination from occurring until the fruit pulp has completely decomposed) being removed from the seed

during passage through the gut (Barnea *et al*. 1991; Cipollini and Levey 1997; Meyer and Witmer 1998; Yagihashi *et al*. 1998; Yagihashi *et al*. 1999; Panetta 2001; Traveset *et al.* 2001; Figueroa and Castro 2002; Robertson *et al.* 2006; Traveset *et al.* 2008; LaFleur *et al.* 2009; Jordaan *et al*. 2011; Mokotjomela *et al*. 2015; Dlamini *et al*. 2018). The fruit pulp is often removed in the gut from a scarification effect (Traveset *et al.* 2008). This is often referred to when comparing both the speed and percentage germination of ingested and un-ingested seeds (Traveset 1998; Traveset *et al.* 2008).

However these studies often compare seeds that have been ingested by birds to seeds that have been manually de-pulped; and these result often show that the two methods have similar effects on seed germination (Barnea *et al.* 1991; Panetta and McKee 1997; Meyer and Witmer 1998; Richardson *et al*. 2000; Panetta 2001), and do not include intact fruit which may result in the actual effect birds have on seed germination not being completely assessed (Robertson *et al*. 2006).

While many studies have shown that, depending on the bird and plant species involved, native plant germination percentages can increase, decrease or remain the same after bird gut passage (Yagihashi *et al*. 1999; Traveset *et al*. 2001; Bartuszevige and Gorchov 2006), the speed at which germination takes place is another key factor to be considered. Seeds that have a faster germination have the benefit that the seeds are less exposed to seed predator species after dispersal on the ground or in the soil; although seeds that only germinate after several weeks have greater seed survival in certain cases, these include instances where the seed is deposited in a microsite that is unsuitable for germination at the time such as during the dry seasons (Traveset *et al.* 2001).

Seed germination can be immediate, but can be delayed as a result of quiescence or dormancy, which can be physical, physiological or morphological (Fenner and Thompson

2006; Cousins *et al*. 2014). Dormant seeds usually preclude germination when conditions are unfavourable (Fenner and Thompson 2006; Cousins *et al*. 2014). Quiescent seeds begin germination as soon as suitable germination conditions are present, while seed dormancy prevents germination from occurring during unfavourable conditions (Fenner and Thompson 2006; Finch-Savage and Leubner-Metzger 2006; Cousins *et al*. 2014).

Seed viability and vigour

Seed vigour has been defined by the International Seed Testing Association (ISTA) and Association of Official Seed Analysts in North America as the seed properties, and the interactions between these biochemical and physiological characteristics, which allow for rapid, constant seedling emergences of plant species (van de Venter 2001).

Seed vigour, sometimes interchanged with viability (Miloševic *et al*. 2010), are tested in order to determine whether seed survivability and germination is better, depending on whether seeds are planted earlier or later within a season (Miloševic *et al*. 2010). Seed vigour is related to both the speed and percentage germination of a seed batch, while seed viability relates to the percentage of seeds that germinate. Seed vigour and seed viability can be tested either mechanically (by measuring seed size and mass when these have already been studied in relation to seed viability – typically larger seeds are viable), physiologically (through growth parameters and germination trials), and/or biochemically, such as through tetrazolium testing (Miloševic *et al*. 2010).

The seed viability of a plant species is an important factor to consider. Some plant species rely on the ingestion of fruit by birds as the seed could lose its viability if the fruit skin and pulp take too long to fully decompose (Panetta and McKee 1997; Yagihashi *et al.* 1998; Panetta 2001; Jordaan *et al*. 2011). Germination tests can be important in determining the viability of seeds (Czabator 1962; Cousins *et al*. 2014). However, the abrasion of the seed

coat by bird gut passage has been found to stimulate germination, but there is also a trade-off of the seed becoming more vulnerable to the chemicals found along the digestion tract of a bird, and, for larger sized seeds, a reduction in viability from gastric juice exposure (Kleyheeg *et al*. 2017).

The state in which the seed is deposited can have an impact on the overall viability of the seed. Seeds deposited with faecal matter can have an increased fertilization effect potentially enhancing seedling emergence, whereas the faecal matter might decrease the overall viability by increasing the facilitation of insect larvae and fungi which may predate/decompose the seed embryo (Meyer and Witmer 1998; Buckley *et al*. 2006; Traveset *et al.* 2008).

Cinnamomum camphora

Cinnamomum camphora (L.) J. Presl. (Lauraceae) is an evergreen tree species that is native to southern Asia (Chen *et al*. 2004; Firth 1979). Over time it has become introduced to various countries and has become an invasive plant species, including Australia, the United States of America and South Africa (Chen *et al*. 2004; Firth 1979; Jordaan *et al*. 2011). There have been several studies on how birds influence the germination of *C. camphora* (Firth 1979; Panetta 2001 Jordaan *et al*. 2011). These studies compared the seeds of *C. camphora* that were manually de-pulped to seeds that had been ingested by birds. The results showed that the manually removed seeds germinated as easily as seeds that had been ingested by birds (Firth 1979; Panetta 2001). Firth (1979) and Panetta (2001) attribute these results to the pericarp of *C. camphora* which has germination inhibitors within the pulp that prevents the seeds from germinating until the fruit pulp has been completely removed. However, these studies did not account for the rate and absolute percentage germination that can occur with

seeds that remain inside the fruit through the different stages from unripe fruit to fruit that has naturally fallen to the ground.

Aim and objectives

The aim of this study was to determine whether bird species had an influence on seed viability and seedling emergence of *C. camphora* in an urbanised area. This was addressed through two objectives; 1) determine the rate of and absolute seedling emergence of *C. camphora* seeds sampled at four different fruit/seed stages: i) Consumed seeds, ii) Ripened fruit, iii) Shed fruit and iv) Unripened fruit, and 2) calculate the seed viability of those *C. camphora* seeds that did not emerge as seedlings from the greenhouse emergence trial in objective 1 using 2, 3, 5-triphenyltetrazolium chloride across the same four fruit/seed stages. It is predicted that *C. camphora* seedling emergence will be significantly increased by bird ingestion of fruit/seeds, as is the case with many other alien invasive species.

Methods

Study site and species

The study took place on the main campus of the University of the Witwatersrand (Wits), Braamfontein (26° 11' 34.8"S, 28° 01' 49.8"E; 1758m above sea level), within highly urbanised central Johannesburg. Fruit and seed sampling took place during the *C. camphora* fruiting season (February to June 2016), and seedling emergence testing occurred from February 2016 to June 2017 using a greenhouse trial of samples collected from the trees. Further viability testing, of those seeds that did not emerge in the trial, took place from February to June 2017. The Wits main campus has numerous indigenous and exotic tree species throughout as well as a high number of both exotic and indigenous bird species from various feeding guilds, including potential dispersers. While highly urbanised, there are a few

green patches around central Johannesburg that could allow for successful dispersal and germination and hence the spread of *C. camphora*.

Cinnamomum camphora (L.) J. Presl. (Lauraceae) is an evergreen tree species that produces large numbers of blue-black berries when mature (Firth 1981). The berries are 7-9 mm in diameter, and each berry contains a single seed that is 6-7.5mm in length (Panetta 2001; Chen *et al*. 2004; Jordaan *et al.* 2011), and covered in a tough, waxy seed coat (Panetta 2001; Jordaan *et al*. 2011). Fruits of *C. camphora* are often dispersed by generalist frugivorous bird species (Panetta 2001) and birds are often observed regurgitating and/or excreting the seeds shortly after consumption (Jordaan *et al*. 2011).

While the seed of a mature *C. camphora* can reportedly remain dormant for around 4-20 weeks (Firth 1981), the seed viability of the species has been noted as of short duration, with the overall viability decreasing to only 1% after 12 months (Panetta 2001).

Six *C. camphora* trees were selected across the campus that were easily accessible, had low canopies (which allowed for easier access to fruit samples) and had high fruit counts, which allowed for consecutive fruit samplings without risking future collections throughout the fruiting season.

Fruit and seed sampling

Fruit and seeds of *C. camphora* were collected from four different stages to determine whether seedling emergence was affected by the maturity stage and status of the seed when planted. The four different stages tested where; (i) unripened fruit - green fruit that was collected directly off the tree, (ii) ripened fruit – blue-black fruit that was collected directly off the trees, samples were only collected if there was little to no resistance by the fruit when pulled it of the pedicle, (iii) shed fruit – intact fruit that had fallen beneath the tree canopy and had no signs of damage to the skin, and (iv) consumed fruit – seeds that were collected

beneath the tree canopy that had been either excreted or regurgitated after ingestion by birds. The study took place for the duration of the fruiting season, from mid-February to mid-July 2016. Fruit and seed sampling occurred once a week (Wednesday or Thursday) from 07hr00 in the morning to prevent any disturbances from affecting the samples such as leaf blowers or high volumes of student traffic. Samples of either five or 10 fruit per tree, depending on availability, were collected per stage, excluding the faecal sample stage (as the actual number of fruit from which they came from could not be counted due to secondary predation by birds or trampling by student traffic) per tree; leading to a total sample of approximately 60 whole fruit samples collected each week for the duration of the fruiting season of approximately 18 weeks, and a grand total of approximately 3240 fruit samples for the whole study.

All samples collected across the four stages were planted individually in seedling trays in a greenhouse trial (each with 30 'pots' per tray (diameter: 45mm, depth: 57.5mm)) within 24 hours of being collected. All samples were planted as collected (intact fruit from the three fruit stages and seeds collected from faecal samples) with each sample being surface-sown in Culterra professional potting mix and left to germinate in the greenhouse on the university grounds under approximately 80% netted shading. Watering occurred every 24 h; 15 min in the morning. The periodic watering also helps to prevent seed death from desiccation (Traveset *et al*. 2008). Seedling emergence counts were recorded every two days from when the first samples had been planted (mid-February 2016) until a year after the last samples had been planted (late-July 2017). A seedling was considered to have successfully emerged once the plumular axis became visible above the soil surface. Observations for seedling emergence were conducted every second day and any emerged seedlings had the pot surface marked with Tipp-ex marking to prevent double counting in subsequent visits.

Seed viability

At the end of the emergence trial, the remaining (un-germinated) seeds still in the soil, were tested for seed viability through the use of 2, 3, 5-triphenyltetrazolium chloride solution (e.g. Mbalo and Witkowski 1997). All seeds after 12 months since being planted were observed and categorized based on the condition of the seed. These conditions included; (a) seedlings that had successfully emerged, (b) seeds that had shown some form of still emerging, such as the appearance of a root, (c) seeds that had rotted away completely, (d) seeds that had died due to predation by insect larvae or fungi, and (e) intact seeds that had not shown any signs of emergence. It was the latter (e) seeds that were tested for viability using 2, 3, 5-triphenyltetrazolium chloride.

Any seeds across the four seed stages used in the seedling emergence trials that had not grown after 12 months since being planted were collected from the trays and had their seed status recorded. Only the intact seeds collected were tested for viability. The seeds were gently rinsed in tap water before the endocarp was cut with a scalpel and the seed carefully split apart as to not damage the embryo as it became exposed. Each seed was then placed in a labelled 5ml vial and covered in 1% 2, 3, 5-triphenyltetrazolium chloride solution for 24 hours. Testing occurred in a room with low light levels to prevent the solution from becoming inert. After 24 hours the vials were removed and the embryo colour observed. The seed was considered viable if the embryo changed to a pale pink colour. If the embryo remained white, then the seed was categorised as unviable.

Data analysis

All collected data were analysed using Microsoft Excel (2013). Different factors were observed, including the total number of seedlings that successfully emerged in the greenhouse trial from the samples collected each calendar month from the start of the 2016

fruiting season (March) to the end of the season the following year (June); and the number of days until successful seedling emergence, based on the month the fruit/seeds were collected from the sample trees as well as between the fruit/seed stages planted, the fruit/seed stage at the time of planting; and the fruit/seed stage at the time of planting within each month of planting.

One-way analysis of variance (ANOVA) tests were performed in order to determine whether the mean number of days until seedling emergence differed significantly between the different fruit/seed stages. These variables included: i) the four different fruit/seed stages tested, ii) the month the fruit/seeds were collected from the sample trees (and then planted in the greenhouse trial) during the fruiting season, and iii) between the four fruit/seed stages within each of the five months in which planting occurred during the 2016 fruiting season of the *C. camphora* trees. If significant differences were found, further testing was conducted using Tukey HSD tests.

Chi-square tests for independence were used to determine whether any significant associations occurred between: i) number of emerged seedlings compared to seeds that did not emerge as seedlings across the four fruit/seed stages, ii) the fates of seeds after the completion of the seedling emergence trial (emerged/still emerging, decomposed and did not emerge) across the fruit/seed stages, iii) between the four ways seeds decomposed during the year, iv) the final fates of seeds after the viability tests and, v) the number of overall viable and unviable seeds across the four fruit/seed stages (germination plus tetrazolium positive seeds).

Results

Fruit/seed sampling

The majority of *C. camphora* seeds (66.9%) did not successfully emerge as seedlings over the 12 months of the emergence trial. The highest successful level of seedling emergence was from the shed fruit stage, with 40.8% of the 590 planted fruits emerging as seedlings (Table 3.1.). This was followed by the consumed seeds and ripened fruit stages with 38.2% and 38.3% of successful seedling emergences respectively (Table 3.1.). The unripened fruit stage had the lowest number of seedling emergences as only 22.6% of planted fruits emerged (Table 3.1.), although the unripened fruit stage had the highest number of planted samples (Table 3.1.). The 4 x 2 Chi-square analysis showed that there was a significant association between the different fruit/seed stages in terms of the proportion of seedling emergence ($\chi^2 = 70.4$, d.f.=3, n=2364, P<0.00001).

Table. 3.1. The total number of *Cinnamomum camphora* fruit/seeds planted in the University of Witwatersrand, Johannesburg greenhouse after collection from and under six *C. camphora* trees, the total number and percentage that successfully emerged as seedlings, and the total number and percentages of seeds that did not emerge across four different fruit/seed stages.

Stage of fruit/seed	Total fruit/seed planted	Total seedling emergence		Total seeds that did not emerge as seedlings	
	n	n	%	n	%
Unripened fruit	880	199	22.6	681	77.4
Ripened fruit	690	264	38.3	426	61.7
Shed fruit	590	241	40.8	349	59.2
Consumed fruit	204	78	38.2	126	61.8
Total	2364	782	33.1	1582	66.9

Seedling emergences of *C. camphora* commenced at three different times in 2016 for the various fruit/seed stages (Table 3.2.). The consumed seeds had emergences commence during August, although one seedling emerged in June 2016. The two on-tree stages (ripened

and unripened fruit) had peak emergences in September 2016, with 86 and 67 seedling emergences respectively (Table 3.2.; Figure 3.1.); and the shed fruit and consumed seed stages had peak emergences in November 2016, with 54 and 27 emergences respectively (Table 3.2.; Figure 3.1.). The three intact fruit stages had reductions in the total number of emergences during October before the number increased again during November 2016 (Figure 3.1.), when the number of consumed seeds increased sharply for the first time (Figure 3.1.). The number for total emergence for the three intact stages decreased at the start of summer (December 2016) (Figure 3.1.). The four stages all took a year or more for emergences to be complete (Table 3.2.; Figure 3.1.). The unripened fruit took the shortest time with only 12 months until emergences finished (Table 3.2.; Figure 3.1.), while the ripened fruit took the longest with 17 months (Table 3.2.; Figure 3.1.).

Table 3.2. The start and end months of successfully emerged *Cinnamomum camphora* seedlings within the University of Witwatersrand, Johannesburg greenhouse, the length of emergences and the peak emergence months for the four different fruit/seed stages.

Stage	First month of emergence	Last month of emergence	Length of emergence	Peak emergence month
Consumed fruit	June/August 2016	July 2017	14 months	November 2016
Ripened fruit	March 2016	July 2017	17 months	September 2016
Shed fruit	May 2016	June 2017	14 months	November 2016
Unripe fruit	May 2016	April 2017	12 months	September 2016

September 2016 had 32.6% of *C. camphora* seeds from ripened fruit and 33.7% of seeds from unripened fruit successfully emerge; while seeds from shed fruit only had 22.4% of seedlings emerge (Figure 3.1.). Seeds that had been consumed by birds had a smaller sample size than the other three stages, but also managed to achieve to have a peak month, namely November 2016, where 35.1% of seeds had successfully emerged (Figure 3.1.).

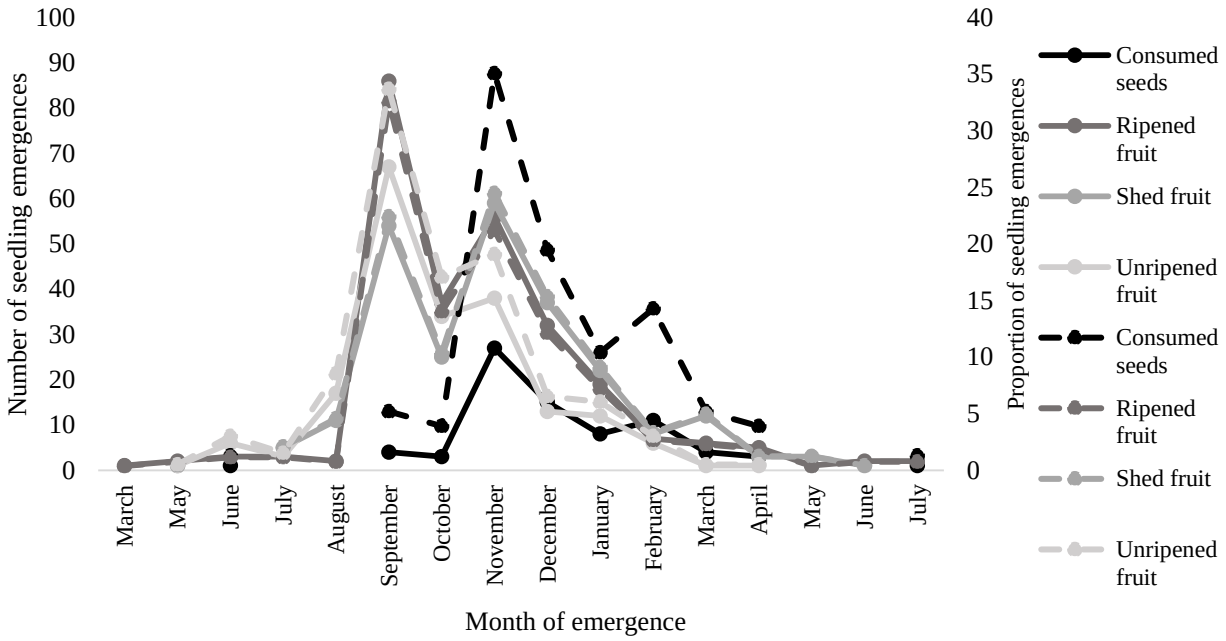


Figure 3.1. The number (solid lines) and proportion (broken lines) of *Cinnamomum camphora* seeds from four different fruit/seed stages that successfully emerged each month over the duration of the 2016/2017 fruit/seed sampling period.

There were significant differences between the four *C. camphora* fruit/seed stages in terms of the rate of emergence (ANOVA: $F_{(3,778)} = 15.8$, P<0.00001; Figure 3.2.). Consumed seeds had took significantly longer mean days for seedlings to emerge, 235 days from being planted; compared with the three intact fruit stages (Tukey HSD P<0.01) (Figure 3.2.). The seedlings from the shed and ripened fruit stages each took ~200 days to emerge and were the only combination that was not significantly different (Tukey HSD P>0.5) (Figure 3.2.). The unripened stage had the fastest emergence time at 188 days (Figure 3.2.); and had a significantly higher seedling emergence than both shed fruit, (Tukey HSD P<0.05); and ripened fruit (Tukey HSD P<0.01) (Figure 3.2.).

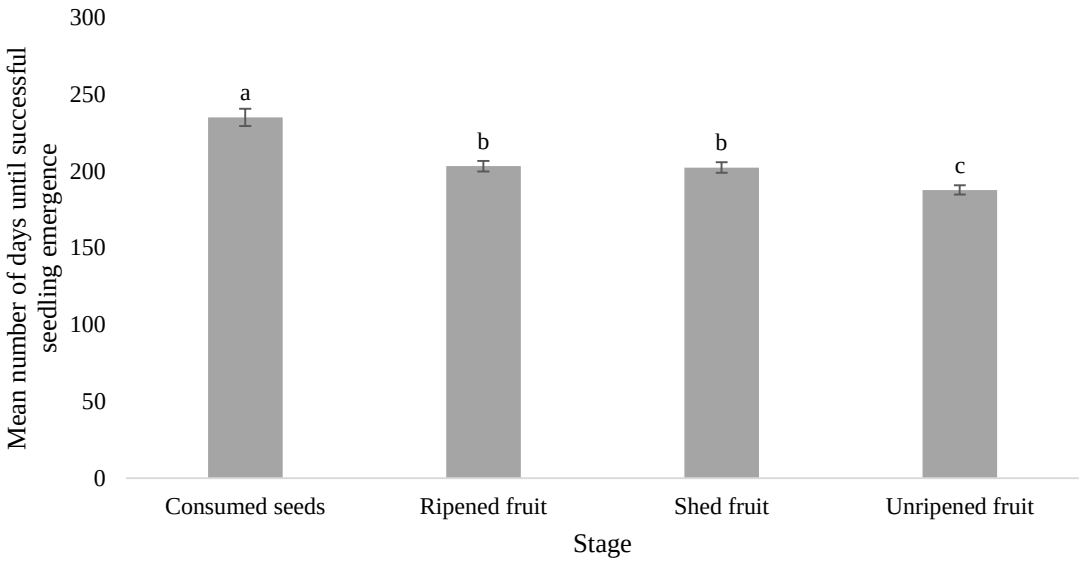


Figure 3.2. Mean number of days (±SE) for *Cinnamomum camphora* seedlings to emerge from four different fruit/seed stages (Tukey HSD P<0.01; P<0.05).

The five months in which *C. camphora* fruit/seeds were planted had significant differences in time to seedling emergence between the four stages (ANOVA: $F_{(4,777)} = 14.5$, P<0.00001; Figure 3.3.). Fruit/seeds planted during February took significantly shorter for seedlings to emerge compared with the other four months (Tukey HSD P<0.01), with seedlings taking only 134 days to emerge (Figure 3.3.). Fruit/seeds planted in April and June 2016 both took 204 days from planting to emergence (Figure 3.3.). The mean number of days until emergence for fruit/seeds planted in June was not significantly different to those planted in March, April or May 2016 (Tukey HSD P>0.01) (Figure 3.3.). Fruit/seeds that were planted in May 2016 took the longest number of 223 days until seedling emergence, and took significantly longer than fruit/seeds planted during March and April 2016 (Tukey HSD P<0.01) (Figure 3.3.). Fruit/seeds planted in March 2016 took 197 days for seedlings to emerge and was not significantly different to fruit/seeds planted in April 2016 (Tukey HSD P>0.5) (Figure 3.3.).

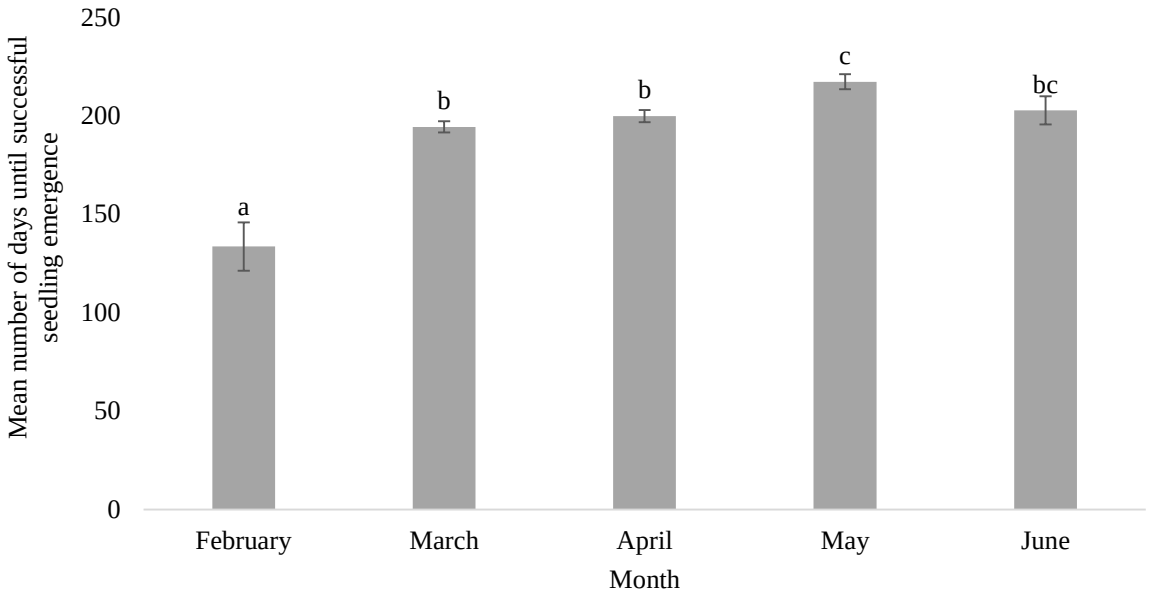


Fig 3.3. Comparison between the five calendar months of the 2016 *Cinnamomum camphora* fruiting season in which fruit/seeds were planted, and the mean number of days (±SE) for seeds to successfully emerge (Tukey HSD P<0.01).

While there were no significant differences between the four *C. camphora* fruit/seed stages within the months of February (ANOVA: $F_{(2,14)} = 1.72$, P=0.21), March (ANOVA: $F_{(3,192)} = 1.31$, P=0.27), and June (ANOVA: $F_{(3,52)} = 2.24$, P=0.09), there were significant differences found for April (ANOVA: $F_{(3,290)} = 5.79$, P=0.0007) and May (ANOVA: $F_{(3,215)} = 6.28$, P=0.0004). Consumed seeds planted in April 2016 took significantly longer for seedlings to emerge than the shed and ripened fruit (Tukey HSD P<0.05) and unripened fruit (Tukey HSD P<0.01) (Table 3.3.). Consumed seeds planted in May 2016 took significantly longer for emergence to occur than the ripened fruit and unripened fruit stages (Tukey HSD P<0.01) (Table 3.3.). The mean days until seedling emergence were not significantly different between consumed seeds and shed fruit that had been planted in May (Tukey HSD P>0.01) (Table 3.3.); and differences of emergence times were not significant between shed fruit and both ripened and unripened fruit (Tukey HSD P>0.01) or between ripened and unripened fruit (Tukey HSD P>0.5).

Table 3.3. Mean ± standard error of the number of days for *Cinnamomum camphora* seedling emergence during greenhouse trials, between the four fruit/seed stages collected from and beneath six *C. camphora* trees, for each month of the 2016 planting period. Months in **bold** had significant differences between the four fruit/seed stages as shown by different letters (Tukey HSD, P<0.05).

	Month				
Stage	February	March	**April**	**May**	June
Consumed seeds	----------	207 ± 11.1[a]	229 ± 7.2[a]	255 ± 11.2[a]	227 ± 20.2[a]
Ripened fruit	112 ± 16.7[a]	199 ± 6.7[a]	201 ± 5.7[b]	225 ± 5.0[ab]	193 ± 18.4[a]
Shed fruit	115 ± 20.3[a]	194 ± 4.3[a]	200 ±6.1[b]	208 ± 8.5[b]	220 ± 6.4[a]
Unripened fruit	157 ± 18.2[a]	188 ± 4.9[a]	184 ±5.1[b]	203 ± 7.1[b]	177 ± 13.5[a]

Seed viability

After the completion of the one year seedling emergence trial, all planted *C. camphora* fruit/seeds were classified into one of four categories depending on the fate of the seed (Table 3.4.). The fate of most of the seeds from unripened fruit was decomposition, with 40.3% of all planted seed having this fate, followed by 36.7% having not emerged and hence were then collected (as they were intact) for viability testing (Table 3.4.). Planted seeds that had originally been consumed by birds had 45.1% of seeds decomposed throughout the year, and only 38.2% of seeds emerged as seedlings (Table 3.4.). Most of the seeds from the shed fruit stage either did not emerge as seedlings (43.7%) or had successfully emerged as seedlings (40.8%) over the year since being planted (Table 3.4.). Seeds that had been planted as ripened fruit had similar numbers of seeds that had emerged as seedlings or had not emerged at all and were collected for viability testing after a year, with 38.3% and 37.5% of seeds for each fate respectively (Table 3.4.). Overall, more seeds did not successfully emerge and were collected for viability testing (36.9%) than seeds that had emerged as seedlings (33.0%) and seeds that had decomposed (29.7%) over the past year for all four of the

fruit/seed stages (Table 3.4.) A significant association was found between the different fates of the four fruit/seed stages (4x3 $\chi^2 = 178.1$, d.f. =6, n=2363, P<0.00001) (Table 3.4.).

Decomposed *C. camphora* seeds were categorized depending on the state the seed was found in, including the seed having rotted away completely, with only the seed coat remaining, the seed was infected by fungus or had been predated by insect larvae (Table 3.5.). The majority of the decomposed seeds from the unripened fruit stage had only the hollow seed coat remaining (55.8%), while 21.4.% of seeds in this category had decomposed from fungus infection and 14.9% had only the seed coat left (Table 3.5.). The seeds that had previously been consumed by birds had the largest percentage of seeds being infected by fungus with 47.8%, followed by seeds that had rotted away completely with 26.1%, while seeds that only had the seed coat left with 19.6%, and the least number of seeds had been predated by insect larvae with 6.5% (Table 3.5.). The ripened and shed fruit stages had similar results, with the larger percentage of seeds having fungal infection (50.3% and 49.5% respectively) and the fewest having only the seed coat left (3.7% and 5.5% respectively) (Table 3.5.) There was a significant association between the causes of seed decomposition between the four fruit/seed stages (4x4 $\chi^2 = 193.8$, d.f. =9, n=701, P<0.00001) (Table 3.5.).

Cutting open the *C. camphora* seeds for viability testing showed that the majority of embryos across three of the four fruit/seed stages were dead (Table 3.6.). The consumed seeds and unripened fruit stages had the highest percentages of dead seeds with 71.9% and 74.6% respectively, followed by seeds that had been planted as shed fruit with 53.5% (Table 3.6.). The remaining seeds for the three stages that had been planted as intact fruit (unripened, ripened and shed fruit) were primarily unviable with 14.9%, 30.1% and 31% of embryos remaining white after testing respectively (Table 3.6.). The consumed seed stage was the only stage that had slightly more viable (18.8%) than unviable seeds (9.4%) after tetrazolium testing (Table 3.6.).

Overall, only 15.5% of tested *C. camphora* seeds across the four stages remained viable while 24% became unviable over the previous year (Table 3.6.). There was a significant association between the four fruit/seed stages in terms of the final fates (dead embryo, unviable embryo or viable embryo) of collected seeds (4x3 $\chi^2 = 53.73$, d.f.=6, n=872, P<0.00001) (Table 3.6.).

Table.3.4. Different fates of the *Cinnamomum camphora* fruit/seeds, at the end of the one year seedling emergence trial, since being planted in the University of Witwatersrand, Johannesburg greenhouse, during 2016, for the four different fruit/seed stages. Percentages are given in parentheses.

Stages	Total number of planted fruit/seeds	Successfully emerged	Still emerging	Decomposed seeds	Did not emerge
Unripened fruit	880 (100)	199 (22.6)	3 (0.3)	355 (40.3)	323 (36.7)
Ripened fruit	690 (100)	264 (38.3)	4 (0.6)	163 (23.6)	259 (37.5)
Shed fruit	590 (100)	241 (40.8)	0 (0)	91 (15.4)	258 (43.7)
Consumed seeds	204 (100)	78 (38.2)	2 (1.0)	92 (45.1)	32 (15.7)
Total	2364 (100)	782 (33.0)	9 (0.4)	701 (29.7)	872 (36.9)

Table 3.5. Breakdown of the different ways *Cinnamomum camphora* seeds, collected from and beneath six *C. camphora* trees, decomposed during the one year seedling emergence trial, with the percentages in parentheses.

Stages	Total number of decomposed seeds	Rotted away seeds	Hollow seed coat	Fungal infection	Predated by insect larvae
Unripened fruit	355 (100)	53 (14.9)	198 (55.8)	76 (21.4)	28 (7.9)
Ripened fruit	163 (100)	50 (30.7)	6 (3.7)	82 (50.3)	25 (15.3)
Shed fruit	91 (100)	32 (35.2)	5 (5.5)	45 (49.5)	9 (9.9)
Consumed seeds	92 (100)	24 (26.1)	18 (19.6)	44 (47.8)	6 (6.5)

Table 3.6. Total number (and percentages in parentheses) of remaining *Cinnamomum camphora* seeds collected after the one year of greenhouse seedling emergence trial, the number of dead embryos found and the total number of embryos that were found to be either viable or unviable after 2,3,5-triphenyltetrazolium chloride testing.

Stages	Total seeds collected after emergence trials	Dead embryos	Total viable embryos	Total unviable embryos
Unripened fruit	323 (100)	241 (74.6)	34 (10.5)	48 (14.9)
Ripened fruit	259 (100)	126 (48.6)	55 (21.2)	78 (30.1)
Shed fruit	258 (100)	138 (53.5)	40 (15.5)	80 (31)
Consumed seeds	32 (100)	23 (71.9)	6 (18.8)	3 (9.4)
Total	872 (100)	528 (60.6)	135 (15.5)	209 (24)

A *C. camphora* seed was considered to be "viable" if it fell into one of three conditions; the seed successfully emerged as a seedling, was still in the process of emerging at the end of the year, or the embryo was viable after viability testing. The unripened fruit stage had the lowest overall "viable" seeds with only 26.8% (Table 3.7.). The other three stages, ripened fruit, shed fruit and consumed seeds, had higher and more similar overall "viable" seed percentages with 46.9%, 47.6% and 42.1% respectively (Table 3.7.). Overall, the percentages of *C. camphora* seeds that had remained "viable" over the 2016/2017 year of

planting was low with only 39.2% of all planted seeds classified as such (Table 3.7.). The Chi-Square analysis showed a significant association between the four fruit/seed stages in terms of whether the seeds were viable or unviable after the seedling emergence trial (4x2 χ^2 = 91.73, d.f.=3, n=2364, P<0.00001) (Table 3.7.).

Table 3.7. Total number of *Cinnamomum camphora* seeds planted for four different fruit/seed stages (collected from and beneath six *C. camphora* trees and germinated in greenhouse trials) and the total number and percentages of the seeds considered as "viable".

Stages	Total seeds planted	Total "viable" seeds	
	n	n	% viability
Unripened fruit	880	236	26.8
Ripened fruit	690	323	46.9
Shed fruit	590	281	47.6
Consumed seeds	204	86	42.1
Total	2364	926	39.2

Discussion

Cinnamomum camphora seeds that had been ingested by birds took significantly longer for seedlings to emerge, while the unripened fruit taken directly off the trees were significantly faster. Dlamini *et al*. (2018) tested ingested fruit, whole fruit and manually de-pulped fruit to determine if native frugivores influenced the germination time and success of two South African invasive plant species, namely the Brazilian pepper tree (*Schinus terebunthifolius*) and the Indian laurel (*Litsea glutinosa*). The two bird species observed feeding were the Red-winged Starlings and Dark-capped Bulbuls (Dlamini *et al*. 2018), the same species observed as major visitors to *C. camphora*. Whole fruit had the longest overall germination time, while ingested and manually de-pulped seeds had similar shorter times (Dlamini *et al*. 2018). The similar germination success between manually de-pulped and ingested seeds also showed that fruit pulp removal on its own, without seed coat abrasion, results in positive germination success (Dlamini *et al*. 2018).

Jordaan *et al*. (2011) found that *C. camphora* seeds ingested by Red-winged Starlings started germinating after around 20 days since being planted. This was significantly earlier than seeds ingested by Dark-capped Bulbuls (Jordaan *et al*. 2011). Dlamini *et al*. (2018) found that Red-winged Starlings also had a significantly higher germination potential than Dark-capped Bulbuls. However, ingested seeds overall did not germinate significantly earlier than manually removed seeds or seeds left inside the fruit (Jordaan *et al*. 2011). Percentage of germinated seeds did not vary amongst bird species (Dlamini *et al*. 2018).

Figueroa and Castro (2002) found that there were no significant differences in the germination proportions of *Gaultheria mucronata* seeds within the fruit pulp and seeds handled by birds outside of the fruit. This indicates that birds were only acting as dispersal agents for *G. mucronata* and were agents that affected seed germination biology (Figueroa and Castro 2002).

Bartuszevige and Gorchov (2006) compared *Lonicera maackii* seeds that were retrieved from faecal samples, manually removed from the fruit pulp and intact fruit. Passage through the gut of frugivorous birds was not necessary for the germination of *L. maackii* seeds, meaning that seeds could still germinate without bird assistance, such as for example by animals stepping on fallen fruit (Bartuszevige and Gorchov 2006).

Thabethe *et al*. (2015) found that, for *C. camphora*, fruit pulp removal leads to better germination as it removes osmotic pressure and germination inhibitors, and that seed coat abrasion was not essential. Thabethe *et al*. (2015) also found that manually de-pulped and turaco-ingested seeds had significantly higher germination success than whole fruit and suggested that this meant that pulp removal was necessary for germination. However, larger seeds (such as *C. camphora*) have been found to have a lower probability of surviving gut passage as the seeds are more likely to be damaged and scarified within the gizzard

(Kleyheeg *et al*. 2017). Larger seeds that do survive had higher germinability, although not significantly (Kleyheeg *et al*. 2017).

However, the low rate of seedling emergence of *C. camphora* in my study was different to Firth (1979) and Panetta (2001), both of which found that the pulp removal of *C. camphora* increased seed germination due to the germination inhibitors in the pulp and the tough, waxy seed coats that do not decompose easily. The reason for the low seedling emergence in the study conducted in this chapter could be due to other unknown factors instead of seed coat inhibition.

This study performed in this chapter excluded comparing the seeds ingested by birds to seeds that had been removed mechanically from the fruit pulp as numerous studies have already been done on that comparison (Barnea *et al.* 1991; Panetta and McKee 1997; Meyer and Witmer 1998; Richardson *et al*. 2000; Panetta 2001). However, often during these studies intact fruit is left out which results in the true effect that birds have on seed germination not being accurately measured (Robertson *et al*. 2006). My study, therefore, included stages in which the seeds remained within the fruit in order to replicate the more natural conditions that would have occurred to fruits that were uneaten by birds and had fallen to the ground with the seed still inside (Barnea *et al.* 1991; Yagihashi *et al*. 1998). Ripe fruit that was still on the tree was also collected to more accurately imitate the timing of when birds would consume the fruit as shed fruit could have been at various stages of decomposition and not often consumed by frugivorous species.

Bird processed seeds are often only available opportunistically (when the birds have not been captured and caged) and so the collected faecal seeds in this study are of unknown age, unknown disperser and it was unknown as to whether the seeds were regurgitated or defecated. There can only be the presumption that the seeds would have been voided from the

frugivorous bird species observed in the trees (Red-winged Starlings (*Onychognathus morio*), Dark-capped Bulbuls (*Pycnonotus tricolor*), Karoo Thrushes (*Turdus smithi*) and Common Mynas (*Acridotheres tristis*)). This is important data to know in the future as Bartuszevige and Gorchov (2006) found that Cedar waxwings (*Bombycilla cedrorum*) were actually detrimental to germination percentage of *L. maackii* seeds. This is also important as Mokotjomela *et al*. (2015) found that Red-eyed Doves (*Streptopelia semitorquata*) resulted in had the highest germination rates of *Acacia cyclops* seeds (note that typically Acacias in general (see Mbalo and Witkowski 1997) have a hard seed coat), that survived grinding in the gut. This indicates that some seeds ingested by doves can survive grinding and pass through intact (Mokotjomela *et al*. 2015).

Only a few samples were successfully collected from birds in and under the canopy of *C. camphora*. While testing the influence of birds on germination using caged individuals from the top visiting species would help solve this, a less stressful (for the birds) way to determine the effect on germination is to use testing similar to Kleyheeg *et al*. (2017). These tests included exposing seeds to gastric juices (using hydrochloric acid (HCl) and pepsin (Pu)) to mimic chemical digestion (Kleyheeg *et al*. 2017), and using intestinal matter collected from recently deceased birds for intestinal digestion effects (Kleyheeg *et al*. 2017).

Not knowing whether birds regurgitated or defecated the *C. camphora* seeds might be something that could be further investigated. Mokotjomela *et al*. (2015) found that *Acacia cyclops* seeds both regurgitated and defecated by Red-winged Starlings had equal germination rates. However, Rose-ringed parakeets were found to influence the germination success of *C. camphora* depending on the method of seed expulsion (Thabethe *et al*. 2015). Seeds that had been regurgitated by birds had both significantly higher germination percentages and seeds that significantly germinated faster than whole, intact fruit (Thabethe

et al. 2015). However, this same method of expulsion also had significantly lower germination proportions compared to whole fruit (Thabethe *et al*. 2015). While Corlett (1998) found that rock pigeons regurgitated a few whole seeds that then successfully germinated, David *et al*. (2015) suggested that this did not mean that these seeds are dispersed by seed predators, as these bird species could be regurgitating the seeds beneath the parent plant.

Seeds of *C. camphora* had the greatest proportion of seedlings starting to emerge during the months of spring. This could indicate that the seeds remained dormant until suitable conditions in the greenhouse reflected suitable conditions found in the field. These conditions in the greenhouse would have included warmth as temperatures started to rise towards the start of September.

The seeds of *C. camphora* have dormancy periods that can last for 4-20 weeks (Firth 1981). However, when observing the mean emergence rates based on the month the seeds were planted, showed that the dormancy of *C. camphora* was longer than what Firth (1981) listed, with the shortest rate of emergence taking around 19 weeks, with many seeds still viable but still not cued to germinate after a year. The long dormancy means that should these conditions occur in urban areas where the source plants are found, more seeds are left vulnerable to consumption by seed predator species.

Frugivores however, can have a variety of behaviours which can influence germination either from ingestion, processing or defecation (Amodeo *et al*. 2017). These processes may increase, decrease or have no effect on germination (Traveset 1998; Traveset et al. 2008; Amodeo *et al*. 2017). Seed germination can also be either increased, decreased or remain unchanged after the seed has passed through a bird's gut (Traveset 1998; Mokotjomela *et al*. 2015; Mokotjomela *et al*. 2016).

There are several, interconnected germination cues that were not observed within this chapter, of which some could be further investigated. The primary factor that connects these cues is soil depth (Panetta 2001; Xia *et al.* 2016; Kameyama and Nakajima 2018). Germination cues, such as soil moisture, light intensity and soil temperature have been found to be dependent on soil depth (Panetta 2001; Xia *et al.* 2016; Kameyama and Nakajima 2018). *Cinnamomum camphora* was found to have a seed germination cue from light only occurring up to and including 2mm below the soil surface, even when plant litter was absent, but could detect temperature signals within deeper soil (Xia *et al.* 2016; Kameyama and Nakajima 2018). Of interest is that once the seedlings have emerged and continue to grow, Kameyama and Nakajima (2018) determined that *C. camphora* seedling survivability and growth can be affected by both light intensity and soil moisture content.

Larger seeds (such as *C. camphora*) were found to become less dependent on light as a germination cue and more dependent on a low water potential and soil temperature fluctuations as soil depth increases (Xia *et al.* 2016; Kameyama and Nakajima 2018). Kameyama and Nakajima (2018) determined that seed germination of *C. camphora* were negatively affected by soil moisture content. This coincides with Panetta (2001), who observed that *C. camphora* seeds that had been surface sown had a higher germination with irrigation, while seeds planted at a depth of 1cm had better germination percentages without irrigation. Twigg *et al*. (2009) found that factors including inhibitory chemicals and adequate soil moisture, light and temperature conditions caused the germination failure of Tetrazolium-positive seeds during a 10-week trial.

Further investigation into soil depth, using similar testing to Witkowski and Wilson (2001), can help determine whether more than currently tested additional soil layers prevents or encourages more seedlings to successfully emerge after germination due to factors such as the weight of soil or soil pressure, and the condition of the soil above the seed (loosely

packed or more compacted soil). Soil type can also be investigated to determine whether the micronutrient composition can influence not only germination percentage, but the rate of germination as well.

Fire is another factor that could prevent or encourage seed germination based on the plant species. However, due to it being a rainforest tree species, *C. camphora* is often found in moist areas not often affected by fire, and therefore does not significantly require fire as a part of its life history (Graham and Taylor 2018). *Cinnamomum camphora* invests a significant amount of its energy on its tap root establishment once germination has been initiated (Graham and Taylor 2018), allowing *C. camphora* seedlings and sometimes saplings to survive, in areas where fire may be a threat, through the processes of resprouting and suckering (Graham and Taylor 2018). Areas in which fire management is possible as a control method, would require further post-fire controls to effectively control *C. camphora* populations (Graham and Taylor 2018). Pulp removal is essential for some plant species by preventing microbial attacks that can form from the decomposing fruit (Figueroa and Castro 2002; Thabethe *et al*. 2015) and by creating shorter germination times, therefore decreasing the probability of seed predation (Thabethe *et al*. 2015).

Seed Viability

The viability tests showed that regardless of whether the seed had passed through the digestive tract of birds or remained in the fruit, 15.5% of the remaining *C. camphora* seeds were still viable after a year of being planted. This is higher than the approximate 1% of surface-sown *C. camphora* seeds Panetta (2001) found to have remained viable after 12 months. This difference could be due to the methods used to test seed viability. Panetta (2001) used a dissecting microscope and counted seeds as unviable if the embryos showed signs of necrosis or discolouration. However, it may have been possible that the discoloured

embryos might have still been viable but were misclassified. The use of solutions such as 2, 3, 5-triphenyltetrazolium chloride (Mbalo and Witkowski 1997) can help prevent misclassification by providing more reliable results (Booth and Hendry 1993; Chen *et al*. 2004; Bartuszevige and Gorchov 2006).

It was unknown how many of the seeds that had been ingested by birds in my study were regurgitated and how many were excreted. The results after the emergence trials showed that any faecal matter that was planted with the seeds did not facilitate the growth of fungi or appearance of insect larvae any more than the decomposing fruit from the other three stages. This indicated that the faecal matter did not appear to influence seed viability, with some studies having found that ingested seeds can actually lose viability, leading to a reduced to no effect on germination success (LaRosa 1985; Wilson and Downs 2012; Thabethe *et al*. 2015).

However, sowing the seeds in the greenhouse trial may have influenced the viability results in regards to the fungi and insect larvae impacts on the seeds. The periodic watering of the fruits/seeds helped to prevent desiccation; but the high moisture and humidity conditions found within the greenhouse may have also encouraged bacteria, fungi and insect larvae to develop/grow and effect the viable seeds that may have been dormant. Gutterman (1994) found that 64-99% of Acacia tree species studied were infested by sand beetles. This high percentage could mean that insects result in a lower number of successful germinations and seedling emergences. Chen *et al*. (2004) found that the viability of *C. camphora* seeds remained at 100% when the moisture content of the surrounding fruit was 46.3%, and that this high viability was maintained even when the fruits/seeds were exposed to open air for a month, even with lower moisture content of 25%. The seeds in the greenhouse were not exposed to open air and were instead within an enclosed environment which may have caused the viability to decrease more rapidly.

An after-ripening period is necessary for *Kumara plicatilis* seeds before germination since after six weeks of germination trials, only 28% of 3-month-old seeds stored under ambient conditions germinated, while 80% of the remaining ungerminated seeds were still viable (Cousins *et al*. 2014). Storage under cold conditions for nine months had similar effects on both germination rate and percentage, and induced dormancy in around 60% of seeds (Cousins *et al*. 2014). It is believed that this could be from physiological dormancy, where an inhibitory mechanism of the embryos prevented radicle emergence (Baskin and Baskin 1998; Cousins *et al*. 2014). Further seed storage tests, such as those done by Cousins *et al*. (2014), could also be conducted to observe how the water content and thus germination and viability of *C. camphora* seeds can be affected by large changes to the environment, such as prolonged winter temperatures or desiccation conditions (high humidity). These tests could also include storing the seeds before any germination trials instead of immediate planting after collection as was done in this research.

The feeding behaviour and morphology of some bird species can also influence the number of dispersed viable seeds (Twigg *et al*. 2009). Parrot and finch species are least likely to disperse viable seeds as they de-husk seeds prior to ingestion and have efficient digestive tracts (Twigg *et al*. 2009). Similarly, very few viable seeds were also recovered from faecal matter of House Sparrows (*Passer domesticus*) and Rock Doves (Krach 1959). Furthermore, the germination of viable seeds that passed through omnivorous/herbivorous birds was reduced when compared with untreated control seeds, implying a reduced establishment of endozoochorous avian-dispersed seeds (Twigg *et al*. 2009).

Further studies on the viability of *C. camphora* can be done by observing whether the method of voiding the seeds (regurgitation or excretion) as well as by determining how the primary dispersers' predominant diets might influence the seed viability as the fertilization

effect can vary between different bird species as factors such as the species' diets and how the faecal matter influences the seeds on the ground (Robertson *et al*. 2006).

Bartuszevige and Gorchov (2006) found that seeds of *Lonicera maackii* that had been voided from Cedar Waxwings (predominantly frugivorous) were only 47% viable while the seeds that had been voided by American Robins (predominantly omnivorous) was much higher at 86% viable. The testing also showed that manually removed seeds had significantly higher viabilities than the waxwings only, while the viability of seeds that remained in the fruit was only significantly lower than the seeds voided by the robins (Bartuszevige and Gorchov 2006). The species of birds visiting *C. camphora* during the fruiting period range from predominant frugivores (Red-winged Starlings) to predominant granivores (Rock Doves) which could have various viability results compared with the original fruit stages as well as to manually de-pulped seeds.

There have been few studies performed to determine whether the different methods of voiding the seeds of the same plant species has any impact on the viability when performed by the same bird species. Meyer and Witmer (1998) found that the variation in the mechanical and chemical treatment of seeds in the guts of frugivorous birds did not differ significantly with regards to the germination percentages. Red-winged Starlings both regurgitate and defecated seeds (Mokotjomela *et al*. 2015) and so using this bird species to see if seed voiding methods have any significant differences on the viability of *C. camphora* could be undertaken in future studies.

McKenzie Pegman *et al*. (2017) found that birds were able to select larger fruit that had viable seeds, but this could be due to birds selecting based on other traits, such as fruit size, which have been correlated with seed viability (McKenzie Pegman *et al*. 2017).

Future testing on viability of *C. camphora* without the influence of bird ingestion could also include X-ray analysis (Al-Turki and Baskin 2017). This testing is a fast, simple, non-destructive method (Al-Turki and Baskin 2017), and can be used to examine a large number of seeds in a relatively short period of time (Gagliardi and Marcos-Filho 2011; Al-Turki and Baskin 2017).

Conclusion

Birds serve as both seed dispersers and germination mediators for some plant species, but only as seed dispersers for other plant species (Barnea *et al*. 1991; Panetta 2001; Figueroa and Castro 2002). Generalist frugivores, in particular, are often both dispersers and mediators of seed germination of invasive plant species that produce fleshy fruit and can enhance the invasion process of these plant species (Barnea *et al*. 1991; Panetta 2001). In the case of *C. camphora* birds act as seed dispersers only as the seeds were able to successfully emerge as seedlings without the need for gut passage. This means that the control of this invasive plant species can become more difficult in certain environments (Bartuszevige and Gorchov 2006), especially as some studies have shown that invasive plant species' seed germination is rapid regardless of whether avian digestion occurs or not (Barnea *et al*. 1991; Jordaan *et al*. 2011; Dlamini *et al*. 2018). As an example, McKenzie Pegman *et al*. (2017) found that germination of *Prumnopitys ferruginea* did not require scarification or de-inhibition for successful germination. This could be due to a number of seed traits that can be further investigated, including seed size, seed coat thickness and seed coat permeability (Kleyheeg *et al*. 2017).

However, bird ingestion did cause seedling emergence to take significantly longer on average compared to the three stages where the seed remained in the camphor fruit. This means that within an urbanised environment, seeds are exposed to seed predator species for longer periods of time, helping to control the spread of the *C. camphora* population. Birds

may increase the resilience of the tree species to seasonally fluctuating weather patterns through more seedling establishment opportunities by spreading the occurrence of germination over a longer time period.

Using the results from the germination and viability experiment trials, seed shadows can be formed for *C. camphora* in future studies by combining the seed quality data with data collected on which dispersers visit the trees, how long those dispersers retain the seeds via their gut passage rates, and the movement of those dispersers through the urban environment (Bartuszevige and Gorchov 2006).

References

Al-Turki, T. A. and Baskin, C. C. 2017. Determination of seed viability of eight wild Saudi Arabian species by germination and X-ray tests. *Saudi Journal of Biological Sciences* 24: 822-829.

Amodeo, M. R., Vázquez, M. B. and Zalba, S. M. 2017. Generalist dispersers promote germination of an alien fleshy-fruited tree invading natural grasslands. *PLoS ONE* 12(2): 1-17.

Barnea, A., Yom-Tov, Y. and Friedman, J. 1991. Does ingestion by birds affect seed germination. *Functional Ecology* 5(3): 394-402.

Bartuszevige, A. M. and Gorchov, D. L. 2006. Avian seed dispersal of an invasive shrub. *Biological Invasions* 8: 1013-1022.

Baskin, C. C. and Baskin, J. M. 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.* Academic Press, San Diego, California.

Booth, R. E. and Hendry, G. A. F. 1993. Seed viability and germination. In: *Methods in Comparative Plant Ecology* (eds. Hendry G. A. F. and Grime, J. P.) pp 10-13. Chapman and Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras.

Bradford, M. G. and Westcott, D. A. 2010. Consequences of southern cassowary (*Casuarius casuarius*, L.) gut passage and deposition pattern on the germination of rainforest seeds. *Austral Ecology* 35: 325-333.

Buckley, Y. M., Anderson, S., Catterall, C. P., Corlett, R. T., Engel, T., Gosper, C. R., Nathan, R., Richardson, D. M., Setter, M., Spiegel, O., Vivian-Smith, G., Voigt, F. A., Weir, J. S. and Westcott, D. A. 2006. Management of plant invasions mediated by frugivore interactions. *Journal of Applied Ecology* 43: 848-857.

Chen, Z.-H., Wu, B., Li, J.-Y., Zhao, J.-G., Zhou, X.-Y. and Zhang, Y.-K. 2004. Germination of the seeds and growth of the seedlings of *Cinnamomum camphora* (L.) Presl. *Plant Species Biology* 19: 55-58.

Cipollini, M. L. and Levey, D. J. 1997. Secondary metabolites of fleshy vertebrate dispersed fruits: adaptive hypotheses and implications for seed dispersal. *American Naturalist* 150: 346-372.

Corlett, R. 1998. Frugivory and seed dispersal by vertebrates in the Oriental (Indomalayan) Region. *Biological Reviews* 73: 413-448.

Cousins, S. R., Witkowski, E. T. F. and Mycock, D. J. 2014. Seed storage and germination in *Kumara plicatilis*, a tree aloe endemic to mountain fynbos in the Boland, south-western Cape, South Africa. *South African Journal of Botany* 94: 190-914.

Czabator, F. J. 1962. Germination value: an index combining speed and completeness of pine seed germination. *Forest Science* 8: 386-396.

D'Avila, G., Gomes-Jr, A., Canary, A. C. and Bugoni, L. 2010. The role of avian frugivores on germination and potential seed dispersal of the Brazilian pepper *Schinus terebinthifoloius*. *Biota Neotropica* 10: 45-51.

David, J. P., Manakadan, R. and Ganesh, T. 2015. Frugivory and seed dispersal by birds and mammals in the coastal tropical dry evergreen forests of southern India: a review. *Tropical Ecology* 56: 41-55.

Díaz Vélez, M. C., Sérsic, A. N., Traveset, A. and Paiaro, V. 2018. The role of frugivorous birds in fruit removal and seed germination of the invasive alien *Cotoneaster franchetii* in central Argentina. *Austral Ecology* 43: 558-566.

Dlamini, P., Zachariades, C. and Down, C. T. 2018. The effect of frugivorous birds on seed dispersal and germination of the invasive Brazilian pepper tree (*Schinus terebinthifolius*) and Indian laurel (*Litsea glutinosa*). *South African Journal of Botany* 114: 61-68.

Fedriani, J. M., Zywiec, M. and Delibes, M. 2012. Thieves or mutualists? Pulp feeders enhance endozoochore local recruitment. *Ecology* 93: 575-587.

Fenner, M. and Thompson, K. 2006. *The Ecology of Seeds.* Cambridge University Press, Cambridge.

Figueroa, J. A. and Castro, S. A. 2002. Effects of bird ingestion on seed germination of four woody species of the temperate rainforest of Chiloé Island, Chile. *Plant Ecology* 160: 17-23.

Finch-Savage, W. E. and Leubner-Metzger, G. 2006. Seed dormancy and the control of germination. *New Phytologist* 171: 501-523.

Firth, D. J. 1979. Ecology of *Cinnamomum camphora* (L.) Nees and Eberm (camphor laurel) in the Richmond-Tweed region of north-eastern New South Wales. Litt. B. Thesis, University of New England, Armidale, New South Wales, Australia.

Firth, D. J. 1981. Camphor laurel (*Cinnamomum camphora*) – a new weed in north-eastern New South Wales. *Australian Weeds* 1: 26-28.

Fricke, E. C., Haak, D. C., Levey, D. J. and Tewksbury, J. J. 2016. Gut passage and secondary metabolites alter the source of post-dispersal predation for bird-dispersed chili seeds. *Oecologia* 181: 905-910.

Gagliardi, B. and Marcos-Filho, J. 2011. Relationship between germination and bell pepper seed structure assessed by the X-ray test. *Science and Agriculture* 86(4): 411-416.

Gioria, M. and Pyšek, P. 2017. Early bird catches the worm: germination as a critical step in plant invasion. *Biological Invasions* 19: 1055-1080.

Graham, M. and Taylor, K. 2018. Fire, weeds and the native vegetation of New South Wales. A report prepared by the Hotspots Fire Project. Nature Conservation Council, New South Wales, Australia.

Gutterman, Y. 1994. Strategies of seed dispersal and germination in plants inhabiting deserts. *The Botanical Review* 60(4): 373-425.

Jordaan, L. A., Johnson, S. D. and Downs, C. T. 2011. The role of avian frugivores in germination of seeds of fleshy-fruited invasive alien plants. *Biological Invasions* 13: 1917-1930.

Kameyama, Y. and Nakajima, H. 2018. Environmental conditions for seed germination and seedling growth of *Cinnamomum camphora* (Lauraceae): the possibility of

regeneration in an abandoned deciduous broad-leaved forest, eastern Japan. *Journal of Forest Research* 23(3): 190-194.

Kleyheeg, E., Claessens, M. and Soons, M. B. 2017. Interactions between seed traits and digestive processes determine the germinability of bird-dispersed seeds. *PLoS ONE* 13(4): 1-15.

Krach, K. E. 1959. The excretion of undigested seeds of clover, grasses and weeds by birds and the effects of passage through the stomach and intestines on viability. *Journal of Agronomy and Crop Science* 107: 405-434.

Krefting, L. W. and Roe, E. I. 1949. The role of some birds and mammals in seed germination. *Ecological Monographs* 19(3): 269-286.

LaFleur, N., Rubega, M. and Parent, J. 2009. Does frugivory by European Starlings (*Sturnus vulgaris*) facilitate germination in invasive plants? *Journal of the Torrey Botanical Society* 136: 332-341.

LaRosa, A. M., Smith, C. W. and Gardener, D. E. 1985. Role of alien and native birds in the dissemination of firetree (*Myricafava Ait.-Myriacaceae*) and associated plants in Hawaii. *Pacific Science* 39: 372-378.

Mbalo, B. A. and Witkowski, E. T. F. 1997. Tolerance to soil temperatures experienced during and after the passage of fire in seeds of *Acacia karroo, A. tortilis* and *Chromolaena odorata*: a laboratory study. *South African Journal of Botany* 63(3): 421-425.

McKenzie Pegman, A. P., Perry, G. L. W. and Clout, M. N. 2017. Size-based fruit selection by a keystone avian frugivore and effects on seed viability. *New Zealand Journal of Botany* 55(2): 118-133.

Meisenburg, M. J. and Fox, A. M. 2002. What role do birds play in dispersal of invasive plants? *Wildland Weeds* 2: 8-14.

Meyer, G. A. and Witmer, M. C. 1998. Influence of seed processing by frugivorous birds on germination success of three North American shrubs. *American Midland Naturalist* 140: 129-139.

Miloševic, M., Vujaković, M. and Karagić, Đ. 2010. Vigour tests as indicators of seed viability. *Genetika* 42(1): 103-118.

Mokotjomela, T. M., Hoffmann, J. H. and Downs, C. T. 2015. The potential for birds to disperse the seeds of *Acacia cyclops*, an invasive alien plant in South Africa. *Ibis* 157: 449-458.

Mokotjomela, T. M., Downs, C. T., Esler, K. and Knight, J. 2016. Seed dispersal effectiveness: a comparison of four bird species feeding on seeds of invasive *Acacia cyclops* in South Africa. *South African Journal of Botany* 105: 259-263.

Panetta, F. D. 2001. Seedling emergence and seed longevity of the tree weeds *Celtis sinensis* and *Cinnamomum camphora. Weed Research* 41: 83-95.

Panetta, F. D. and McKee, J. 1997. Recruitment of the invasive ornamental, *Schinus terebinthifolius,* is dependent on frugivores. *Australian Journal of Ecology* 22: 432 438.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. and Rejmanek, M. 2000. Plant invasions – the role of mutualisms. *Biological Review* 75: 65-93.

Robertson, A. W., Trass, A., Ladley, J. J. and Kelly, D. 2006. Assessing the benefits of frugivory for seed germination: the importance of the de-inhibition effect. *Functional Ecology* 20: 58-66.

Roxburgh, L. 2007. The effect of gut processing on the quality of mistletoe seed dispersal. *Journal of Tropical Ecology* 23: 377-380.

Schupp, E. 1993. Quantity, quality and the effectiveness of seed dispersal by animals. *Vegetatio* 107/108: 15-29.

Schupp, E. W., Jordano, P. and Gomez, J. M. 2010. Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* 188: 333-353.

Thabethe, V., Wilson, A-L., Hart, L. A. and Downs, C. T. 2015. Ingestion by an invasive parakeet species reduces germination success of invasive alien plants relative to ingestion by indigenous turaco species in South Africa. *Biological Invasions* 17: 3029-3039.

Traveset, A. 1998. Effect of seed passage through vertebrate frugivore's guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics* 1/2: 151-190.

Traveset, A. and Richardson, D. M. 2014. Mutualistic interactions and biological invasions. *Annual Review of Ecology, Evolution and Systematics* 45: 89-113.

Traveset, A., Riera, N. and Mas, R. E. 2001. Passage through bird guts causes interspecific differences in seed germination characteristics. *Functional Ecology* 15: 669-675.

Traveset, A., Rodríguez-Pérez, J. and Pías, B. 2008. Seed trait changes in dispersers' guts and consequences for germination and seedling growth. *Ecology* 89(1): 95-106.

Twigg, L. E., Lowe, T. J., Taylor, C. M., Calver, M. C., Martin, G. R., Stevenson, C. and Howe, R. 2009. The potential of seed-eating birds to spread viable seeds of weeds and other undesirable plants. *Austral Ecology* 34: 805-820.

van de Venter, A. 2001. What is seed vigour? *Journal of New Seeds* 2(3): 67-72.

Wilson, A. L. and Downs, C. T. 2012. Knysna turacos (*Tauraco corythaix*) do not improve seed germination of ingested fruit of some indigenous South African tree species. *South African Journal of Botany* 78: 55-62.

Witkowski, E. T. F. and Wilson, M. 2001. Changes in density, biomass, seed production and soil seed banks of the non-native invasive plant, *Chromolaena odorata*, along a 15 year chronosequence. *Plant Ecology* 152: 13-27.

Xia, Q., Ando, M. and Seiwa, K. 2016. Interaction of seed size with light quality and temperature regimes as germination cues in 10 temperate pioneer tree species. *Functional Ecology* 30: 866-874.

Yagihashi, T., Hayashida, M. and Miyamoto, T. 1998. Effects of bird ingestion on seed germination of *Sorbus commixta*. *Oecologia* 114: 209-212.

Yagihashi, T., Hayashida, M. and Miyamoto, T. 1999. Effects of bird ingestion on seed germination of two *Prunus* species with different fruit-ripening seasons. *Ecological Research* 14: 71-76.

CHAPTER FOUR

GENERAL CONCLUSION

Alien plant species often rely on mutualisms with local dispersers in order to disperse seeds, as dispersal is one of the only means for plant species to "move" through and colonize new environments (Cronk and Fuller 1995; Richardson *et al.* 2000; Jordaan *et al*. 2011, Jordano *et al*. 2011). However, these plant species can become invasive should populations not be monitored and controlled (Mokotjomela *et al*. 2015). *Cinnamomum camphora* (L.) J. Presl. (Lauraceae) could be one such species that may be expanding its range through a mutualism with local bird dispersers within the Gauteng province as its invasive status has not been classified by NEMBA Act 10 (Invasive Species Compendium 2015)

This study assessed how birds affect the seed dispersal of *C. camphora* in an urbanised area across both the quantitative (avian frugivory) and qualitative (seed viability/germination and seedling emergence) components, focusing on which species are primary visitors and consumers of *C. camphora* fruit, and how visiting bird species that consume *C. camphora* fruit impact on seedling emergence in comparison to seeds in intact, unconsumed fruit.

Avian frugivory

Cinnamomum camphora produced a large amount of fruit over the 2016 fruiting season, with nearly 5000 ripened fruits/tree produced on the 12 sample trees on the University of Witwatersrand Braamfontein campus during the peak period. Over time, the increasing number of ripe *C. camphora* fruit over the fruit production period resulted in a concomitant increase in the number of birds visiting the trees. This large number of visiting birds was due to the fruit size being small enough (7.0-9.5mm in diameter; Panetta 2001,

Chen *et al*. 2004) for the majority of bird gape sizes, as well as a large amount of fruit that is produced by *C. camphora* during the fruiting period. Birds are often 'gape-limited' (Levey 1986; Symes and Downs 2001), meaning that smaller fruit can attract more bird species which increases the chance of potential dispersal (Jordano 2000; Burns 2013; Wenny *et al*. 2016; Sebastián-González 2017). Another reason for a large number of bird species was possibly due to the lipid-rich and sugar-poor nutritional content of *C. camphora,* allowing certain bird species to meet their daily energetic requirements (Jordaan *et al*. 2011b). The major bird species found in *C. camphora* tree canopies were a combination of four predominately omnivorous species (Rock Dove (26.4%), Karoo Thrush (19.1%), Common Myna (14.3%), and Red-eyed Dove (8.9%)) and two predominantly frugivorous species (Red-winged Starling (10.1%), and Dark-capped Bulbul (9.4%)). However, observing the seed consumption behaviour and seed condition after consumption, showed that only the two dove species were seed predators while the remaining four major bird species were legitimate seed dispersers.

The two bird activities that were observed occurring the most were the birds resting perched to the branches of the *C. camphora* trees (between 8 and 54% of observations), and birds feeding on ripened *C. camphora* fruit (between 35 and 51%). The Rock Dove, the species with the greatest number of visitors to *C. camphora*, was observed to also be performing secondary predation on *C. camphora* seeds that had been voided by the other bird species found in the *C. camphora* canopies. The high proportion of observations of birds resting in the trees across the six bird species was important to note for urban areas as those species could remain in the *C. camphora* for the duration of the seed retention times (including the small percentage of intact seeds that pass through seed predator gut intact; Mokotjomela *et al*. 2015), which would lead to seeds being voided beneath the source plants and either secondarily predated on the ground, trampled by human traffic, or seedlings

removed by gardening services, should the seeds manage to fall into the small soil spaces beneath the trees.

Seedling emergence

An important result from seedling emergence tests was that seeds from intact fruit still had successful seedling emergences even when it was previously found that the fruit pulp contained germination inhibitors (Firth 1979, Panetta 2001). Seedlings from *C. camphora* seeds that had been voided by visiting bird species took significantly longer to emerge when compared with seedlings from seeds that remained within intact fruit for the duration of the various fruit stages (unripened, ripened and shed), with unripened fruit taking the significantly shortest time for seedlings to emerge of the three fruit stages. The five calendar months in which the seeds were planted also had significant effects on the seedling emergence times, with seeds sown in February taking a significantly shorter time for seedlings to emerge. For two of the six fruiting months, April and May 2016, there were significant differences in the time to seedling emergence between the four fruit/seed stages. Consumed seeds planted during April took significantly longer for seedlings to emerge from than the shed and ripened fruit as well as the unripened fruit. Consumed seeds planted during May took significantly longer for a seedling to emerge compared with the ripened and unripened fruit stages only.

Seed viability testing showed that only 15.5% of seeds from across all four fruit/seed stages that had not emerged within the 12 first months remained viable in the greenhouse pot soil. The remaining seeds that did not emerge within those 12 months were either unviable or the seed/embryo had been decomposed due to fates such as predation by insect larvae, fungal infection or the seed had completely rotted away. The non-significant seed viability between the four fruit/seed stages, as well as the reduced germination emergence “rate” that resulted

from bird ingestion indicated that birds were reducing the seed vigour of *C. camphora* instead of increasing it. These results indicate that unlike the results from other studies on *C. camphora*, birds served as *C. camphora* dispersal agents only and not germination mediators as well as dispersers, making the control of *C. camphora* more difficult to monitor within certain habitats.

Recommendations for future studies

Seed shadows

The measurement of seed retention time of visiting bird species in relation to those species' major interactions with *C. camphora* trees, combined with the movement of the dispersers between visits can help determine the seed shadow of *C. camphora* within the urban area. Measurements of seed retention time can be done through captive bird species (seed predator species should also be included as intact seeds have passed through in a few instances (Mokotjomela *et al*. 2015)) being fed *C. camphora* fruit and timed from consumption of fruit until the voiding of the seed. These data would be combined with the distances visiting bird species travel during the time in which fruits are available to create an understanding on possible seasonal seed shadows.

A further study could include longer, more committed observation times for the 12 *C. camphora* trees and their primary bird visitors in order to accurately determine both the both feeding rates and the exact time certain species spent in the *C. camphora* trees. Symes and Downs (2001) found that two frugivorous bird species had peak feeding rates in the morning while dedicating the rest of the day to other activities. Observations of the 12 trees but for longer periods of time (for example a whole day per tree) will not only allow for determination of how long the major visiting species spend feeding in the *C. camphora* tree canopies, but also, when combined with knowledge about seed retention time for each

dispersing bird species, if the bird species are successfully dispersing the seeds away from the source plant or dropping seeds beneath it.

The movement of dispersers visiting *C. camphora* can be observed through two methods, visual observation and tracking via GPS (Côrtes and Uriarte 2013). GPS-telemetry and accelerometers can be used to monitor behavioural changes remotely (Côrtes and Uriarte 2013). This can help determine when birds are inactive in roosts or foraging in trees (Côrtes and Uriarte 2013). Using these tools can help determine disperser behaviours such as resting, feeding and flying (Côrtes and Uriarte 2013). Visual observations from both beneath the canopy and at a higher location would give a broad idea as to where the dispersers are moving to after feeding, while GPS tracking would allow for more accurate readings for larger bird species.

These new measurements along with the results obtained from this study on the different bird activities observed while birds are in *C. camphora* tree caopiess would show whether seeds of *C. camphora* have a greater chance of being successfully dispersed at varying distances away from the source plant, or if seeds are more likely to be voided directly beneath the source plant with a lower chance of subsequent germination and subsequent seedling emergence.

Seedling emergence: ingestion versus intact fruit stages

Seeds that are processed by birds are often only available for collection opportunistically and so faecal samples that can be collected are often of various ages, from different bird species and voided through unknown means. Jordano (1992) observed that regurgitation of seeds was more rapid than seeds excreted. A way to ensure seeds of similar or equal age and state in future studies, captive-held birds could be used as done by several previous studies (Barnea *et al*. 1991; Traveset *et al*. 2001; Bartuszevige and Gorchov 2006;

Bradford *et al.* 2010). These studies also often compared the voided seeds to seeds that had been manually de-pulped and/or scarified (Barnea *et al*. 1991; Traveset *et al*. 2001; Bartuszevige and Gorchov 2006; Bradford *et al.* 2010). A study could be performed that includes all of the ways that seedling emergence of *C. camphora* could be effected, including whether the de-inhibition (removal of the fruit pulp, which contains inhibitory chemicals, inside of the gut, Robertson *et al*. 2006) or scarification of the seed coat effects seedling emergence differently.

Seedling emergence differences between and within bird species

Different bird species have different effects on the number of viable seeds voided and could be further investigated. Bartuszevige and Gorchov (2006) found that seeds voided from Cedar Waxwings were only 47% viable compared with seeds that had been voided by American Robins which were 86% viable. Further studies could also be done to determine how the method of voiding the seed for particular bird species affects seedling emergence. Factors such as whether the seed was regurgitated or defecated (could impact scarification of the seed coat differently), the predominant diets of the major visiting species and how seeds react when they hit the ground could all have some influence on seedling emergence (Robertson *et al.* 2006). Red-winged Starlings both regurgitated and defecated seeds (Mokotjomela *et al*. 2015), and so using this bird species to see if different means of seed voiding affects viability of *C. camphora* should be used in future studies.

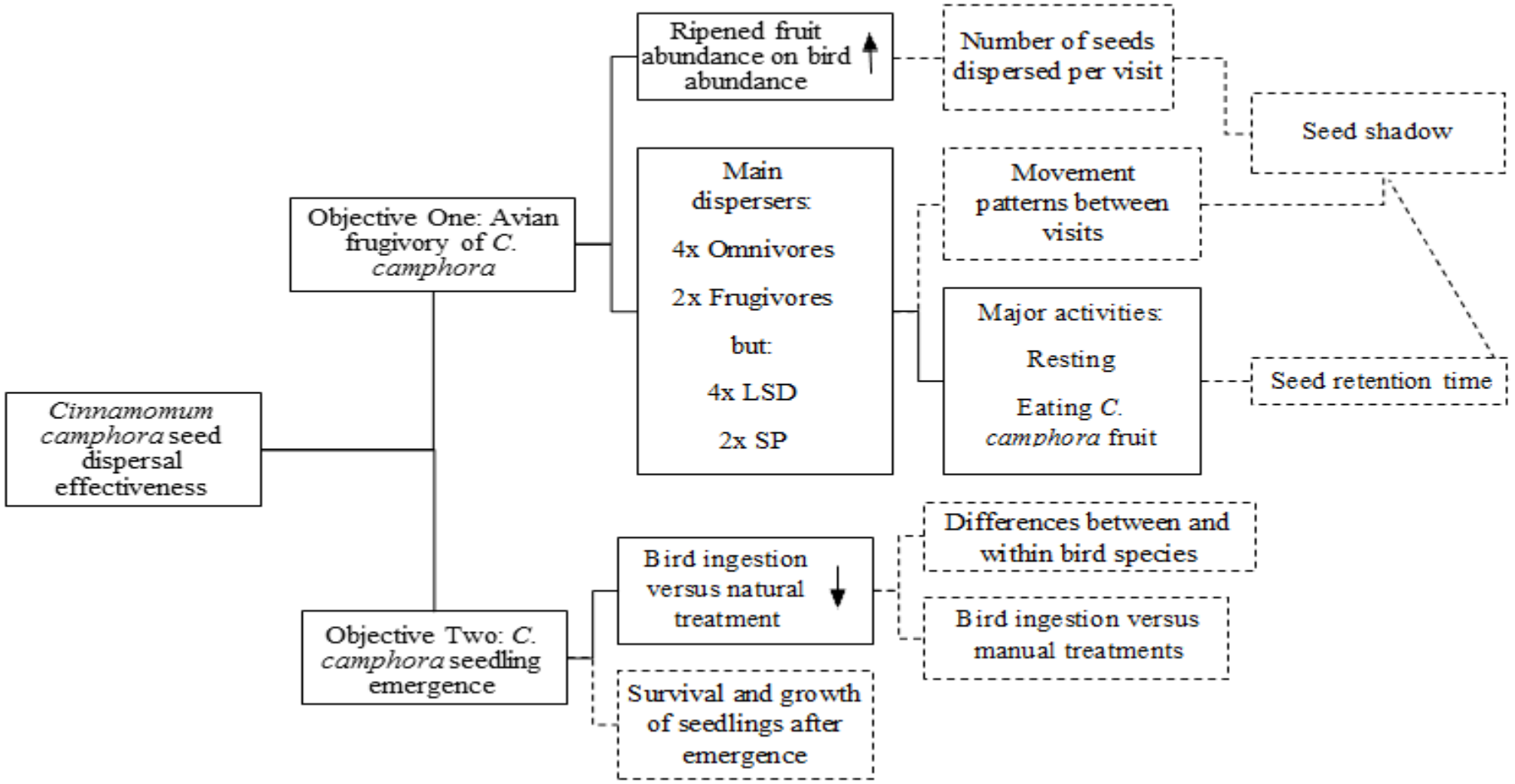


Fig. 4.1. Broad summary of the results obtained from this study. Solid boxes represent the findings from this study with arrows within boxes indicating the main effects (↑ = significance increase and ↓ = significance decrease). Dashed boxes represent factors that can be researched in future studies of the seed dispersal effectiveness of *Cinnamomum camphora*.

Conclusion

It is important to understand the role that bird species have on the seed dispersal of the alien plant species *C. camphora*. Understanding this role will assist in the future management and distribution predictions before populations become sufficiently large that control efforts become very difficult (Mokotjomela *et al*. 2015). Even though the major bird species (both seed predators and legitimate seed dispersers) are able to successfully disperse *C .camphora* seed, the bird species do not promote seed germination (as previously reported), as seedling emergence is not significantly affected by ingestion when compared with germination from seed directly shed onto the ground (without bird dispersal) and subsequent seedling emergence. Bird ingested seed also resulted in a reduced germination rate, indicating that birds can potentially reduce the seed vigour of *C. camphora*. It would be important to further observe how these results would differ when repeated in suburban and rural areas. Rural areas would be predicted to be more suitable for the establishment of *C .camphora* as seed predators such as the Rock Dove are fewer in number and the various habitats with varying levels of disturbance are often found to promote invasive plant species growth and establishment (Firth 1979; Neilan *et al*. 2006).

Additional studies on the other subcomponents that lead to a seed shadow, such as disperser movement patterns between feeding sites and mean retention times, and further comparison between the scarification and de-inhibition effects on seedling emergence can improve the overall understanding of the seed dispersal effectiveness of *C. camphora*. The relationship that exists between *C. camphora*'s and the various seed dispersing bird species creates the potential for *C. camphora* to become an invasive plant species in South Africa.

References

Barnea, A., Yom-Tov, Y. and Friedman, J. 1991. Does ingestion by birds affect seed germintion. *Functional Ecology* 5(3): 394-402.

Bartuszevige, A. M. and Gorchov, D. L. 2006. Avian seed dispersal of an invasive shrub. *Biological Invasions* 8: 1013-1022.

Bradford, M. G. and Westcott, D. A. 2010. Consequences of southern cassowary (*Casuarius casuarius*, L.) gut passage and deposition pattern on the germination of rainforest seeds. *Austral Ecology* 35: 325-333.

Burns, K. C. 2013. What causes size coupling in fruit-frugivore interaction webs? *Ecology* 94: 295-300.

Chen, Z-H., Wu, B., Li, J-Y., Zhao, J-G., Zhou, X-Y. and Zhang, Y-K. 2004. Germination of the seeds and growth of the seedlings of *Cinnamomum camphora* (L.) Presl. *Plant Species Biology* 19: 55-58.

Côrtes, M. C. and Uriarte, M. 2013. Integrating frugivory and animal movement: a review of the evidence and implications for scaling seed dispersal. *Biological Reviews* 88: 255-272.

Cronk, Q. C. B. and Fuller, J. L. 1995. *Plant Invaders.* London: Chapman and Hall.

Firth, D. J. 1979. Ecology of *Cinnamomum camphora* (L.) Nees and Eberm (camphor laurel) in the Richmond-Tweed region of north-eastern New South Wales. Litt. B. Thesis, University of New England, Armidale, New South Wales, Australia.

Invasive Species Compendium. 2015. *Cinnamomum camphora* (camphor laurel). Cabi.org,http://www.cabi.org/isc/datasheet/13519

Jordaan, L. A., Johnson, S. D. and Downs, C. T. 2011b. Digestion of fruit of invasive alien plants by three southern African avian frugivores. *Ibis* 153: 863-867.

Jordano, P. 1992. Fruits and frugivory, in: Fenner, M. (Ed.), *Seeds: the Ecology of Regeneration in Plant Communities.* CAB International, Wallingford, pp. 105-156.

Jordano, P. and Schupp, E. W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* 70(4): 591 615.

Jordano, P., Forget, P-M., Lambert, J. E., Böhning-Gaese, K., Traveset, A. and Wright, S. J. 2011. Frugivores and seed dispersal: mechanisms and consequences for biodiversity of a key ecological interaction. *Biology Letters* 7: 321-323.

Mokotjomela, T. M., Hoffmann, J. H. and Downs, C. T. 2015. The potential for birds to disperse the seeds of *Acacia cyclops*, an invasive alien plant in South Africa. *Ibis* 157: 449-458.

Neilan, W., Catterall, C. P., Kanowski, J. and McKenna, S. 2006. Do frugivorous birds assist rainforest succession in weed dominated oldfield regrowth of subtropical Australia? *Biological Conservation* 129: 393-407.

Panetta, F. D. 2001. Seedling emergence and seed longevity of the tree weeds *Celtis sinensis* and *Cinnamomum camphora*. *Weed Research* 41: 83-95.

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J. and Rejmanek, M. 2000. Plant invasions – the role of mutualisms. *Biological Review* 75: 65-93.

Robertson, A. W., Trass, A., Ladley, J. J. and Kelly, D. 2006. Assessing the benefits of frugivory for seed germination: the importance of the de-inhibition effect. *Functional Ecology* 20: 58-66.

Sebastián-González, E. 2017. Drivers of species' role in avian seed-dispersal mutualistic networks. *Journal of Animal Ecology* 86: 878-887.

Symes, C. T. and Downs, C. T. 2001. Feeding and energy intake in two avian frugivores, the Black-eyed Bulbul *Pycnonotus barbartus* (Passeriformes: Pycnonotidae) and Speckled Mousebird *Colius striatus* (Passeriformes: Coliidae). *Durban Museum Novitates* 26: 20-24.

Traveset, A., Riera, N. and Mas, R. E. 2001. Passage through bird guts causes interspecific differences in seed germiantion characteristics. *Functional Ecology* 15: 669-675.

Wenny, D. G., Şekercioğlu, Ç. H., Cordeiro, N. J., Rogers, H. S. and Kelly, D. 2016. Chapter 5: Seed Dispersal by Fruit-Eating Birds, in Şekercioğlu, Ç. H., Wenny, D. G. and Whelan, C. J. (Eds.). *Why Birds Matter: Avian Ecological Function and Ecosystem Services*. The University of Chicago Press, Chicago. Pp. 107-146.