

**REPRODUCTIVE ECOLOGY OF *PYRACANTHA ANGUSTIFOLIA* IN
AFROMONTANE GRASSLANDS OF THE EASTERN FREE STATE**

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2012067514

Dissertation submitted in fulfilment of the requirement for the degree of Master of
Science majoring in Botany, Department of Plant Sciences, Faculty of Natural and
Agricultural Sciences at the University of the Free State



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April 2020

DECLARATION

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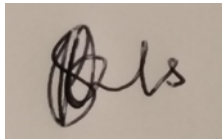
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ABSTRACT

Invasion by alien plants is one of biodiversity's major threats. Several plant traits are associated with invasiveness, such as fast growth rate, having long flowering and fruiting periods, higher fecundity and higher specific leaf area and efficient seed dispersal. Most of these traits are reproductive traits such as effective seed production, dispersal and pollination. It is important to study such traits in order to understand the process of invasion. These invaders are capable of modifying ecosystem functioning and disrupt ecosystem services, changing the plant community diversity. There are also economic losses such as loss in crop production. In South Africa, invasive fleshy-fruited shrubs in the Maloti-Drakensberg Mountains use native frugivores to disperse their seed. They also transform the habitat and threaten the agricultural productivity of the region. One of these invasive shrubs is the notorious *Pyracantha angustifolia*. This species was introduced to South Africa for ornamental purposes and used as hedgerows along highways. It has escaped cultivation and invaded grasslands. Scientifically sound information is needed in order to advise on invasive alien plant management strategies. The aim of this study was to determine the reproductive ecology and associated mechanisms of spread of *Pyracantha angustifolia* (Rosaceae) in the eastern Free State. Objectives of the study included determining: (1) the seed biology of the plant, (2) the effect of ingestion by frugivorous birds on germination rate and success, and (3) the role of floral visitors in pollination and reproductive success. Seed biology was determined through seed production, dispersal and soil seed bank studies. Fruit estimates indicated that the shrub produces enormous amounts of fruit (>5 million per square metre) available to frugivores for dispersal. Observations and seed traps confirmed frugivorous birds, livestock and wildlife to be seed dispersers. Soil cores revealed the soil seed bank to be more dense under ($46400 \text{ seed per m}^2 \pm 8934 \text{ SE}$) and near ($10000 \text{ seed per m}^2 \pm 3611 \text{ SE}$) source plants. Seed away from the parent plants were largely not viable. Seed traps indicated that seeds do also reach far distances under perching structures. Aviary trials involving feeding fruits to frugivorous birds and germination trials with defecated seeds were performed to investigate the effect of ingestion on germination. Ingestion by the four frugivorous birds, Dark-capped Bulbuls (*Pycnonotus tricolor*), Purple-crested Turacos (*Gallirex porphyreolophus*), Speckled Mousebirds (*Colius striatus*), and Red-winged Starlings (*Onychognathus morio*), under study did not affect germination rate and success; instead, the birds facilitate the spread and germination of seeds by removing the fruit pulp. Cape White-eyes (*Zosterops virens*) refused to feed on fruits. Overall, native South African birds were shown to play a pivotal role in the spread

and germination success of this invasive alien plant. Larger birds are important in dispersing *Pyracantha angustifolia* seeds further distances and smaller birds deposit seed at a faster rate. Floral visitor observations, pollinator exclusion of flowers and pollen load counts on insect floral visitors addressed the effect of insect floral visitors on pollination and reproductive success. The study showed that hymenopterans, specifically *Apis mellifera*, were the most important pollinating agents. *Pyracantha angustifolia* pollen dominated pollen loads of all insect floral visitors in the study site. Results revealed that this invader might be sharing pollinators with important crops. *Pyracantha angustifolia* produced healthy seeds without the aid of pollen vectors but the fruit yield was increased in the presence of a pollen vector. This plant used generalist pollinators to ensure increased fruit yield and thus it is likely to attract pollen vectors in newly invaded ecosystems. It uses native bird species as the main seed dispersers; they enhance germination by removing the pulp. Despite high fruit and seed production rates, this species' spread may be hampered by potential seed predation by rodents and low longevity of seeds in the soil seed bank.

Keywords: fleshy-fruited plants, germination, invasive alien plants, pollination, seed bank, seed production, seed dispersal

ACKNOWLEDGEMENTS

I am humbly grateful for supervision and guidance that I have received from my three supervisors, namely Dr S-L Steenhuisen, Dr VR Clark and Dr G Martin. With the same breath, I send my gratitude to the ground-forces, my field assistants, Albert, Dimakatso, Lindokuhle for being dedicated and patient in the field. I thank Mr Duncan Martin and Wyndford holiday farm for providing access to their properties for me to collect data. I acknowledge funding from University of the Free State Postgraduate Tuition Fees bursary for 2018 academic year. The financial assistance of the UFS Afromontane Research Unit (ARU) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the ARU. This work is based on the research supported in part by the National Research Foundation of South Africa (Grant number: SFH180531338186). I acknowledge the DST-NRF Centre for Excellence in Invasion Biology for providing running costs in the 2019 academic year. I acknowledge the South African National Botanical Institute for providing me with an internship and allowing me to complete my dissertation write-up while on an internship. Special thanks for the support that I have received from my family, your prayers kept me afloat. The Zion Christian Church Students' Fellowship should also receive special thanks for being my "home away from home", with you I have never felt alone in a new environment of Phuthaditjhaba. Motshela Mafiri, a best friend I have never had, thank you for always reminding me of whom I am and your words of encouragement through tough times. Valuable friendships from Dipuo Mosea and Albert Malefane will never go unnoticed, Thank you guys for being good company. Many thanks to Dr Thabiso Mokotjomela for always igniting discussions and questions particularly on seed dispersal and sharing research material. I thank Mr Dino Giovannoni (Rhodes University) for helping with fruit estimates data analysis and modelling in Python. Humble acknowledgements to Serero Modise and Dr Bredenhand from the Department of Entomology and Zoology (UFS) for insect identification. Many thanks to the Department of Plant Sciences staff and postgraduate students (UFS) for technical assistance and support. A token of appreciation to Sellwane Moloji for assisting with data analysis on germination trials, proofreading and dissertation layout. I appreciate Prof Downs for allowing me to use the animal house at UKZN, Vuyisile and Abi for assistance with acclimatisation of birds, feeding trials, germination trials and collecting germination records. Special thanks to Nasiphi (Dokotela) for sharing reading material on fleshy-fruited plants and companionship. My deep gratitude to Transport department UFS for always taking me to the field, Anthony and Nick for

assisting in bird watching and Journal club for contributing to my research critical thinking. I thank Lesly Henderson for sharing the SAPIA database.

Special thanks to myself for all the efforts and not thinking about quitting. Exceptional thanks to God of Mount Zion for spiritual guidance, reaching into places that I could not reach and making it possible for completion of this study.

“Keep faith and never lose hope in the God of Mt Zion” – His Grace The Right Rev Bishop Dr B. E. Lekganyane

DEDICATION

This dissertation is dedicated to my awesome parents, Nthabiseng and John Adams. It has not being easy at all, but your support to the study that you did not even understand kept me going. Thank you for allowing me to pursue the career of my choice.

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CHAPTER 1

GENERAL INTRODUCTION

For millennia, humans have moved organisms both accidentally and purposefully around the world (Hulme, 2009). With globalisation and economic development, the number of organisms moving and the speed by which they move has vastly increased (Chapman et al., 2017). Such human-aided movements are reshaping the Earth's biota, and this qualifies biological invasions as one of the most important features of the Anthropocene (Hui and Richardson, 2017; Richardson, 2011). Humans do not only facilitate the spread of alien species but also alter invasion mechanisms (Kueffer, 2017). An alien species is a species/taxon that has been introduced by humans to a distribution/spatial range outside of its natural distribution (CABI, 2012; Richardson et al., 2000; Hui and Richardson, 2017).

Invasion science investigates the artificial introduction of organisms, their ability to naturalise and become invasive, and their interactions with the indigenous biota (Fallis, 2011; Latombe et al., 2017). Due to dramatic impacts that invading organisms have on the global resident biota and ecosystem functioning, biological invasions have received increasing attention from scientists, policymakers and practitioners around the world (Canavan et al., 2019; Kesner and Kumschick, 2018; Richardson, 2011). Although timing has been debated (Chew., 2011), this field of science dates back to as early as the 1950s, through the work of English ecologist Charles Elton (1900–1991), with his famous and widely acknowledged work (e.g. Godoy, 2019;; Richardson, 2011; Vitousek et al., 2007) published in *The ecology of invasions by animals and plants* (Elton, 1958). This chapter presents a review on fleshy-fruited plant invasions with *Pyracantha angustifolia* as a focus study species. It highlights the knowledge gaps identified for the study species. The chapter also introduces the broader aspects of biological invasions such as the impacts of invasions, mountain invasions, and drivers of invasion. It concludes with outlining the motivation and objectives of the current study.

1.1. Invasive alien plants

Introduction of a species implies that the species has been transported by humans across major geographical barriers into a novel environment for that species (Pyšek et al., 2017;

Richardson et al., 2000). However, such a species is said to have naturalised if it has overcome abiotic and biotic barriers to regular unaided reproduction (Richardson, 2011; Richardson et al., 2000). Then invasion occurs when the introduced species produces viable offspring in areas that are more than 100m from the primary place of introduction in less than 50 years (Richardson et al., 2000). There are different ways in which an alien species can be introduced into a new area out of its native range (Faulkner et al., 2016). These includes release in nature (e.g. as biological control), escape from confinement (e.g. horticulture escape plants), transport contaminant (e.g. seed contamination), transport stowaway (e.g. hitchhiking on ship or boat), corridor (e.g. interconnected waterways) or unaided where the introduction occurred thorough the previously mentioned pathways (Harrower et al., 2018).

Invasive alien plants (IAPs) do not only impose negative effects on human well-being, but also positive benefits (Milanović et al., 2020). Alien plant species are those that have been introduced to non-native areas (Pyšek et al., 2017; Seebens et al., 2018). Reasons for introductions include forestry, landscaping and horticulture, such as *Eucalyptus*, *Pinus* and *Acacia* species commercially introduced for timber (Hulme et al., 2008). Some plants are introduced for pharmaceutical purposes due to their possession of cosmetic compounds (Scott, 2010). Furthermore, other alien plant species were introduced for ecological restoration (Galatowitsch et al., 2016) and planted to form wind breaks (Hassan and Anzar, 2020). Many herbaceous species have been introduced for aesthetic value in botanical and private gardens (van Kleunen et al., 2018). The number of alien plants escaping cultivation is steadily increasing at a global scale (Seebens et al., 2018; van Kleunen et al., 2018), to an extent that ornamental horticulture is regarded as the primary alien plant introduction pathway (Hulme et al., 2018).

Invasive alien plants benefit from various evolutionary and ecological histories compared to the resident biota (Pyšek et al., 2017; Richardson et al., 2000). They might possess traits that are not present in the invaded ecosystem (novel weapons hypothesis) (Callaway and Ridenour, 2004) or lack natural enemies (enemy release hypothesis) (Keane and Crawley, 2002). Several plant traits are associated with invasiveness (Funk et al., 2016), some alien plants are not invasive due to traits that do not allow them to spread and reproduce efficiently(Mathakutha et al., 2019). Such traits include, to mention a few, flowering period, clonality and height (Milanović et al., 2020; Van Kleunen et al., 2014, 2010). Other

traits include increased growth rate, having longer flowering and fruiting periods, higher fecundity, higher specific leaf area, and more efficient seed dispersal (Seebens et al., 2017). It has been stressed by Foxcroft et al. (2019) that scientifically sound information is needed in order to advise on IAP management strategies.

1.2. Impacts of invasion

Invasive alien plants are capable of substantially modifying ecosystem functioning and disrupting invaded ecosystem services (Nock et al., 2016; Vilà et al., 2011). They modify ecosystem functioning by disrupting carbon and nutrient cycles (Chamier et al., 2012). This results in an altered ecosystem that resident organisms cannot adapt to properly (Basnou et al., 2015). Competition for resources is also one of the most reported negative ecosystem impacts of IAPs on resident flora (Kumschick, 2016; Kumschick et al., 2015). Invasive alien plants also change the diversity of plant communities (Pyšek et al., 2012; Yapi et al., 2018). Such competition might result in resident biota being outcompeted by IAPs (Samant et al., 1998), thus lowering the biodiversity. Biodiversity is an important asset and biodiversity maintenance offers substantial benefits for humans (Ngorima and Shackleton, 2018). Biodiversity plays a critical role in the support of ecosystem services and functioning, like providing food, nutrient cycling and regulation of microclimate (Milanović et al., 2020). Changing biodiversity composition thus affects the functioning of that particular ecosystem (Potgieter et al., 2019). Besides unbalancing water reserves (Le Maitre et al., 2000), timber production IAPs are known to release toxic allelopathic compounds (Foxcroft et al., 2019; Holmes et al., 2009). These allelopathic compounds hinder growth and development of surrounding vegetation (Herrera et al., 2016). Therefore, lowering biodiversity and disrupting ecosystem functions that maintain biodiversity (Adomako et al., 2019). Invasive alien plants can also cause detrimental health problems (Reaser et al., 2007; Richardson, 2011). For example, *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) causes severe allergies in humans (Nentwig and Mebs, 2017; Sladonja et al., 2015).

Invasion by IAPs has a major negative impact on agricultural crops, rangelands and pastures. These invaders cause enormous direct and indirect economic losses. Economic losses associated with IAPs include losses in crop production (Milanović et al., 2020), and the costs to mitigate the effects brought about by IAPs such as eradication, ecosystem restoration and other forms of mitigation measures. With all these impacts mentioned, it is

therefore necessary to study IAPs in order to find a better way to control the spread and mitigate the impacts of invasion (van Wilgen et al., 2020a).

1.3. Invasive alien plants in South Africa

South Africa is one of the most biodiverse countries in the world (Government of South Africa, 2015). The vision of the National Biodiversity Strategy and Action Plan (Raimondo, 2015) is to ensure the use of South African biodiversity in a sustainable manner, and to conserve and manage it for the benefit of all South Africans (Government of South Africa, 2015). One of South Africa's major threats to achieving this is biological invasions (van Wilgen et al., 2020a). Although there is no adopted standard inventory for recording IAPs (van Wilgen et al., 2020a; Wilson et al., 2017), the national legislation lists 327 invasive plant taxa (Department of Environmental Affairs, 2014). In South Africa, 759 non-indigenous plant taxa have naturalised, spanning 126 families and 418 genera. At least half of these plants are shrubs or trees (Henderson and Wilson, 2017; van Wilgen et al., 2020a). The Southern African Plant Invaders Atlas (SAPIA) has been providing an assessment of the extent of invasion at a national scale (Henderson and Wilson, 2017). The SAPIA database only collects general distribution patterns and accurate methods for estimating the size of invaded areas is lacking (Henderson, 2007). One of South Africa's Strategy for Plant Conservation objectives is to assist in conserving plant diversity by managing invaded areas and to prevent new biological invasions (Wilson et al., 2013). South Africa has successfully managed numerous extremely problematic IAPs: for example, the biological control of various Cactaceae and *Acacia longifolia* (Andr.) Willd (Fabaceae) (Wilson et al., 2018, 2017, van Wilgen et al., 2018). The main challenge has been the establishment of strong linkages between planning, implementation, monitoring and reporting, and the long periods needed for screening and trialling potential biocontrol agents (Wilson et al., 2018). The Working for Water Programme (WfW) has led the way in the physical and chemical management of prominent IAPs (notably *Acacia dealbata* Link and *A. mearnsii* De Wild (both Fabaceae)); this is a Natural Resource Management programme that combines a science-based approach to IAP management with a labour-intensive (and therefore job creation) implementation philosophy (Wilson et al., 2013). This programme also established the Invasive Species Programme at the South African National Biodiversity Institute (SANBI), which deals with early detection, post-border introductions, risk assessment, and management of alien species (Wilson et al., 2013).

1.4. Drivers of invasion

Apart from globalisation playing a key role in the spread of IAPs, evolutionary processes have also shaped how biological invasions play out at a regional scale (Zenni et al., 2017). Populations experience phenotypic and ecological divergence (Mathakutha et al., 2019) or convergence from their native sources in the process of becoming invasive (Zenni et al., 2017). Plant functional traits have been associated with invasion in many studies (Mathakutha et al., 2019; Peoples and Goforth, 2017) and have been incorporated into management frameworks (Nunez-Mir et al., 2019). Hence, not all alien plants are invasive and not all invasive plants are aliens (Richardson et al., 2000). When they are functionally different from natives, IAPs can tap into unused niches to ensure invasion success (Dellinger et al., 2016) – such as woody temperate species invading open temperate habitats. Functional traits refer to structural, morphological, biochemical and physiological characteristics that influence the performance or fitness of an organism (Nock et al., 2016). Scientific understanding of functional traits in all areas of ecology is fragmented (te Beest et al., 2015). However, scientists have been using functional traits to investigate the impact of IAPs and their spread (Hulme and Bernard-Verdier, 2018). The majority of plant functional traits associated with increased invasion levels are plant reproductive traits. These include seed density (Yannelli et al., 2018), seed dispersal (de Sá Dechoum et al., 2015; Mokotjomela et al., 2016; Thabethe et al., 2015a), pollination (Dietzsch et al., 2011; Timóteo et al., 2018), germination (Molefe et al., 2019) and fruiting and flowering phenologies (Corlett, 2005; Gallinat et al., 2018). These are some of the functional traits investigated in this study in order to gain knowledge on the spread of the study species.

1.5. Seed biology

The processes involved in the dispersal dynamics of fleshy-fruited species are of importance to invasive species ecologists (Díaz Vélez et al., 2018; Thabethe et al., 2015a, 2015b; Vergara-Tabares et al., 2018). These processes includes fruit production (Melin et al., 2014), fruit removal (Díaz Vélez et al., 2018), seed rain (Prather et al., 2017), seed predation (Mokotjomela, 2016), seed bank dynamics (Holmes and Cowling, 1997) and germination (Tiebel et al., 2018). Pollination has also been highlighted as one of the important processes in seed dispersal dynamics, as it plays a critical role in seed production

(Milton et al., 2010; Rambuda and Johnson, 2004; Wang and Smith, 2002). As seed-bearing plants often have the capacity to survive harsh conditions as seed in the soil (Ferrerias et al., 2015), understanding seed bank dynamics is a critically important component in studying and managing in biological invasions (Ferrerias et al., 2015). Seed bank dynamics therefore have direct relevance for vegetation restoration (Yannelli et al., 2017), predicting the future of the vegetation community (Drury et al., 2019), and tracking changes in the community structure (Gioria and Pyšek, 2015). Investigating the capacity to form persistent soil seed banks is critical to determine an IAP's invasion potential in an area of novel introduction (Gioria et al., 2019; Gioria and Pyšek, 2015). Soil seed bank analysis also assesses the chances of re-establishment of cleared IAP populations (Gioria and Pyšek, 2015; Marchante et al., 2010). It is thus important to incorporate seed bank analysis with functional traits in order to provide the complete process of invasion and factors enhancing invasion (Yannelli et al., 2017).

1.6. Seed dispersal

Fleshy-fruits have been consumed by vertebrates for a long time, and birds have been reported as more common dispersal agents than mammals in all angiosperm lineages (Fleming and Kress, 2011). A classic example of a fleshy-fruited plant adapted to dispersal by vertebrates is the Fig tree *Ficus* spp. (Moraceae) (Kaufmann et al., 2008). Fleshy-fruited invasive alien plants use native frugivorous bird species to disperse their seed and thus enhance invasion (D L Vergara-Tabares et al., 2016). It is important to investigate such interactions in invasion biology so that the ecological dynamics of plant invasions are better understood (Hui and Richardson, 2017). After a seed survives primary dispersal, which is being moved from a branch by the movement of the branch or removal by a dispersal agent, it has to survive often harsh post-dispersal conditions (Holmes and Cowling, 1997). For example, a seed has to survive post-dispersal predation, reported to be a more limiting factor for vegetation establishment than drought (Chilpa-Galvan et al., 2017). Hence, it is important to study this crucial process as it affects plant population dynamics. Agents of seed predation include birds, insect and small mammals (Williams et al., 2000; Wróbel and Zwolak, 2017). The effect of fleshy-fruit ingestion by vertebrates has been well studied, including research on ingestion by mammals (Bobadilla et al., 2016), reptiles (Valido and Nogales, 1994), primates (Stevenson et al., 2002), rodents (Garcia-iriarte et al., 2016) and birds (Thabethe et al., 2015a). Such studies are often lacking for the effect of fruit ingestion on seed germination of many IAPs.

1.7. Pollinator-plant interactions

The effect of IAPs on ecological interactions continues to be of great concern (CABI, 2012; Hui and Richardson, 2017; New, 2017). One of these important ecological interactions affected by IAPs includes pollinator-plant interactions of native species (Issaly et al., 2019; Milton et al., 2010; Stiers and Triest, 2017; Stout and Tiedeken, 2016; Traveset and Richardson, 2006). It has been reported that IAPs are capable of reducing pollinator visitation rates (Bjerknes et al., 2007; Dietzsch et al., 2011) and disrupting native mutualistic relationships (Stout and Morales, 2009) among indigenous and endemic species. Razanajatovo et al. (2015) shows that naturalised aliens may have more flower visitors than non-naturalised aliens. Such plants are sometimes referred to as pollination competitors (Bjerknes et al., 2007). They may sometimes provide valuable food sources for pollinators (Bjerknes et al., 2007) and have different flowering patterns from native plants (Godoy et al., 2009; Tiedeken and Stout, 2015). Some IAPs produce large numbers of flowers (Nielsen et al., 2008), an attribute that can give an IAP a competitive advantage over native species, and lead to IAPs ‘stealing’ native pollinators. For example, *Robinia pseudoacacias* L. (Fabaceae) large, fragrant flowers compete with native plants for pollinating bees in South Africa (Henderson, 2001).

The capacity to produce high seed set without pollinators can also be an advantage for IAPs (Roux et al., 2010; Shivanna, 2015; Yan et al., 2016). This has been observed in *Rosa rubiginosa* whereby the species still produces high quality fruits through wind- and self-pollination, if not pollinated by its usual insect visitors (Mazzolari et al., 2016). Although Razanajatovo and van Kleunen (2016) indicated that pollen limitation might not have a major impact on the spread of IAPs once they naturalise, Burns et al. (2019) claim that IAPs have generalized floral displays to compensate for pollen limitation. This adaptation would help the IAPs to use generalist pollinators and increase reproductive output, compared to highly specialised angiosperms whereby a specific pollinator is needed in order to transfer pollen. In trying to understand the effect of IAPs on pollination systems, several studies have focussed on pollinator abundance (Nienhuis et al., 2009) and flower visitation rates (Nielsen et al., 2008). Jakobsson et al. (2009) highlighted the risk of overstating the impacts of IAPs on pollination systems in invaded sites in small case studies; therefore considering spatial scale in any study of invasions of IAPs is very important. It was found that floral visitors do not respond the same to all IAPs (Stout and Tiedeken, 2016).

1.8. Biological invasions in Southern African mountains

Mountains host considerable proportions of Earth's biodiversity and thus by virtue are important habitats that need preservation and sustainable management (Antonelli et al., 2018; Brand et al., 2012; Carboni et al., 2017; Carbutt, 2019; Noroozi et al., 2018). The same mountains also provide freshwater to communities (du Plessis, 2019), contributing to livelihoods of people living near them, an example being the southern African Great Escarpment (Bentley et al., 2018; Nel et al., 2013). They also harbour a variety of endemic biota adapted to their unique climate and environmental conditions (Amori et al., 2019; Antonelli et al., 2018). The Drakensburg Mountain Centre (DMC) in South Africa holds a record of 334 endemic and 595 near-endemic angiosperms (Carbutt, 2019). After a period of 13 years, a study by Carbutt (2019) indicated that there was 4% reduction of endemic plant species. This was from those recorded in an earlier study by Carbutt and Edwards (2006) in the DMC. The reduction is purported to be a result of poor land management practices such as overgrazing and unseasonal burning and also uncontrolled invasions of alien plant species (Brand et al., 2012; Carbutt, 2019). Thus far, mountains are not suffering from large-scale invasion, but land-use and climate change are expected to break the 'invasion barrier' (Carboni et al., 2017).

1.9. *Pyracantha* genus and study species *Pyracantha angustifolia*

The genus *Pyracantha* M.Roemer consists of eleven species of evergreen shrubs and small trees naturally occurring in China (Cuizhi and Spongengberg, 2003; Serviss, 2020). Branches are pubescent and thorny with small buds (Henderson, 2001). They have simple, fascicled or alternate leaves (Cuizhi and Spongengberg, 2003), with leaf venation being camptondrous, and the margins entire, crenulated or serrulate. Inflorescences are compound, with flowers having five sepals and five petals (usually suborbicular, white and spreading) (Cuizhi and Spongengberg, 2003).

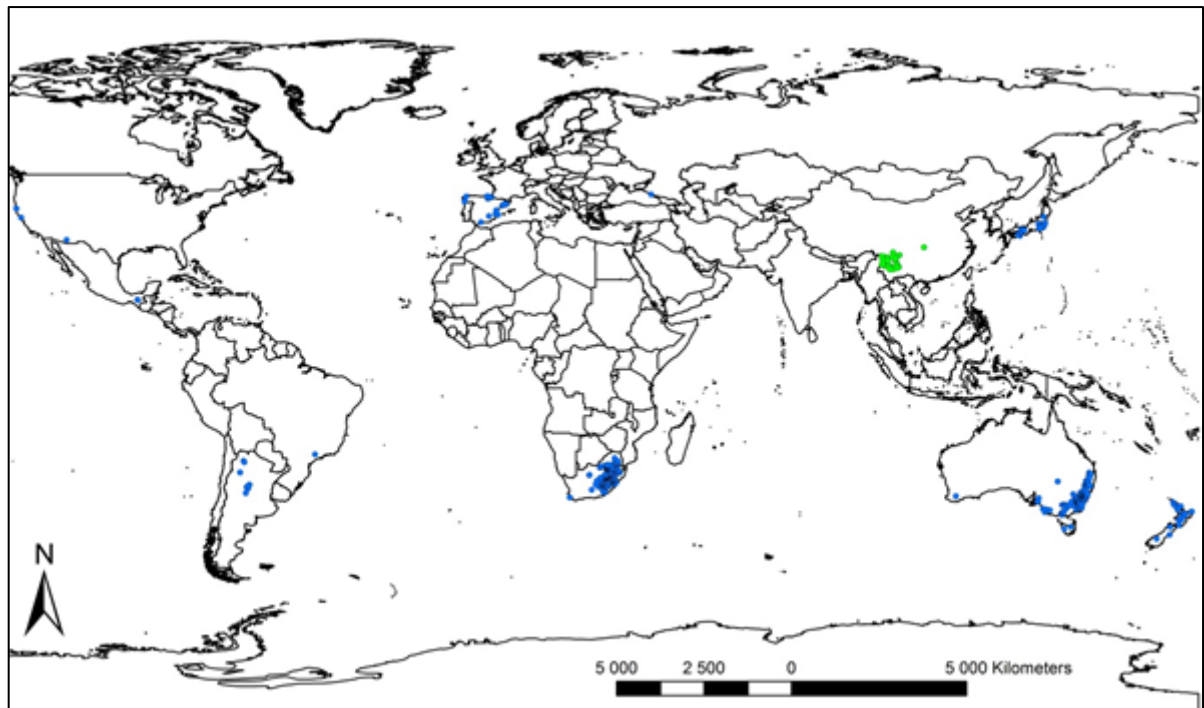


Figure 1. 1 Global distribution of *Pyracantha angustifolia* representing native (green) and invasive populations (blue). The species distribution was mapped using Geographic Information System (GIS) software, ArcGIS 10.5.1 (Price, 2010). Distribution data were obtained from (GBIF (2019) and SAPIA (Henderson, 2019)

Pyracantha angustifolia (Franch.) C.K.Schneid (Rosaceae), is native to south-western China (Cuizhi and Spongengberg, 2003) (Figure 1.1). *Pyracantha angustifolia* has been widely planted and has subsequently become naturalised in many countries. At least 19 countries are invaded by *Pyracantha* species. This includes: Argentina, Australia, Brazil, Canada, France, French Polynesia, Germany, Italy, Japan, Lesotho, Mexico, New Zealand, Portugal, Russia, South Africa, Spain, eSwatini, United Kingdom and United States of America (Andreu, 2011; Brandle and Brandl, 2012; de Villalobos et al., 2010; Guix, 2007a; Henderson, 2007, 2001; Moreschi et al., 2019; Urcelay et al., 2018).

Pyracantha angustifolia, commonly known as Yellow firethorn, has naturalised and become invasive in much of the high elevation interior of South Africa (Henderson, 2001). Its young shoots are covered in thick yellowish down with woody sharp-pointed spines (Cuizhi and Spongengberg, 2003). The shrubs bear narrowly elongate leaves that are dull dark-green above and grey-downy beneath, with entire margins and a leaf tip with a small notch (Henderson, 2001). White flowers (5mm long) are produced in October to December

and orange-red or orange-yellow berries (5-8mm across) are produced in large numbers (Figure 1.2).



Figure 1. 2 (A) *Pyracantha angustifolia* in Clarens, eastern Free State, South Africa. (D) White flowers (5mm long) are produced in October to December and (A, B and C) orange-red or orange-yellow berries (5-8mm across) are produced in large numbers from April to October

Pyracantha angustifolia has a broad distribution in South Africa, with records from all nine provinces (Figure 1.3). Although it is occasionally found in large areas of its invaded range, it is particularly abundant in Gauteng, Free State, Eastern Cape and KwaZulu-Natal Provinces (Figure 1.4).

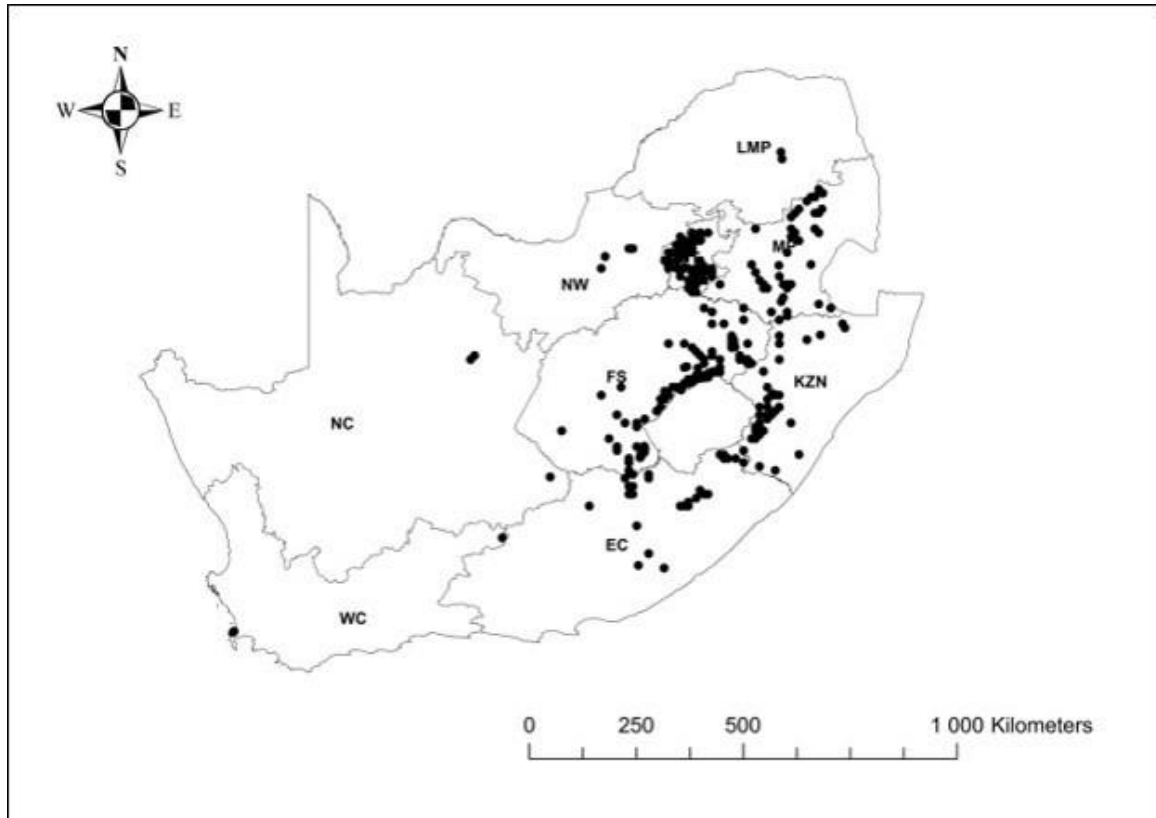


Figure 1. 3 *Pyracantha angustifolia* distribution in South Africa. Provinces includes Western Cape (WC), Northern Cape (NC), North West (NW), Limpopo (LMP), Free State (FS), Eastern Cape (EC), Mpumalanga (MP), Gauteng (GP) and KwaZulu-Natal (KZN). *Pyracantha angustifolia* distribution was mapped using Geographic Information System (GIS) software, ArcGIS 10.5.1. Distribution data was obtain from GBIF (2019) and SAPIA (2019)

In South Africa, the plant is predominantly found along river courses and rocky ridges along the South Africa - Lesotho border in the Free State Province, with large infestations near the town of Ficksburg and Fouriesburg (Figure 1.4). It is invading fallow lands and roadsides in Gauteng province (L. Henderson, personal communication; Personal observations).

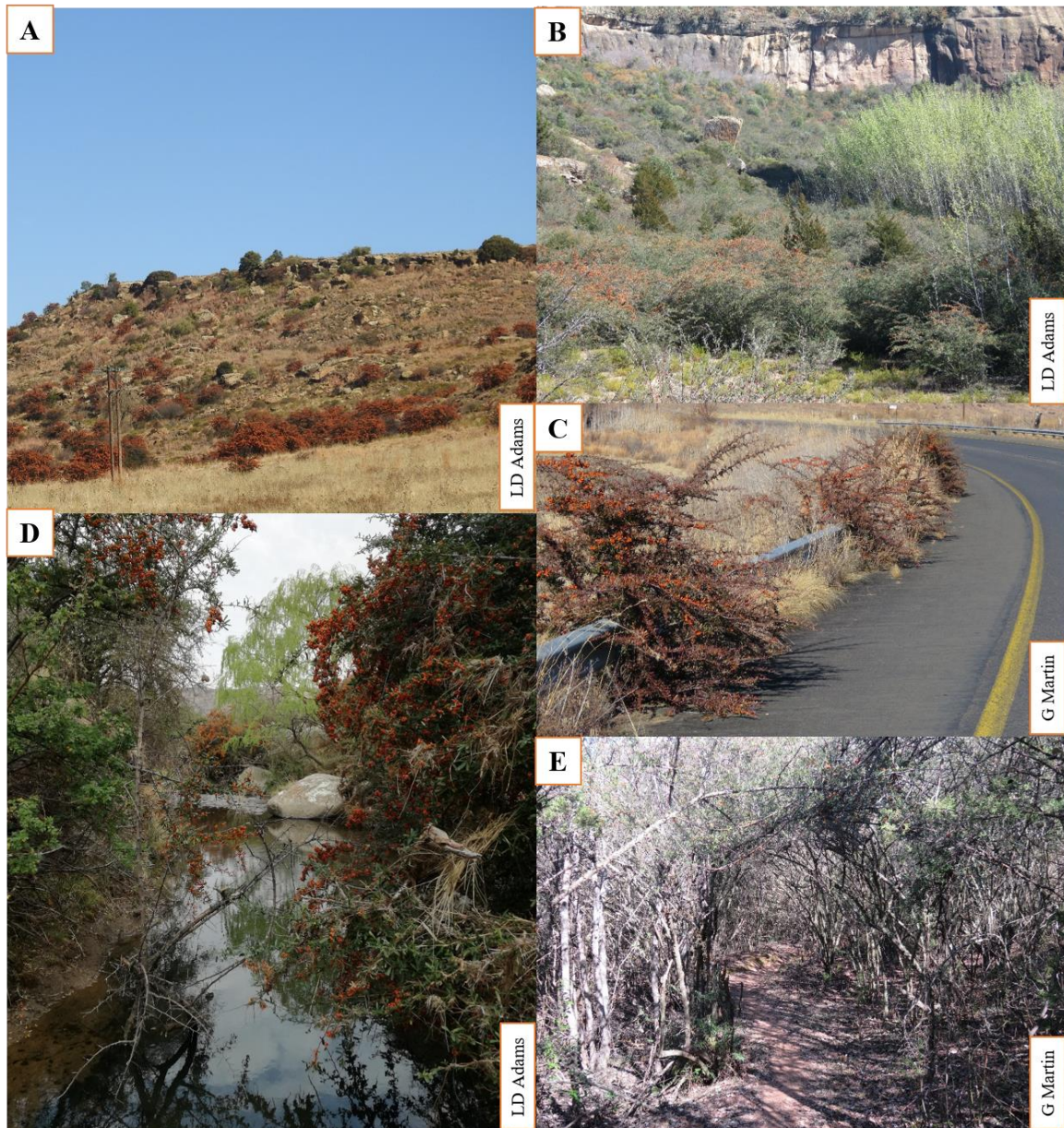


Figure 1. 4 *Pyracantha angustifolia* invasions in eastern Free State, South Africa. Such invasions included (A) rocky hills on farms, (B) open grasslands showing complete invasion (orange-tinted bushes), (C) road sides resulting in difficulties in observing on-coming traffic for vehicle drivers, (D) riparian systems and (E) open grassland converted to woody habitat around Clarens, Bethlehem and Fouriesburg towns

Five species of *Pyracantha* have been introduced to South Africa as ornamental plants, with *P. angustifolia* being the most aggressive invader (Henderson, 2019). As indicated by Alien and Invasive Species Regulations of the National Environmental Management: Biodiversity Act, Act 10 of 2004 (NEM:BA Alien and invasive species regulations),

Pyracantha angustifolia, *P. coccinea* M.Roem, *P. crenatoserrata* (Hance) Rehder, *P. crenulata* (D.Don) M.Roem and *P. koidzumii* (Hayata) Rehder are included as 1b category IAPs (Department of Environmental Affairs, 2014). Species under category 1b should be controlled or eradicated where possible. *Pyracantha angustifolia*, *P. coccinea*, *P. fortuneana* (Maxim.) H.L. L and *P. rogersiana* Bean have been reported to have naturalised in the Free State Province of South Africa (Henderson, 1989). *Pyracantha angustifolia* is one of the most prominent invaders along the roadsides, which often makes it difficult for drivers to observe oncoming traffic on curved roads (Henderson, 1999). In the Eastern Cape Nama Karoo Biome, *P. angustifolia* occurs in lower abundance in false Karoo, at an altitude of up to 3000 m.a.s.l (Henderson, 1992). In the southern and south Western Cape Region, *P. angustifolia* invades the Fynbos Biome in mountains reaching a peak elevation of over 2000 m.a.s.l (Henderson, 1998). It is clear that *P. angustifolia* invasion is not only a threat in the Grassland Biome but also the Fynbos Biome.

1.10. Impacts of *Pyracantha* invasion

By producing fruits when natives are not fruiting, *P. angustifolia* fruits become a supplementary food source to frugivores (Ballari et al., 2015; Vergara-Tabares et al., 2018). Migrating birds also feed on *P. fortuneana* and *P. coccinea*, especially in winter (Guix, 2007a). *Pyracantha angustifolia* has been reported to enhance woody species recruitment. Higher sapling survival of invasive *Ligustrum* seedlings under *Pyracantha* canopies was found to be due to a plant-nurse effect in one study, as there was no effect of cage and canopy treatments (Tecco et al., 2007). *Pyracantha angustifolia* provided significantly shadier conditions than native woody species both in summer and winter in Cordoba, Argentina mountains (Tecco et al., 2006). Soils under *P. angustifolia* canopies were cooler and moister with higher ammonium levels (Tecco et al., 2006). Tecco et al (2006) recorded increased species richness under *Pyracantha* overall. A biome-scale assessment indicated that *P. angustifolia* had a moderately high impact on grazing potential and moderate impact on biodiversity in South Africa (van Wilgen et al., 2008).

Pyracantha species have been reported multiple times to introduce various types of pests into their place of introduction globally (Kollár et al., 2009). This has happened in various countries in Europe (Łagowska et al., 2017). *Pyracantha coccinea* has facilitated the introduction of the moth *Phyllonorycter leucographellus* (Lepidoptera, Lithocolletidae) to

Europe as a primary host plant (Kollár et al., 2009). *Phyllonorycter leucographellus* is native to western China and southern Europe (Walczak et al., 2018). This moth selects hosts based on the specific leaf area (SLA) and the infestation increases with an increase in SLA. *Pyracantha* bushes damaged by *P. leucographellus* may look extremely affected in winter by heavy infestations but normally recover following Spring or early Summer (Backhaus et al., 2000).

In Norway, a new weevil, *Otiorhynchus armadillo* (Coleoptera: Curculioidea), has been reported to use *P. coccinea* as a host. Weevil adults feed on the host's leaves while the larvae feed on the roots (Staverl et al., 2010). These case studies highlight the possible dangers of pest introductions facilitated by *Pyracantha* species invasions. Although the case studies present pest introductions in countries other than South Africa, such introductions might also occur in places of invasion in future and cause damage to native flora and crops.

1.11. Problem statement and motivation

Due to its rapid spread, Henderson (1989b) suggested that *P. angustifolia* was one of the IAPs that might be problematic in future. Carbutt (2012) also warned against the dangers of emerging invasive alien plants in the Maloti-Drakensberg. He referred to *P. angustifolia* as a current emerging invasive plant and *P. crenulata* as a future emerging invasive alien plant in the Maloti-Drakensberg (Carbutt, 2012). Recently, Moffett (2018) regarded *P. angustifolia* as a prominent invader in the Maloti-Drakensberg (Figure 1.4). These researchers highlight the need for urgent attention on this invasive alien plant for better management. The lack of knowledge pertaining to the ecology, physiology and biology of an IAP concerned serves as an encouragement for this study, as such concepts should be studied in order to gain a better understanding of the invasive potential of *P. angustifolia* (Hui and Richardson, 2017).

Only 44.5% of the total extent of the Eastern Free State Clay Grassland remains; it is poorly protected and classified as an Endangered ecosystem (Mucina and Rutherford, 2006). There is a need to study the mechanisms of spread of *P. angustifolia* in this region for better management of the species. This invader reduces the grazing capacity of the invaded sites and therefore needs serious attention (Giantomasi et al., 2008; Tecco et al., 2016). There is

an obvious niche in southern Africa for a focused research effort on IAPs in mountain systems, including the reproductive ecology of extant and emerging IAPs under increasing land-use impacts and climate change (Giantomasi et al., 2008; Moreschi et al., 2019; Tecco et al., 2010, 2007). Studies have been conducted around the world to quantify fruit production in order to examine the invasive potential of different fleshy-fruited IAPs (Gosper and Vivian-Smith, 2010). This study is the first to quantify the number of fruits produced by *P. angustifolia* allowing us to understand the quantity of fruit available to frugivorous birds and other possible seed dispersers. The concept of fruit quantification and frugivore visitation rates allows us to explore the accessibility of fruits to frugivorous birds and ultimately follow the fate of seeds.

1.12. Aims and objectives

The main aim of this study was to determine factors enhancing the spread of *P. angustifolia* by studying the species' reproductive ecology.

Objectives of the study included to determine:

1. The seed biology of *P. angustifolia*
2. The effect of ingestion by frugivorous birds on seed germination rate and success.
3. The role of floral visitors in pollination and reproductive success.

The first objective (1) was achieved through estimating the fruit/seed production, soil seed bank analysis, seed dispersal and seed rain. The second objective (2) was achieved through aviary bird feeding trials and assessing germination rates in ingested seed in a greenhouse. The last objective (3) was achieved by determining floral visitors and visitation rates, pollen purity and investigating breeding systems.

1.13. Study area

The data were collected in farms around Clarens (28°32'8"S; 28°25'2"E) in the eastern Free State Province of South Africa (Figure 1.4 and 1.5). The study area is typified by private farmland with some patches of natural grassland, rocky outcrops, and sandstone cliffs deeply incised by rivers. The area is surrounded by agro-industry activities to the north, east and west; these include croplands, fallow agricultural fields that are no longer arable, and some cattle ranching (Mucina and Rutherford, 2006). To the south, it borders Lesotho.

The area receives an average rainfall of 600-1000 mm per annum and considerable snow and frost in winter. The annual average minimum and maximum temperatures are 6 °C and 26 °C respectively, although frost usually reduce the temperature to well below freezing (SAEON, 2020). Cold spells have been experienced occasionally (approximately twice a year) (SAEON, 2020). The area falls under the Grassland Biome (Mesic Highveld Grassland) (Mucina and Rutherford, 2006). Its original extent comprised mostly two vegetation types: Eastern Free State Clay Grassland and Eastern Free State Sandy Grassland, which are now mostly transformed by agro-industry (Mucina and Rutherford, 2006).

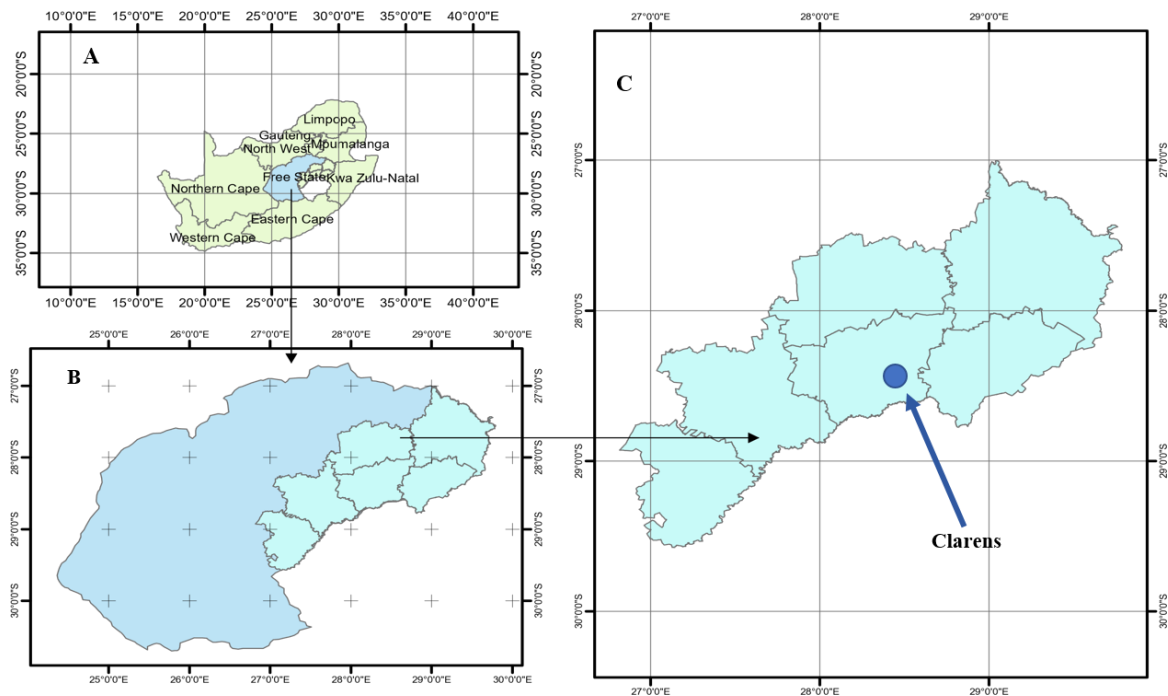


Figure 1. 5 Study area located in (A) South Africa (A), (B) eastern Free State Province in (C) Thabo Mofutsanyana district) in farms around the town of Clarens. (C) Dark blue circle indicates Clarens town and the light blue demographics demarcates town districts in the eastern Free State

1.14. Seed dispersal conceptual framework

As seed dispersal links propagule production to the recruitment of mature plants, it is essential to consider *Pyracantha* reproductive ecology in the form of a continuous life cycle (Wang and Smith, 2002) (Figure 1.6). Therefore, concepts such as ingestion of seeds by frugivores, fruit availability and germination are intermediary and form part of the bigger process. Presenting such concepts in isolation would be difficult if not impossible because they are all linked to a fruiting plant life cycle.

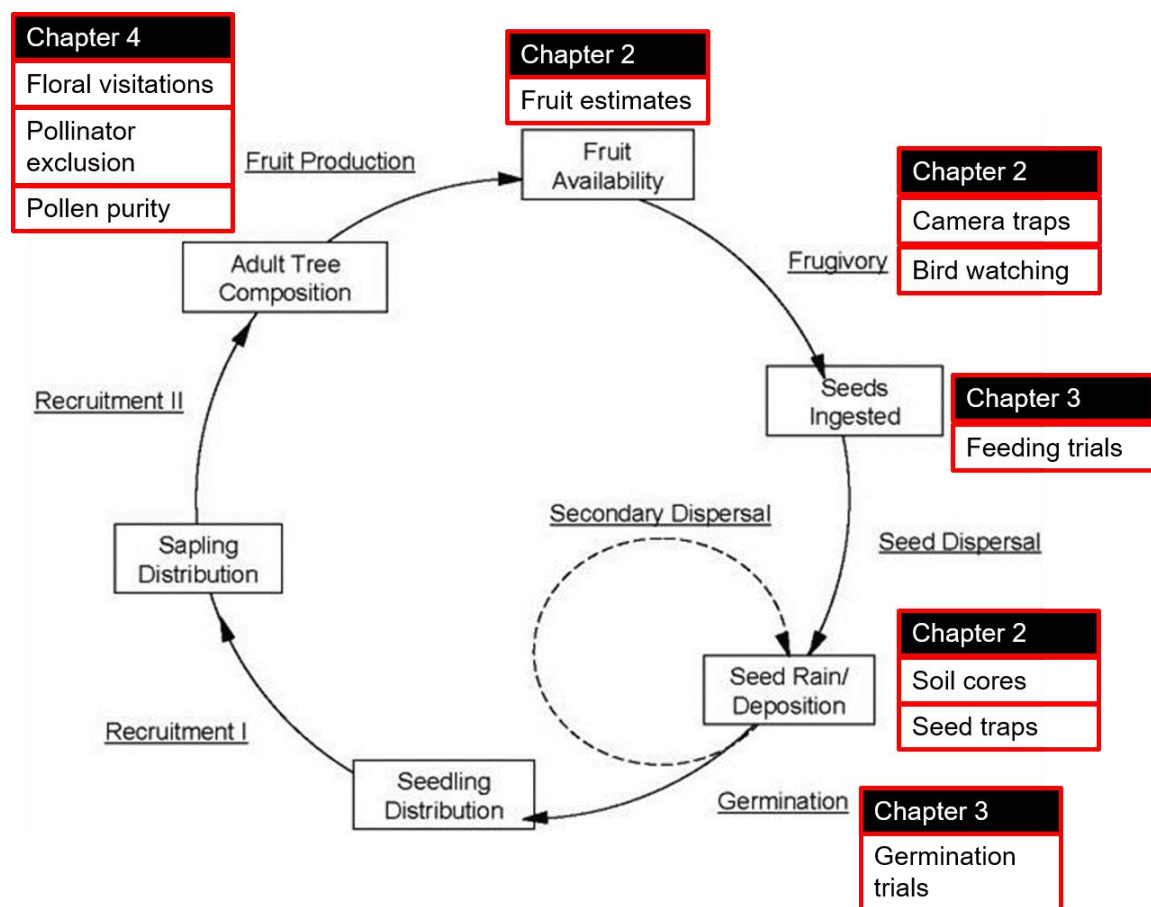


Figure 1. 6 Seed dispersal model developed by Wang and Smith (2002) to highlight the importance of linking seed dispersal and adult vegetation structure. The diagram was used in this dissertation to ensure ordering of concepts and linking them to specific life stages of a fleshy-fruited invasive alien plant *Pyracantha angustifolia*, thus linking concepts to methods. Orange boxes indicate specific chapters in the dissertation and the main methods used to address specific concepts in the diagram

1.15. Dissertation structure

The structure of the following dissertation resembles that of scientific journal articles. Chapter 1 presents a general introduction and Chapter 5 presents a general discussion and conclusion. Chapters 2, 3 and 4 resemble the format of manuscripts with the aim of publication. All the experiments used *Pyracantha angustifolia* as a focal study species. The first chapter introduces concepts, study species, rationale and literature review on topics presented in the dissertation (Chapter 1). The second chapter investigates seed biology; and includes a study on seed production estimates, dispersal, the soil seed bank and seed rain (Chapter 2). The third chapter examines the effect of ingestion by frugivorous birds on germination rate and success (Chapter 3). The fourth chapter focuses on breeding systems, and determining the effect of floral visitors on pollination and seed set (Chapter 4). The last chapter provides a general discussion to link each experimental chapter, recommendations for management authorities, and improvements on the current study (Chapter 5).

CHAPTER 2

SEED BIOLOGY: SEED DYNAMICS OF *PYRACANTHA ANGUSTIFOLIA* IN SOUTH AFRICA

2.1. Introduction

The incursion of native ecosystems by invasive alien plants (IAPs) and animals has been and continues to be amongst the major threats to biodiversity and ecosystem functioning globally (Mostert et al., 2017; Richardson and Van Wilgen, 2004; van Wilgen et al., 2008). The grassland biome of South Africa is of no exception and is under threat from a number of non-native shrub and tree species, including the red-berried (fleshy-fruited) shrub species including *Pyracantha angustifolia*, *Rosa* L. spp. and *Cotoneaster* Medik. spp. (all Rosaceae) (Carbutt, 2012; Dellafiore et al., 2015). This is particularly concerning as the biome is of economic value to South Africa as it provides valuable grazing to livestock (van Wilgen et al., 2008) and is the most valuable ecosystem with regard to South African water security (Nel et al., 2013).

It is assumed that the main driver of fleshy-fruited shrub species invasions in the grassland biome are birds, however this remains to be determined (Aslan and Rejmánek, 2012). An important mutualistic relationship exists between birds and fleshy-fruited plants (Tecco et al., 2006; Vergara-Tabares et al., 2018; White and Vivian-Smith, 2011). In this particular ecological interaction, freshly-fruited plants are able to expand their distribution through birds' movement as they eat the fruits and defecate them into favourable microsites away from the parent plant (Milton et al., 2007; Witkowski and Wilson, 2003). In return, birds receive a source of food when digesting the fruit wall of the ingested fruit (Calviño-Cancela, 2004; Izhaki and Safriel, 1990). Some of these fleshy-fruited plants are those that have been distributed out of their natural range and start to invade pristine areas (Ballari et al., 2015; Dean et al., 2002; Milton et al., 2007). They use the same mutualistic relationship to expand their range and explore new niches, and some of them have detrimental environmental effects post-invasion (Holmes and Cowling, 1997; Nsikani et al., 2017).

The effect of birds as seed dispersers in invasion biology has been well-studied across the globe as they play a role in the exacerbation of IAPs spread (Aslan and Rejmánek, 2012; Milton et al., 2007; Panetta, 1997). However, the role of rodents in the dispersal of IAPs

has been overlooked, thus it is important to examine the rodent activity around a plant under study when assessing seed dispersal models (Wennyi, 2000). Most fleshy-fruited plants invest a lot of energy in the production of fruit flesh in order to increase the chances of being dispersed (Stapanian, 1982). This investment in fruit production does not only focus on the development of the fruit but also into the increased number of seeds per plant (Aslan and Rejmánek, 2012). A portion of these seeds would end up forming part of the soil seed bank (Tiebel et al., 2018). Not only is it important to examine the soil seed bank, but also to determine how seeds reach the soil (Guix, 2007b; Panetta, 1997). Some seeds are dispersed by birds, bats and mammals, as they gradually defecate them into various microhabitats (Howe, 1989; Mokotjomela et al., 2016; Vergara-Tabares et al., 2018).

As much as small rodents are regarded as granivores destroying seeds during consumption, some studies have reported improvement on seed germination after ingestion by small rodents (Yang et al., 2019). This indicates that small rodents can also be seed dispersers than predators (Holmes, 1990). Pulp consumers help remove the fruit wall, then the seed can be dropped to the ground and probably germinate (Pena-Egana et al., 2018). Effective dispersers are those that increase the chances of seed reaching a favourable site and successfully germinate (Pena-Egana et al., 2018). Thus, it is important to take into consideration the method of feeding (grainivory or pulp feeding) of a potential disperser when investigating seed dispersal.

It is well-known that seed-eating animals deposit seeds in patterns with respect to the movement behaviour of the animal (Corlett, 2005; Milton et al., 2007). Such movements, referred to as seed rain, determine the survival of seedlings because different seedlings survive under different environmental conditions (Emsweller et al., 2018; Wennyi, 2000). Determining aforementioned dispersal patterns and seed dispersal rates will enable better understanding of biological invasion by fleshy-fruited plants. Plants may survive harsh conditions via the persistence of seeds in the soil seed bank (Holmes and Cowling, 1997; Marchante et al., 2010). Seed persistence is very important in unpredictable environments (e.g. uncontrolled bushfires), since plants surviving through underground seed persistence are better adapted to re-establish after harsh conditions. Lack of having the latter survival characteristic might lead to a population dying out due to harsh environmental conditions.

In general, fleshy-fruits are known to have low seed persistence in the soil (Tiebel et al., 2018) and therefore it is not expected that *P. angustifolia* seeds will have a persistent seed bank. Thus it is important to examine the persistence of seeds in the soil in order to determine the possibility of an IAP re-establishing post-clearance (Gioria and Pyšek, 2015). The aim of this chapter was to determine the seed biology of *P. angustifolia*. In order to achieve the abovementioned aim, the following objectives were set for this chapter: (1) determining the amount of seed produced per annum through fruit estimates; (2) assessing the contribution of native fauna to the dispersal of *P. angustifolia* seeds using camera traps and direct observations, (3) examining the soil seed bank by analysing soil cores and (4) determining the seed rain using seed traps.

2.2. Materials and methods

Fruit estimates, observations and soil cores were used in this chapter to investigate fruit production, fruit availability, frugivory, seed bank, secondary dispersal and predation by rodents (Figure 1.6). Based on roadside surveys and literature three representative sites were selected for the study (Carbutt, 2012). Three different sites (Figure 2.1), namely in open grassland (Figure 2.1A), a rocky hillside (Figure 2.1B) and a riverine site (Figure 2.1C) were selected. At each site, 40 shrubs were sampled. In the open grassland sites (28°32'8"S; 28°25'2"E), the species had invaded an open grassland area in private property where the land was occasionally used for grazing livestock. The second site was a rocky outcrop (28°32'9"S; 28°25'9"E), where the species had invaded a rock-dominated hillside. At the riverine site (28°32'2"S; 28°31'5"E) the species had invaded the banks of a perennial river. All three sites were located in farms, and *P. angustifolia* stands were not in the cultivated areas but in natural areas with low disturbance compared to cultivated areas (Figure 1.4). Within each site, mature shrubs (plants with fruits) were chosen randomly. Sampling efforts included varying sizes of plants within the population in order to avoid sampling only plants of one size. Fruit production was estimated towards the end of the fruiting season (August to September 2019).

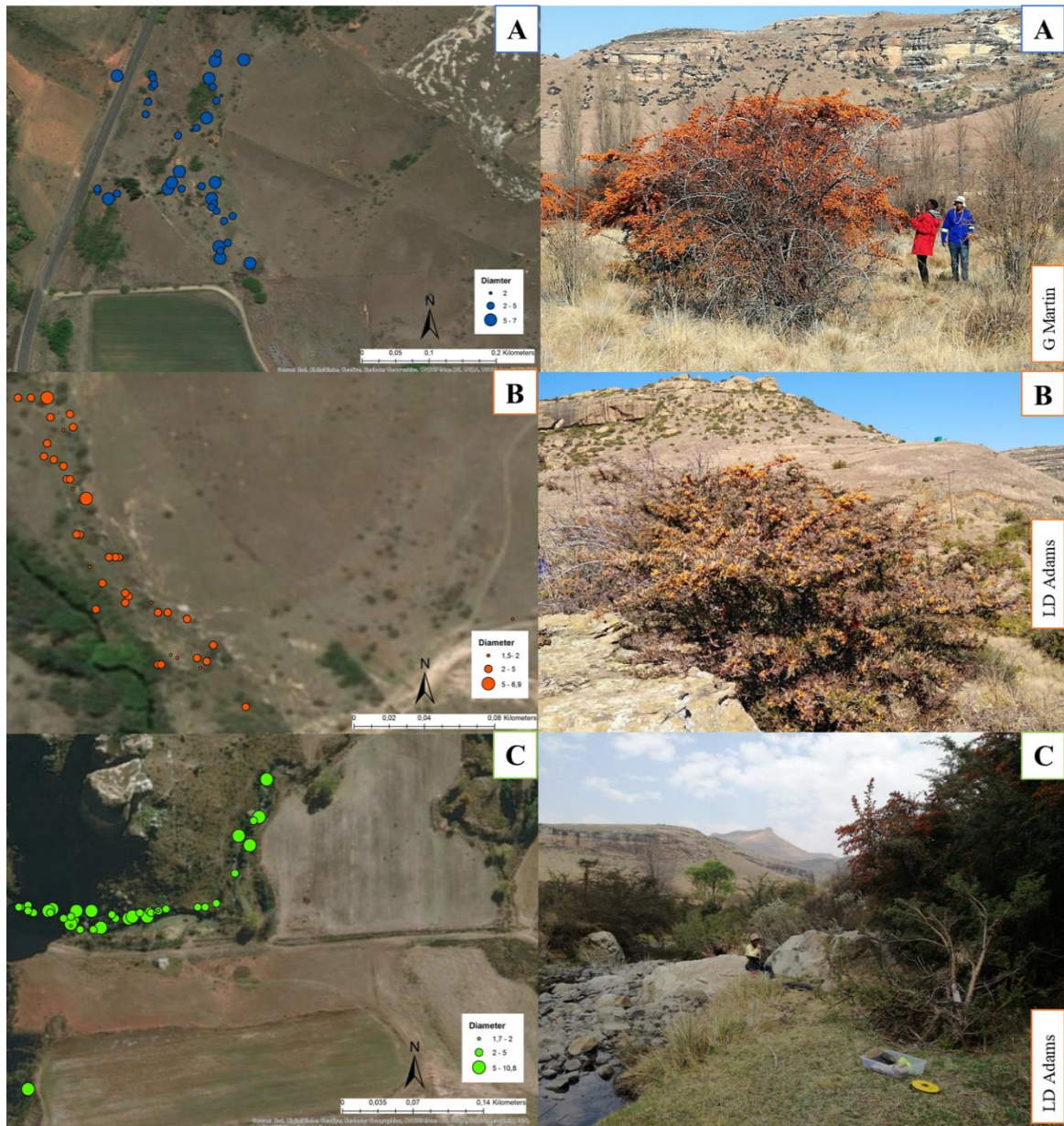


Figure 2. 1 Field sites used to collect fruit production data around Clarens town in eastern Free State including nearest neighbour plot summaries. Sites included: (A) open grassland, (B) rocky outcrop and (C) riverine habitats. Dots on pictures on the left represent only those individual *Pyracantha angustifolia* shrubs sampled for fruit quantifications. Size of dots indicates plant diameter in metres.

2.2.1. Fruit production

To investigate the amount of fruits available to frugivores, the amount of seed produced per annum through fruit estimates was determined. This was achieved by examining the relationship between plant volume and the number of seeds. Due to the high number of

fruits per shrub, it was necessary to calculate the number of fruits and seeds per shrub. The following method was used to estimate the number of fruits per shrub. Firstly, the size of the shrub on which the amount of fruits was to be calculated was measured. This was done by measuring two diameters: one in a north to south direction and one east to west. These measurements were taken at the widest point of the shrub in both directions. Plant height (ground level to the highest leaves) was also measured. The first quadrant was the southwest quarter of the shrub, second quadrant northwest, third quadrant northeast and fourth quadrant on the southeast (Figure 2.2). The fruiting distance within each quadrant was then determined by placing a measuring pole into each quadrant towards the centre of the shrub and measuring the distance from the outermost berries until the innermost berries. Following this the amount of fruits per quadrant was estimated by randomly placing a wire cube in the fruiting area and counting fruits inside the cube ($10 \times 10 \times 10 \text{cm} = 1000 \text{cm}^3 = 0.001 \text{m}^3$). Five random cube samples were taken per quadrant. The total number of fruits produced per shrub was then extrapolated (see Statistics section below, Figure 2.2).

In order to determine the average number of seeds per fruit, 10 fruits were collected in each quadrant per shrub and stored in paper bags. In the laboratory the fruit wall of the collected fruits was removed and seeds inside were counted and noted within seven days of collection. The average number of seeds produced per fruit was then used to calculate the number of seeds produced per sampled shrub using the extrapolated total number of fruits per shrub. In order to determine the average shrub density and the plant demography at each of the sites (rocky, river and open) five randomly placed 5x5m quadrates were selected. Within each quadrats, all shrubs and herbs found growing in the quadrat were identified and measured.

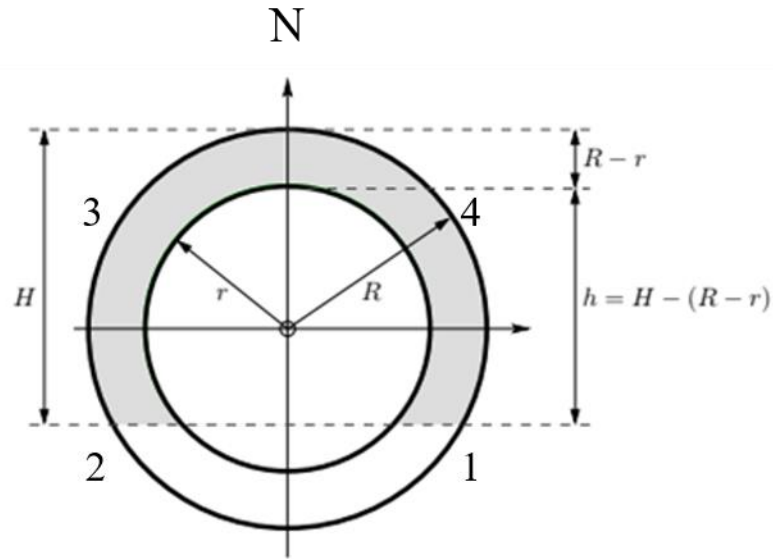
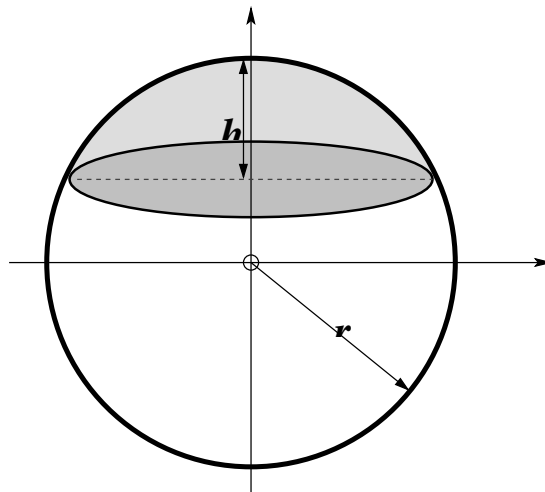


Figure 2. 2 Shrub's (circle) side view assuming round shape of the shrub indicating the effective fruiting distance ($R - r$, in grey) and non-fruiting height ($h = H - (R - r)$) of a spherically shaped plant using a Python mathematical model. The numbers around the shrub indicate the quadrants (numbers 1 to 4), N indicates geographical direction of sampling, R = plant radius, r = plant radius excluding fruiting distance (in grey), h = radius excluding fruiting distance and H = height of the shrub

Python programming (Stevens and Boucher, 2015) was used to design a model that allowed calculations of fruit estimates, and ultimately seed production by a shrub. The following steps were followed in calculating fruit estimates:

For a sector of a sphere as shown:



The volume of a spherical segment is given by:

$$V = \frac{1}{3}\pi h^2(3r - h) \quad (1)$$

As a special case, with $h = 2r$, we obtain

$$V = \frac{1}{3}\pi(2r)^2(3r - 2r) = \frac{4}{3}\pi r^3 \quad (2)$$

which is the volume of a sphere. The surface area of the spherical part of the surface is given by

$$A = 2\pi r h \quad (3)$$

Again, as a special case (Figure 2.2), $h = 2r$, we obtain the total surface area of a sphere

$$A = 4\pi r^2. \quad (4)$$

For a spherical shell with an outer radius R and an inner radius r , the volume of the shell is then

$$\begin{aligned} V(R, r, H) &= \frac{1}{3}\pi H^2(3R - H) - \frac{1}{3}\pi h^2(3r - h) \\ &= \frac{1}{3}\pi [-R^3 + 3HR^2 + 3r^2R - 3r^2H - 2r^3] \end{aligned}$$

For the uncertainties, the following derivatives were used:

$$\begin{aligned} \frac{\partial V}{\partial R} &= \pi(2HR + r^2 - R^2) \\ \frac{\partial V}{\partial r} &= 2\pi(r(R - r) - Hr) \\ \frac{\partial V}{\partial H} &= \pi(R^2 - r^2) \end{aligned}$$

These formulae reduce error since cumulative standard errors from each measurement taken adds to bigger error.

To determine the amount of seeds produced at each site (open, rock, river) the following calculation was used

The average fruit count per tree was calculated:

1. n_q ($q = 1, 2, 3, 4$) in a quadrant - based on 5 samples per quadrant.
2. The density per quadrant was then calculated:

$$\rho_q = n_q \times 5 \times 1000$$

where 1000 is to convert to m³.

3. The fruiting area on each tree was then calculated, H and average radius, \bar{R} from the data. Use the fruiting distance, d from the data to calculate the inner radius r_q for each quadrant. These were then used to calculate the volume for each quadrant using (6),

$$V_q = \frac{1}{4}V(\bar{R}, r_q, H)$$

4. The total seed count (in thousands) is then calculated using::

$$\bar{N} = \frac{1}{1000} \sum_q V_q \times \rho_q$$

Seed Count Model

The average number of seeds could then modelled using R as a proxy for age

$$N(R) = \begin{cases} 0 & \text{for } R < r^* \\ a(R - r^*)^3 & \text{for } R > r^* \end{cases}$$

For a radius R less than some value r^* , the shrub will not produce any seeds. Only when the size $R > r^*$ (i.e. has reached a certain age) will the shrub produce seeds. From that point the number of seeds must scale as the volume, i.e. $N \sim (R - r^*)^3$. Only sexually mature shrubs were included in the study. Parameters used for calculations are illustrated in Figure 2.2 showing the shrub's (circle) side view assuming round shape of the shrub indicating the effective fruiting distance ($R - r$, in grey) and non-fruiting height ($h = H - (R - r)$) of a spherically shaped plant using a Python mathematical model. The numbers around the shrub indicate the quadrants (numbers 1 to 4), N indicates geographical direction of sampling, R_r = plant radius, rR = plant radius excluding fruiting distance (in grey), h = radius excluding fruiting distance and H = height of the shrub (Figure 2.2).

To determine Mean shrub size across each of the sampled sites the following calculation was used:

$$f(x; \lambda, k) = k (x/\lambda)^{k-1} e^{-(x/\lambda)^k}$$

The Weibull distribution function (Fleming, 2001) was used to determine the probability of finding seeds per/m² with each of the of the three sites using the exponential distribution function:

$$fE(x; \lambda) = \lambda e^{-\lambda x}$$

The number of seeds produced per meter of the invaded populations are presented as a probability where 0.5 is high probability and 0 is low probability. Probability refers to the chances of finding specific number of seeds in an invaded area.

2.2.2. Seed dispersal

Remote cameras (Bushnell Trophy Cam HD Max-Colour LCD, Bushnell Outdoor Products, Overland Park, KS, USA) were used following the methods of Zoeller et al. (2016) to identify vertebrate plant visitors, and the timing and frequency of their visits to feed on fruits. The remote cameras were triggered by heat or motion. Then the remote cameras would take snapshots and films. When triggered, the cameras filmed the visitor's activity for 60 seconds and it could be re-triggered after a 10-second interval. Cameras were used to observe foraging vertebrates throughout the fruiting season of *P. angustifolia* for two months (July – September 2018). Three Bushnell remote cameras were mounted on tripods and aimed at fruiting branches of focal plants. Each camera was placed between 25cm to 1m away from the plant (depending on the model and optimum focal distance of the camera). Camera storage capacity and battery strength were checked at least once a week and data downloaded on a laptop. From the videos, the seed dispersers or predators were identified using birds (Hockey et al., 2005; Newman, 2013) and rodents (De Graaff, 1981) and South Africa field guide books and the following factors were documented: behaviour of seed dispersers and predators, duration of foraging, number of fruits eaten and the abundance of foragers. Predation of fallen seeds/fruits on the ground was assessed by installing cameras aiming at bowls containing *P. angustifolia* fruits (approximately 100 fruits).

2.2.3. Direct observations (Opportunistic and times observations)

Observations were conducted to supplement the data captured by the camera traps. Opportunistic observations were conducted in the study sites (open grassland, rocky outcrop and river) while collecting data on other methods used in this study (Figure 2.1). Vertebrate visitors to *P. angustifolia* were identified using birds field guide books (Hockey et al., 2005; Newman, 2013) and their behaviour observed. Only birds observed feeding on *P. angustifolia* fruits were noted on opportunistic observations.

Timed observations were also done to increase the area of observation while adding to the data collected by camera traps. Wynford holiday farm, located between Fouriesburg and

the Lesotho border, was used for the observations. The former grassland area has been converted to dense woodland encroached by woody plant species including *P. angustifolia* (Figure 1.4E). Timed observations were done during peak fruiting periods of *P. angustifolia*. Observation periods spanned 6 hours a day, these refers to 3 hours of peak bird activity after sunrise (07:00–10:00) and 3 hours of peak activity prior to sunset (14:00–17:00) over 3 days (total of 54 hours) in June 2019. Three mature shrubs (plants with fruits) were randomly chosen for observations. Observations were made using binoculars and if possible a photo was also taken. Three days of observations were done on each shrub. Every bird species feeding on *P. angustifolia* fruits was recorded. Each observation consisted of foraging bird arrival and departure time, and birds were identified to species level using field guide books (Hockey et al., 2005; Newman, 2013) and behaviour (feeding or resting) was noted.

2.2.4. Soil seed bank

To determine the seed density in the soil around the source plant, soil cores were taken during the peak fruiting season of *P. angustifolia* (August 2018). Soil cores were taken from under the plants, at intervals from 20 randomly chosen shrubs that had fruits on them and were at least 16 m away from another *P. angustifolia* plant in the sampling direction so as not to sample fruits fallen from neighbouring shrubs. Six soil samples (using a soil auger 7.5 cm diameter × 20 cm depth) were taken in one random direction away from each of the 20 focal plants, thus 120 soil core samples were taken. This experimental design only determines the soil seed bank, not the seed viability.

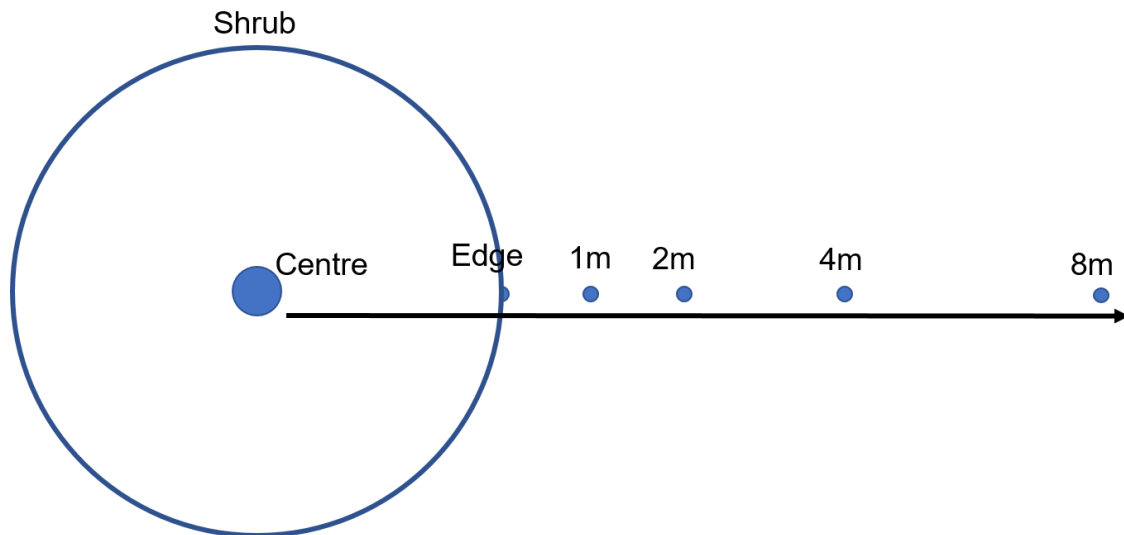


Figure 2. 3 Soil core sampling design with distances from the centre of the shrub and the edge. The circle represents the top view of a shrub with the circumference being the edge of the shrub. The centre represents the stem of the shrub and the small blue solid circles indicate the soil core sampling points and their distance relative to the edge of the shrub

The first sample was taken directly under the shrub canopy, the second one at the edge of the canopy, and the remaining four samples at 1 m, 2 m, 4 m and 8 m from the canopy edge (Figure 2.3). Soil samples were collected for seed bank assessments using modified methods of Holmes (2002). Soil samples were passed through a sieve (1 mm) and fine particles were removed only seeds and large particles in the sieve. The remaining *P. angustifolia* seeds could be clearly distinguished. Seeds collected from the field were stored in the laboratory inside paper bags until the analysis was done. The average number of seeds per area was calculated and plotted on a graph. Intact seeds were assumed to be viable, that is, they have the capacity to germinate if they are planted in a suitable soil. Unviable seeds were those that would not germinate if planted in a favourable soil, especially those that were easily destroyed by hand. No viability tests were performed to determine the seed viability.

2.2.5. Seed rain

Seed rain below the trees was calculated per tree at from the open grassland. Seed traps (n=55), comprising shallow perforated plastic containers (diameter 30cm x 15 cm deep) covered with wire netting to exclude rodents and birds, were secured to the ground with

metal pegs, beneath tree canopies to estimate the density of seed arriving on that surface (Figure 2.4). Two traps were placed under mature *P. angustifolia* at each site. In addition the same seed traps were placed randomly under surrounding shrubs (*Rosa rubiginosa* L. and *Leucosidea sericea* Eckl. & Zeyh, both Rosaceae) as well as under nearby fence lines. It was assumed that *P. angustifolia* seed found in seed traps under other tree species and fence had been dispersed there by birds or mammals. Seed traps were emptied monthly, seed was cleaned and sorted, and the numbers of seeds arriving in each trap were counted. For whole fruits emptied from the traps, the number of fruits was multiplied by the average number of seeds to calculate the total number of seeds in the trap. Forty four seed traps, two per tree, were placed under *P. angustifolia* (n = 44), one trap per tree placed under two *L. sericea* shrubs (n = 2), six traps under *R. rubiginosa* (n = 6) shrubs and one under fence (n = 1). The total amount of seed traps amounts to 53 seed traps. Seed traps were placed in the field for 12 months to cover all the seasons (October 2018 to September 2019).



Figure 2. 4 Seed trap made from bowl covered with wire mesh pinned to the ground under *Pyracantha angustifolia* shrub

Two perpendicular tree diameters were measured and canopy area was calculated. Seed rain (SR) was calculated using the following formula (Iponga et al., 2009):

$$SR = (SF/TA) \times CA$$

Seed fall (SF) refers to the number of seeds counted in the seed trap and total area (TA) refers to the sum of seed traps area. Canopy area (CA) was the tree canopy area calculates using the following formula:

$$CA = (\pi/4) \times \text{canopy1} \times \text{canopy2}$$

Where canopy1 and canopy2 were the two perpendicular diameters of the trees.

Ultimately, mean annual seed rain (Mean \pm SD) in seed per square metre was determined for loose seed, seed in intact fruits and total seed number for seed traps under difference canopies and under fence. Statistically significant differences were also determined in One-Way ANOVA using GraphPad Prism 5. Data were checked for normality D' Agostino & Pearson omnibus normality test before the analyses.

2.3. Results

Results indicated that *P. angustifolia* produces millions of seeds per square metre; these seeds are dispersed by livestock and native frugivorous birds and also consumed by rodents on the ground.

2.3.1. Fruit production

Fruit production was presented in form of seed density taking into account shrub size and the amount of seeds produced. The mean shrub radius (diameter/2) in each of the populations was determined. It was shown that the mean size of shrubs growing in rocky environments was smaller than the mean size found in riverine and open environments (Figure 2.5). The riverine study area had the highest peak radius of 2.40m, followed by 2.17m from open grassland and lastly 1.46m from rocky outcrops (Figure 2.5). Mean shrub radiuses from the three study fields were 2.63, 2.23 and 1.78m for river, open grassland and rocky outcrop in decreasing order respectively.

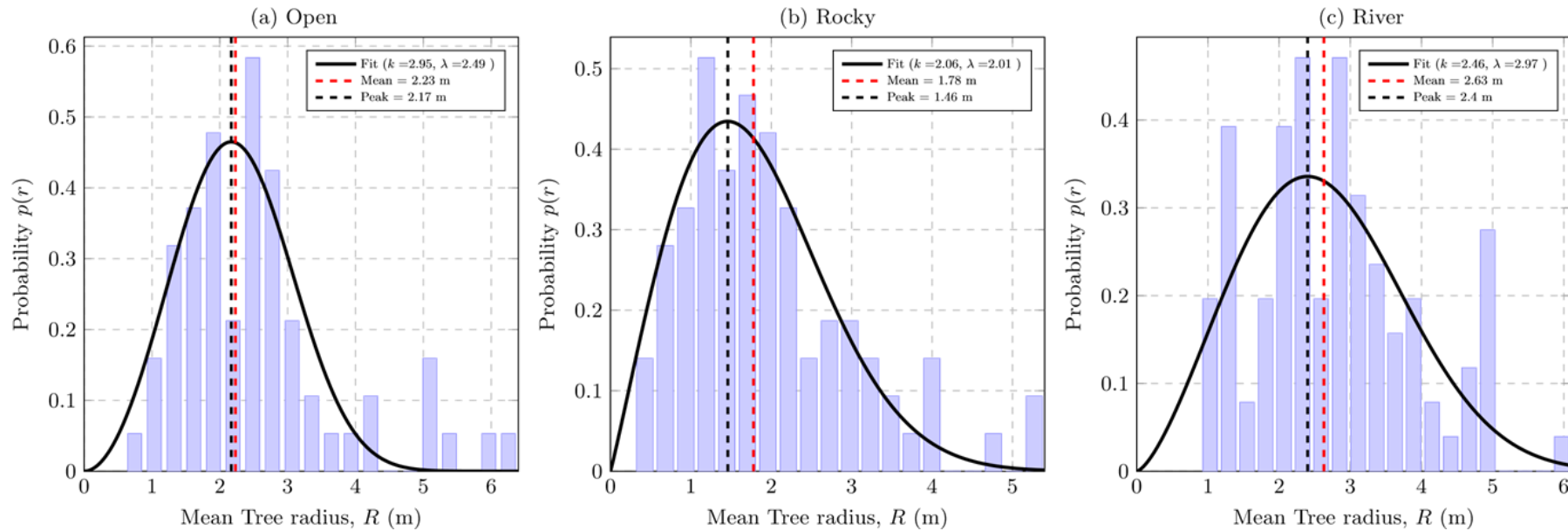


Figure 2. 5 Probability $p(r)$ against mean tree radius R (m) from three field sites (a) open grassland, (b) rocky outcrop and (c) river. Black dotted vertical line represent peak radius, the red dotted lines represent mean radius values and the black solid line represents trendline

The densities of the trees growing within the three environments were expressed as shrubs per square meter. Riverine environments were shown to have the highest density, while open habitats have the lowest density (Figure 2.6).

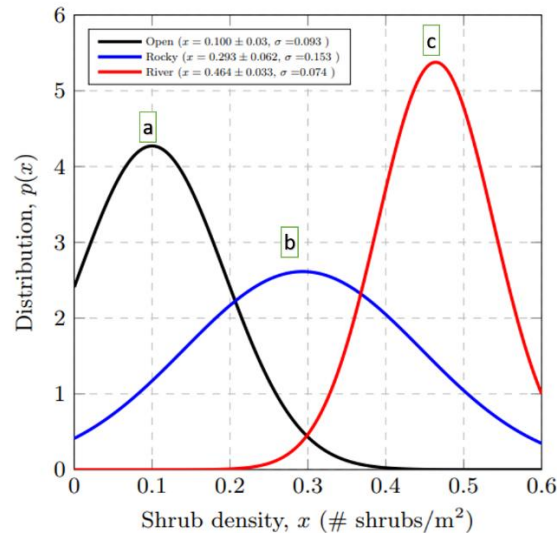


Figure 2. 6 The mean density of sampled *Pyracantha angustifolia* trees recorded growing within the three populations in (a) grassland (black line), (b) rocky outcrops (blue line) and (c) riverine (red line) habitats

Shrub densities were calculated with the same shrubs used for fruit production. Each fruit produced a range of 3-6 seeds, with the majority (at least 70%) of fruits producing five seeds. There was an exponential increase in the number of seeds produced with an increase in plant radius (Figure 2.7). Small shrubs with a radius of 0.75m are already producing fruits. At least 47% of shrubs from the open grassland produced over five million seeds, while 25 and 2.5% of shrubs from river and rocky outcrop habitats, respectively produced over five million seeds. Larger trees in open grassland can carry up to 20 million seeds per tree. Trees in rocky and river environments produce fewer seeds (approximately less than 50% compared to open) but still numbering in the millions. This indicates that shrubs in open grassland produce more seeds than other study areas. In open grassland, 55% of shrubs have a radius of over 2m, shrubs from rocky outcrop and river 12.5% and 60% respectively (Figure 2.6).

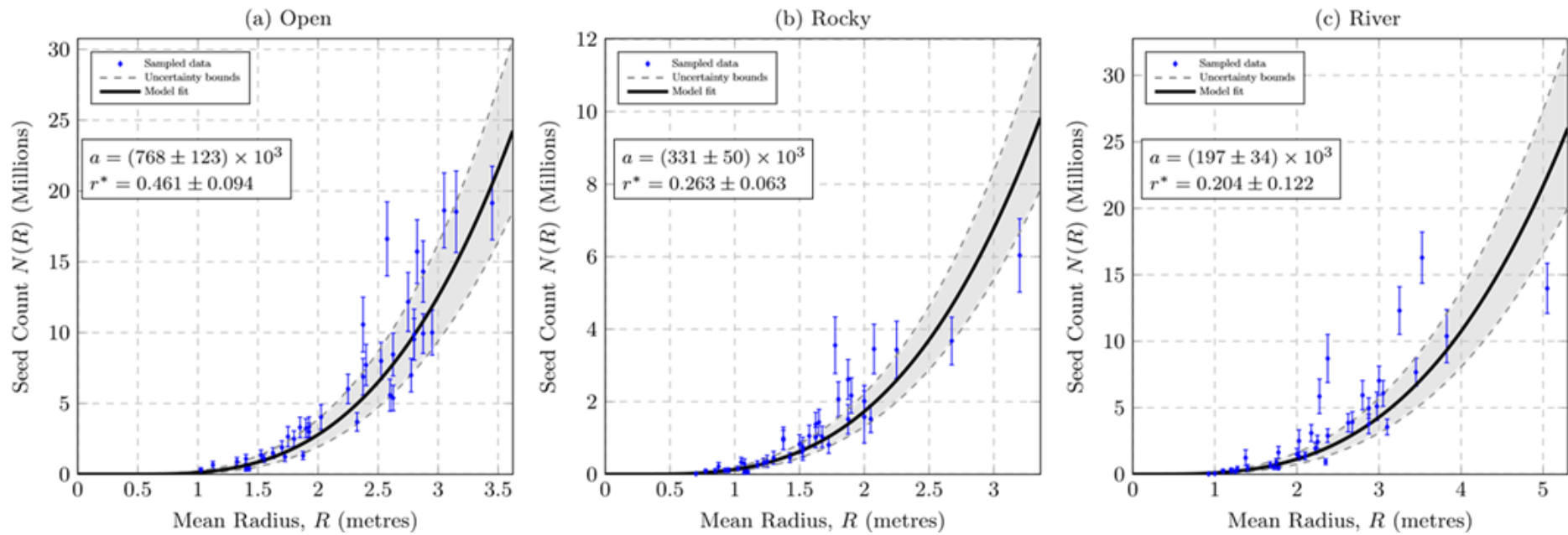


Figure 2. 7 The mean radius of sampled *Pyracantha angustifolia* shrubs in relation to the mean number of seeds produced in thousands for the three sampled field sites in (a) grassland, (b) rocky outcrops and (c) riverine habitats. Dots on each graph show mean number of seeds and error bars indicate minimum and maximum seed count values

Once the average tree density, size and amount of seeds per tree was determined it was possible to determine the amount of seeds per metre. Seed density (million seeds/m²) for invaded populations were presented in the form of a probability index p (p) against seed density where 0.5 is high probability and 0 is low probability (Figure 2.8). The probability index indicates the probability of finding a seed within an invaded area. These graphs show that there are at least one million seeds being produced in each square metre of the invaded area in all the three field sites. The highest probability ($P(p)=4$) for both open grassland and rocky outcrop habitats is at 1 million seeds/m² and 0.27 for river habitat. This means that shrubs in both open grassland and rocky outcrop habitats have high chances of producing the same amount of seed per fruiting season per given area in the field.

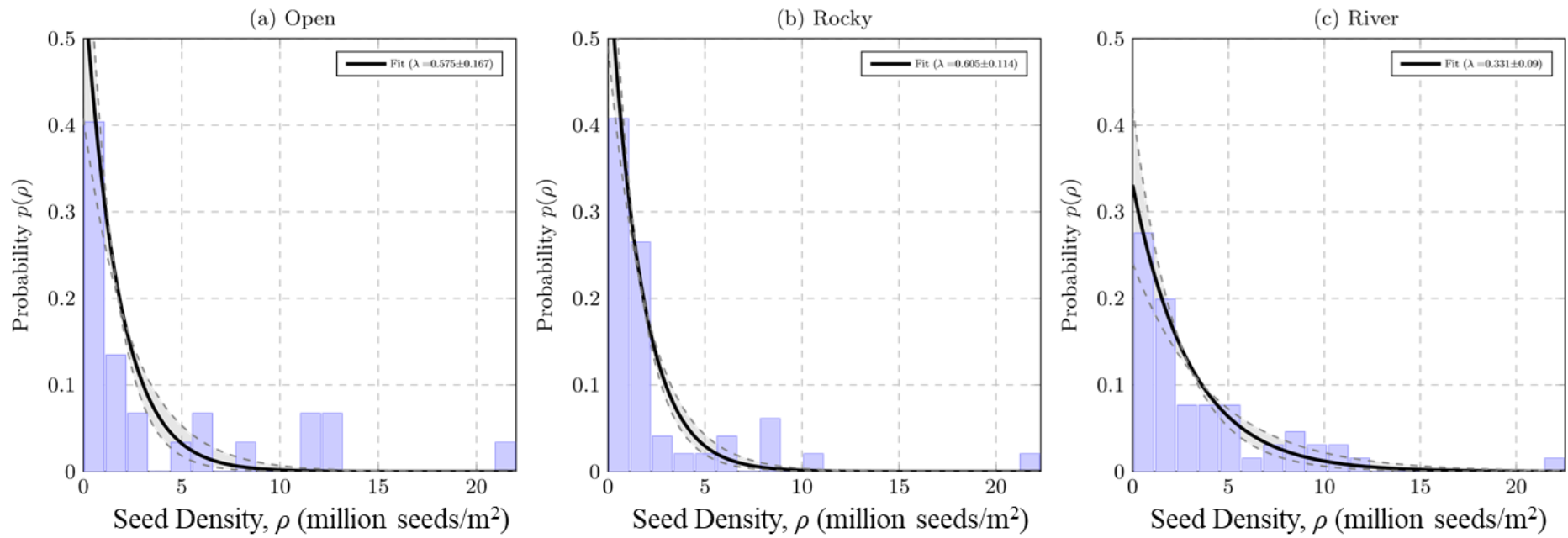


Figure 2. 8 The relationship between probability p (p) and seed density (million seeds/m²) from three field sites in (a) Open grassland, (b) Rocky outcrop and (c) River habitats for seed production. The probability index (p) indicates the probability of finding a seed within an invaded area.

2.3.2. Seed dispersal

To assess seed dispersal in the form of fruit consumption by animals, remote cameras were placed in the field and direct observations were made to improve the results. Remote cameras indicated that small rodents are the main fruit and/or seed consumers once the fruits have fallen from the trees and birds are the main fruit consumers feeding on fruits directly from the fruiting branch (Table 2.1).

Table 2. 1 Bird and mammal species captured feeding on *Pyracantha angustifolia* seeds that were provided. In addition, the total time and mean time spent feeding as indicated

Species	Camera aiming position	Total time spent feeding (%)
<i>Cossypha caffra</i> (Cape Robin Chat)	Bowl	2.70 (n = 6)
<i>Otomys unisulcatus</i> (Karoo bush rat)	Bowl	51 (n = 6)
<i>Rhabdomys pumilio</i> (Four-striped mouse)	Bowl	83 (n = 37)
<i>Pycnonotus nigricans</i> (African Red-eyed Bulbul)	Branch	61 (n = 13)

Remote cameras aiming at the fruiting branches revealed that birds visiting fruiting branches included the African Red-eyed Bulbul (*Pycnonotus nigricans*) and Buff-streaked Chat (*Oenanthe bifasciata*) but only the African Red-eyed Bulbul fed on fruits. African Red-eyed Bulbul fed on whole fruits directly from the fruiting branches (Table 2.1). The African Red-eyed Bulbuls spent 61% of their branch visiting time feeding on fruits, spending an average feeding time of 16.38 seconds per single visit (n=13) and feeding on 2.14 fruits per minute (Table 2.1). Buff-streaked Chats only used *P. angustifolia* shrubs for perching.

Remote cameras aiming at bowls full of fresh *P. angustifolia* fruits captured three animal and one bird species (Figure 2.9). This includes two rodents, the Four-striped mouse (*Rhabdomys pumilio*) and Karoo bush rat (*Otomys unisulcatus*) and one bird, the Cape Robin Chat (*Cossypha caffra*) (Table 2.1). Four-striped mice removed the fruit pulp and ate the seeds, eating fruit pulp partially while Karoo bush rats fed on both fruit and seed. Number of seeds/fruits consumed could not be counted for the two rodents, as they ate too fast and the cameras were placed at an angle that was not suitable to allow counting because the rodents obscured the view (Figure 2.9). Karoo bush rat only fed on one fruit while searching on the ground. Four-striped mice spent 83.0% of their time on the videos captured feeding on *P. angustifolia* seeds while Karoo bush rats and Cape Robin Chats spent 51.0 and 2.7% of their time feeding on fruits, respectively.



Figure 2. 9 Video snapshots of birds and rodents, captured by remote cameras during field observations on and under *Pyracantha angustifolia* shrubs, feeding on seeds and/or fruits

Opportunistic data on animals feeding on *P. angustifolia* seeds/fruits were collected while collecting other data in the study area. Four bird species and two domestic animal species were observed feeding on *P. angustifolia* seeds/fruits. All livestock observed feeding on *P. angustifolia* did not feed on vegetative material of the adults and seedlings (Adams, pers.obs). Bird species included African Red-eyed Bulbul (*Pycnonotus nigricans*), Speckled Mousebird (*Colius striatus*), African Pied Starling (*Lamprotornis bicolor*), Crested Barbet (*Trachyphonus vaillantii*); and domestic animals included goats and horses (Figure 2.10). *Pyracantha angustifolia* seeds were also spotted in Chacma baboon (*Papio ursinus*) dung during surveys. These results informed the choice of bird species used in feeding trials to investigate the effect of a bird's digestive system on the seed germination rate of *P. angustifolia* (see chapter 4).



Figure 2. 10 (A) Livestock spotted feeding on *Pyracantha angustifolia* fruits and (B) fruits spotted in baboon dung during opportunistic observations

Timed observations were conducted to examine visitation frequencies and visitor behaviour (feeding/perching) on *P. angustifolia* fruiting branches over 3 days (total of 54 hours) in June 2019. Cape Bunting (*Emberiza capensis*), Cape Rock Thrush (*Monticola rupestris*), Cape White-eye (*Zosterops virens*), Speckled Mousebird (*Colius striatus*), Dark-Capped Bulbul (*Pycnonotus tricolor*), Dusky Flycatcher (*Muscicapa adusta*), Fiscal Shrike (*Lanius collaris*), Familiar Chat (*Cercomela familiaris*) and African Red-eyed Bulbul (*Pycnonotus nigricans*) were nine bird species observed visiting the shrubs (Figure 2.11). One-Way ANOVA indicated that there were no significant differences ($P \geq 0.05$) between visitation lengths between shrub visitors ($P = 0.1616$). Only one species, Speckled Mousebird, fed on one fruit while all other bird species used the shrubs only for perching.

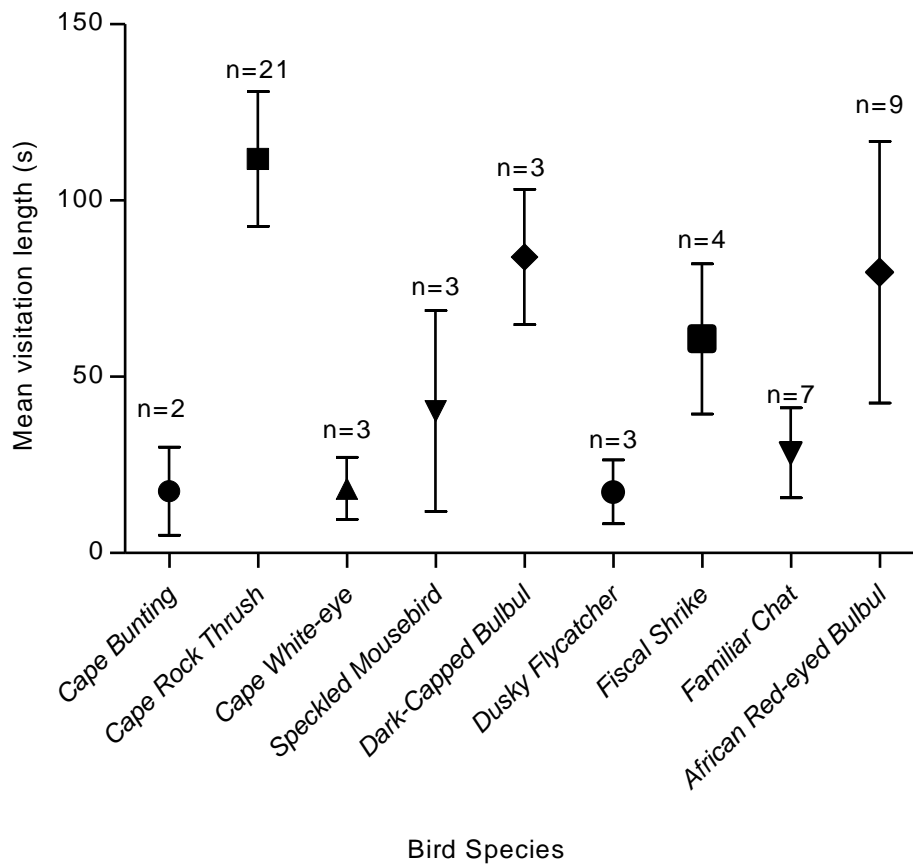


Figure 2. 11 Mean standard error visitation length (seconds) of different bird species that visited *Pyracantha angustifolia* shrubs during the timed observations ($P = 0.1616$, $F\text{-value} = 1,566$)

2.3.3. Soil seed bank

Soil cores from under and at the edges of the canopies of shrubs contained a large number of intact or partially eaten fruits, whereas very few or no fruits/seeds were found at distances away from the trees (1, 2, 4, and 8 m from the canopy; Table 2.2). There was a substantial decrease in mean number of seeds with an increase in distance away from the centre of the shrub (Table 2.2). The majority (more than 60%) of seeds found in the soil cores were decaying (easily broken when pressed between fingertips). Nonetheless, there were viable seeds and their densities are represented in Table 2.2. There were significant differences ($P < 0.001$) in overall samples and Tukey's posthoc test showed that there are significant differences ($P < 0.05$) between the centre and the other treatments but not between the rest of the treatments (Table 2.2).

Table 2. 2 Soil seed bank densities (mean \pm SE seed/m²) from samples taken at different positions under and away from the *Pyracantha angustifolia* shrub using soil auger. Different letters on the last row indicates the statistical significance between treatments (P<0.05). GraphPad Prism 5 One-Way Analysis ANOVA was used to test for significant differences

	Seed density	Significant difference (P<0.05)
Centre	46400 \pm 8934	A
Edge	10000 \pm 3611	B
1m	430.1 \pm 229.8	B
2m	33.95 \pm 33.95	B
4m	22.64 \pm 15.58	B
8m	0	B

2.3.4. Seed rain

Seed traps were used to determine seed rain. High number of loose (73.45 seed per m² \pm 11.32 SE) and intact seeds (3297 \pm 282.1) were found under *P. angustifolia* canopy (Table 2.3). Intact seeds refer to seeds that are still in whole fruits. Lesser number of both intact and loose seed were found under *Rosa* and *Leucosidea* canopies. Seed from intact fruits collected were (533.8 seed per m² \pm 522.1 SE) and (12.50 \pm 12.50) under *Rosa* and *Leucosidea* respectively. Seed traps under fence collected the smallest amount of seed (7 seeds) and no intact seeds. Only seed traps under *P. angustifolia* passed the D'Agostino & Pearson omnibus normality test, while *L. sericea* and *R. rubiginosa* samples were too small (n=2 and n=6 respectively).

Table 2. 3 Annual *Pyracantha angustifolia* seed rain in seed per square metre (Mean \pm SD) under *P. angustifolia*, *Rosa rubiginosa* and *Leucosidea sericea* and the fence. Intact and loose seed were counted separately. Mean standard error was calculated using GraphPad Prism 5 for annual seed rain using formulae from Iponga et al. (2009)

	<i>P. angustifolia</i> (n=44)	<i>L. sericea</i> (n=2)	<i>R. rubiginosa</i> (n=6)	Fence (n = 1)	F value	P-value
Total seed rain	56770 \pm 7735	71.12 \pm 55.1	2310 \pm 2244	7	6.1	0.0054
Seed rain in intact fruit	3297 \pm 282.1	12.50 \pm 12.5	533.8 \pm 522.1	0	12.8	0.0001
Loose seed rain	73.5 \pm 11.3	1.5 \pm 0.5	14.50 \pm 11.85	7	12.5	0.0329

2.4. Discussion

In this chapter, it was shown that the density and abundance of trees differed per site effectively influencing the amount of seeds being produced in each site. Even though there were differences between seed numbers, it was shown that no matter the site *P. angustifolia* produces a high number of seeds that are then available to enter the environment. A number of birds and livestock then consume these seeds, which are potentially contributing to the spread of the species, together with rodents acting as seed predators. A number of seeds do however fall to the ground but they primarily land near the base of the tree and are often not found too far from directly from the parent tree.

2.4.1. Fruit production

Shrubs with a radius as small as 0.75m (1.5 m canopy width, smallest height value recorded was 1.7 m) produce fruits, potentially contributing to the spread of the species as well as having implications for management. (Figure 2.4). However, additional research on growth rate is still required. Shrubs in open grassland area produced more seeds than shrubs in rocky outcrop and river habitats when plotted against shrub size but open grassland and rocky outcrops produced similar amount of seed per given area in the field (Figure 2.7). Larger shrubs generally had more seeds, which might be due to reproductive maturity and larger shrubs could have been more mature than the smaller sized shrubs. This leads to shrubs with larger size producing higher seed numbers compared to less matured ones (Witkowski and Wilson, 2001). It is also worth noting that high seed production plays a role in the probability of recruitment as it increases the chances of a seed reaching a favourable site for germination and seedling survival (Gioria and Pyšek, 2017).

In addition, the large number of seeds produced increases the availability and attractiveness of fruits to the frugivores as densely presented *P. angustifolia* fruits would be attractive to frugivores (Aslan and Rejmánek, 2012; Klinerová et al., 2018). *Pyracantha angustifolia* fruits persist on the shrub through winter and summer seasons, in large populations, and shrubs start fruiting while the previous season's fruits are still on the shrub (pers. obs.). The observed persistence of fruits on shrubs implies that the fruits are available for frugivorous birds almost all year round (pers.obs). As compared to other invaded systems, the shrubs also produce fruits 'out of season' (Vergara-Tabares et al., 2016) compared to native summer-rainfall species in the eastern Free State, such as rusty-leaf currant *Searsia*

divaricata (Eckl. & Zeyh.) Moffett (Anacardiaceae) which fruits from October to January (Moffett, 2018).

Pyracantha angustifolia fruits stay on the shrub until the next fruiting season (early autumn). The extended fruiting periods suggests that while native fruits are not available for frugivorous birds, native frugivorous birds would feed on IAP fruits (Lediuk et al., 2014; David L. Vergara-Tabares et al., 2016). This would be advantageous for native frugivorous birds as they will be able to supplement their diet with alien plant fruits without having to travel long distances (Vergara-Tabares et al., 2018). It would also be advantageous for the IAP as they can exploit native frugivores as seed dispersers without competing with native fleshy-fruited plant species. At the same time, this might pose a threat to native fleshy-fruited plants with overlapping fruiting seasons as the native frugivorous birds might prefer IAP's fruits over the native fruits (Aslan and Rejmánek, 2012). Such preference would lead to a reduction in seed dispersal and ultimately of native plant diversity as an IAP would be out-competing native fleshy-fruited plants in their native environment.

Native frugivorous birds might even prefer *P. angustifolia* fruits over native species since they are bigger and potentially more rewarding than native shrubs, i.e. *P. angustifolia* fruits are much fleshier than those of *S. divaricata* (Lambert and Marshall, 1991). In addition, *P. angustifolia* produces larger quantities and more brightly coloured fruits compared to *S. divaricata*. The brightness contributes to visibility of fruits to frugivorous birds whereby the bright coloured fruits would be easily visible compared to dull ones. In this case, the orange berries of *P. angustifolia* may be more attractive than the brownish to green fruits of *S. divaricata*. Red-fruited plants have been linked with large-scale dispersal events (Lu et al., 2019). The propagule pressure of this species is not limited by individual fruit production in Afromontane environments of the Eastern Free State. Fruit production is very high all over the invaded landscape, despite some differences in shrub densities and sizes between environments.

2.4.2. Seed dispersal

As remote cameras have revealed, small rodents are the main fruit and/or seed consumers on the ground and birds are the main fruit consumers feeding on fruits directly from the fruiting branch (Figure 2.8). This implies that birds could be regarded as seed dispersers

and rodents as seed predators. Rodents observed feeding on fruits and/or seed were regarded as predators since seeds cannot survive digestive tract of small rodents (Campos et al., 2008). This study has shown that the Four-striped mouse and Karoo bush rat are the main seed predators of *P. angustifolia* in the eastern Free State. This might occur in every area where the aforementioned two rodents occur, as they are widely distributed (Campos et al., 2016).

South African grasslands have a high diversity of seed feeding rodents species of which only a few were captured by the cameras (Avenant, 2011). While larger rodents have been proven to be secondary seed dispersers through seed caching and scatter hoarding (Wang and Smith, 2002), small rodents have been reported to be seed predators due to the fact that seeds do not survive their gut conditions (Howe, 1989; Wróbel and Zwolak, 2017). Predation by small rodents might be one of the factors limiting the spread of this notorious plant invader as rodents might destroy many of the seeds (Holmes, 1990). Such a phenomenon is also seen in studies by Wennyi (2000) where small rodents are major seed predators on the ground as they are opportunistic frugivores (Campos et al., 2016). This suggests that small rodents may play an important ecological role in hindering huge amounts of seeds from being covered by litter and forming an established soil seed bank. In this way, the spread of *P. angustifolia* may be slowed by seed predation by small rodents. It has been reported that *Pyracantha* species are dispersed mainly by birds (Ballari et al., 2015; Corlett, 2005; Henderson, 1999; Vergara-Tabares et al., 2018) and the results in this study concur with this claim, as remote cameras captured African Red-eyed Bulbul feeding on *P. angustifolia* fruits (Table 2.1). Three other species were also spotted during opportunistic observations, including Speckled Mousebird, African Pied Starling and Crested Barbet. All birds are frugivorous, with Crested Barbet being omnivorous.

Not only birds have been proven to have fed on *P. angustifolia* fruits, since the seeds were also spotted in Chacma baboon dung in the riverine habitat and livestock feeding on fruits in the open grassland habitat were also observed as part of opportunistic data collection (Figure 2.10). This adds to seed dispersal diversity, increasing seed dispersal vectors and thus improving seed dispersal. Timed observations recorded nine bird species (Figure 2.11), but only one species fed on one fruit during *P. angustifolia* shrub visit while the rest of the birds used the shrub for perching. Only four bird species out of nine were truly frugivorous, this includes African Red-eyed Bulbuls, Cape White-eyes, Dark-Capped

Bulbuls and Speckled Mousebirds. This shows that *P. angustifolia* is also used by birds for perching as it would be expected with any shrub. Such results on timed observations were not expected as most of the shrub visitors were expected to feed on the fruits. It might be that there were other fruiting plants around as the observations were done in June or the observation time started late (07h00 am).

2.4.3. Soil seed bank

The decrease of the soil seed bank density with an increase in distance away from the parent plant has also been revealed by other studies (White and Vivian-Smith, 2011; Witkowski and Wilson, 2003). Seed density also decreased with increase in distance away from the source plant in *P. angustifolia* seed banks (Table 2.2), with significantly ($P < 0.05$) higher numbers under the canopy towards the centre of the shrub. Irrespective of small rodents feeding on large amounts of *P. angustifolia* seeds (Figure 2.9). The seeds in the soil suggest that the plants are producing enough fruit to saturate predation, ensuring that seeds are still making it into the soil to form part of the soil seed bank or alternatively germinate. The chances of *P. angustifolia* re-establishment through the soil seed bank post-clearing are small because of the seeds having low survival rates in the soil. The viability of the seeds was not tested but a high proportion of seeds crumbled under light pressure when handled, suggesting that they were not viable and susceptible to decomposition. Still, seed viability needs to be tested.

2.4.4. Seed rain

The use of *P. angustifolia* as perching sites for birds (Figure 2.10) might also result in recruitment of other fleshy-fruited IAPs as birds might defecate them while perching on *P. angustifolia* branches (Tecco et al., 2006; Milton et al., 2007). This may facilitate the establishment of other species (potentially invasive species) within the invaded site as the thorny nature of the shrub protects developing seedlings growing close to the tree from the harsh sunlight as well as grazing from livestock. A similar mutualistic relationship was showing in a secondary shrubland in central Argentina mountains where *P. angustifolia* was shown to play an important role in the enhancement of invasion by other IAPs. High seed rain densities were observed under *P. angustifolia* canopy. High proportions of seed from intact fruits under *P. angustifolia* canopy indicates that many seeds reached the ground in intact fruits as confirmed by the soil seed bank analysis. The presence of *P.*

angustifolia seed under *R. rubiginosa* and *L. sericea* proves that birds using the latter as perching structures also use *P. angustifolia*. In addition, the presence of these seeds under the fence confirms that the seeds are bird dispersed, as they would have egested such seeds while perching on the fence. Unexpectedly, there were whole fruits collected from seed traps under *R. rubiginosa* and *L. sericea* canopies. This might be a result of short dispersal distances near *Pyracantha* bushes.

2.5. Conclusions

Pyracantha angustifolia produces an enormous amount of fruits (>5 million /m²), and birds are the main dispersers of these seeds. Small rodents are the main seed predators of fruits and seeds fallen on the ground, which qualifies rodents for potentially hindering the spread of *P. angustifolia*. The most important factors exacerbating the spread of *P. angustifolia* is the production of large quantities of fruits and use of native birds, baboons and livestock to disperse the seeds.

CHAPTER 3

THE EFFECT OF INGESTION BY BIRDS ON GERMINATION OF *PYRACANTHA ANGUSTIFOLIA*

3.1. Introduction

There is a mutualistic relationship that exists between frugivorous birds and fleshy-fruited plants (Fricke et al., 2019; Jordaan and Downs, 2012). This interaction allows fleshy-fruited plants to disperse their propagules away from the source plant (Tabassum and Leishman, 2018). In return, the frugivorous bird digests the fruit flesh as an energy reward (Tiebel et al., 2018). In addition, frugivores play an important role in improving germination of the dispersed seeds (Dlamini et al., 2018; Paulsen and Högstedt, 2002). Such enhancement is believed to be a result of the fruit flesh and skin being removed and the breaking of seed dormancy by the digestive juices of the frugivores' gut (Cimi and Campbell, 2017; Traveset et al., 2001). Several studies show no effect on germination while others show lower germination rate post-ingestion by birds (Mora and Smith-Ramirez, 2016; Thabethe et al., 2015a). Enhancement of germination by the passage of seeds through the gut is not guaranteed since some birds' digestive conditions are not suitable for some seeds (Valido and Nogales, 1994).

Dispersal of fleshy fruited plants is enhanced by the spreading of seeds by frugivores and sometimes the enhancement of germination (Chama et al., 2013). Some invasive alien plants can use this interaction to assist them in spreading into new environments (Fricke et al., 2019; Wotton and Mcalpine, 2015), and allow the seedlings to access new niches and avoid resource competition with the source plant. For example, in South Africa , the germination of the invasive plant Brazilian pepper tree *Schinus terebinthifolius* Raddi (Anacardiaceae) was improved by ingestion by birds (Dlamini et al., 2018). Since the invasive *Pyracantha angustifolia* (Yellow firethorn) seeds are dispersed mainly by frugivorous birds in South Africa (Chapter 2). It would be expected that after ingestion, the germination rate of the seeds would be improved. Pulp removal might be the factor enhancing germination (Thibault et al., 2018; Traveset et al., 2001). This does not necessarily have to be through ingestion by birds, as some smaller bird species may just eat the flesh and not ingest the seeds. The relationship between the plant species and frugivorous birds is also important with regard to invasive alien plants (Mokotjomela et al.,

2015; Tabassum and Leishman, 2018). Different bird species may facilitate their spread within and between sites (Prather et al., 2017).

Understanding the plant-animal interactions is fundamental to finding solutions to assist in management (Fricke et al., 2019). In this chapter, the role of avian frugivorous in enhancing the spread of *P. angustifolia* in the grassland of South Africa is determined. This was achieved through determining the ingestion effect of various frugivorous bird species on seed germination. Germination rate and success were compared among non-ingested fruits, ingested fruits and manually depulped seeds.

3.2. Materials and methods

To determine the effect of ingestion by frugivorous birds on *P. angustifolia* germination rate and success, feeding and germination trials were run using experimental methods used by Dlamini et al. (2018) with some adaptations (Figure 3.1). Ethical clearance (Protocol reference number: 020/15/Animal) was obtained for the use of aviary birds in the study.

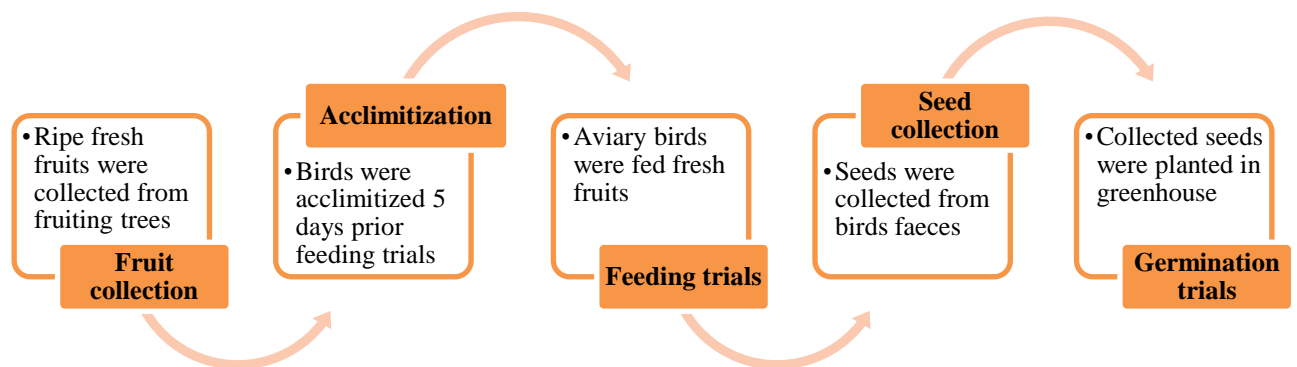


Figure 3. 1 Processes indicating major steps taken to conduct the germination study investigating the effect of ingestion by birds on germination of *Pyracantha angustifolia* seeds. Seed treatments included manually depulped seeds and whole fruits for comparison

3.2.1. Bird species

To investigate the effect of ingestion by birds on seed germination, *P. angustifolia* fruits were fed to five South African frugivorous bird species in controlled aviary conditions. Bird species used in the study included five Dark-capped Bulbuls (*Pycnonotus tricolor*), two Purple-crested Turacos (*Gallirex porphyreolophus*), three Speckled Mousebirds (*Colius striatus*), five Red-winged Starlings (*Onychognathus morio*) and nine Cape White-

eyes (*Zosterops virens*). All birds have been observed feeding on *P. angustifolia* fruits in the field except for Purple-crested Turacos and Cape White-eyes. Purple-crested Turacos and Red-winged Starlings were regarded as “large” birds with regard to their mass weighing on average 285 and 140g respectively and Speckled Mousebirds, Dark-capped Bulbuls and Cape White-eyes as “small birds” weighing on average 55, 39 and 9g respectively (Chittenden et al., 2009; Newman, 2013; Table 3.1). Purple-crested Turacos do not naturally occur in the study area, and they are a surrogate species for other larger bird species that might consume the fruits in the study area, like the African olive pigeon (*Columba arquatrix*).

Table 3. 1 Aviary bird species kept in the animal house at the University of KwaZulu-Natal for the study of avian ingestion on seed germination, and their mass, length, habitat (in South Africa) and diet (Chittenden et al., 2009; Newman, 2013)

Species	Mass (g)	Length (cm)	Habitat and diet
Purple-crested Turaco	285	42	Woodlands, forest, riparian margins and suburban gardens. Eats fruits and leaf buds.
Red-winged Starling	140	30	Prefers mountainous and rocky regions. Eats mainly fruits and insects.
Speckled Mousebird	55	33	Wide range of wooded habitats and urban areas. Eats wide range of fruits and also leaves, buds, flowers and nectar.
Dark-capped Bulbul	39	20	All woodland habitats in moister eastern regions and common in suburban gardens. Eats mainly fruits, also insects and flower petals.
Cape White-eye	9	12	Common endemic. Inhabit mainly riverine woodland, but also in all wooded habits, including parks and gardens. Eats fruits, nectar and insects.

3.2.2. Fruit collection and storage

Fresh, ripe *P. angustifolia* (Franch.) C.K.Schneid. (Rosaceae) fruits were collected from 20 shrubs from the Clarens region (28°32'69"S; 28°25'20"E) during the fruiting season (August 2019). Branches with fruits were collected to keep them as fresh as possible. Upon arrival at the Animal House at the University of KwaZulu-Natal (UKZN), fruits were removed from branches by hand and placed in plastic bags. Fruits from all shrubs were pooled, in order to standardize any differences associated with local seed origin as well as incorporating genetic variability. After collection, fruits were placed in the refrigerator and the feeding trials started a day after collection.

3.2.3. Acclimatization of birds prior to feeding trials

Birds used in the study went through five days acclimatization so that they got used to a new environment prior to commencement of the experiment (Figure 3.2). Aviary birds were typically kept in outside cages (1 x 2.12 x 2.66 m) at the UKZN Animal house (29°37'0"S; 28°23'0"E), KwaZulu-Natal Province, South Africa (Figure 3.3). Birds used in this study were then acclimatized in experimental indoor cages for a period of five days prior to the commencement of feeding trials. They were fed maintenance food which included apples, pears, bananas, and oranges, together with a bird protein supplement of AviPlus Softbill/Mynah pellets. The acclimatization was included in order for the birds to get used to staying in the experimental cages in a constant environment room with 12L: 12D photoperiod at $25 \pm 1^\circ\text{C}$.



Figure 3. 2 Five Dark-capped Bulbuls (*Pycnonotus tricolor*) and three Speckled Mousebirds (*Colius striatus*) kept in indoor experimental cages during acclimatization of five days before feeding trials commenced at the University of KwaZulu-Natal Animal house. Birds were fed apples, bananas, oranges and supplementary food and water was provided *ad libitum*

3.2.4. Feeding trials

In order to emulate a natural scenario whereby birds feed on fruits and disperse seeds, fruits were fed to the birds and the germination success of defecated seeds was investigated. Feeding trials were conducted for three days after the acclimatization period. Birds were given their supplementary food that included five *P. angustifolia* fruits. Supplementary food was removed from the cages at 5:00 pm so that the birds would be hungry in the following morning for the feeding trials. Feeding trials began at 8:00 am where birds were provided with *P. angustifolia* fruits to feed on (Figure 3.3). Water was provided to birds *ad libitum*.



Figure 3. 3 Purple-crested Turaco (*Gallirex porphyreolophus*) feeding on *Pyracantha angustifolia* fruits during the feeding trials in experimental cages at the University of KwaZulu-Natal's Animal house, Pietermaritzburg

3.2.5. Seed retention time

To inform on potential seed dispersal distances, seed retention data were collected in order to predict the effect of seed dispersal distances. The seed retention time was determined for each bird species to investigate how long seeds take to pass through the birds' gut. This measure would help us predict spatial implications of seed dispersed by birds. Seed retention time referred to the time from when the bird consumed the first fruit/seed until the first seed was defecated by the bird.

3.2.6. Germination success rate

Different germination treatments were used in order to compare the germination success and rate. Treatments included seeds ingested by birds, manually depulped seeds and intact fruits. After being collected straight from the feeding trials, seeds defecated by birds were sown within 24 hours of collection in a greenhouse (Figure 3.4)



Figure 3. 4 Events highlighting the processes that were taken during germination trials. Seeds were (A) hand-picked after being defecated by birds from the cages and planted in the (C) greenhouse using (B) seedling trays

Seeds ingested by birds treatment emulated the natural environment whereby the seeds are ingested as fruits. Birds were provided with whole fresh fruits, then seeds were collected from the cages and planted. Newspapers were placed under the bird cages to trap all seeds defecated by birds. All seeds were collected and kept in paper bags for planting (Figure 3.5A). Seeds collected from bird faeces were planted in potting soil in seedling trays (265 × 180 × 75 mm) (Figure 3.5B) and kept in a greenhouse at 26 °C maximum temperature (Figure 3.5C) (Jordaan et al., 2011). Seeds were planted within 24 hours of collection from the faeces (Barnea and Friedman, 1992). The sown seeds were watered daily and were regarded to have germinated when the radicle appears above the soil. After being counted, such seedlings were removed from the seedling tray to avoid recounts (Dlamini et al., 2018).

In de-pulped seed treatment, the treatment using de-pulped seeds emulated a condition where the fruit wall is removed by other factors but not birds' ingestion. For example, chapter 2 showed field mice removing the seed pulp and birds that only eat the fruit pulp and drop the seeds on the ground. Such an experiment excludes the digestive juices in the gut of frugivores as one of the factors that might trigger germination. A total number of 200 seeds were planted from 40 manually depulped fruits. Un-ingested intact fruits were sown in the same conditions as other treatments to serve as a standard of comparison (control) for the experiment. This treatment emulates the natural environment where seeds will fall directly to the soil without been eaten by animals, probably being buried under the soil or litter while intact. One hundred fruits were planted in four separate planting trays, totalling approximately 500 seeds (average of 5 seeds per fruit, Chapter 2).

3.2.7. Data analysis

Mean cumulative seedling percentages for ingested, manually depulped and whole fruits were calculated using Excel 2013. Time taken for seedlings to emerge in each treatment was compared using Analysis of variance (ANOVA). Mean seedling emergence times for seeds ingested by different bird species and manually depulped and whole fruits (control) were compared using post-hoc Tukey tests for multiple comparisons. Seedling emergence time referred to the time it takes for a single seedling to emerge on top of the soil. The significance level for this analysis was set at $\alpha = 0.05$ and the One-Way ANOVA analysis was performed using GraphPad Prism 5 statistical software (Motulsky, 1999). Two-tailed Chi-square test was also done at 95% confidence interval to compare the proportion of seeds that germinated from each treatment. Null hypothesis was that there are no significant differences on days of germination between the treatments. Data normality was tested using D'Agostino and Pearson omnibus normality test.

3.3. Results

All birds, except Cape White-eyes, attempted to eat the fruits within the first five minutes of the start of the feeding trials. Only Purple-crested Turacos managed eat the fruit whole. Other birds struggled to feed on the fruits. Red-winged Starlings and Dark-capped Bulbuls struggled at first but after a short while got accustomed to handling the fruits. Speckled Mousebirds were able to hold the fruits with their claws and feed on the fruit wall. Cape White-eyes initially did not eat the fruits, but they fed on fruit pulp in small quantities.

3.3.1. Germination trials

The number of seeds collected from the feeding trials was 443 seeds from the Red-winged Starlings, 298 from Purple-crested Turacos, 133 from Dark-capped Bulbuls and 44 from Speckled Mousebirds.

3.3.2. Seed retention times

Birds with larger mass had longer seed retention times (i.e. Purple-crested Turacos and Red-winged Starlings had longer retention times (Mean \pm SE) 24.5 ± 0.5 min and 28.8 ± 1.39 min respectively; (Table 3.1). Smaller birds generally had shorter seed retention times with Dark-capped Bulbuls and Speckled Mousebirds having 21.67 ± 2.03 min and 11.33 ± 5.84 min respectively. Tukey's *post hoc* multiple comparison test indicated that there was a significant difference in retention times between Red-winged Starlings and Speckled Mousebirds ($F_{3,9} = 6.51$, $P < 0.05$) but no difference ($P > 0.05$) in retention times between other bird species treatments (Figure 3.5).

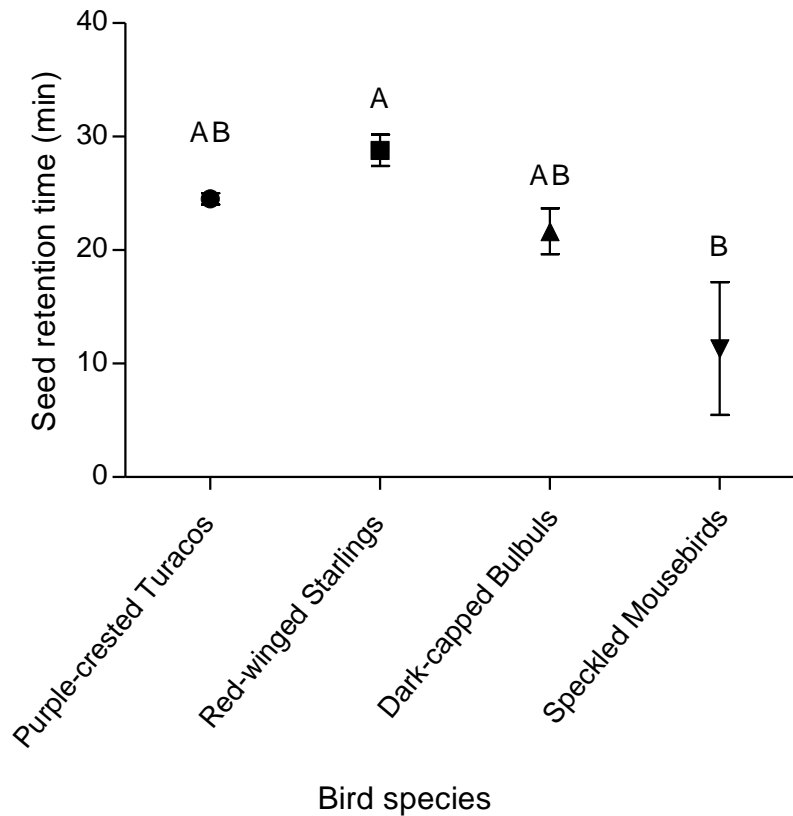


Figure 3. 5 Seed retention times (Mean and Standard error of mean) of four bird species which were fed *Pyracantha angustifolia* seeds, namely Purple-crested Turacos (n=2), Red-winged Starlings (n=5), Dark-capped Bulbuls (n=3) and Speckled Mousebirds (n=3). Retention time was measured as the time from when the bird ate the first fruit to when the first seed was defecated. Different letters over bars indicate statistically significant differences between treatments ($P = 0.0124$, F value = 6.506). One-Way ANOVA was used to determine statistical significance differences between mean retention times. Sample size for seed retention times were too small for statistics, therefore the results from this analysis are inconclusive.

3.3.3. Rate of seedling emergence

One-way ANOVA revealed an overall significant difference ($P < 0.0001$) between three treatments. There was a slower rate of germination in seeds planted as whole fruits compared to the manually depulped seeds and those ingested by birds (Figure 3.6). Mean seedling emergence times for all birds, depulped and whole fruits were 31, 32 and 38 days respectively (Table 3.1). There was no significant difference in the seedling emergence

time between the manually depulped and ingested seeds ($F_{2,1039} = 28.15$, $P > 0.05$) but there was a significant difference ($P < 0.05$) between ingested and manually depulped treatments when compared to whole fruits. Seeds planted as whole fruits took a longer time to germinate compared to depulped and ingested seeds.

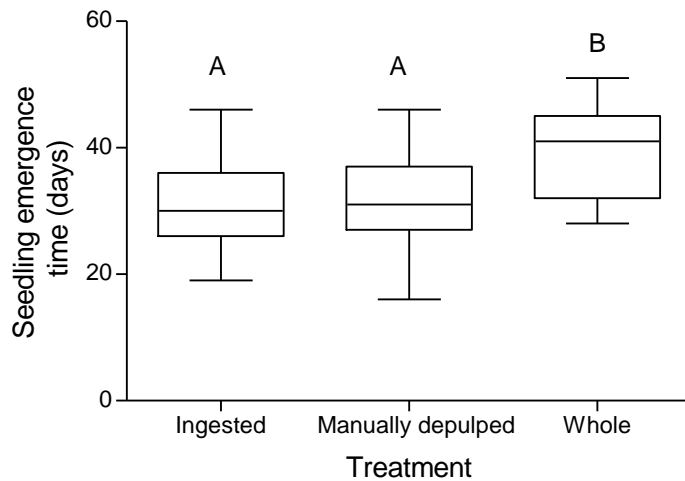


Figure 3. 6 Box and whisker plot (minimum to maximum) of *Pyracantha angustifolia* seedling emergence time in days for three treatments, namely ingested by birds (n=805), manually depulped by hand (n=199) and whole fruits (n=38). Boxes represent mean with standard error of the mean (SE). Different letters on top of boxes indicate statistical significance ($P < 0.05$, $df = 2$, F value = 38.27) and error bars indicate minimum and maximum values

Emergence times of seedlings from seed ingested by the four bird species were compared using One-Way ANOVA. Seedling emergence time referred to the time it takes for a single seedling to emerge on top of the soil. Seeds defecated by small birds (Speckled Mousebirds and Dark-capped Bulbuls) germinated faster (approximately 4 days earlier) compared to larger birds (Purple-crested Turacos vs Dark-capped Bulbuls, Purple-crested Turacos vs Speckled Mousebirds, Red-winged Starlings vs Dark-capped Bulbuls and Red-winged Starlings vs Speckled Mousebirds) but there was no significant difference in seedling emergence rates among larger birds and smaller birds (Purple-crested Turacos vs Red-winged Starlings and Dark-capped Bulbuls vs Speckled Mousebirds) ($F_{3,801} = 11.08$, $P < 0.05$).

3.3.4. Germination success rate

Chi-square test ($X^2 = 1041$, $df = 5$) indicated that there were significant ($P < 0.01$) on days of germination between the treatments. Null hypothesis was rejected, therefore there were significant differences on days of germination between the treatments. Germination success of both depulped and ingested seeds was high (Purple-crested Turacos = 93.95%, Red-winged Starlings = 81.94%, Speckled Mousebirds = 88.64, Dark-capped Bulbuls = 94.74%, depulped = 99.50%) compared to whole fruits (whole fruit = 7.6%) (Figure 3.8). Seed ingested by small birds (Speckled Mousebirds and Dark-capped Bulbuls) had rapid rates of seedling emergence during a specific period in the germination trials (19 – 34 days).

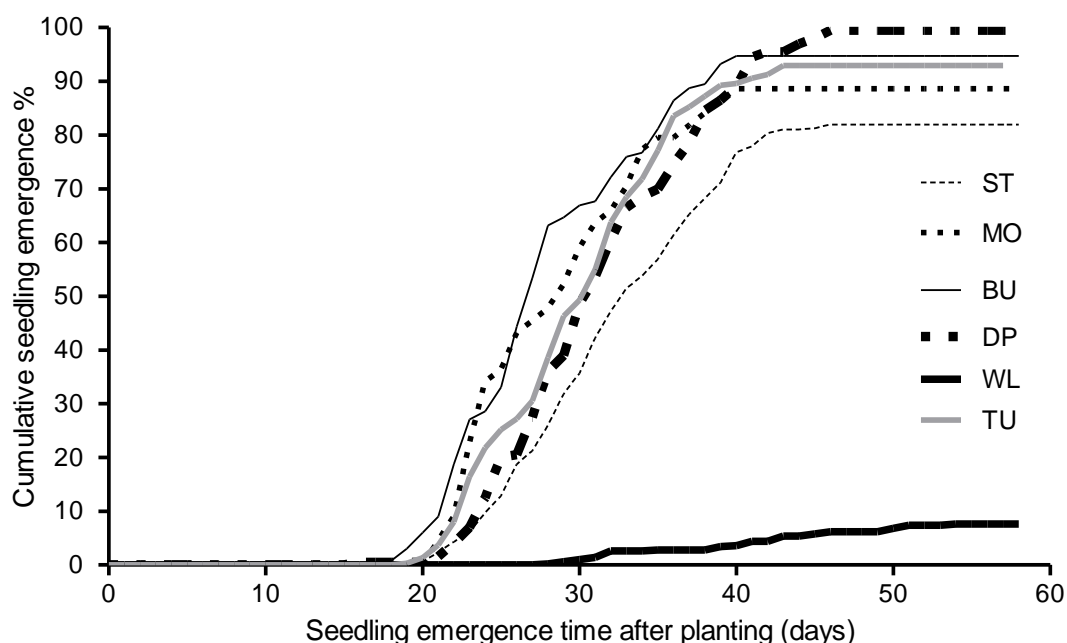


Figure 3. 7 Cumulative *Pyracantha angustifolia* seedling emergence percentage against number of days after planting for seeds defecated by Purple-crested Turacos (TU), Red-winged Starlings (ST), Speckled Mousebirds (MO) and Dark-capped Bulbuls (BU) together with seeds from manually depulped (DP) fruits and seeds planted as whole fruits (WL). Seedling emergence was recorded until the 58th day

3.4. Discussion

The results from this study indicated that fruit ingestion by birds does not enhance germination success compared to depulped seeds but the removal of the fruit wall by any means greatly increase germination rates. All treatments had high germination success rates, except for whole fruits.

Non-consumption of *P. angustifolia* seeds by Cape White-eyes indicates that small birds probably do not consume entire *P. angustifolia* fruits. Cape White-eyes were similarly unable to consume larger fruits of another invasive alien plant, Indian laurel (*Litsea glutinosa* (Lour.) C.B.Rob., Lauraceae) (Dlamini et al., 2018). The same behaviour of feeding on only the fruit wall when presented to the Cape White-eyes was observed in studies by Dlamini et al. (2018) as they also ate *L. glutinosa*'s pulp. This then excludes Cape White-eyes as potential long-distance seed dispersers of *P. angustifolia* as they do not ingest the seeds. However, eating the fruit pulp would still assist in germination when these depulped seeds fall to the ground.

3.4.1. Seed retention times

Retention time appeared to have an effect on rate of germination of ingested seeds although not significant (Figure 3.5). Smaller birds, Dark-capped Bulbuls and Speckled Mousebirds, had shorter seed retention with faster rate of germination than larger birds. Purple-crested Turacos and Red-winged Starlings had longer seed retention and slower rate of seed germination. This indicates that bigger birds would deposit seeds at longer distances away from source plants compared to small birds with shorter retention times. However, small birds would deposit more seeds in shorter time (Figure 3.6). Thus, temporal aspect of dispersal is more important in small birds than the spatial aspect of dispersal in larger birds. These data suggest that small birds contribute more on rate of *P. angustifolia* spread as they would deposit more seed per time and seeds would germinate faster (Figure 3.8). While, larger birds are expected to contribute more on area of spread than rate of spread as they feed on more seeds and have longer seed retention times. Retention times did not have an effect on germination success since all ingested seeds had high germination success together with the manually depulped seeds (Figure 3.9).

Seed retention times were contrary to the results found by Dlamini et al. (2018) as Speckled Mousebirds had significantly higher retention time (25 minutes) compared to Dark-capped Bulbuls (13 min) when fed on *L. glutinosa* but in this study Speckled Mousebirds had slightly lower retention times (11.3 minutes) than Dark-capped Bulbuls (21.67 minutes). Jordaan et al. (2011) found similar results to those of Dlamini et al. (2018) when feeding Speckled Mousebirds and Dark-capped Bulbuls with *Solanum mauritianum* Scop. (Solanaceae), *Psidium guajava* L. (Myrtaceae), and *Lantana camara* L. (Verbenaceae)

fruits. Such results might be influenced by the handling of fruits by the digestive systems of the latter birds, indicating that a Speckled Mousebird's digestive system digests the fruit wall of *P. angustifolia* better than the fruit wall of *L. glutinosa* while a Dark-capped Bulbul's digestive system handles *L. glutinosa* seeds quite well.

Another factor that might have affected the results might be that Speckled Mousebirds "squashed" *P. angustifolia* fruits with their feed before feeding on fruit pulp and seeds (L. Adams, pers. obs.). Seeds might have been ingested by mistake because fruit pulp might have been what the Speckled Mousebirds aimed for when feeding on fruits.

3.4.2. Rate of seedling emergence

As there was no difference between the germination rates of manually depulped and bird ingested seeds, it implies that ingestion by birds does not inhibit or enhance germination but rather facilitates germination by mechanically removing the fruit wall. Similar results have been reported for germination of *S. mauritianum*, *Cinnamomum camphora* (L.) J.Presl (Lauraceae), *L. camara* and *P. guajava* after being ingested by Red-winged Starlings, Speckled Mousebirds, and Dark-capped Bulbuls (Jordaan et al., 2011). Although ingestion in this study did not have an effect on the rate of germination, birds do help with the dispersal of the seed away from the source plant and therefore reduce competition between seedlings, provided that the seed reaches a favourable site for germination (Dellafiore et al., 2015).

The low rate of germination observed in seeds planted as whole fruits was an expected result (Figure 3.6). Slower rate of seedling emergence from intact fruits has been reported by numerous studies (Dlamini et al., 2018; Jordaan et al., 2011; Mokotjomela et al., 2016; Thabethe et al., 2015a). The fruit wall of fallen fruits, found in seed traps in Chapter 2, has to be decomposed by soil microorganisms first before the seedling emerges. This typically takes longer than when the fruit wall is removed by a bird as it takes less than an hour for a bird to remove the fruit wall but it takes days for the soil microorganisms to digest the fruit wall (Holmes, 1989). This implies that seeds fallen as whole fruits have lower germination success and rates than those dispersed by birds. Such a phenomenon shows that birds remain important seed dispersers of *P. angustifolia*.

The results of this study conform to those presented in many studies whereby seeds ingested by birds germinate significantly faster than those in intact fruits. The number of seeds planted was controlled in some studies investigating effect of ingestion by birds on seed germination (Díaz Vélez et al., 2018; Mokotjomela et al., 2016) but not controlled in others (Jordaan et al., 2011; Thabethe et al., 2015b).

3.4.3. Germination success rate

Dellafiore et al. (2015) found that *Pyracantha atalantoides* germination was lower for bird-ingested seeds compared to manually depulped seed. This might be because the seeds were collected from birds' excreta in the field, while in this study the seeds were collected immediately after defaecation and in controlled conditions, while still fresh. Seeds collected from faeces in the field might have already been exposed to harsh environmental conditions such as sunlight. Cumulative germination percentage in *P. angustifolia* was high in both ingested and depulped seeds compared to whole fruits (Figure 3.9). This shows that germination success was not affected by ingestion as in studies by Lafleur et al. (2009) where European Starlings (*Sturnus vulgaris*) increased germination success of *Elaeagnus umbellata* Thunb. (Elaeagnaceae) and *Celastrus orbiculatus* Thunb. (Celastraceae) compared to manually depulped seeds.

The same results were observed when *Solanum mauritianum*, *C. camphora*, *P. guajava*, and *Morus alba* L. (Moraceae), were fed to Knysna (*Tauraco corythaix*) and Purple-crested Turacos in which germination success was unaffected (Thabethe et al., 2015a). Contrary results were revealed when Castro et al. (2016) found that germination success of glossy pivot tree *Ligustrum lucidum* W.T.Aiton. (Oleaceae) was reduced after ingestion by Austral Thrush (*Turdus falcklandii*). In contrast, Mora & Smith-Ramirez (2016) found that there was an increase in germination success of the invasive alien plant *Aristotelia chilensis* (Molina) Stuntz. (Elaeocarpaceae) post-ingestion by frugivorous austral thrushes while the same bird species had no effect of germination success on *Rubus ulmifolius* Schott. (Rosaceae) and *Ugni molinae* Turcz. (Myrtaceae).

Such contrasts show that seeds of different plant species respond differently to ingestion by different bird species and therefore have different effects on germination. Findings presented in this study highlight the importance of frugivorous birds in facilitating the spread of IAPs (Castro et al., 2016). Factors that might have affected the results are

highlighted in a review by Traveset (1998), and include size and thickness of the seed coat, differences in gut passage times, diets of birds and also seed age.

3.5. Conclusions

Pyracantha angustifolia seed ingestion by the four frugivorous birds under study did not affect germination rate and success; instead, the birds facilitate the spread and germination of seeds by removing the fruit pulp. Overall, native South African birds were shown to play a role in the spread and germination success of this IAP.

CHAPTER 4

POLLINATION ECOLOGY OF *PYRACANTHA ANGUSTIFOLIA* IN SOUTH AFRICA

4.1. Introduction

Pollination as it is a key ecological process in flowering plants (Buchholz and Kowarik, 2019). In the quest of trying to understand plant-pollinator dynamics of IAPs, researchers have studied such networks from different perspectives (Aminatun et al., 2019; Nel et al., 2017). When plants flower simultaneously, they might influence each other's pollinator visitation rates and the quality and/or quantity of the transferred pollen by interacting with each other indirectly (Yang et al., 2011). Such effects depend on how the plants present their resources (i.e. pollen amount, nectar accessibility, flower structure and colour) and how the flower visitors respond to such presented resources (Ojija et al., 2019; Trøjelsgaard et al., 2019). Understanding such effects is very important and numerous studies have been conducted to assess the effect of plant invasions on plant-pollinator mutualisms at different levels (Hallett et al., 2017; Stout and Tiedeken, 2016; Tiedeken and Stout, 2015). This includes flower visitation frequencies (Dohzono et al., 2008), floral visitors overlapping (Gibson et al., 2013) and pollen load on floral visitors (Brown and Mitchell, 2001).

In this Chapter, visitation frequencies, fruit yield without pollinators, and pollen loads of floral visitors were determined for *P. angustifolia*. The latter methods investigated to what extent *P. angustifolia* uses native pollinators to enhance pollination success, which has implications for its reproductive success and invasive potential. Aspects of reproductive ecology that have been previously studied for *P. angustifolia* include mainly seedling recruitment and plant density effects (Giantomasi et al., 2008; Tecco et al., 2006, 2007). However, it appears that there has been no specific study done in the pursuit of addressing breeding systems in *Pyracantha* species.

This study aimed to address part of this gap in literature by performing various experiments on the pollination and breeding ecology of *P. angustifolia*. The aim of this chapter was to determine the role of floral visitors in *P. angustifolia* pollination. Objectives of the study were to: (1) determine floral visitors and their visitation rates, (2) measure the frequency of floral visitors to flowering branches, (3) determine whether *P. angustifolia* can set fruit

without the aid of pollinators and (4) determine pollen loads on floral visitors' bodies by collecting pollen from floral visitors.

4.2. Materials and methods

Pollinator observations, the capture of floral visitors, and pollinator exclusion experiments were performed in December 2018 at Clifton farm near Clarens in the eastern Free State (28°32'8"S; 28°25'2"E). Mature shrubs of *P. angustifolia* that were already producing fruits and possessed a round shrub shape (mature) instead of an elliptic shape (immature) were used for the experiments.

4.2.1. Observations of individual floral visitors

To investigate the visitation rates of insect visitors to flowers of *P. angustifolia*, observations of individual insect visitors were performed on flowering shrubs (Figure 4.1). Each insect was observed for a period of one minute and the number of flowers visited was noted. Eighty observations were done on different shrubs randomly, and included insects of different species. Visitation frequencies were expressed as the number of flowers visited per minute. One-way Analysis of Variance (ANOVA) was performed to investigate the significant differences ($P < 0.05$) in mean visitation frequencies between species of insect visitors. Analysis was done using GraphPad Prism 5 statistical software (Motulsky, 1999). Insects were identified to genus level if possible; otherwise, morpho-species level identifications were used. Data normality was tested using D'Agostino and Pearson omnibus normality test.



Figure 4. 1 Floral insect visitors observed on *Pyracantha angustifolia* flowers during the flowering period of December 2018 at Clifton farm near Clarens, eastern Free State Province, South Africa.

4.2.2. Observations of floral visitors to flowering branches

To determine the visitation frequency of floral visitors to *P. angustifolia* shrubs, flowering branches were observed for a set period and the number of all visitors noted. Each branch was observed for five minutes and different individual insects visiting the flowers were noted and counted. Flowering branches were randomly chosen, and branch size was not taken into consideration. A total of twenty branches were consecutively observed for five minutes each, totalling 100 minutes of observation over two days. Visitation frequencies for floral visitors calculated as the average number of individual insects observed visiting per minute. Insect species were not identified, only the number of insect individuals visiting the flowers were counted.

4.2.3. Pollinator exclusion

To determine natural fruit set and if pollinators are needed for sexual reproduction in *P. angustifolia*, the effect of excluding floral visitors from flowers was investigated. A total of 22 mature *P. angustifolia* shrubs from the open grassland were chosen randomly. Branch lengths of approximately 30cm were used in this experiment. It was late in the field season and branches with closed buds were difficult to locate. Thus only 8 branches on four shrubs could be bagged to exclude floral visitors. The four branch tips (n=8) with flower buds used in the pollinator exclusion treatment were bagged with fine nylon mesh to exclude all floral visitors (Figure 4.2). This tested for autogamous pollination and seed set. The bags were not removed to avoid fruits falling on the ground or being eaten by birds. The number of buds were counted and the point to which these were counted along the branch was marked with fluorescent tape.



Figure 4. 2 *Pyracantha angustifolia* branch tip bagged with a fabric bag to exclude pollinators from visiting flowers. A pink tag indicates the point to which 46 initial buds were bagged

Similarly, for investigating natural fruit set from open pollination and to compare natural fruit set to bagged branches, the number of flowers were noted for each of 44 flowering branch tips and marked with a plastic band (Figure 4.3). The flowers were left open to allow any pollinator to visit the open flowers. During the early development of the fruits, branches were bagged to avoid fruits falling off or being eaten by birds directly from the branches.



Figure 4. 3 Open *Pyracantha angustifolia* flowers on a flowering branch with a pink tag indicating the number of 71 initial flowers counted before developing into fruits. An arrow indicates the branch length from the tag to the tip of the branch.

Fruit set on bagged and open branches was compared using a Generalised Estimating Equation (GEE) in IBM SPSS Statistics version 25 software. The GEE was used because it allows one to account for plant effects. Plant was used as the subject variable to account for any variation in flower number between plants. The model followed a binomial distribution transformed with a logit link function, and the model employed an exchangeable correlation matrix. Ten fruits per branch were cut open and the number of seeds inside were counted in order to determine if fruits contained seeds and how many were produced per fruit on average.

4.2.4. Pollen loads

Pollen load, referring to the amount and type of pollen carried by a floral visitor, was determined in order to assess the amount of *P. angustifolia* pollen transferred to visiting insects and if these insects has visited other plant species or were showing constancy (i.e. only visiting *P. angustifolia*). In order to examine the pollen load on insects visiting *P. angustifolia* flowers, insects visiting flowers were caught with an insect net and placed in 5ml microcentrifuge tubes (Figure 4.4A) during the flowering period (December 2018). Insects were dabbed with Fuchsin gel (Beattie, 1971) to collect pollen that might have been deposited on the insect's body (Figure 4.4B). Microscope slides were made by melting the Fuchsin gel (Figure 4.4C) and covering the melted gel with a glass cover slip (Figure 4.4D). The slides were heated with a lighter at a distance of at least 5cm to avoid smoke on the slide that could make the slide blurry. Pollen grains were counted and identified to genus level (where possible) under a light microscope at 40X magnification (Figure 4.4E). All insects were pinned for identification and reference (Figure 4.4F).

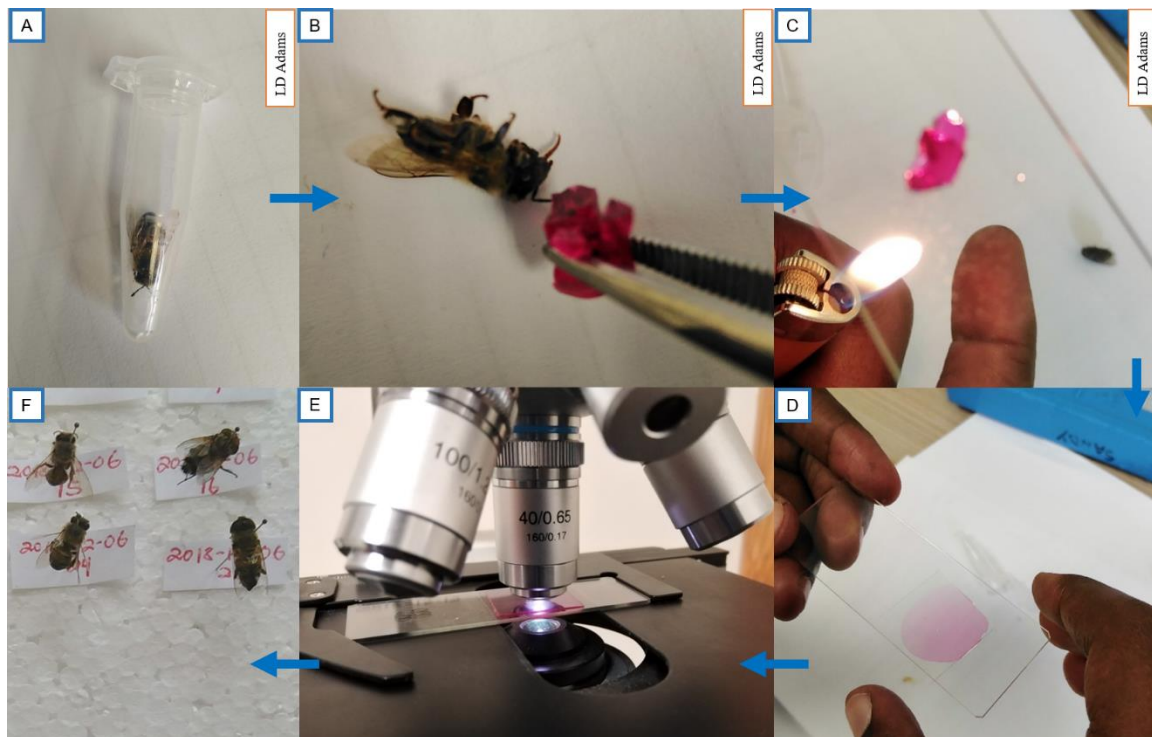


Figure 4. 4 (A) Insects visiting *Pyracantha angustifolia* flowers were collected and placed in a microcentrifuge tube and (B) frozen until dabbed with Fuchsin gel to collect pollen from the insect's body. (C) A slide was made by melting the gel and (D) covering the melted gel with a cover slip. (E) Pollen grains were counted and identified under a light microscope at 40X magnification. (F) All insects were pinned for identification

Total *P. angustifolia* pollen was counted, and the mean number of pollen grains per insect species was calculated. Pollen grains from other plant species were similarly identified and counted. Mean pollen number with standard error (Mean \pm SE) was determined for insect species for which more than one individual was caught. The exact value of pollen grains was presented for insect species for which only one individual was caught. Insect species were identified to genus or species level where possible by the Department of Zoology and Entomology, University of the Free State QwaQwa campus. The mean number of *P. angustifolia* pollen grains between insect species was compared using one-way Analysis of variance (ANOVA) on GraphPad Prism 5 for any insect species for which more than one individual had been caught. Mean number of foreign pollen grains (pollen from other plant species flowering sympatrically) carried by each insect were also determined for each insect species. Percentage *P. angustifolia* pollen purity (Pp) (how pure the pollen collected from insects was) was determine using the following formula:

$$Pp = \frac{\text{Total *Pyracantha angustifolia* pollen}}{\text{Total *Pyracantha angustifolia* pollen} + \text{total other species' pollen}} \times 100\%$$

The higher the P_p value, the more *P. angustifolia* pollen was found on the insects' body relative to pollen from other plant species.

4.3. Results

4.3.1. Observations of individual floral visitors

One-minute observations revealed that four insect species visited *P. angustifolia* flowers frequently (Figure 4.5). One-Way ANOVA ($F = 72.50$, $df = 79$) indicated strong significant ($P < 0.001$) differences. The highest mean \pm SE visitation frequency was 8.9 ± 3.7 flowers.min⁻¹ for *Apis mellifera* (Apidae), followed by *Lucilia* sp. (Calliphoridae) with 3.1 ± 2.0 flowers.min⁻¹, 0.2 ± 0.7 flowers.min⁻¹ for *Calliphora* sp. (Calliphoridae) and *Bellardia* sp. (Calliphoridae) and 0.05 ± 0.2 flowers min⁻¹ for an unidentified small bee species.

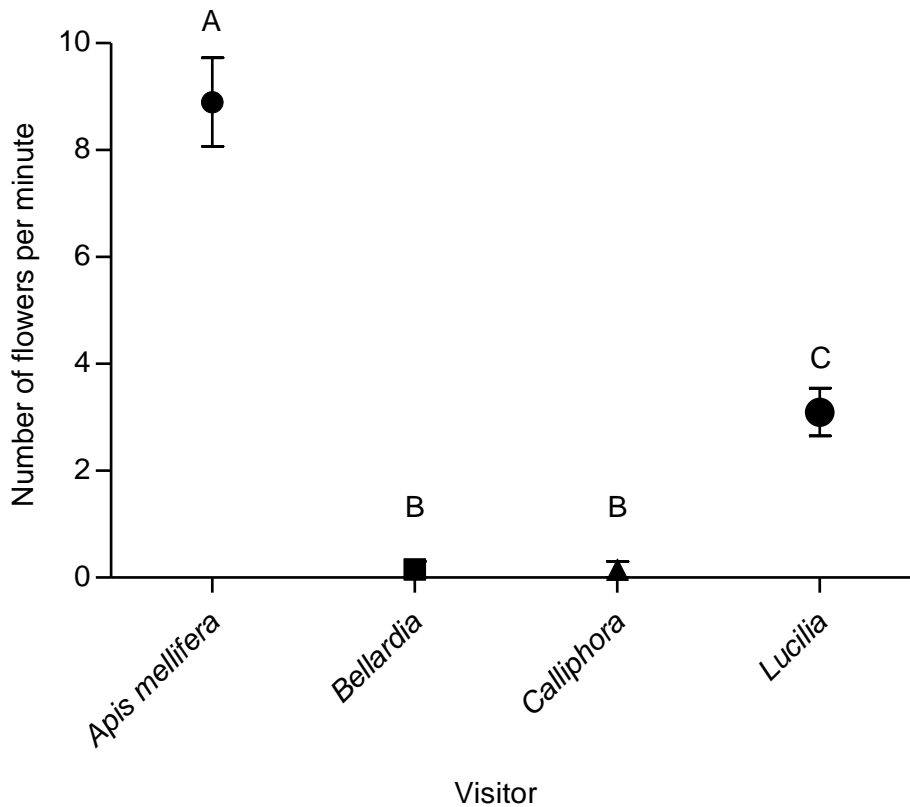


Figure 4. 5 Number (Mean \pm SE) of flowers visited per minute by various insects on *Pyracantha angustifolia* in the eastern Free State. Error bars indicate standard error of the mean and different letters indicate statistical significance ($P < 0.05$, F value = 72.50, ANOVA)

Only *A. mellifera* ($P = 0.9069$) and *Lucilia* ($P = 0.5665$) passed the normality test and *Bellardia* and *Calliphora* did not pass ($P < 0.0001$). Krauskal-Wallis non-parametric was used to determine the significant differences between the insect groups.

4.3.2. Observations of floral visitors to flowering branches

Five-minute observations ($n=20$) totalling to 400 minutes revealed 128 different individuals visiting flowering branches. A mean \pm SE of 1.28 ± 0.19 individuals visited flowering branches per minute.

4.3.3. Pollinator exclusion

Floral visitor exclusions were conducted through bagging flower buds before they opened to determine if *P. angustifolia* could produce fruits without the aid of pollinators. The

sample size of bagged branches was very small, resulting in a large standard error. However, results indicated that *P. angustifolia* plants were capable of setting fruit without pollen vectors (i.e. by autogamy and/or facilitated self-pollination). Although the tapered branches/thorns may have prevented the bags from excessively touching the flowers and potentially moving pollen around within flowers, self-pollen may have been moved from anthers to the stigma within a flower. There were no significant differences between means ($P=0.31$) of the two treatments, with open having high mean fruit yield of 0.91 (± 2.43 SE, $n=44$) compared to bagged with 0.66 mean yield (± 5.10 SE, $n=8$). All fruits sampled ($n = 394$) from both treatments produced normal numbers of seeds (5 seeds per fruit) (Figure 4.6).

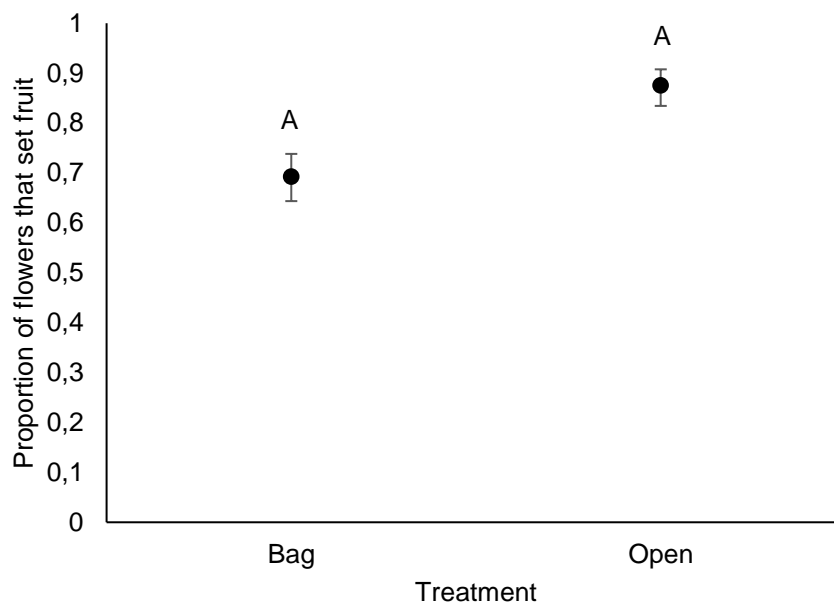


Figure 4. 6 Back-transformed mean (\pm SE) proportion of seed set per branch tip from bagged and open flower buds. Different letters above the bars indicate statistical significance ($P<0.05$)

4.3.4. Pollen loads

Fifty-seven individual insects comprising twelve different species were collected from *P. angustifolia* flowers. Insects included *A. mellifera* (Apidae), *Bellardia* (Calliphoridae), *Calliphora* (Calliphoridae), *Chrysomya* (Calliphoridae), *Dysmachus* (Asilidae), *Eristalinus*

(Syrphidae), *Eristalis* (Syrphidae), *Pollenia* (Calliphoridae), *Syritta* (Syrphidae), *Sarcophaga* (Sarcophagidae), *Spilostethus* (Lygaeidae) and *Lucilia* (Calliphoridae) (Table 4.1). Diptera was the diverse floral visitor group, and the largest number of species of any visiting insect family were from the Calliphoridae (Table 4.1). The largest number of insects caught visiting the flowers were *A. mellifera* (n=24 individuals), followed by *Calliphora* sp. (n=11), then *Lucilia* (n=9). *Eristalis* sp. carried the highest amount of *P. angustifolia* pollen (>1000 pollen grains) followed by *Eristalinus* sp. (467.8), and then *Bellardia* sp. (370.0).

To assess the level of purity of pollen carried by insects (i.e the proportion of *P. angustifolia* contamination by other species' pollen) the mean number of foreign plant species pollen grains was determined. *Bellardia* sp. carried the highest amount of pollen from other plant species (30.4 pollen grains), followed by *Eristalis* sp. (15.9), and then *Eristalinus* sp. (14.9). *Chrysomya*, *Spilostethus* and *Syritta* had the lowest amount of foreign pollen grains on their bodies carrying means of 0.4, 0.7, and 1.2 pollen grains respectively. All insects carried high proportions of *P. angustifolia* pollen (> 70% purity) but *Chrysomya*, *Spilostethus*, *A. mellifera* and *Lucilia* had the highest purity of 99.1, 97.5, 97.4 and 97.4% respectively (Table 4.1).

Table 4. 1 Number of pollen grains found on each floral visiting insect collected from flowering *Pyracantha angustifolia* shrubs during the flowering period (December 2018)

Order	Family	Subfamily	Species	Mean (\pm SE) pollen grains	Mean Other pollen grains	Pyracantha pollen purity (<i>Pp</i> in %)
Hymenoptera	Apidae	Apinae	<i>Apis mellifera</i> (n=24)	333.6 \pm 71.1	8.9	97.4
Diptera	Calliphoridae	Calliphorinae	<i>Calliphora</i> sp. 1 (n=11)	70.2 \pm 27.0	3.2	95.7
			<i>Chrysomya</i> sp. 1 (n=2)	49.0 \pm 34.0	0.4	99.1
			<i>Lucilia</i> sp. 1 (n=9)	85.9 \pm 44.2	2.3	97.4
		Polleniinae	<i>Pollenia</i> sp. 1 (n=1)	68.0	5.6	92.4
		<i>Bellardia</i> sp. 1 (n=1)	370.0	30.4	92.4	
	Syrphidae	Eristalinae	<i>Eristalinus</i> sp. 1 (n=4)	467.8 \pm 253.9	14.9	96.9
			<i>Eristalis</i> sp. 1 (n=1)	>1 000.0	15.9	98.4
			<i>Syritta</i> sp. 1 (n=1)	10.0	1.2	89.1
	Asilidae		<i>Dysmachus</i> sp. 1 (n=1)	7.0	2.7	72.4
	Sarcophagidae		<i>Sarcophaga</i> sp. 1 (n=1)	32.0	1.6	95.4
Hemiptera	Lygaeidae		<i>Spilostethus</i> sp. 1 (n=1)	26.0	0.7	97.5

4.4. Discussion

All floral visitors observed in this study belonged to three insect orders, namely Hymenoptera, Diptera and Hemiptera (Figure 4.5). The most frequent insect visitors to *P. angustifolia* flowers were *A. mellifera* (Hymenoptera). *Eristalinus* (Diptera) individuals carried the largest pollen loads, a high proportion of which was *P. angustifolia* pollen. As the species observed (especially *A. mellifera*) are typical generalist pollinators of many plant species in South Africa, this plant species is thus highly likely not to be pollen limited outside of its native range. Nevertheless, visitation does not always equate to pollen deposition and successful reproduction, and a more in depth study of the contribution of these insect visitors to seed set needs to be conducted. The pollinator exclusion experiment did reveal that these plants are capable of setting fruit and producing seeds without pollination – but further experimentation is needed with larger sample sizes to determine if they produce fruit autonomously or by selfing, as the bags may have facilitated movement of self-pollen. However, the results of this study do strongly suggest that *P. angustifolia* is self-compatible.

Order Hymenoptera is the only order known to include loyal pollen and nectar feeders as it consists of wasps and bees (Picker, 2012). The high visitation frequencies observed is probably because *A. mellifera* bees show constancy (i.e. are loyal pollen feeders). Orders such as Hemiptera do not contain many loyal pollen feeders; hence, they were not expected to visit the flowers frequently (except for preying on insects that frequently visited the flowers). Order Hemiptera is comprised of bugs that feeds on a range of animal and plant foods. In family Lygaeidae, they are mostly herbivores, feeding mainly on seed and only a few of them feed on insects. Genus *Spilostethus* is common in grasslands, feeding on flower heads of yellow daisies and their developing seeds (Picker, 2012). It is clear that members from this genus are usually not legitimate floral visitors judging from their feeding behaviour and because they were not recorded during all observational periods of floral visitors to *P. angustifolia* (Figure 4.5).

A flowering branch was visited by an average of 1.28 individual insects per minute. This means that a mature plant with 50 flowering branches would receive visits from 64 individuals per minute, equating to an extrapolated 3 840 insect visitors per hour on an ideal day when environmental factors (i.e. weather) are suitable for floral visitor activity. These

results show that *P. angustifolia* may be attracting a large proportion of the generalist pollen vectors in the study area and might be competing with other flowering plants for potential pollinators. Despite the high numbers of visitors, results indicated that *P. angustifolia* plants are capable of setting fruit without pollen vectors (i.e. by autogamy and/or self-pollination). Due to the large numbers of flowers on each shrub, insect visitors spend a lot of time on a single shrub and visit flowers of the same shrub consecutively, more likely picking and depositing self-pollen during this time. Since *P. angustifolia* is most likely self-compatible, selfing from these pollinators is still resulting in high fruit and seed set.

A high proportion of open-pollinated flowers produced fruits (89%) compared to those excluded from pollinators (67%). This implies that floral visitors also enhanced pollination since the flowers were left open for floral visitors. The 100% seed production (all fruits produced five seeds per fruit from both treatments) showed that reproduction was successful with and without floral visitors. Seeds appeared healthy but viability tests could affirm this in future experiments. This is an advantageous evolutionary adaptation, as *P. angustifolia* would reproduce successfully without insects if it happens that floral visitors such as *A. mellifera* are not available in an invaded area.

Overall, the most frequently caught and diverse group of insects were Dipterans, carrying sometimes large loads of almost pure *P. angustifolia* pollen. The high overall percentages of purity in all insects' pollen loads showed that either *P. angustifolia* flowers produced larger amounts of pollen or flowers than other plant species that were flowering at that time or floral visitors preferred *P. angustifolia* flowers over other species. Promoting pure pollen loads on insect visitors would be advantageous for *P. angustifolia* as more pollen of its own kind would be transferred to flowers for pollination, with less stigma clogging from other plant species' pollen, thus increasing the chances of pure pollen reaching as many different flower stigmas of that species as possible.

Floral visitors with high visitation rates also carried very pure pollen loads. *Apis mellifera* had both the highest floral visitation frequency of 8.9 flowers.min⁻¹ and highest pollen purity at 97.4%, followed by *Lucilia* (3.1 flowers.min⁻¹; 97.4%). These results suggest that *A. mellifera* is the most important pollinator of *P. angustifolia* as it visits many flowers per minute (Figure 4.5), carries a relatively large amount of pollen and the pollen purity is high

(Table 4.1). For the same reasons, *Lucilia* qualifies as the second most important pollinator and lastly both *Calliphora* and *Bellardia* although the latter two genera had the lowest visitation frequencies. Although *Chrysomya* sp. carried the purest (99.1%) pollen load, the species was not recorded in floral observation periods and therefore the study observations should be extended in order to cover as many visitors as possible. Compared to species having highly specialised mating systems whereby only specific insects can ensure successful pollination, *P. angustifolia* seemed to be a rather non-specialised plant that uses generalist pollinators to enhance its reproductive capacity. A typical example of a specialised flowering plant would be non-invasive *Verticordia nitens*, which produces flowers that are not attractive to other pollinators except for the solitary bee *Euryglossa aureophila* (Houston et al., 1993). Being a specialised alien plant is not one of the characteristics that would allow an alien plant to flourish in an invaded ecosystem (Sutherland, 2004). The ability to find pollinators in a foreign geographical area indicates that pollination is not one of the factors that could possibly hinder the spread of *P. angustifolia*. In addition, this plant is capable of producing viable seeds without the aid of an insect pollinator.

It cannot be ignored that some of the floral visitors observed visiting *P. angustifolia* are important crop pollinators at a global scale (Brittain et al., 2013; Melin et al., 2014).. the presence of *P. angustifolia* in the area might facilitate pollination by attracting pollinators to the area. *Apis mellifera* has been reported to be effective in pollination of important crops such as avocados *Persea americana* Mill. (Ish-Am and Lahav, 2011), apples *Malus domestica* (Carvalho et al., 2011), mangos *Mangifera indica* (Carvalho et al., 2010), rooibos tea seed *Aspalathus linearis* (Gess and Gess, 1994) and sunflowers *Helianthus annuus* (Carvalho et al., 2011). By virtue of *P. angustifolia* sharing such important pollinators with crops of economic importance, there are two possibilities regarding the effects of *P. angustifolia* invasions on ecosystem services. The Free State Province accounts for about 14.5% of commercial farming in South Africa (Free State Development Corporation, 2020). The first possibility would be that *P. angustifolia* is stealing crop pollinators, enhancing its reproductive output while reducing floral visitation and seed set of crops. The second possibility would be that *P. angustifolia* attracts pollinators at a time when crops do not need them and thus play a role in sustaining pollinator populations during a time when the crops do not. A scenario like this occurred in Germany, where wild

bees were found to increase crop yield by 150% compared to commercial bees in cherry production (Holzschuh et al., 2012). Farmers were then advised to conserve semi-natural habitats in their landscapes to increase pollination success of cherry production and provide resources to wild pollinator populations (Holzschuh et al., 2012). Similar advice on Macadamia (*Macadamia integrifolia*) orchards were also made (Grass et al., 2018).

Pyracantha angustifolia invasions along farming landscapes, as threatening as they may be, might be therefore be assisting in sustaining important pollinator populations for crops such as cherries in the eastern Free State (Carvalho et al., 2011). The possible effects of *P. angustifolia* on cherry pollination should not be overlooked, since 90% of cherry crops in South Africa are produced in the eastern Free State (Department of Agriculture Forestry and Fisheries, 2008; Free State Development Corporation, 2020). Nevertheless, these possibilities are yet to be tested, and the potential benefits of having this invasive species as part of the landscape need to be carefully weighed up against the disadvantages, especially since *P. angustifolia* is a transformer, having the capacity to change grassland into bushy habitat through invasion.

4.5. Conclusion

Hymenopterans, specifically *A. mellifera* are the most important *P. angustifolia* pollination agents. All insect visitors carried high purity loads of *Pyracantha angustifolia* pollen. Sharing pollinators with important crops increases the importance of *P. angustifolia* as an IAP that could either be competing with crops for pollinators or sustaining large pollinator populations when crops are not flowering. This IAP produces seed without the aid of pollen vectors but the fruit yield increased when exposed to pollen vectors. This plant uses generalist pollinators to ensure increased fruit yield and thus it is likely to attract pollen vectors in newly invaded ecosystems.

CHAPTER 5

GENERAL SYNTHESIS

5.1. Introduction

The main aim of this study was to determine factors exacerbating the spread of *Pyracantha angustifolia* through studying the species' reproductive ecology. The objectives included: determining the seed biology (Chapter 2); the effect of ingestion by frugivorous birds on seed germination rate and success (Chapter 3); and the role of floral visitors in pollination and reproductive success (Chapter 4). In this chapter, general discussion, conclusions, management implications and recommendations are provided. The aim of this chapter is to provide a summary of the dissertation and bring all the chapters into perspective.

5.2. General discussion

Results from this study indicated that this IAP produced at least one million seeds per square metre in all the invaded sites (Chapter 2) and field observations revealed that birds and livestock are fruit consumers. Rodents are potential seed predators feeding on fruit and/or seed that fall to the ground and thus hinder the spread of the species. Irrespective of high seed predation occurring under the canopy, seeds still manage to form part of the soil seed bank. Soil cores indicated that seed density was higher under the canopy compared to various distances from the source. The longevity of seed in the soil is still not known. Birds were the main seed dispersers, based on field observations (Chapter 2). In addition to field observations, aviary feeding and germination trials were conducted to investigate the effect of ingestion of seeds by frugivorous birds on germination rate and success. Findings from this study showed that frugivorous birds did not have an effect on the rate of germination (Chapter 3). Such results implied that ingestion only assisted in removing the fruit pulp, and defecating the seed into different and more distant sites. Although no effect on germination was observed, smaller birds had lower seed retention and larger birds ate more fruit than smaller birds. Both small and larger bird sizes are efficient dispersers when taking either time or distance into consideration for seed dispersal. It can be postulated that small birds disperse more seed per unit time due to their shorter seed retention time – and by implication, closer to parent plants. In contrast, larger birds disperse seed at low rate due to longer retention times, but probably much further from parent plants. In a long run, both small and larger birds disperse similar amount of seeds since larger birds consume more

seeds than smaller birds. Germination success of both manually depulped and bird ingested seeds was high (above 80% germination) compared to whole fruits (Chapter 3). Feeding and germination trials have shown Red-winged Starlings, Speckled Mousebirds and Dark-capped Bulbuls to be legitimate seed dispersers of *P. angustifolia* seeds.

The involvement of faunal species in a fruiting plant's spread did not only include seed dispersers but also pollen vectors (Chapter 2 and 4). Pollination studies revealed that *A. mellifera* was the most frequent floral visitor (Chapter 4). A high diversity of insects visited flowers and all collected insects carried high amounts of pollen, most of which included a high proportion of *P. angustifolia* pollen. Pollinator exclusion experiments revealed that *P. angustifolia* could produce healthy fruits with seeds without the aid of insects as pollen vectors. Although healthy fruits were produced without insect visitation, fruit yield was higher in the presence of floral visitors.

The rationale for this study was to enhance our understanding of the ecology of *P. angustifolia* for better management. The ultimate aim of management would be sustainable control through, for example, the use of suitable biological control agents. The efficiency, safety and low costs of this control method makes it more preferable compared to its counterparts of chemical and mechanical control (Moran et al., 2013). In addition, the method has a proven record of managing other IAPs in South Africa (Kaplan et al., 2017; Moran et al., 2013) and has been reported to have high return on investment (van Wilgen et al., 2020b). For now, the study has helped us gain knowledge on the species and will help us manage it in time by identifying potential life stages that could be targeted during control. Currently, there seems to be no registered herbicide or biological control agent released to manage *P. angustifolia* invasion and this might be the case in other countries as well across the globe where this plant invades. This is of great concern, herbicide registration for this species would contribute to how it is managed (Lazzaro et al., 2019). However, there is a global trend, including South Africa, of using less herbicide. This is due to their negative impacts on the environment. For example, the use of glyphosate has been banned in European agriculture (Böcker et al., 2019).

This study was one of the few across the globe to study *P. angustifolia* invasion mechanisms. The study is also one of a few in South Africa to study the reproductive

ecology of this particular IAP in depth. Knowledge of such invasion mechanisms help researchers and managers to identify characteristics that allow this IAP to flourish and also factors hindering its spread so that management practices would focus on the most important characteristics (Hui and Richardson, 2017; Richardson, 2011).

5.3. Management implications and recommendations

Control measures should consider a biological agent that will affect plant survival like herbivorous insects (Hoffmann and Moran, 1998) since the shrub is not seed limited. The potential to control this species using fire should be looked into as *Pyracantha* seeds have been reported to be prone to fire (Moreschi et al., 2019). Therefore, integrated control methods should be employed to effectively manage the species. Although seed disperser observations were conducted (Chapter 2), observations should be made for the rest of the fruiting period as different birds might be visiting the plants in different seasons (Mokotjomela et al., 2016). While predation by rodents might be one of the factors limiting the spread of this notorious plant invader (Chapter 2), this plant might be attracting rodents to the invaded sites as it provides food for them. This might increase rodent populations on the invaded sites and might have an impact on crop production as these rodents might become pests (Prakash, 2018). Further studies could confirm the damage to *P. angustifolia* seeds by small rodents by running feeding trials with small rodents (Garcia-Iriarte et al., 2016).

The probability of species re-establishment post-management is due to the suspected lack of persistence of viable seed in the soil (Chapter 2), ensuring that control measures do not have to focus on the soil seed bank. However, seed viability tests should be done to confirm this prediction (Izuddin et al., 2019). In Chapter 3, germination trials ran for 58 days only, which might have been too short for seeds in whole fruits to germinate, thus it is recommended that a longer period of observation should be used in germination trials. Seed could be buried under the soil and harvested monthly to check the rates of seed decay so that seed persistence can also be investigated (Marchante et al., 2010). This would also confirm the suspected lack of seed persistence in the soil from the soil seed bank analysis (Chapter 2). Considering the high seed densities in the soil beneath shrubs and up to 4m away from seeders (Table 2.2), the seed rain beneath other plants and fences (Table 2.3), and the germination success of depulped seeds (Figure 3.8). It is recommend that actions

should be taken to control re-establishment from seeds in the soil after clearing bushes. The high regrowth ability of damaged *P. angustifolia* shrubs should also be taken into account. It is also important not to overrate the importance of birds as seed dispersers. Dispersal efficiency of both birds and other vectors should be studied, taking into consideration vector movements. Large animals' movement might be prohibited by physical barriers such as fences.

This IAP seems to share pollinators with important agricultural crops (Chapter 4). Studies should be conducted to investigate the direct impact of *P. angustifolia* invasion on crop pollinators within the invasion area. This recommended study would clarify if the invasion impacts were either positive or negative. A negative impact would be reducing the crop fruit yield by lowering a crop's floral visitor frequencies; while a positive impact would be through increasing crop yield by attracting and sustaining larger populations of wild pollinators than would be present otherwise (Melin et al., 2014; Moritz et al., 2005). The use of generalist pollinators' boosts *P. angustifolia* invasive potential. This species is able to acquire pollinators in the invaded areas, thus ensuring reproductive success. Therefore, more fruits will be produced. These fruits will be consumed by native frugivores and large amounts of seed will be dispersed. Thus, the use of generalist pollinators and frugivores is one of the traits posing indirect challenges on the management of the species.

There is a lack of emphasis on invasions of mountainous regions by *P. angustifolia* in South Africa compared to other countries. This species is a very dangerous invader and it is potentially spreading at an alarming rate facilitated by birds. There are also other fleshy-fruited invaders in the Maloti-Drakensberg mountains. These include shrub species of *Cotoneaster*, *Rubus* and *Rosa* (all Rosaceae). Further thorough investigations are needed on the ecology and interactions between all of these species in order to formulate viable management options.

5.4. General conclusions

The spread of *P. angustifolia* is enhanced by the production of large numbers of fruits; by having a wide range of dispersal agents; and having fruits remaining for long periods of time on the parent tree (thus increasing the fruit availability for frugivores). Birds have been confirmed to be important seed dispersers, and domestic livestock also feed on the fruits.

However, seed dispersal is hindered by predation of seed on the ground by rodents, and a lack of persistence of seed in the soil. Although this species can produce seed without floral visitors, insect floral visitors play a critical role in fruit yield as the presence of floral visitors increases fruit yield. Pollination success was achieved through the services of generalist pollinators.

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