Plant stress and the prevalence of pests and pathogens associated with a native and an invasive alien legume tree in the Cape Floristic Region, South Africa

by

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Declaration

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Abstract

Invasive alien plant species have devastating effects on the environments that they invade. Australian Acacias, a group of plants that has been planted globally for a range of uses, but has escape plantation areas and became invasive in many countries, are particularly problematic. *Acacia mearnsii* is one of these invasive alien plant species and in South Africa it is also an important forestry species. It is currently the fifth most widespread invasive alien plant in South Africa, only restricted by the very arid Karoo, thus it is important to assess the different habitats that it enters. The Afromontane forest complex in South Africa is highly fragmented and is one of the most threatened Biomes in the country. The widespread forest margin tree *Virgilia divaricata* occurs within these forest margins. It is ecologically similar to *A. mearnsii* as these two species share many characteristics (nodulating legumes, forest pioneer species, fast growing and fire adapted). These species occur sympatrically within invaded forest margins and within these sites, there is a potential for biological exchanges of associated pests and pathogens in the form of arthropods and fungal species. We hypothesize that these two species have different interactions with their pests and pathogens in accordance with the Enemy Release Hypothesis (ERH) and the Biotic Resistance Hypothesis (BRH), respectively. We first compared arthropod associates between these two tree species and found that they share many arthropod species. The native tree did, however, have much higher abundances of herbivores and overall arthropod associates than the invasive tree species, which supports the predictions of the ERH. The distribution of these two species also had an effect on their arthropod assemblages. We assessed their ophiostomatoid fungal associates and herbivore loads and then determined how these pests and pathogens were influenced by environmental conditions along a water gradient. We also compared the effect of plant nutrient content of the two tree species on pest and pathogen loads. *A. mearnsii* was unaffected by water limitation along this gradient, while $\delta^{12}C/\ \delta^{13}C$ analyses showed that *V. divaricata* trees experienced drought within drier sites. *V. divaricata* also had higher herbivore loads in drier sites. *A. mearnsii* had higher herbivore loads on nutrient deficient trees and higher disease development in trees with sufficient nutrient levels. Comparisons of the nutrient economies of the two legume trees showed that they had similar leaf nutrient contents and resorption efficiencies, but they differed in the use of Biological Nitrogen Fixation (BNF). The native tree utilized BNF more than the invasive. We also tested the physiological effects of a native fungal species on the two tree species. We found the infection elicited more response from the invasive, while the native plant was almost non-

responsive. Both plants had significantly longer lesions on infected seedlings than on control plants after inoculation with this pathogen. This difference in response offers a measure of support to the BRH, as the invasive may be more vulnerable to infection. The importance of using related, ecologically similar species in the assessment of the impacts of invasive alien plants is highlighted here. This may provide more information on the actual ecological interaction between native and invasive species within invaded ranges. Forest margins are very vulnerable and dynamic habitats. The influx of a new species into this habitat in the form of an invasive alien plant may therefore have much negative effects. We found support for the exchange of pest and pathogens where these two tree species co-occur. The two host species were very similar in their nutrient economies, creating a potential for competition for similar resources between *A. mearnsii* and *V. divaricata*. The environment had an influence on how these plants responded to pest and pathogens and this may be important under the predicted scenario of future climate change.

Opsomming

Uitheemse indringer plant spesies het vernietigende effekte op die omgewings waarbinne hulle indring. Australiese Acacias, ‘n groep plante wat reg oor die wêreld aangeplant is vir ‘n reeks gebruike, maar wat uit plantasie areas ontsnap het en indringers geword het in baie lande, is besonder problematies. *Acacia mearnsii* is een van hierdie indringer uitheemse plant spesies, en in Suid Afrika is ook ‘n belangrike bosbou spesie. Dit is tans die vyfde mees wydverspreide uitheemse indringer plant in Suid Afrika, en word slegs beperk deur die baie droë Karoo, so dit is belangrik om die verskillende habitatte wat dit binnedring te ondersoek. Woudrandte, the grense van die Afromontane woudkompleks in Suid Afrika, is hoogs gefragmenteerd en is dus een van die mees bedreigde Biome in die land. Die wydverspreide woudrand boom *Virgilia divaricata* kom in hierdie woudrandte voor. Dit is ekologies eenders aan *A. mearnsii*, aangesien hierdie twee spesies baie kenmerke deel (wortelknop-vormende peulplante, woudpionier spesies, vining groeiend, aangepas tot brande). Hierdie spesies kom simpatries voor binne woudrandte wat deur *A. mearnsii* ingedring is, en in hierdie lokaliteite bestaan daar die potensiaal vir biologiese uitruiling van geassosieerde peste en patogene in die vorm van geleedpotiges en fungi spesies. Ons stel die hipotese dat hierdie twee spesies verkillende interaksies met hulle peste en patogene het, in ooreenstemming met die Vyand-Vrystellingshipotese (VVH) en die Biologiese-Weerstandshipotese (BWH), onderskeidelik. Ons het eers die geleedpotige assosieasie tussen hierdie twee boom spesies vergelyk en het bevind dat hulle baie geleedpotige spesies deel. Die inheemse boom het egter baie hoër getalle herbivore en algehele geleedpotige-assosiasies gehad as die indringer boom spesie, wat die voorspellings van die VVH ondersteun. Die verspreiding van hierdie twee spesies het ook ‘n effek gehad op hulle geleedpotige samestellings. Ons het ook hulle geassosieerde ophiostomatiede fungus assosiate en hulle herbivoor ladings bestudeer, en het bepaal hoe hierdie peste en patogene deur omgewingstoestande beinvloed is langs ‘n water gradient. Ons het ook die effek van hierdie peste en patogene op die voedingstof-inhoud van hierdie twee spesies vergelyk. *A. meansii* is nie geaffekteer deur waterbeperkings langs hierdie gradient nie, terwyl $\delta^{12}C$/ $\delta^{13}C$ analises aangedui het dat *V. divaricata* bome droogte stres in droër lokaliteite ervaar het. *V. divaricata* het ook hoër herbivoorladings gehad in die droër lokaliteite. *A. meanrsii* het hoër herbivoorladings gehad op voedingstof-beperkte bome, en daar was verhoogde siekte-ontwikkeling in bome met genoegsame voiding. Vergelykings van die voedingstof-ekonomië van die twee peulplant bome het aangedui dat hulle eenderse blaarvoedingstof-inhoude en resorpsie effektiwiteite het, maar het verskil in die gebruik van

Biologiese Stikstof Fiksasie (BSF). Die inheemse boom het meer van BSF gebruik gemaak as die indringer. Ons het ook die fisiologiese effekte van ‘n inheemse fungus spesie op die twee boomspesies getoets. Ons het bevind dat infeksie ‘n sterker reaksie in die indringer ontlok het, terwyl die inheemse plant feitlik glad nie op infeksie gereageer het nie. Beide plante het beduidend langer wondmerke in geinfekteerde saailinge ontwikkel as in kontrole plante na innokulasie met die patogeen. Hierdie verskil in reaksie verleen ‘n mate van ondersteuning aan die BWH, aangesien die indringer meer vatbaar mag wees teen infeksie. Die belang daarvan om verwante, ekologies soortgelyke spesies te gebruik in die bepaling van die effekte van uitheemse indringer spesies word hier beklemtoon. Dit mag meer inligting verskaf oor die werklike ekologiese interaksie tussen inheemse en indringer spesies binne verspreidings wat binnegedring is. Woudrandte is baie weerlose en dinamiese habitatte. Die invoer van nuwe spesies in hierdie habitat in die vorm van ‘n uitheemse indringer plant mag daarom baie negatiewe effekte hê. Ons het ondersteuning gevind vir die uitruiling van peste en patogene waar hierdie twee spesies saam voorkom. Hierdie spesies was baie eenders in terme van hulle voedingstof-ekonomië, wat die potensiaal skep vir kompetisie tussen *A. mearnsii* en *V. divaricata*. Die omgewing het ‘n effek gehad op hoe hierdie plante gereageer het op peste en patogene, en dit mag belangrik wees onder die huidig voorspelde senarios van toekomstige klimaatsverandering.

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Dedication

I dedicate this thesis to my grandfather, George Goliath, whom always wanted to see me in a graduation gown, receiving my degree. The degree illustrates knowledge gained, as he believed knowledge is power and it is the only thing in this ever changing world that cannot be taken from you once you have it.

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Chapter 1

Plant stress and the prevalence of pests and pathogens associated with a native and an invasive alien legume in the Cape Floristic Region, South Africa

1.General introduction

Invasive alien (IA) species threaten native species by competition and predation as well as by the potential of hybridization and ecosystem changes. These species are known to have negative effects on ecosystem integrity in their invaded ranges (Drake *et al.*, 1989; Mack & D'Antonio, 1998; Pimentel *et al.*, 2000). They also have multiple other impacts as they affect agriculture, forestry and human health (Van Wilgen *et al.*, 2008). Today, IA species are classified as the second largest international threat to biodiversity (Mooney & Hobbs, 2000; Secretariat on the Convention on Biological Diversity, 2001).

Globally there are close to 120 000 species that have invaded the United States, United Kingdom, Australia, South Africa, India and Brazil (Pimentel *et al.*, 2001). The cost to control, manage and attempt to eradicate these species are immense and cost countries millions of US $ per annum (Pimentel *et al.*, 2001). Most IA species were initially introduced in new areas to provide a service. For example, often pest control organisms were introduced intentionally to control other pests. The introduction of the cane toad in Australia (Froggatt, 1936; Tyler, 2003) was intended to control the native grey-backed cane beetle (*Dermolepida albohirtum*) and Frenchi beetle (*Lepidiota frenchi*) (Froggatt, 1936). However, their numbers soon exploded, which led to major ecological problems as they not only deplete native biodiversity by their feeding activities, but they also kill animals that feed on them (Tyler, 2003). The lack of proper research of the intended biological control agent, the pests it was intended to control and the environment in which it was released, resulted in disaster, still prevalent today (Froggatt, 1936; Tyler, 2003, Lettoof *et al.*, 2013).

Australia has been invaded by a large number of additional organisms (Dickman, 1996; Eldridge & Myers, 2001; Moseby *et al.*, 2009, Taylor & Kumar, 2013), but it is also a source of many IA plants, specifically legumous *Acacia* species (Le Roux *et al.*, 2011; Miller *et al.*, 2011; Richardson *et al.*, 2011). Australian acacias have been introduced to many countries in the world for the extraction of tannins, for their production of high value, short fiber wood used in the pulp and fuel industries and for aspects such as dune stabilization and fodder (Maslin, 2001; Marchante *et al.*, 2008; Kull & Rangan, 2008; Kull *et al.*, 2011; Griffin *et al.*, 2011; Richardson *et al.*, 2011). However, an unforeseen consequence of the cultivation of these species was their escape from plantations into the natural environment (Miller *et al.*, 2011). Today *ca*. twenty-three species are invasive in various countries (Richardson & Rejmánek, 2011), including the African continent. A recent review on plant invasions in Africa has highlighted the extensive effects of these Australian acacias (Matthews & Brand, 2004). *Acacia mearnsii* De Wild., for example is one of the most notorious invaders, but has great value as an important forestry species (Henderson, 2007; DEA, 2009; Le Maitre *et al.*, 2011; Morris *et al.*, 2011; Tye & Drake, 2012).

A. mearnsii is a legume within the subfamily Mimosoidaeae of the Fabaceae (Orchard & Wilson, 2001; Kyalangalilwa *et al.*, 2013). It is characterized by bi-pinnate adult foliage and has yellow flower heads in an elongated raceme (Searle, 1997). It is an evergreen tree that produces copious numbers of seeds and generates suckers, resulting in monotypic thickets (Nyoka, 2003). In its native ranges it flowers during winter, while within South Africa it flowers from July to October (Nyoka, 2003). It is a nodulating legume and has a range of rhizobial associates driving biological nitrogen fixation (BNF) (Joubert, 2003). This tree is a fast-growing, short-lived, pioneer species in its native ranges and reaches its maximum height after three to five years of growth (6-20 m) (Searle, 1997; Campbell, 2000). As a pioneer species it plays a role in the transformation between forest succession stages, but unlike typical pioneer species it is also present within climax forests (Nyoka, 2003).

A. mearnsii was introduced to South Africa in 1863 to use for a range of functions in the forestry sector (Stinson *et al.*, 2006, Griffin *et al.*, 2011). It was thus wildly planted and at some stage covered an area of 324 000 ha (Sherry, 1971). Today plantations of *A. mearnsii* cover a much reduced area (DWAF, 1997; DEA, 2009). However, it escaped from plantations and became invasive in most of the country (DWAF, 1997, Henderson, 2007), where it is only restricted by the very dry desert areas in the Karoo (Mucina & Rutherford,

2006). Its invasiveness is related to its ability to generate many small seeds that can persist for many years in soil, building a large seed bank over time (Milton, 1980; Holmes, 1989). It also has a short juvenile phase (Rejmanek, 1995). In South Africa it invades most biomes, especially along roadsides, riparian zones and along forest and plantation margins (Musil, 1993; DWAF, 1997).

Invasion into forests by *A. mearnsii* is limited, since this species is shade intolerant (Sherry, 1971; Searle, 1997; Geldenhuys, 1986; Geldenhuys, 2004). Shade intolerant invasive tree species like this are more likely to invade in forest margins (Geldenhuys, 2004). This is important, as forests in South Africa are highly fragmented presenting many potential areas to occupy (Mucina & Rutherford, 2006). The largest forest complex in South Africa is the afrotemperate Knysna forest complex that is located in the southern Cape (including parts of the Western Cape & Eastern Cape Provinces) (Geldenhuys, 1994). It is included in the Cape Floristic Region (CFR), a global biodiversity hot spot (Goldblatt & Manning, 2002; Linder, 2003). The CFR also includes biomes such as Fynbos and Thicket (Geldenhuys, 1997; Turpie *et al.* 2003; Mucina & Rutherford, 2006).

The forest biome is the smallest in South Africa and covers about 0.5 million hectares, which equates to 0.5% of the total land cover (Mucina & Rutherford, 2006). These forests are also considered one of the most threatened biomes in the country as it persists as fragments (Van der Merwe *et al.*, 2011). Natural forests can be described as having multi-layered vegetation that is dominated by large evergreen and/or semi-deciduous trees with overlapping crowns (Geldenhuys, 2004). These forest fragments are separated with areas covered by Fynbos vegetation. Fynbos is characterized by sclerophyllous evergreen shrubs (Goldblatt & Manning, 2000) that are adapted to frequent fires (10- 12 years). This is in contrast to forests, which are fire resistant (Shackleton *et al.*, 1999; Geldenhuys, 1994). Forest margins consist of a mixture of forest and fynbos species and are fire prone (Manders *et al.*, 1992; Shackleton *et al.*, 1999; Geldenhuys, 1994; 2004).

The leguminous tree *Virgilia divaricata* Adamson occurs within many forest margins (Phillips, 1926). It is part of the subfamily Papilionoideae in the Fabaceae and is endemic to the southern regions of the CFR (Van der Bank *et al.*, 1996) from George in the Western Cape Province to Port Elisabeth in the Eastern Cape Province (Mbambezeli & Notten, 2003). It is a small to medium tree reaching a height of 10 m when fully grown. This species is short lived, with an average lifespan of 12 to 20 years. The tree has pinnately compound leaves

with pea-shaped flowers in dense terminal sprays. Flowers are pinkish mauve to violet-pink and are formed from August to November. Like *A. mearnsii* it is a forest pioneer species, providing a nursing ground for later succession trees (Phillips, 1926). It is fast-growing and can establish without shade (Phillips, 1926; Geldenhuys, 1994, Mbambezeli & Notten, 2003). It is fire adapted as its seeds need fire for germination and they can remain dormant for 230 years (Geldenhuys, 1994). Therefore this tree species share many ecological characteristics with *A. mearnsii* with which it often share this CFR forest margin habitat.

Within these forest margins and particularly in plantations, *A. mearnsii* encounters many pests and pathogens (Roux & Wingfield, 1997; Govender, 2007). The effects of diseases and pests in natural forests vs. plantations are vastly different (Wingfield, 2003; Drenth, 2004; Wingfield *et al.*, 2011). In natural forests, trees are keystone or foundational species (Henry & Stevens, 2009; Loo, 2009). When pests and pathogens attack a specific tree species and functionally remove it from the ecosystem, it can result in a cascade effect in other organisms dependent on that specific tree (Loo, 2009). An example of the impact of a pest and how it shapes and changes forest structure and function is the southern pine beetle (*Dendroctonus frontalis* Zimmermann – Coleoptera, Curculionidae, Scolytinae). In the south-eastern coniferous forests of the United States of America a range of pine species (*Pinus palustris* Mill. (longleaf pine), *Pinus echinata* Mill. (shortleaf pine), *Pinus taeda* L. (loblolly pine) and *Pinus elliottii* Engelm. (slash pine) are hosts to this beetle (Schowalter *et al.*, 1981). When the beetle occurs in high abundances longleaf and slash pine thrive, while in low abundances shortleaf and loblolly pines have a competitive advantage (Walker, 1992).

Natural forests may have some individuals that have the capacity to defend against disease and pest attack based on their genetic composition (Drenth, 2004). Plantation forests have a more uniform genetic base in comparison to the surrounding natural vegetation (Drenth, 2004). This makes these plantations more vulnerable to epidemic development (Drenth, 2004, Wingfield *et al*, 2011). Non-native plantations have been somewhat more successful, as they grow in the absence of their natural enemies (Bright, 1998; Wingfield, *et al*., 2000, 2001; Wolfe, 2002; Siemann & Rogers 2003; Wingfield, 2003). This is especially the case in plantations in the tropics and the Southern Hemisphere where acacias, eucalyptus and pines are widely planted (DEA, 2009). Pests and pathogens from adjacent native vegetation (Coetzee *et al.*, 2000, Crous, 2002; Coetzee *et al.*, 2005; Sinclair & Lyon, 2005) and from

accidental introductions (Barnes *et al.*, 2004; Gibson, 1972; Hunter *et al.*, 2008, 2009) gradually build up in numbers within these plantations.

The ophiostomatoid fungi are an important group of pathogens and disease-causing organisms in plantations and natural forests. This group of fungi is phylogenetically unrelated, but they are grouped together based on convergent evolution to arthropod dispersal (Wingfield *et al.*, 1993). They are ascomycete fungi that share morphological characteristics of dark, globose ascomata with elongated necks giving rise to sticky spores at their apices (Upadhyay, 1981; Wingfield *et al.*, 1993; Wingfield & Van Wyk, 1993), which assist in arthropod dispersal. The ophiostomatoid fungi are known for their associations with various arthropod species. These may include bark beetles, ambrosia beetles, nitidulid beetles and mites (Six & Wingfield, 2011; Kirisits *et al.*, 2009). Ophiostomatoid fungi have been studied internationally as a result of their associations with commercially valuable hosts as well as their devastating effect on native populations (Hawksworth, 2001). Some genera included in the group are *Ceratocystis* Ellis & Halst. (Wingfield *et al.*, 1993), *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Grosmannia* Goid., *Ophiostoma* Syd. & P.Syd. (Zipfel *et al.*, 2006) and *Knoxdaviesia* M.J. Wingf., P.S. van Wyk & Marasas (Réblova *et al.*, 2011). Species found within these genera are diverse in their functional traits and range from pathogens (Matusick & Eckhardt, 2010) to saprobes (fungi that colonize dead wood or dead organic material) (Wingfield *et al.*, 1988, Lee *et al.*, 2004). Saprophytes may cause blue–staining of timber (Seifert 1993; Uzunovic & Webber 1998; Harrington, 2005), while pathogens can cause cankers, wilting, vascular staining and rot diseases (Bretz, 1952; Sinclair *et al.* 1987; Kile, 1993; Wingfield *et al.*, 1993, Barnes *et al.*, 2005; Roux *et al.*, 2005; Brasier, 2008). Different species within the same genus may be a pathogen to some host plant species and a saprobe on others (Roux *et al.*, 2007). Most of the pathogenic species have been identified as vascular pathogens that cause vascular stains and tree wilting (Pegg, 1985).

A. mearnsii have been associated with a range of these fungal species within its plantation distribution in southern Africa as well as within its native ranges (Wingfield & Kemp, 1993, Roux & Wingfield, 1997). Previously many disease symptoms were recorded in *A. mearnsii* with no proper aetiological characterization (Roux & Wingfield, 1997). However, there has been an increase in studies to identify the causative agents of disease. Two of the main diseases associated with *A. mearnsii* are black butt disease caused by *Phytophthora nicotianiae* var. *parasitic* (Dastur.) Waterhouse (Zeijlemaker, 1971; Zeijlemaker & Margot,

1971) and wattle wilt caused by the native *Ceratocystis albifundus* De Beer, Wingfield & Morris (Morris *et al*., 1993). *C. albifundus* causes tree wilt, die-back, discoloured lesions on the stems and branches, blisters and discoloration of wood (Morris *et al.*, 1993; Wingfield and Kemp, 1993; De Beer, 1994). In other African countries such as Uganda *Ophiostoma quercus* (Georgevitch) Nannfeldt has also been isolated from wounds of *A. mearnsii* in plantations (Kamgan *et al.*, 2008a). In its native ranges there are few studies that have assessed ophiostomatoid fungi associated with this tree. As the result of a recent study in Australia, *Pesotum australi* sp. nov, a new ophiostomatoid fungal species associated with *A. mearnsii*, was discovered (Kamgan *et al.*, 2008a). Thus in its native ranges it also encounters these types of pathogens. Little is known about the ophiostomatoid fungal associates of *A. mearnsii* within its invaded ranges.

Recently, diseased and dying *V. divaricata* trees were observed in their natural ranges (Machingambi *et al.*, 2013). These diseases were associated with a range of fungal pathogens, both native and exotic, as well as their vectoring beetles (Machingambi *et al.*, 2013). It was found that this species is also associated with a range of ophiostomatoid species such as *Ceratocystis tsitsikammensis* Kamgan & Roux that was isolated from the larval tunnels of *Leto venus* Cramer (ghost moth). This fungus was previously isolated from the native tree *Rapaneae melanophloeos* (L) Mez. on which it is a confirmed pathogen (Kamgan *et al.*, 2008). Pathogenicity tests confirmed the pathogenicity of this species to *V. divaricata* (Machingambi *et al.*, 2013). As *V. divaricata* and *A. mearnsii* grow sympatrically and they are fairly closely related, the possibility exists that this native fungus may have also moved onto *A. mearnsii* in its invasive range. If proven to be pathogenic to *A. mearnsii* too, it can have severe consequences when reaching plantations of this species.

Apart from fungal associates, *A. mearnsii* and *V. divaricata* are also associated with folivores that cause damage to the photosynthetic machinery of the trees (Kozlov *et al.*, 2009). Within plantations *A. mearnsii* is associated with a range of arthropods e.g. fire blight beetles and boring beetles that cause defoliation and wounding (Govender, 2008). In its invaded ranges few studies have investigated its associated pests (Proches *et al.*, 2008). The application of biological control agents against this species has received much more focus, but is a very controversial issue (invasiveness vs. plantation uses) (Impson *et al.*, 2009). Pest associated with *V. divaricata* is currently unknown.

The response of trees to pests and pathogens in the natural environment is dependent on the effects of the pathogen/pests itself, the tree species identity and environmental conditions (Agios, 2005; McMahon, 2007; Huber *et al.*, 2012). This concept is the basis of the disease triangle model as proposed by Huber *et al.* (2012). How plants utilize their environment, and whether nutrients are limited or available in excess determine how effective they can defend against pests and diseases (Tiaz & Zeiger, 2006; Huber *et al.*, 2012). In the CFR, plants are exposed to a heterogeneous environment in terms of soil fertility, water availability and temperature gradients (Goldblatt & Manning, 2000; Mucina & Rutherford, 2006). *A. mearnsii* originates from Australia from a much drier and more nutrient poor environment and is therefore pre-adapted to the conditions in the CFR (Sherry, 1971; Searle, 1997; Orchard & Wilson, 2001). Both tree species are legumes that may provide them with some advantages and/or disadvantages in this nutrient poor environment (Power *et al.*, 2010). Nitrogen fixing plants are less dependent on nitrogen capture from the soil. When soil nitrogen is limited they can capture nitrogen via Biological Nitrogen Fixation (BNF) (Hardy & Burns, 1968). While both tree species can make use of BNF (Orchard & Wilson, 2001; Van der Bank *et al.*, 1996), this process is phosphorous limited (Qiao *et al.*, 2007), a nutrient that is very limited within CFR soils (Lambers *et al.*, 2007) and the process is energetically costly.

Nutrition has been shown to affect the ability of a plant to defend against pests and pathogens as it influences plant vigor (Agios, 2005). Nutrient stress may cause a reduction in plant vigor and some individuals may be susceptible to disease and/or herbivore attack (Entry 1986; Huber & Hanekleus, 2007; McMahon, 2012). Resistance to infection and herbivore attack is determined by the genetic composition of the plant, but the generic ability of a plant can only be expressed in the presence of adequate resources (Huber & Jones, 2013). Resistance to disease is thus spread along a continuous scale.

In plants with resistance to disease and/or herbivory, plants produce defense molecules when their defense system is activated (Agios, 2005; Pamela *et al.*, 2008). Nutrient limitations can reduce the quantity and quality of these defense compounds (Spectrum, 2013). There is also evidence that high nutrient levels, specifically N, beyond what is needed may also cause a reduction in defense molecules (Spectrum, 2013). Excess N causes an increase in the free amino acids in the plant tissue, making it available to folivores (McMahon, 2012; Spectrum,

2013). The high N level causes morphological and physiological changes in the plant that can benefit herbivore activities (Agios, 2005; Spectrum, 2013).

How a plant utilizes what is available in the environment, eventually determines its own nutritional content (Tiaz & Zeiger, 2006). Nutrient cycling as a technique can be used to follow the flow of nutrients from the soil to the plant and back into the soil (Reed *et al.* 2012). This process is very complex and is influenced by many factors (Tiaz & Zeiger, 2006; Huber & Hanekleus, 2007). Many techniques have been developed to study the movement of nutrients in the environment and two will be highlighted here, namely the use of N and C isotopes (Farquhar & Richards, 1984; Richards, 1996) and nutrient stoichiometry (Reed *et al.*, 2012). δ^{15}N isotope is used to determine the dependence a legume on BNF and is widely used in natural systems and within agriculture (Isaac *et al.*, 2012), while δ^{13}C is used as in indicator of drought stress in plants (Condon *et al.*, 1987). Nutrient stoichiometry utilizes measures of plant resorption efficiency, which provides information on how nutrients are retained by the plant before leaf abscission (Reed *et al.*, 2012). This is important as these nutrients are immediately available to the plant and may be important in habitats with limited resources (Clark, 1977; Turner, 1977; Vitousek, 1982; Aerts & Chapin, 2000; Franklin & Agren, 2002).

Knowing a plant's ability to capture nutrients in a changing world is important if one considers the interactions of plants with their pests and pathogens. Ophiostomatoid fungi are prevalent in plantations as well as in natural forest systems. How these organism affect plant physiology is of interest to both foresters and invasive species researchers (Pegg, 1985). This is especially important as this group of fungi infects both *A. mearnsii* and *V. divaricata* (Kamgan *et al.*, 2008; Machingambi *et al.*, 2012). Plant pathogens have been shown to cause changes in plant physiology from a decrease photosynthetic ability of the plant to driving changes in resource allocation (Pegg, 1985; Omari *et al.*, 2001; Agios, 2005). Typical vascular infection results in drought symptoms as these species cause blockage of the xylem vesicles and hinders water transportation (Roux & Wingfield, 1997; Agios, 2005). Ophiostomatoid genera known to be involved in vascular infection include *Ceratocystis* and *Ophiostoma* (Agios, 2005).

1.1 Problem statement and Research question

With the increase in globalization (Wingfield *et al*., 2000) and the onset of a changing climate (IPPC, 1996; 2001; Ayres & Lombardero, 2000), it is important to know how current pest and pathogen associates of native and invasive alien plants interact with their host plants. Understanding how these interactions change over space and along nutrient and water gradients in the natural environment is important, as these conditions may change in the near future (IPPC, 1996; 2001; Ayres & Lombardero, 2000; Wingfield *et al.*, 2000). It is important to know how *A. mearnsii* and *V. divaricata* compare when considering their nutritional economies and response to disease and arthropod attack, as they are ecologically very similar and occupy the same niche in their respective native and invasive populations. This information is particularly important in forest margins, as these are the frontiers of forest expansion. For example, if *A. mearnsii* has a competitive advantage over *V. divaricata* in a changing environment, it may hinder future forest development and recovery after fire.

We hypothesize that the origin of these two plant species (invasive or native) will determine their number of interactions with pests and pathogens, following the Enemy Release Hypothesis (ERH), that states that the success of an invasive species is partially related to its release from its native enemies (Wolfe 2002; Siemann & Rogers 2003). Contrary to this, as the two plants are fairly closely related, there may be some support for the Biotic Resistance Hypothesis (BRH) that states that when a native and invasive plant are closely related, herbivory/pathogen attack will be higher on the invasive, as a long preceding co-evolutionary processes is absent, making the invasive a suitable, defenseless host (Futuyma *et al*., 1995; Maron & Vilà, 2001; Agrawal & Kotanen, 2003; Frenzel & Brandl, 2003).

1.2 Main aims and objectives of this study

1. Compare foliar associated arthropods between *A. mearnsii* and *V. divaricata*
2. Identify ophiostomatoid fungi associated with artificial wounds on bark and compare these fungi between the two host tree species
3. Assess how environmental factors and plant nutrient content influence disease development and herbivore attack
4. Assess and compare nutrient cycling and BNF in the two tree species
5. Assess the physiological effects of a fungal infection on *A. mearnsii* and *V. divaricata* under controlled conditions

1.3 Outline of thesis chapters

CHAPTER 1 gives a general introduction to invasive alien plants, the two study species and their interaction with the environment and their pest and pathogens.

CHAPTER 2 investigates the overlap in arthropod associates between *Acacia mearnsii* and *Virgilia divaricata* within forest margins where these species occur sympatrically. Foliage associated arthropods are sampled and identified to feeding guild and taxonomic order level. Analyses are done to determine differences in arthropod richness, abundances and assemblage composition between the two tree species. Beta diversity is assessed making use of the analysis PERMDISP.

CHAPTER 3 sets out to assess the influence environmental factors (e.g. soil water availability and plant nutrient content) have on disease development as measured by lesion length and pest loads in the form of herbivore abundances. Environmental parameters are first compared between the two tree species. Then Generalized Linear Models are used to assess the relationships between response and predictor variables within each tree species separately.

CHAPTER 4 assesses the nutrient economies of these two tree species within forest margins. Then plant nutrient content, resorption efficiency and biological nitrogen fixation by assessing $\delta\ ^{15}N/\ \delta\ ^{14}N$ isotope ratio are compared between the two tree species. We assess soil nutrition and compare the resorption of these two tree species with the nutrients available in the soil.

CHAPTER 5 assesses how these two tree species respond to infection by a known ophiostomatoid vascular infecting fungus of *V. divaricata*. We measure as range of physiological responses in a controlled environment within seedlings of these two tree species.

CHAPTER 6 the thesis is concluded with the synthesis of all knowledge gained during this assessment of these two legumous trees within forest margins in the CFR of South Africa and their interactions with their pests and pathogens and the environment.

2.References

Aerts, R. & Chapin, F.S. III. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research 30: 1–67.

Agios, G.N. 2005. Plant Pathology. UK: Elsevier Academic Press Publication.

Agrawal, A., & Kotanen, P.2003. Herbivores and the success of exotic plants: a phylogenetically controlled experiment. Ecology Letters 6: 712–715.

Ayres, M.P. & Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance form herbivore and pathogens. The Science of the Total Environment 262: 263 – 286.

Barnes , I. , Crous , P.W. , Wingfield , B.D. & Wingfield , M.J. 2004. Multigene phylogene reveal that red band needle blight of *Pinus* is caused by two distinct species of Dothistroma , D. septosporum and D. pini . Studies in Mycology 50: 551 – 565.

Barnes, I., Nakabonge, G., Roux, J., Wingfield, B.D. & Wingfield, M.J. 2005. Blackwell Publishing, Ltd.

Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathology 57: 792-808.

Bretz, T.W. 1952. The ascigerous stage of the oak wilt fungus. Phytopathology 42: 435-437.

Bright , C. 1998. Life Out of Bounds. Bio-invasion in a Borderless World . WW Norten , New York.

Campbell, P., 2000. Wattle control. Plant Protection Research Institute, handbook no. 3, Pretoria.

Coetzee, M.P.A., Wingfield , B.D., Bloomer, P. & Wingfield, M.J. 2005. Phylogenetic analyses of DNA sequences reveal species partitions amongst isolates of *Armillaria* from Africa. Mycological Research 109: 1223 – 1234.

Coetzee , M.P.A. , Wingfield , B.D. , Coutinho , T.A. & Wingfield , M.J. 2000. Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa . Mycologia, 92: 777 – 785.

Condon, A.G., Richards, R.A. & Farquhar, G.D. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. Crop Science 27: 996–1001.

Crous , P.W. & Groenewald , J.Z. 2005. Hosts, species and genotypes: opinions versus data. Australasian Plant Pathology 34: 463 – 470.

Lettoof, D.C., Matthew J. Greenlees, Michelle Stockwell, Richard Shine, Do invasive cane toads affect the parasite burdens of native Australian frogs?, International Journal for Parasitology: Parasites and Wildlife, Volume 2, December 2013: 155–164

Darwin, E. 1800. Phytologia. Published., London.

De Beer, C., 1994. *Ceratocystis fimbriata* with special reference to its occurrence as a pathogen on Acacia mearnsii in South Africa. MSc. Thesis, University of the Orange Free State. Bloemfontein. South Africa

Department of Agriculture, Forestry and Fisheries. 2009. State of the forest report.

Department of Water Affairs and Forestry. 1997. The Working for Water Programme Annual Report 1996/97. Department of Water Affairs and Forestry, Pretoria.

Del Lungo, A., Ball, J. & Carle, J. 2006. Global planted forests thematic study: results and analysis. Planted Forests and Trees Working paper 38. FAO, Rome

Drake, J.A., Mooney, H.A., Di Castri, F., Groves, R.H., Kruger, F.J., Rejma´ nek, M. & Williamson, M., 1989. Biological Invasions: A Global Perspective. Wiley, Chichester.

Drenth , A. 2004. Fungal epidemics – does spatial structure matter? New Phytologist, 163: 4 – 7.

Dickman, C.R., 1996. Impact of exotic generalist predators on the native fauna of Australia. Wildlife Biology. 2: 185–195.

Eldridge, D.J., Myers, C.A., 2001. The impact of warrens of the European rabbit (Oryctolagus cuniculus L.) on soil and ecological processes in a semi-arid Australian woodland. Journal of Arid Environments. 47: 325–337.

Entry, J.A., Martin, N.E., Cromack Jr. & K., Stafford, S.G. 1986. Light and nutrient limitation in *Pinus monticola*: Seedling susceptibility to *Armillaria* infection. Forest Ecology and Management 17: 189–198.

Farquhar, G.D. & Richards, A.R. 1984. Isotope composition of plant carbon correlates with water use efficiency of wheat genotypes. Australian Plant Physiology 11: 539–552.

Franklin, O. & Agren, G.I. 2002. Leaf senescence and resorption as mechanisms of maximizing photosynthetic production during canopy development at N limitation. Functional Ecology 16: 727–733.

Frenzel, M. & Brandl, R. 2003. Diversity and abundance patterns of phytophagous insect communities on alien and native host plants in the Brassicaceae. Ecography 26: 723–730.

Froggatt, W.W .1936 .The introduction of the great Mexican toad Bufo marinus into Australia. The Australian Naturalist 9:163–164.

Futuyma D. J., Keese M. C. & Funk D. J. 1995. Genetic constrains on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. Evolution 49: 797–809.

Geldenhuys, C.J. 1986. Costs and benefits of the Australian blackwood Acacia melanoxylon in South African forestry. In I.A.W. Macdonald, F.J. Kruger, & A.A. Ferrar, eds. The ecology and management of biological invasions in southern Africa, 275–284. Cape Town, South Africa, Oxford University Press.

Geldenhuys, C.J., 1994, Bergwind fires and location pattern of forest patches in the Southern Cape landscape, South Africa, Journal of Biogeography 21: 49–62.

Geldenhuys, C.J., 2004. Concepts and Process to Control Invader Plants in and around Natural Evergreen Forest in South Africa. Weed Technology 18: 1386-1391.

Gibson , I.A.S. 1972. Dothistroma blight of *Pinus radiata*. Annual Review of Phytopathology 10: 51 – 72.

Goldblatt, P. & Manning, C, 1943, 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria: National Botanical Institute, Pretoria

Govender, P. 2007. Status of seedling establishment pest of Acacia mearnsii De Wild. (Mimosaceae) in South Africa. South African Journal of Science 103: 141–147.

Hardy, W.F. & Burns, R.C. 1968. Biological Nitrogen Fixation. Annual Review of Biochemistry 37: 331–358.

Harrington TC, 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega FE & Blackwell M (eds.) Ecological and Evolutionary Advances in Insect-Fungal Associations, Oxford University Press, 257–291.

Hawksworth, D.L. 2001. The magnitude of fugal diversity: the 1.5 million species estimate revisited. Mycology Research.105(12): 1422–1432.

Henderson, L. 2007. Invasive, naturalized and casual alien plats in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37(2): 215–248.

Henry, M. & Stevens, H. 2009. A Primer of Ecology with R. Monograph. Springer: New York

Holmes, P. M. 1989. Decay rates in buried alien *Acacia* seed populations of different density. – South African Journal of Botany 55: 299–303.

Huber. D.M. & Hanekleus, S. 2007. Managing nutrition to control plant disease. 2007. Landbauforschung Volkenrode, 57: 313–322.

Huber, D.M. & Jones, J.B. 2013. The role of magnesium in plant disease. Plant and Soil, 368: 73-85.

Huber, D.M, Romheld, V. & Weinmann, M. 2012. Relationship between nutrition, plant diseases and pests. In Nutritional Physiology. Marschner's Mineral Nutrition of Higher Plants.

Hunter, G.C. , van der Merwe , N.A. , Burgess , T.I. , *et al*.. 2008. Global movement and population biology of *Mycosphaerella nubilosa* infecting leaves of cold – tolerant *Eucalyptus globulus* and *E. nitens*. Plant Pathology, 57: 235–242.

Hunter, G.C., Crous, P.W., Carnegie, A.J. & Wingfield, M.J. 2009. *Teratosphaeria nubilosa* a serious leaf disease pathogen of *Eucalyptus* spp. in native and introduced areas. Molecular Plant Pathology 10: 1–14.

Impson, F., Hoffmann, J. & Kleinjan, C. 2009. In Biological control of tropical weeds using arthropods. Ed. Muiappan, R., Reddy., D.V.P. & Raman, A. Cambridge University Press. :38 – 62.

IPCC, Climate Change. 1996. Climate change 1995—the science of climate change. *Edited by* J.T. Houghton, L.G. Meira Filho, B.A. Callander, N. Harris, A. Kattenberg, and K. Maskell. Contribution of WG1 to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, U.K.

IPCC, Climate Change 2001, The scientific basis, Contribution of Working Group I to the third assessment report of the IPCC, Houghton J.T., Ding Y., Griggs D.J., Noguer M., Van derLinder P.J., Dai X., Maskell K., Johnson C.A. (Eds.), Cambridge University Press, Cambridge, UK, 2001.

Isaac, M.E., Hinsinger, P. & Harmand, J.M. 2012. Nitrogen and phosphorus economy of a legume tree-cereal intercropping system under controlled conditions. Science of the Total Environment. 434: 71–78.

Joubert, C. 2003. Rhizobia associated with Australian Acacia species (*Acacia mearnsii*, *Acacia dealbata* & *Acacia decurrents*) in South Africa as determined by Sodium Dodecyl-Sulphate Polyacrylamide Gel Electrophoresis. MSc thesis. University of Pretoria.

Kamgan, N.G., Jacobs, K., de Beer, Z.W., Wingfield, M.J. and Roux, J. 2008. *Ceratocystis* and *Ophiostoma* species including three new taxa, associated with wounds on native South African trees. Fungal Diversity 29: 37-59.

Kamgan, N.G., Jacobs, K., de Beer, Z.W., Wingfield, M.J. & Roux, J. 2008a. Pesotum australi sp. nov. and Ophiostoma quercus associated with Acacia mearnsii trees in Australia and Uganda, respectively. Australas Plant Pathology 37: 406–416

Kile GA, 1993. Plant diseases caused by species of Ceratocystis sensu strica and Chalara. In:

Wingfiled MJ, Seifert KA, Webbe JF (eds.), Ceratocystis and Ophiostoma: Taxonomy, ecology and pathogenicity. APS Press, St. Paul, Minnesota, 173–183.

Kirisits T .2004. Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: *Bark and wood boring insects in living trees in Europe, a synthesis*. (Lieutier F, ed.). Kluwer Academic Press, The Netherlands: 1–55.

Kozlov, M. V. Zvereva, E.L. & Zverev, V.E. 2009. Insect herbivory. Impacts of Point Polluters on Terrestrial Biota. 15: 297 – 322.

Kull, C.A. & Rangan, H. 2008. Acacia exchanges: Wattles, thorn trees, and the study of plant movements. Geoforum 39: 1258 –1272.

Kull, C.A., Shackleton, C.M., Cunningham, P.S., Ducatillon, C., Dufour Dror, J.-M., Esler, K.J., Friday, J.B., Gouveia, A.C., Griffin, A.R., Marchante, E.M., Midgley, S.J., Pauchard, A., Rangan, H., Richardson, D.M., Rinaudo, T., Tassin, J., Urgenson, L.S., von Maltitz, G.P., Zenni, R.D. & Zylstra, M.J. 2011. Adoption, use, and perception of Australian acacias around the world. Diversity and Distributions, 17: 822–836.

Kyalangalilwa, B., Boatwright, J.S., Daru, B.H., Maurin, O., van der Bank, M., 2013. Phylogenetic position and revised classification of Acacia s.l. (Fabaceae: *Mimosoideae*) in Africa, including new combinations in Vachellia and Senegalia. Botanical Journal of the Linnean Society 172, 500–523.

Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E. 2007. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23, 95–102.

Le Maitre, D.C., Gaertner, M., Marchante, E., Ens, E.-J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J. & Richardson, D.M. 2011. Impacts of introduced Australian acacias: implications for management and restoration. Diversity and Distributions, 17:1015–1029.

Lee S, Mel'nik V, Taylor JE, Crous PW, 2004. Diversity of saprobic hyphomycetes on Proteaceae and Restionaceae from South Africa. Fungal Diversity 17: 91–114

Le Maitre, D.C., Richardson, D.M., Chapman, R.A., 2004. Biological invasions in South Africa: driving forces and the human dimension. South African Journal of Science 100: 103–112.

Le Roux, J.J., Brown, G.K., Byrne, M., Ndlovu, J., Richardson, D.M., Thompson, G.D., Wilson, J.R.U., 2011. Phylogeographic consequences of different introduction histories of invasive Australian Acacia species and Paraserianthes lophantha (Fabaceae) in South Africa. Diversity and Distributions 17: 861–871.

Linder, H.P. 2003. The radiation of the Cape flora, southern Africa. Biological Reviews 78: 597–638.

Mack, M.C., & D'Antonio, C.M., 1998. Impacts of biologicalinvasions on disturbance regimes. Trends in Ecology and Evolution 13 (5): 195–198.

Machingambi, N.M., Roets, F., Dreyer, L. L. & Roux, J. 2013. An investigation into the death of native Virgilia trees in the Cape Floristic Region of South Africa. Master Thesis. Stellenbosch: University of Stellenbosch.

Manders, P.T., Richardson, D.M. & Masson, P.H., 1992, Is fynbos a stage in succession to forest? Analysis of the perceived ecological distinction between two communities, in B.W. van Wilgen, D.M. Richardson, F.J. Kruger & H.J. van Hensbergen (eds.), Fire in South African Mountain Fynbos: Ecosystem, community and species response at Swartboskloof, Springer Verlag, Berlin. Ecological Studies 93. 81–107.

Marchante, E., Kjøller, A., Struwe, S. & Freitas, H. 2008. Short- and long-term impacts of Acacia longifolia invasion on the below ground processes of a Mediterranean coastal dune ecosystem. Applied Soil Ecology, 40: 210–217.

Maron J. L. & Vilà M. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95: 361–73.

Maslin, B.R. 2001. Introduction to Acacia. Flora of Australia volume 11A. Mimosaceae, Acacia part 1 (ed. by A.E. Orchard and A.J.G. Wilson), 3–13. CSIRO Publishing, Melbourne.

Matthews, S. & Brand, K. 2004. Africa Invaded: The Global Invasive Species Programme.

Matusick, G., Eckhardt, L.G., 2010. The pathogenicity and virulence of four Ophiostomatoid fungi on young Longleaf pine trees. Canadian Journal of Plant Pathology 32: 170–176.

Mbambezeli, G. & Notten, A. 2003. http://www.plantzafrica.com/planttuv/virgilia.htm

Miller, J.T., Murphy, D.J., Brown, G.K., Richardson, D.M. & Gonza´lez-Orozco, C.E. 2011. The evolution and phylogenetic placement of invasive Australian Acacia species. Diversity and Distributions, 17: 848–860.

McMahon, P. 2012. Effect of Nutrition and Soil Function on Pathogens of Tropical Tree Crops, Plant Pathology, Dr. Christian Joseph Cumagun (Ed.), ISBN: 978-953-51-0489-6, InTech, DOI: 10.5772/32490.

Milton, S.J. 1980. Studies on Australian acacias in the south-western Cape, South Africa . MSc thesis, University of Cape Town.

Morris T. L. Esler K.J. Barger N.N. Jacobs S.M & Cramer M D. 2011. Ecophysiological traits associated with the competitive ability of invasive Australian acacias. Diversity and Distributions. 17: 898–910.

Morris, J. Wingfield. M.J. De Beer. C. 1993. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathology 42(8): 14-8 17.

Mooney, H.A. & Hobbs, R.J. 2000. Invasive Species in a Changing World. Island Press, Washington, DC.

Moseby, K.E., Hill, B.M., Read, J.L., 2009. Arid recovery – a comparison of reptile and small mammal populations inside and outside a large rabbit, cat and fox-proof exclosure in arid South Australia. Austral Ecology. 34: 156–169.

Mucina, L. & Rutherford. M. 2006. The vegetation of South Africa, Lesotho and Swaziland.

Musil, C.F., 1993. Effects of Invasive Australian *Acacias* on the Regeneration, Growth and Nutrient Chemistry of South African Lowland Fynbos. Journal of Applied Ecology., 30: 361-372.

Nyoka, B.I., 2003. Biosecurity in Forestry: A case study on the status of invasive forest trees species in Southern Africa. Forest Biosecurity Working Paper FBS/1E. Forestry Department. FAO, Rome.

Orchard, A.E. & Wilson, A.J.G. 2001. Flora of Australia. Vol. 11A, Mimosaceae, *Acacia*. Part 1. (ABRS/CSIRO Publishing: Melbourne)

Pamela, P., Dubois, T., Coyne,D. & Viljoen,A. 2008. Defense-Related Gene Expression in Susceptible and Tolerant Bananas (*Musa* Spp.) Following Inoculation with Non-Pathogenic *Fusarium Oxysporum* Endophytes and Challenge with *Radopholus Similis*. Physiological and Molecular Plant Pathology 71: 149–57.

Pegg, G.F. 1985. Life in a black hole-The micro-environment of the vascular pathogen. Transactions of the British Mycological Society. 85:1-20.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. 2000. Environmental and economic costs associated with non-indigenous species in the United States. Bioscience 50: 53–65.

Power, S.C., Cramer, M.D., Verboom, G.A. & Chimphango, S.B.M. 2010. Does phosphate acquisition constrain legumes in the fynbos of the Cape Floristic Region? Plant Soil 334: 33–46.

Qiao, Y., Tang, C., Han, X., Miao, S., 2007. Phosphorus Deficiency Delays the Onset of Nodule Function in Soybean. Journal of Plant Nutrition 30: 1341–1353.

Reblova, M., Gams, W. & Seifert, K.A. 2011. *Monilochaetes* and allied genera of *Glomerellales*, and a reconsideration of families in the *Microascales*. Studies in Mycology. 68: 163–191.

Reed, S.C., Townsend, A.R., Davidson, E.A. & Cleveland, C.C. 2012. Stoichiometric patterns in foliar nutrient resorption across multiple scales. New Phytologist. 1–8.

Rejmanek, M., 1995. What Makes a Species Invasive? Plant Invasions SPB Academic Publishing, The Hague, Netherlands.

Richards, R.A. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regulation 20: 157–166.

Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Robertson, M.P., Rouget, M., Le Roux, J.J. & Wilson, J.R.U. 2011. Human-mediated introductions of Australian acacias— a global experiment in biogeography. Diversity and Distributions, 17: 771–787.

Richardson, D.M. & Rejmánek, M. 2011. A global review of trees and shrubs as invasive species. Diversity and Distributions, 17: 788–809.

Riley, C. V. 1893. Parasitic and predaceous insects in applied entomology. Insect Life 6: 130-41.

Riley, W. A. 1931. Erasmus Darwin and the biologic control of insects. Science 73: 475–6.

Roux J, Meke G, Kanyi B, Mwangi L, Mbaga A, Hunter GC, Nakabonge G, Heath RN, Wingfield MJ, 2005. Diseases of plantation forestry trees species in Eastern and Southern Africa. South African Journal of Science 101: 409–413.

Roux, J., Heath, R.N., Labuschagne, L., Nkuekam, G.K. & Wingfield,M.J. 2007. Occurrence of the wattle wilt *pathogen, Ceratocystis albifundus* on native South African trees. Forestry Pathology. 37: 292–302.

Roux, J., & Wingfield, M.J. 1997. Survey and virulence of fungi occurring on diseased *A. mearnsii* in South Africa. Forest Ecology and Management 99: 327–336.

Searle, S., 1997. *A. mearnsii* De Wild.(black wattle) in Australia. Black wattle and its utilizations. Barton, ACT: Rural Industries Research and Development Corporation, 1-10.

Secretariat of the Convention on Biological Diversity. 2001. The Value of Forest Ecosystems. Montreal, SCBD Technical Series No. 4.

Schowalter, T,D., Coulson, R.N. & Crossley, Jr. D.A. 1981. Role of southern Pine beetle and fire in maintenance of structure and function of the southern coniferous forest. Environmental Entomology 10: 821–825.

Shackleton, C. M., Cawe, S. G. & Geldenhuys, C. J. 1999. Review of the Definitions and Classifications of South African Indigenous Forests and Woodlands. Report ENV-P-C 99007. Pretoria, South Africa: CSIR, Division of Water, Environment and Forestry Technology. 33

Siemann, E. & Rogers W. E. 2003. Herbivory, disease, recruitment limitation, and success of alien and native tree species. Ecology 84: 1489–505.

Sherry, S.P., 1971. The black wattle (*Acacia mearnsii* de Wild.). University of Natal Press. Pietermatitzburg.

Sinclair WA, Johnson WT, Lyon H, 1987. Diseases of trees and shrubs. Cornell University Press, Ithaca, New York, USA.574.

Sinclair, W.A. & Lyon, H.H. 2005. *Diseases of Trees and Shrubs*, 2nd ed. Cornell University Press , Ithaca.

Six, D.L., Wingfield, M.J. 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm. Annual Review of Entomology 56: 255-272.

Spectrum. 2013. The relationship between nutrients and other elements to plant diseases. Agronomic library. Spectrum Analytic excellence testing. www.spectrumanalytic.com

Stinson, K.A., K. Campbell, J.R. Powell, B.E. Wolfe, 66 R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati and J.N. Klironomos, 2006. Invasive Plant Suppresses the Growth of Native Tree Seedlings by Disrupting Belowground Mutualisms. PLOS Biology, Vol 4.

Streletzia 19, Pretoria: South African National Biodiversity Institute: 77: 93–94: 181–182.

Taylor, S & Kumar, L.2013. Potential distribution of an invasive species under climate change scenarios using CLIMEX and soil drainage: A case study of Lantana camara L. in Queensland, Australia. Journal of Environmental Management114: 414–422

Taiz, L. & Zeiger, E. 2006. Plant Physiology Sunderland, Mass: Sinauer Associates, Sunderland, Mass.

Turner, J. 1977. Effects of nitrogen availability on nitrogen cycling in a Douglas fir stand. Forest Science 23: 307–316.

Turpie JK, Heydenrych BJ, Lamberth SJ, 2003. Economic value of terrestrial and marine biodiversity in the Cape Gloristic Region: implications for defining effective and socially optimal conservation strategies. Biological Conservation 112: 233–251.

Tye, D.R.C., Drake, D.C. 2012. An exotic Australian Acacia fixes more N than a coexisting indigenous Acacia in a South African riparian zone. Plant Ecology 213: 251–257.

Tyler. M. 1975. The cane toad (*Bufo marinus*). CSIRO. 2003

Upadhyay HP .1981.A monograph of the genus *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press: Athens.

Van Der Bank, M., Van Der Bank, F.H., Van Wyk, B.1996. Speciation in Virgilia (Fabaceae): Allopatric divergence followed by introgression? *Plant Systematics and Evolution* 201: 57-73.

Van der Merwe, I. 2011, Green heritage: Trees, forests and woodlands of South Africa, Department of Agriculture, Forestry and Fisheries, Pretoria.

Van Wilgen, B.W., Reyers, B., Le Maitre, D.C., Richardson, D.M. & Schonegevel, L. 2008. A biome-scale assessment of the impact of invasive alien plants on ecosystem services in South Africa. Journal of environmental management 89: 336–349.

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. American Naturalist 119: 553–572.

Walker, L.C. 1962. The coastal plain southern pine region. In: Barret JW (ed.) Regional silviculture of the Unites States. The Ronald Press Co., Bew York: 246-295.

Wingfield , M.J. 2003. Daniel McAlpine memorial lecture. Increasing threat of disease to exotic plantation forests in the southern hemisphere: lessons from *Cryphonectria* canker. Australasian Plant Pathology 32: 133–139.

Wingfield, M.J. & Kemp. G.H.J. 1993. Disease of Pines. Eucalypts and Wattle. In: Van der Sijde. H.A. (Ed.). South African Forestry Handbook. The South African Association of Forestry. Pretoria. South Africa: 231–248.

Wingfield, M.J., Seifert, K.A. & Webber, J.F. eds. 1993. *Ceratocystis* and *Ophiostoma*: *Taxonomy Ecology* and *Pathogenicity*. St. Paul: Am. Phytopathology. Soc. Press

Wingfield, M.J., Slippers , B. , Roux J. & Wingfield , B.D. 2000. World - wide movement of exotic forest fungi, especially in the tropics and Southern Hemisphere. Bioscience 51:134–140.

Wingfield, M.J., Slippers, B., Roux, J. & Wingfield, B.D.2011. Fifty year of tree pest and pathogen invasions, increasingly threatening world forests. In (Ed.Richardson, D.M.) Fifty years of invasion ecology: The legacy of Charles Elton: Blackwell Publishing 89.

Wingfield , M.J. , Roux , J. , Coutinho , T.A. , Govender , P. & Wingfield , B.D. 2001. Plantation disease and pest management in the next century. South African Forestry Journal 190: 67 – 72.

Wingfield, M.J., Wyk, P.S.V., Marasas, W.F.O. 1988. *Ceratocystiopsis proteae sp. nov.*, with a New Anamorph Genus. Mycologia 80: 23–30.

Wolfe L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. American Naturalist 160: 705–11.

Zeijlemaker. F.C.J. 1971. Black butt disease of black wattle caused by *Phytophthora nicotianiae var. parasitic.* Phytopatholagy 61: 144–145.

Zeijlemaker. F.C.J. & Margot P. I971. Black butt disease of black wattle. Wattle Research Institute University of Natal. South Africa. Report for l970–197: 19–50.

Zipfel RD, de Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ. 2006. Multigene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. *Stud. Mycol.* 55:75–97.

Chapter 2

Remarkable overlap of arthropod communities between a native and an invasive alien tree growing sympatrically

Abstract

The negative effects of alien invasive plants on habitats have been well-documented. However, the exchange of organisms between these and native taxa have received far less research attention even though the consequences can be ecologically and economically devastating. Here we assess the potential exchanges of arthropod associates of a native (*Virgilia divaricata*) and an invasive (*Acacia mearnsii*) legume tree within the ecotone between forest and fynbos vegetation within the Cape Floristic Region of South Africa. Arthropods species richness, abundance, species assemblage composition and measures of beta-diversity were assessed between these two legume species where they grow sympatrically within the Garden Route National Park in the Western and Eastern Cape provinces. Except for spiders and ants, arthropod species richness did not differ significantly between these two tree taxa. However, overall the abundance of arthropods was significantly higher on the native tree species. This pattern was strongly driven by herbivores as is consistent with predictions of the Enemy Release Hypothesis, which envisage lower herbivore pressure on invasive plants in their invaded ranges. When excluding rare taxa, over 75% of all arthropod species collected in this study were associated with both host trees, a pattern reflected by most guilds and taxa. However, arthropod community composition differed significantly between the two host plant taxa, largely due to differences between their herbivore communities. PERMDISP analyses indicated that arthropod community changeover (a measure of beta diversity) was high on the native host, with arthropod communities on the invasive host being much more homogenous across the sampling range. This is likely due to the more isolated distribution of populations of the native plant as compared to the invasive species that has a much more uniform distribution across the range. These results indicate that there are numerous exchanges of arthropods between this native and invasive plant. The invasive plant may provide

arthropods with a pathway to other habitats between previously isolated native populations. This will have significant implications for biodiversity conservation at the habitat-, species- and population level.

Key words: Biological invasions, plant insect interaction, feeding guilds, Cape Floristic Region

1.Introduction

Biological invasion has been classified as the second largest global threat to biodiversity (Mooney and Hobbs, 2000; Secretariat on the Convention on Biological Diversity, 2001). Biological invasions may also result in enormous financial expenditure by governments to control, manage and where possible eradicate these species (Pimentel *et al.*, 2001). In South Africa, Australian acacias are among the most notorious invasive alien plants (IAP's) as they can transform the ecosystems that they invade (Drake *et al.*, 1989). This results in a decline in native species diversity (Richardson *et al.*, 1989; Holmes & Cowling, 1997; Marchante *et al.*, 2003), decrease in water availability (Enright, 2000; Dye *et al.*, 2001), altered nutrient cycling (Yelenik *et al.*, 2004) and even changes in fire regimes (Van Wilgen & Richardson, 1985). The success of Australian acacias as invaders are attributed to many factors such as their ability to capture more resources and grow much larger than the native species in their invaded ranges (Milton & Siegfried, 1981). This accumulation of resources allows them to produce large numbers of nutrient rich seeds, which feeds a persistent seed bank (Milton, 1980; Holmes, 1989; Siemann & Rogers 2003; Gioria *et al*., 2012). Another reason for the success of these IAP's in general is the release from their native predators, particularly their associated arthropods (Rejmánek *et al*., 2005), a phenomenon known as the Enemy Release Hypothesis (ERH) (Wolfe 2002; Siemann & Rogers, 2003). Therefore, one of the main aims of biological control of IAP's is to re-unite them with their control agents from their native ranges (Darwin, 1800; Riley, 1893; 1931). However, the success of an IAP species is not only influenced by lack of herbivores (Ghazoul 2002; Traveset & Richardson 2006), but also by the availability of more beneficial organisms such as pollinators (Richardson *et al*., 2000; Traveset & Richardson 2006).

A few studies have found herbivores to be more sensitive to host plant identity than any other arthropod feeding guilds (Strong *et al*., 1984; Keane and Crawley 2002; Proches *et al.*, 2008). Therefore, invasive species usually experience much lower herbivory than related or ecologically equivalent native plants (Southwood *et al*., 1982; Olckers & Hulley, 1991). However, very few studies have compared insect diversity and abundance on IAP's and co-occurring native plants (McEvoy 2002; Tallamy 2004) and more such studies are needed to elucidate the role of insect herbivory in invasion success (Colautti *et al*., 2004; Harris *et al*., 2004). When the ERH is refuted, support may be found for the Biotic Resistance Hypothesis (BRH) (Agrawal & Kotanen,

2003), which is usually applicable when plants are closely related. It states that when a native and an invasive plant are closely related, herbivory can be higher on the invasive than the native plant (Agrawal & Kotanen 2003; Frenzel & Brandl, 2003). The close relatedness makes the invasive plant an appropriate host for the herbivores associated with the native plants and, as native plants may have better defences against these herbivores, the invasive species may be a much easier target due to a lack of long preceding co-evolutionary processes between the invasive plant and new native herbivores (Futuyma *et al*., 1995; Maron & Vilà, 2001).

The Australian *Acacia mearnsii* De Wild., a notorious IAP in South Africa, is distributed over most of the country excluding the arid Karoo (Searle, 1997; Henderson, 2007). In the southern Cape Floristic Region (CFR) *A. mearnsii* occurs in fynbos vegetation, growing between forest fragments and also within forest margins (Henderson, 2007). *A. mearnsii* is a fast growing, legume, forest pioneer tree species, which has the ability to adapt to a range of soils and has a good level of drought tolerance (Searle, 1997). *A. mearnsii* thrives in disturbed areas such as fire disturbed areas within fynbos vegetation and within ecotones (interaction zone between fynbos and adjacent vegetation types (forests). When *A. mearnsii* invades a habitat it forms monocultures and exclude most native species (Boucher, 1978; Henderson, 2007).

Acacia mearnsii often shares its invaded range within forest margin habitats of the CFR with the native *Virgilia divaricata* Adamson (Geldenhuys, 1994; Henderson, 2007), also a fire adapted forest pioneer legume tree (Palgrave 1983, 2002; Palmer & Pitman, 1972). *V. divaricata* is endemic to the southern Cape (Goldblatt & Manning, 2000) and is the most wide spread forest margin species in South Africa (Phillips, 1926, 1928; Geldenhuys, 1994). *Virgilia* is a monophyletic genus within Papilionoideae ,a subfamily within Fabaceae (Adamson, 1934) and includes two species, *Virgilia divaricata* and *Virgilia oroboides* (Berg.) Salter. *V. divaricata* is distributed eastward from George to Port Elizabeth and *V. oroboides* (two subspecies - *Virgilia oroboides* (Berg.) Salter subsp. *oroboides* and *Virgilia oroboides* (Berg.) Salter subsp. *ferruginea* B.E van Wyk) westward from George to the Cape Peninsula (Palgrave, 1983; Palmer & Pitman, 1972). *V. divaricata* rarely grows within fynbos vegetation, but is often found within forest margins, the ecotone between fynbos and afromontane forests in South Africa (Geldenhuys, 1994). Afromontane forests persist as fragments throughout the CFR, and *V. divaricata* borders these fragmented indigenous forests, also rendering the distribution of this species fragmented

(Geldenhuys, 1986; Geldenhuys, 1994) with highly localized populations (covering areas between 1-5 hectars) (Van der Bank *et al.*, 1996). This fragmented distribution has kept populations of arthropods and other organisms associated with this tree isolated (Van der Bank *et al.*, 1996). This pattern is likely different for organisms associated with *A. mearnsii*, as it has a more even distribution over the landscape.

The co-occurrence of these two legumes may assist the exchange of their associated arthropods. When arthropods normally associated with *V. divaricata* find *A. mearnsii* as a suitable host, the IAP may also assist arthropod range expansion to populations that had previously been isolated by their fragmented distribution (Geldenhuys, 2000). The arthropod associates of *A. mearnsii* in its invaded ranges are poorly known and in need of focussed attention (DEA, 2009; Gibson *et al.*, 2013; Kleinjan & Hoffmann, 2013). Understanding the potential exchange of associated arthropods between these ecologically similar species is also important given that *A. mearnsii* is largely associated with native arthropods within plantations (Govender, 2007; DEA, 2009). The native arthropod pests associated with *A. mearnsii* in plantations mostly occurred in low abundances before colonization and exploitation of this tree species (Govender, 2007). The utilization of this invasive tree could result in pest epidemics that may affect entire plantations as well as neighbouring native vegetation (Ohmart, 1989). The monoculture stands utilized in extensive forestry (Govender, 2007) and the monoculture stands formed during invasion (Khanna, 1997) are ecologically similar and therefore may be affected similarly by the influx of native arthropods.

A recent study by Proches *et al.*, (2008) set out to compare arthropods associated with IAP's from Australia and phylogenetically closely related native tree species within South Africa. Few differences were observed between arthropods associated with the tree species within different feeding guilds, except for herbivores. Higher herbivore abundances were observed on the native trees even when excluding biological control agents. However, sampling was conducted at different sites for the respective plant taxa even though care was taken to minimize site differences. The potential effect of microclimatic conditions on arthropod beta-diversity is very important, especially in the CFR, where large changes are noted over short distances (Pryke *et al.*, 2013, Goldblatt & Manning, 2000) and need to be minimised in comparative studies such as these.

Arthropod assemblages associated with *V. divaricata* and *A. mearnsii* within forest margins are unknown. It is possible that the ERH may be expressed in this situation and that *A. mearnsii* may benefit from having a lower herbivore load, increasing its invasive potential (Williamson, 1996; Crawley, 1997; Keane and Crawley, 2002, Colautti *et al*., 2004; Liu and Stiling, 2006). Alternatively, if the BRH is expressed, *A. mearnsii* may be attracting previous specialist herbivores of *V. divaricata*, disrupting evolutionary processes (Agrawal and Kotanen, 2003; Parker and Hay, 2005; Lombardero *et al*., 2008; Carrillo-Gavilán *et al*., 2012, Roques *et al*., 2006, Elton, 1958; Maron and Vilá, 2001). Here we investigate the foliage arthropods associated with the native *V. divaricata* and the invasive *A. mearnsii* at sites where these grow sympatrically. We compare their complements of arthropod species richness, abundance and species assemblage composition with regards to feeding guild structure and the main taxonomic groups collected. In addition we determine the number of shared taxa between the two host trees, to investigate the extent of species being shared and potential range expansions for associated arthropods. Based on the ERH, we hypothesis that the native tree will have higher arthropod richness and abundances than the invasive tree. Secondly we hypothesise that the native tree will have distinct arthropod assemblages associated with it within each population as a result of its fragmented distribution, while the invasive plant will have a more uniform complement of associated arthropods due to its more uniform distribution across the landscape.

2.Materials and methods

2.1 Study site and arthropod collection

This study was conducted in the southern part of the Western and Eastern Cape Province including the Garden Route National Park of the Cape Floristic Region, South Africa (Figure 2-1). Rainfall in the region varies from an average of 500 mm to 1200 mm per year, peaking during autumn and early summer and is at its lowest during December. Temperatures are mild ranging from 7 ˚C to 19 ˚C during June and 15 ˚C to 26 ˚C during January (Bond, 1981). Soils are largely derived from quartzitic sandstone of the Table Mountain Group. The vegetation in the area is isolated Afromontane forest patches bordered by fire prone fynbos and in some cases forestry plantations (*Pinus* species) (Geldenhuys, 1997, DEA, 2009). These forests form part of the largest forest complex in South Africa (DEA, 2009, Geldenhuys, 1994). Fynbos is dominated by low-growing sclerophyllous shrubs (Goldblatt & Manning, 2000), while Afromontane forests are dominated by evergreen trees, with multi-layered vegetation underneath (Low & Rebelo, 1996).

Sampling sites were focused within Afromontane forest margins as this is where *A. mearnsii* grows sympatrically with *V. divaricata*. Six sites, with a minimum interspacing of ±20 km to prevent pseudo-replication, were selected (Figure 2-1, Table 2-1) between Knysna (33˚9805 S 23˚0464 E) in the west and Stormsrivier in the east (33˚9901 S 23˚8979 E). At each site, ten mature individual trees per tree species were selected based on similarity in tree diameters (18 – 30 cm for *V. divaricata* and 18 – 28 cm for *A. mearnsii*) and proximity to individuals of the other focal tree taxon (individual interspacing distance no more than 3 meters). As arthropod abundance is known to peak during spring/summer in the CFR following the flowering season (Gibson *et al.*, 2012; Mucina & Rutherford, 2006), we conducted our sampling during November 2013. During this time both tree species were reaching the end of their flowering period (Sherry, 1971; Mbambezeli & Notten, 2013), but pollinators could still be sampled at the Keurboom site.

Arthropods associated with foliage and flowers were collected using a vacuum sampler constructed from a Stihl SH 86 leaf blower/vacuum (Stihl, Germany) with a 15 cm diameter nozzle fitted with a collection net as is described by Stewart & Wright (1995). Individual branches were placed into the mouth of the vacuum nozzle for 3 seconds and the process was repeated for 50 different branches per tree individual (Richmond & Graham, 1969). After

sampling each tree individually, the contents from the collection net was emptied into a re-sealable plastic bag and frozen until further analyses. All arthropods were sorted and assigned to a morpho-species (Oliver & Beattie, 1996) and feeding guild based on their taxonomic group and mouthparts (Labandeira, 1997). A voucher collection of all collected morpho-species is housed at the Stellenbosch University Entomology Collection, Stellenbosch, South Africa (USEC).

2.2 Statistical analyses

Species richness was assessed using three non-parametric species estimators, as arthropod assemblages normally include many rare species (Bunge & Fitzpatrick, 1993; Novotny & Basset, 2000; Hortal *et al*., 2006). The Incident-based Coverage Estimator (ICE) was chosen as it is a highly robust estimator of species richness (Chazdon *et al.*, 1998), and the Choa2 and Second-order Jackknife estimators were chosen as these provide the least biased estimates with small sample sizes (Chao, 1984; Colwell & Coddington, 1994). For the Chao estimator the classic method was used as the estimated incidence distribution was <0.5. Species estimations were calculated using Estimate S (Cowell, 2006).

To compare arthropod species richness and abundances between the two host plants we used Generalized Linear Models (GLZs) with Poisson distribution (with log-link functions as this is count data) (O'Hara, 2009; Zuur *et al.*, 2010) in SAS 9.1 (SAS Institute Inc. Cary, USA). Analyses were conducted for overall (combining all arthropods taxa collected per host plant), different feeding guilds (Herbivores, Detritivores, Nectar feeders, Parasites, Predators and Formicidae) and the seven most abundant taxonomic groupings (Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Formicidae). These GLZs were calculated for all data, as means were <5 and the minimum numbers of successes and failures were <5 (Bolker *et al.*, 2009). The Wald x^2 (Z) statistic was calculated using the quasi-likelihood technique, given that the analysis showed no over-dispersion of variances compared to the models (Bolker *et al.*, 2009).

Multivariate analyses using Permutational Multivariate Analysis of Variance (PERMANOVA) (Anderson, 2001; 2005) were used to calculate the distance-based pseudo F-statistic and p-value for the similarity of the arthropod assemblages (using abundance data) between the two host plant species (irrespective of collection site) for overall, different feeding guilds as well as for the

seven most abundant taxonomic groups. Analyses were performed using Bray-Curtis similarity measures, with data fourth root transformed to reduce the weight of common species (PRIMER 6 -PRIMER –E 2008). These analyses were also performed for different sites (irrespective of plant taxa sampled) and for the effect of collection site on the assemblage composition for each host plant species individually. Further, to remove the influence of site on groupings (feeding guilds and/or taxanomic groupings), the analyses were repeated for each group significantly influenced by host plant identity using only single site data and comparing the arthropod assemblage composition between the two species within each site.

To compare differences in the homogeneity of arthropod assemblages between the two host plants (a measure of beta-diversity) by incorporating only presence/absence data, we used a permutational test for multivariate dispersion – PERMDISP in PRIMER 6. PERMDISP firstly plots multivariate dispersion within each pre-defined factor (i.e. the arthropods associated with a particular host species at a particular site). It then calculates the mean distance of each factor to the centroid of each factor group (i.e. that for *A. mearnsii* or *V. divaricata*, respectively) and uses ANOVA to calculate F and P values. Thus, in our analyses PERMDISP analyses determined whether there was a significant change-over of arthropod assemblages on each plant species when moving between sites using only presence/absence data. We used Jaccard similarity measures and the procedure was conducted with 5000 permutations (Anderson, 2004, 2006).

Species contributing the most to observed differences between the two host plants were identified using the analysis SIMPER in PRIMER 6. This procedure calculates the average Bray-Curtis dissimilarity between all pairs of inter-group samples (i.e. all species associated with *A. mearnsii* against all species associated with *V. divaricata*). A good discriminating species can be identified as one that contributes heavily to inter-host dissimilarity and has a small standard deviation (Clarke & Gorley, 2006). For these analyses the data were square-root transformed as this down-weights the importance of rare species, highlighting common species across the groups (Yoshioka, 2008) and limiting the effect of singletons (Zuur *et al.*, 2007).

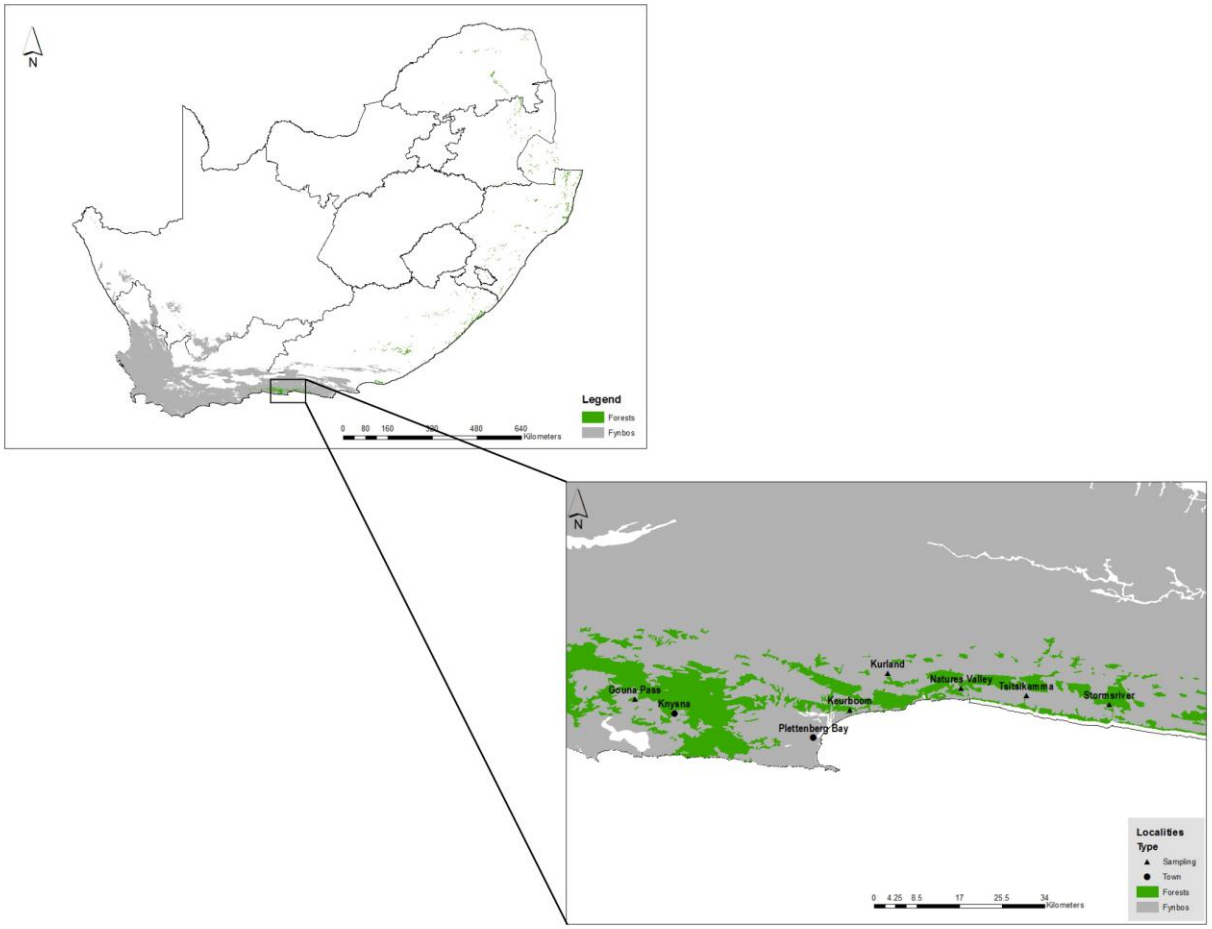

Figure 2-1 Localities sampled for arthropod associates of the invasive tree *Acacia mearnsii* and the native tree *Virgilia divaricata* within forest margins, located in the Garden Route National Park (Western Cape & Eastern Cape provinces), South Africa.

Table 2-1 Location, site description and mean stem diameter (standard error in bracts) of the sampling sites from which arthropods were collected from *A. mearnsii* and *V. divaricata*.

Site name	**GPS co-ordinates**	**Mean stem diameter *V. divaricata* (cm)**	**Mean stem diameter *A. mearnsii* (cm)**	**Site description**
Gouna	33˚9805 S 23˚0464 E	23.14 (± 3.28)	18.97 (± 1.66)	The site borders the Gouna indigenous forest. *V. divaricata* is interspersed by fynbos shrubs with *A. mearnsii* growing in a thick stand within a few meters of the widely distributed *V. divaricata* trees. Past clearing activities are evident, but none of the trees sampled during was affected by these activities.
Keurboom	34˚0002 S 23˚4323 E	30.22 (± 3.46)	28.30 (± 1.40)	This site is located closer to the coastal area with *V. divaricata* and *A. mearnsii* interspersed. The under growth at this site was dense and trees where located in close proximity to each other.
Kurland	33˚9346 S 23˚5006 E	23.22 (± 0.89)	17.50 (± 0.85)	Trees were located along the border of a pine plantation with *V. divaricata* and *A. mearnsii* interspersed. Some past clearing activities were evident, but no recent work was observed. None of the trees that were selected to be sampled were affected by these clearing activities.
Natures Valley	33˚9613 S 23˚6319 E	30.29 (± 4.17)	27.66 (± 7.35)	This site was located along the boundary of a pine plantation, with *V. divaricata* and *A. mearnsii* interspersed, with some individuals further away. These individuals were only sampled if no other options where available.
Tsitsikamma	33˚9743 S 23˚7492 E	20.80 (± 8.60)	22.42 (± 3.20)	This site was located along the boundary of a pine plantation. The majority of *V. divaricata* trees occurred clumped together, with some *A. mearnsii* trees growing close by
Stormsriver	33˚9901 S 23˚8979 E	28.59 (± 9.64)	20.27 (± 4.81)	The site was in close proximity to a large *Eucalyptus* stand. *V. divaricata* trees were densely spaced, while *A. mearnsii* trees were scattered more sparsely. The undergrowth was dominated by grasses.

3.Results

3.1 Arthropod alpha-diversity

A total of 2875 arthropod individuals were collected and assigned to 300 morpho-species. They belonged to fourteen different orders, of which the seven most abundant orders (Figure 2-2) were used for further analyses (Araneae (57), Coleoptera (67), Diptera (45), Hemiptera (37), Hymenoptera (50), Lepidoptera (12) and Formicidae (11)) and the six most abundant feeding guilds (Herbivores (98), Detritivores (43), Nectar feeders (37), Parasites (44), Predators (67) and Formicidae (11)). The remaining orders were lumped together into a category named "other (25)" and included the Neuroptera, Phasmatodea, Thysanoptera, Orthoptera, Tricoptera, Acari and Dermaptera. Observed and estimated species richness figures are presented in Table 2. In total, *V. divaricata* hosted 220 observed species, while *A. mearnsii* hosted only 182 species (Table 2-2). For most groupings the estimated number of species was well-above the observed number of species, indicating that sampling was not exhaustive. Estimated species richness was higher on the native *V. divaricata* than the invasive *A. mearnsii* (Table 2-2). However, GLZ analyses indicated that this difference was non-significant (Table 2-3). This was true for comparisons between the respective host plants for all feeding guilds and all taxa, except for the Araneae and Formicidae that had higher species richness on the native tree species than on the invasive host plant species (Table 2-3).

Overall arthropod abundance was significantly higher on *V. divaricata* than on *A. mearnsii* (Table 2-3). Herbivores occurred in higher abundance on the native host plant, while Detritivores had higher abundances on the invasive host. Other feeding guilds were unaffected by host plant identity (native or invasive). Within taxonomic groupings, the Araneae, Lepidoptera and Formicidae occurred in higher abundances on the native host plant, while other taxonomic groupings were unaffected by host plant identity (Table 2-3).

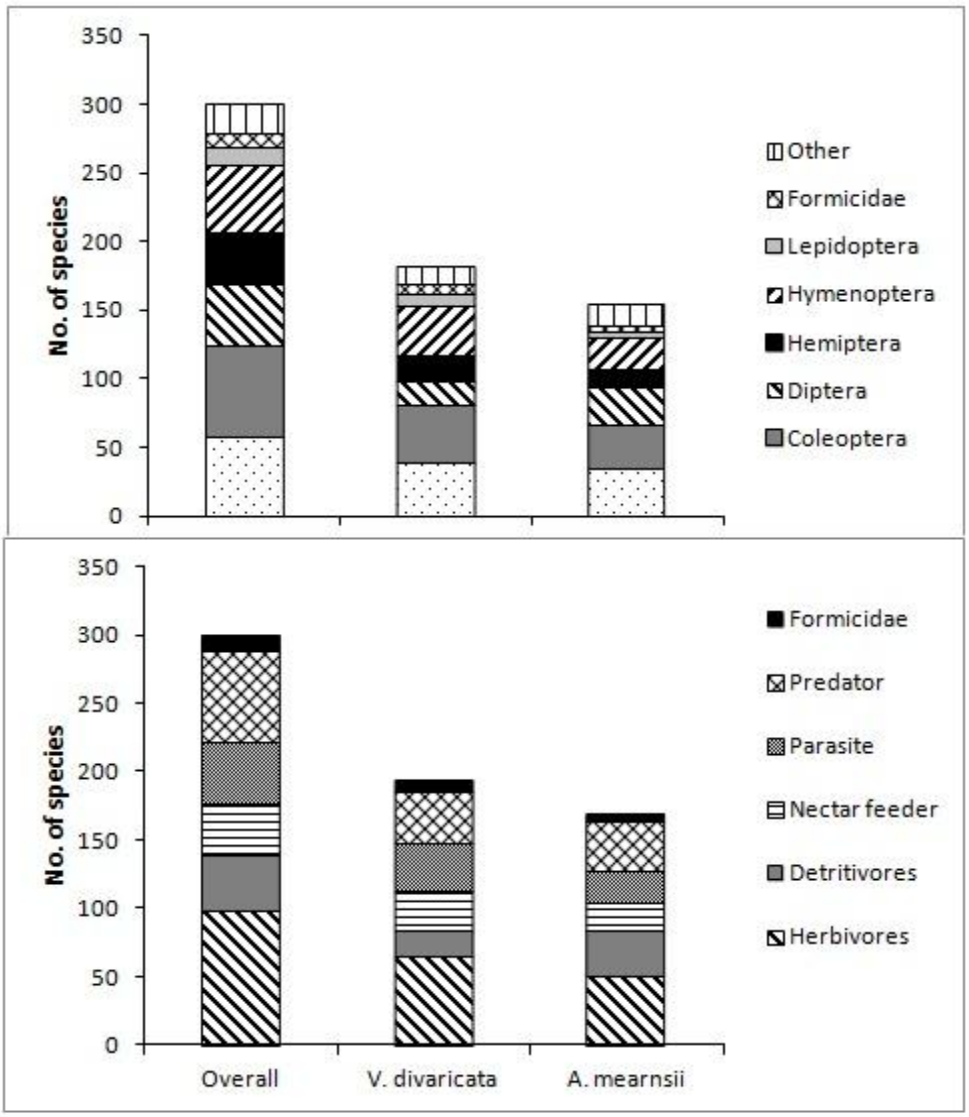

Figure 2-2 Observed arthropod numbers collected from *Virgilia divaricata* and *Acacia mearnsii* respectively and combined, based on feeding guild and taxonomic grouping.

Table 2-2 Observed and estimated arthropod species richness as calculated by ICE, Chao 2 and Jack 2 species estimators for overall, different feeding guilds and main taxonomic groupings for each host plant respectively (*Virgilia divaricata* and *Acacia mearnsii*).

	Observed spp.	Individuals	ICE	Chao2	Jack2
Overall (both hosts combined)	300	2875	634.61	525 (SD ± 42.09)	556.27
Overall *Virgilia divaricata*	194	2022	568.92	463.72 (SD ± 54.44)	436.63
Guilds					
Herbivore	65	1247	156.38	125.54 (SD ± 24.63)	125.07
Detritivore	19	70	74.85	74.33 (SD ± 46.76)	43.93
Nectar feeder	29	98	72.24	63.33 (SD ± 21.86)	57.33
Parasite	34	73	158.35	122.6 (SD ±55.36)	76.37
Predator	39	203	99.49	79.97 (SD ±20.78)	78.23
Formicidae	8	243	22.61	17.50 (SD ± 8.55)	17.93
Taxa					
Araneae	39	149	86.23	85.4 (SD ± 25.67)	76.3
Coleoptera	41	316	118.51	91.06 (SD ± 27.45)	80.77
Diptera	18	77	71.08	64.14 (SD ± 30.92)	47.9
Hemiptera	18	262	69.09	52.25 (SD ± 24.84)	41.9
Hymenoptera	37	85	156.72	118.08 (SD ± 46.8)	81.83
Lepidoptera	8	41	12.27	10.67 (SD ± 2.27)	12.33
Formicidae	8	243	22.61	17.5 (SD ± 8.55)	17.93
Other	13	849	48.85	49.33 (SD ± 25.76)	28.93
Overall *Acacia mearnsii*	169	853	422.94	336.04 (SD ± 37.2)	346.67
Guilds					
Herbivore	51	304	125.58	108.09 (SD ± 23.15)	107.13
Detritivore	33	158	123.41	291.42 (SD ± 280.9)	67.97
Nectar feeder	20	89	25.89	24.44 (SD ± 4.2)	28.8
Parasite	24	46	29.07	25.07 (SD ± 2.85)	29.63
Predator	36	114	120.18	101.2 (SD ±30.77)	86.77
Formicidae	5	20	30.38	14.33(SD ± 8.43)	13.5
Taxa					
Araneae	34	99	83.88	72.33 (SD ± 25.72)	62.33
Coleoptera	32	309	73.19	59.78 (SD ± 17.63)	58.8
Diptera	28	148	156.69	312.67(SD ± 303.0)	69.47
Hemiptera	12	117	29.46	24.44 (SD ± 7.61)	27.37
Hymenoptera[a]	23	56	26.79	23.67 (SD ± 2.63)	27.67
Lepidoptera	5	28	13.72	11 (SD ± 5.92)	12
Formicidae	5	20	30.38	14.33 (SD ± 8.43)	13.5
Other	15	76	35.46	30.44 (SD ± 8.61)	31.36

Observed species and number of individuals are given for all groups sampled (excluding group "other"). ICE = Incidence-based Coverage Estimator, Chao 2 = Second order Chao estimator, Jack2 = Second order Jack knife estimator. [a] All members of Hymenoptera with the exception of Formicidae. SD = standard deviation

Table 2-3 Summary results of Generalized Linear Models (with Poisson distribution and log-link function) on species richness and abundance data for the five most abundant feeding guilds and the seven most abundant taxonomic groups between the two host plants (*V. divaricata* and *A. mearnsii*).

	Mean comparisons	Wald statistic	*P* value
Species richness			
Overall	Native = Invasive	1.99	0.1584
Feeding guilds			
Herbivore	Native = Invasive	3.33	0.068
Detritivore	Invasive= Native	1.93	0.1649
Nectar feeder	Native = Invasive	0.93	0.3355
Parasite	Native = Invasive	0.03	0.8628
Predator	Native = Invasive	0.07	0.7977
Taxa			
Araneae	**Native > Invasive**	5.94	**0.0148**
Coleoptera	Native= Invasive	0.41	0.5213
Diptera	Invasive = Native	0.11	0.7389
Hemiptera	Native = Invasive	0.65	0.4196
Hymenoptera[a]	Native = Invasive	1.24	0.2651
Lepidoptera	Native = Invasive	0.24	0.6223
Formicidae	**Native > Invasive**	3.96	**0.0467**
Abundance			
Overall	**Native > Invasive**	**332.85**	**<0.0001**
Feeding guilds			
Herbivore	**Native > Invasive**	**495.41**	**<.0001**
Detritivore	**Invasive > Native**	**17.44**	**<.0001**
Nectar feeder	Invasive = Native	0.61	0.4334
Parasite	Native = Invasive	0.6	0.4373
Predator	Native = Invasive	1.57	0.2098
Taxa			
Araneae	**Native > Invasive**	**22.12**	**<.0001**
Coleoptera	Invasive = Native	0.01	9312
Diptera	Invasive = Native	5.26	0.219
Hemiptera	Native = Invasive	1.42	0.2342
Hymenoptera[a]	Invasive = Native	0.11	0.7389
Lepidoptera	**Native > Invasive**	**6.34**	**0.0118**
Formicidae	**Native > Invasive**	**114.46**	**<.0001**

Host plants are ordered with the highest mean on the left and the lowest on the right. [a] All members of Hymenoptera with the exception of Formicidae. "=" signifies no significant difference in means between the two host plants and ">" signifies that the mean on the left is significantly higher than the mean on the right. Bold values signify significant differences between the two host plants.

3.2 Shared arthropod communities

A large number of arthropods were shared by the two host plants; overall 102 (34%) species occurred on both *V. divaricata* and *A. mearnsii*, while 118 (39 %) species were unique to *V. divaricata* and 80 (26%) species were unique to *A. mearnsii* (Table 2-4). When removing rare taxa (in our study those with less than 4 individuals collected), this pattern became even more evident, with close to 70% of the common taxa occurring on both plants. Sixty percent of common herbivores were found on both tree taxa (Table 2-4). When considering common species of other guilds, nearly all guilds shared more than 80% of taxa (Table 2-4). This pattern of high levels of shared common morpho-species was also observed for the separate taxonomic groups (Table 2-4). The Coleoptera that contained many herbivorous members only shared 52% of common species between the two hosts. We identified five species to contribute the most to the differences observed between the two host plants. These were identified according to feeding guild and taxonomic grouping. Of significance is that all species identified were herbivores, three of which were beetles, again emphasising the importance of these groups in this study (Table 2-5).

Table 2-4 Number of unique and shared arthropod species from total arthropods species collected and within feeding guilds and orders collected from the two host plants. Percentage of total in parenthesis and including species occurring in abundances of higher than 4 individuals.

	Shared	>4	Unique *V. divaricata*	>4	Unique *A. mearnsii*	>4
Total	102 (34)	69 (77.6)	118 (39.3)	10 (11.2)	80 (26.7)	10 (11.2)
Feeding guild						
Herbivores	26 (26.8)	20 (60.6)	41 (42.3)	4 (12.1)	27 (27.8)	9 (27.3)
Detritivores	13 (31.7)	8 (88.9)	8 (19.5)	0(0)	20 (47.8)	1 (0.1)
Nectar feeder	18 (47.4)	11 (84.6)	16 (42.1)	2 (15.4)	4 (10.5)	0 (0)
Parasite	16 (35.5)	9 (100)	21 (46.7)	0 (0)	8 (17.8)	0 (0)
Predator	23 (34.3)	15 (88.2)	26 (38.8)	2 (11.8)	18 (26.9)	0 (0)
Formicidae	4 (36.4)	4 (66.7)	6 (54.4)	2 (33.3)	1 (0.1)	0 (0)
Order						
Araneae	20 (35.1)	14 (93.3)	22 (38.6)	1 (0.1)	15 (6.7)	0 (0)
Coleoptera	19 (28.4)	11 (52.4)	27 (40.3)	4 (19.0)	21 (31.3)	6 (28.6)
Diptera	13 (28.9)	8 (88.9)	12 (26.7)	0 (0)	20 (40.8)	1 (11.1)
Hemiptera	11 (29.7)	10 (71.4)	17 (45.9)	1 (7.1)	9 (24.3)	3 (21.4)
Hymenoptera	20 (40)	11 (100)	25 (50)	0 (0)	5 (10)	0 (0)
Lepidoptera	4 (33.3)	3 (75)	6 (50)	1 (25)	2 (16.7)	0 (0)
Formicidae	4 (36.4)	4 (66.7)	6 (54.5)	2 (33.3)	1 (9.0)	0 (0)
Other	11 (52.4)	8 (88.9)	3 (14.3)	1 (11.1)	7 (33.3)	0 (0)

Table 2-5 Identification of morpho-species contributing the most to the differences observed between *A. mearnsii* and *V. divaricata*, along with their taxonomic grouping, feeding guild and average abundances within each host plants, and their percentage contribution to the dissimilarity.

Species code	Taxonomic order	Feeding guild	Ave. abundance – *V. divaricata*	Ave. abundance - *A. mearnsii*	Dissimilarity /SD	% Contribution
sp70	Thysanoptera	Herbivore	0.97	0.03	0.82	4.74
sp32	Hemiptera	Herbivore	0.74	0.28	0.99	3.94
sp42	Coleoptera	Herbivore	0.53	0.63	0.98	3.66
sp2	Coleoptera	Herbivore	0.54	0.05	0.81	2.74
sp3	Coleoptera	Herbivore	0.02	0.46	0.7	2.46

3.3 Arthropod beta-diversity

Arthropod assemblage composition based on PERMANOVA analyses indicated that there was an overall significant difference in arthropod assemblages based on site (when disregarding plant taxa) (Table 2-6). This was reflected for all feeding guilds and some of the taxonomic groups. Overall there were also differences in arthropod assemblage composition between the two host plant taxa when disregarding collection site (Figure 2-3). The herbivore and nectar feeder assemblages differed significantly between the two hosts (Table 6). When taxonomic groups were considered, only the Coleoptera and Diptera assemblages differed significantly between the two hosts (Figure 2-3; Table 2-6). When considering the influence of host plant identity on arthropod assemblages at different sites, (as there was a significant interaction between site and host plant) (Table 2-6), significant differences were still observed between the two hosts for overall assemblages and herbivore assemblages, while nectar feeder assemblages were only significantly different at a single site (Keurboom). Similarly, the Coleoptera and Araneae assemblages were significantly different between the two host plants at most individual sites.

PERMDISP analyses revealed that overall, *V. divaricata* showed a significant beta-diversity response when considering its associated arthropods from different sites (Table 2-7). This was also reflected in the taxonomic groups Araneae and Coleoptera. No beta-diversity effect was found for any of the groupings of arthropods associated with *A. mearnsii* (Table 2-6).

Table 2-6 Summary statistics for Permutational Multivariate Analysis of Variance (PERMANOVA) analyses for arthropod assemblages associated with *A. mearnsii* and *V. divaricata* for overall assemblages, different feeding guilds and different taxa. Separate analyses were conducted for site data (host taxa combined), the interaction of site and host plant, host plant (sites combined) as well as for the two host plants at separate sites.

	Site (host taxi combined)	Site x Host plant	Host plant (sites combined)	Host plant within site
Overall	**3.04****	**2.47****	**3.04****	**
Guilds				
Herbivores	**2.83****	**2.34****	**9.55****	*
Detritivores	**2.64****	**2.01****	1.63	-
Nectar feeder	**1.66****	**1.64****	**1.74***	***(excluding TS, G, K, NV, S)**
Parasite	**1.41****	1.13	0.81	-
Predator	**1.62****	**1.52***	1	-
Formidea	0.69	1.01	0.84	-
Orders				
Araneae	1.21	**1.39***	0.79	-
Coleoptera	**2.75****	**2.15****	**8.82****	*** (excluding TS, S)**
Diptera	**1.55***	**1.87****	**2.11***	*** (excluding NV, S)**
Hemiptera	1.06	0.87	1.06	-
Hymenoptera	**1.37****	1.08	0.85	-
Lepidoptera	1.06	0.81	1.96	-
Formidea	0.69	1.01	0.84	-

Figures represent F-values, number of permutations for each analysis = 9,999. [a] All members of Hymenoptera (except Formicidae). Bold values indicate significant differences, *****p-value < 0.05 and ****** p-value <0.01, "-" not included in analyses.

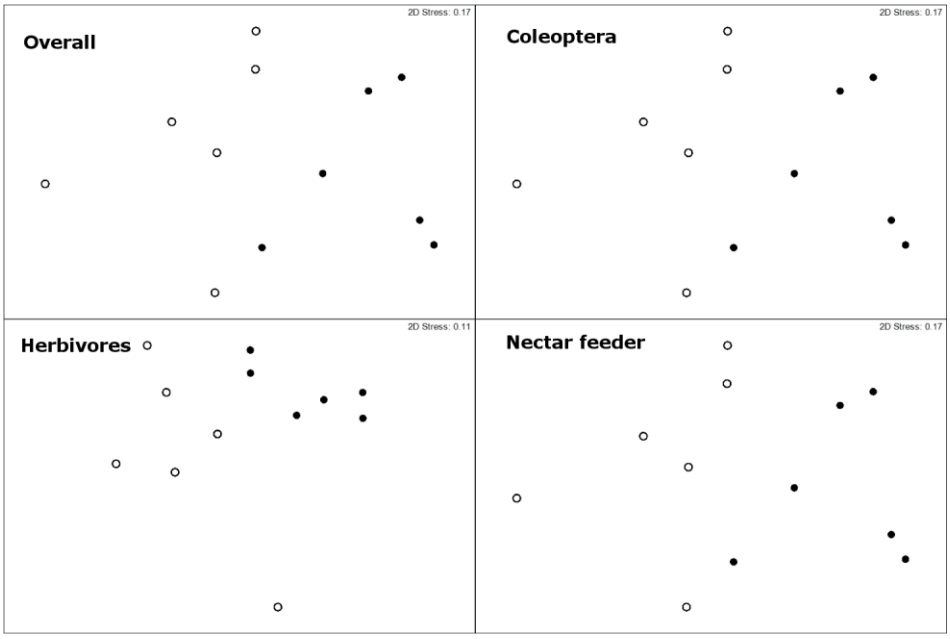

Figure 2-3 Non-metric MDS ordination of arthropod assemblages for the all arthropods collected in the study on each of the host plants *V. divaricata* (open circles) and *A. mearnsii* (closed circles) respectively Overall, the herbivore assemblage, the nectar feeding assemblage and for the Coleoptera.

Table 2-7 Results of PERMDISP analyses of arthropod beta-diversity associated with *V. divaricata* and *A. mearnsii*. Assemblages are compared for overall assemblages, within feeding guilds and between the seven most abundant taxonomic groups.

	PERMDISP	
	Virgilia divaricata	*Acacia mearnsii*
Overall	**3.02***	0.68
Feeding guilds		
Herbivores	0.47	1.92
Detritivores	4.56	0.88
Nectar feeder	0.83	0.84
Parasite	2.44	0.81
Predator	2.48	2.31
Taxonomic orders		
Araneae	**4.77***	1.36
Coleoptera	**9.73****	2.21
Diptera	1.48	0.40
Hemiptera	1.33	1.38
Hymenoptera[a]	3.10	2.24
Lepidoptera	1.94	6.61
Formicidae	2.43	5.25

Figures represent F-values, number of permutations for each analysis = 9,999. [a] All members of Hymenoptera except Formicidae. Bold values indicate significant differences, *p-value <0.05 and **p=value< 0.01.

4.Discussion

Results of this study showed that there is a remarkable overlap in the arthropod assemblages shared between an invasive and native leguminous tree where they occur sympatrically in the CFR. Overall species richness was very similar between the two host plants. This is in contrast to previous studies in which native species tended to house more species than their invasive counterparts (Simao & Rudgers, 2010, Roets & Pryke, 2013). However, some of our results reflect those of a recent study on the arthropod assemblages between the same invasive species (*A. mearnsii*) and a different native leguminous tree (*A. karroo* Hayne) (Proches *et al.*, 2008) in the CFR. *Acacia karroo* is phylogenetically more closely related to *A. mearnsii* (both in the Mimosoideae) than to *V. divaricata* that resides in the Papilionoideae (Van der Bank *et al.*, 1996). Our results therefore signify that plant taxa need not necessarily be phylogenetically very closely related for many arthropods to utilise both. This highlights the important impact that ecologically similar species can have within invaded ranges in the absence of congenerics.

Spiders and ants showed significantly higher species richness and abundance on the native plant than on the invasive species. All spiders and probably most ants collected in this study are predatory and would likely be attracted to areas with abundant food. In this study the native plant also showed greater numbers of arthropods (abundances) associated with it and may therefore account for the higher numbers of spiders and ants. Unexpectedly, we found no significant difference in species richness between the native and invasive host for the herbivore guild. This is in contrast to the study of Proches *et al.* (2008) that showed that the native *A. karroo* had higher herbivore species richness than *A. mearnsii*. Potential explanations for these conflicting findings may include the difference in sampling technique (sweep netting vs. vacuum sampling) and the difference in localities of host plant sampling in the earlier study. A vacuum sampler has been shown to be more effective in collecting arthropods from various habitats than sweep netting. The latter method would be especially limiting in sampling arthropods from trees (Buffington & Redak, 1998). Fewer herbivores, and maybe also only particular groups of herbivores, may therefore have been collected using sweep netting. However, as mentioned by Proches *et al.* (2008), a limitation to their work was that the distance between sites for comparative studies on native and invasive species were fairly large. As they sampled in different sites, one would expect that sites with two invasive species may be more degraded than sites with only a single IAP species or sites

without any IAP's present. In our study we were able to control for this by sampling at sites where only one invasive species occurred (*A. mearnsii*) and that it shares a habitat with the native species at all sites included. Also, arthropod communities are known to have a large changeover between sites (beta-diversity) (Samways, 1990; Pryke *et al.*, 2013), further confounding differences observed between arthropod groups sampled in the previous study. Furthermore the compositions of arthropod assemblages are also important when comparing arthropod communities, not just their richness and abundances (Anderson, 2001; 2005).

The higher abundance of arthropods on the native species was largely driven by the herbivore guild, providing indirect support for the ERH. The herbivore guild also contained numerous species that were found on both plant taxa. It therefore seems likely that, although many species could potentially utilise both plant taxa, the native species is preferred over the invasive (Cappuccino & Carpenter, 2005; Han *et al.*, 2008; Ridenour *et al.*, 2008; Tallamy *et al.*, 2010), as a result of leaf palatability and/ or higher nutritional value of the native plant. Recent research found no significant difference in leaf nutrient concentration between these two species, indicating no nutritional benefit (Van der Colff *et al.*, 2013; Chapter 3). *V. divaricata* may potentially have other chemical and/or physical characteristics (Franceschi *et al*., 2005; Zas *et al*., 2011) not measured here that are different to *A. mearnsii*. Chemical differences have not yet been studied between these two hosts, but difference in leaf size between the two species may account for at least some of these preferences as the leaves of *A. mearnsii* are much smaller than those of *V. divaricata*. Other factors accounting for higher abundance of herbivores on the native plant may include species area relationship factors, time since introduction of the alien species and taxonomic isolation between the host taxa (Strong *et al.*, 1984). However, the invasive range of *A. mearnsii* has become very extensive since its introduction almost 150 years ago (Stirton, 1978; Henderson, 2007; Poynton, 2009). The invasive plant could also have novel weapons that are absent in the native (Callaway & Ridenour, 2004) and may not be recognized as a suitable host plant in accordance to the ERH (Keane & Crawley, 2002) by native herbivores.

Host plant identity had a significant influence on overall arthropod community composition, the herbivore community composition and the composition of nectar feeding communities. These differences were significant also at all separate sites for the overall and herbivore communities, but only at one site for the nectar feeders. This is due to the fact that this was

the only site where the hosts were still in flower. Differences in the assemblages of nectar feeders can be expected as the two hosts have very different flower morphologies. *A. mearnsii* has yellow flower heads with flowers in elongated racemes (Sherry, 1971; Searle, 1997), while *V. divaricata* have larger pinkish pea-shaped flowers (Mbambezeli & Notten, 2003). Additionally, *V. divaricata* produces rich nectar (Mbambezeli & Notten, 2003), while nectar is absent from the flowers of *A. mearnsii*. However it produces large amounts of pollen that may be utilized my bees (Sherry, 1971; Searle, 1997). At the taxonomic level host plant identity significantly influenced only the Diptera and Coleoptera communities. These groups comprise mostly of nectar feeders and herbivores, respectively. Community composition of all other taxa and guilds were very similar between these two hosts.

PERMDISP analyses indicated that *A. mearnsii* had a much more homogenous arthropod assemblage across the sampled landscape than *V. divaricata*. The higher observed beta diversity for arthropods associated with the native tree highlights the importance of viewing these fragmented populations of *V. divaricata* as separate biodiversity conservation units (Secretariat on the Convention on Biological Diversity, 2001; Geldenhuys, 1994). Isolation of populations promotes speciation and other evolutionary processes (Chen & He, 2009). The current distribution of *V. divaricata* is determined by forest distribution and forest distribution by prevailing bergwinds and its interaction with terrain physiography and fire (Geldenhuys, 1994). These are natural determinants and have been maintained before and after anthropogenic influences on the system (e.g. plantations) (Geldenhuys, 1994; DEA, 2009). At the habitat level, *V. divaricata* trees are restricted to forest margins and are interspaced within these margins with fynbos and these trees do not transverse fynbos vegetation (Coetsee & Wigley, 2013). In contrast *A. mearnsii* spread readily between forest fragments and invade fynbos vegetation regularly (Henderson, 2007). This invader may thus provide a pathway between previously isolated populations. Since the invasive *A. mearnsii* seems to act as a suitable alternative host for many taxa, it may be able to assist the movement of arthropods between different *V. divaricata* populations that have previously been isolated. This seems particularly plausible given that *A. mearnsii* has a fairly even distribution in the region where sampling was conducted. This may also be the reason why the arthropod assemblages of *A. mearnsii* are more homogenous (Henderson, 2007). It is therefore important not only to conserve all populations, but also to maintain separation between natural forest fragments, as this would conserve evolutionary processes. We

therefore propose that this invasive species provides a corridor between forest margins increasing habitat connectivity in the process. Management activities relating to the clearing of *A. mearnsii* should thus also note the connectivity of the landscape in relation to *A. mearnsii* distribution.

5.References

Adamson, R.S. 1934. Novitates Africanae. Journal of Botany. 72: 43.

Agrawal, A. & Kotanen, P. 2003. Herbivores and the success of exotic plants: a phylogenetically controlled experiment. Ecology Letters 6: 712–715.

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

Anderson, M.J. 2004. PERMDISP: A Fortran Computer Program for Permutational Analysis of Multivariate Dispersion (for any Two-Factor Anova Design) using Permutational Tests. Department of Statistics, University of Auckland, New Zealand.

Anderson, M.J. 2005. Permutational multivariate analysis of variance. Department of Statistics, University of Auckland, Auckland.

Anderson, M.J. 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62: 245–253.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24: 127–135.

Bond, W.J., 1981, Vegetation gradients in the southern Cape mountains. MSc thesis, University of Cape Town.

Boucher, C. 1978. Black wattle, in: Stirton, C.H. (eds.) Plant invaders, beautiful but dangerous. Department of Nature and Environmental Conservation, Cape Provincial Administration, Cape Town. 48–51.

Buffington, M. & Redak, R. 1998. A comparison of vacuum sampling versus sweep-netting for arthropod biodiversity measurements in California coastal sage scrub. Journal of Insect Conservation 2: 99–106.

Bunge, J. & Fitzpatrick, M. 1993. Estimating the number of species: a review. Journal of the American Statistical Association 88: 364–373.

Callaway, R.M. & Ridenour, W.M. 2004. Novel weapons: invasive success and evolution of increased competitive ability. Frontiers in Ecology and the Environment 2: 436-443.

Cappuccino, N. & Carpenter, D. 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. Biological Letters 1: 435–438.

Carrillo-Gavilán, A., Moreira, X., Zas, R., Vilà, M. & Sampedro, L. 2012. Early resistance of alien and native pines against two native generalist insect herbivores: no support for the natural enemy hypothesis. Functional Ecology. 26: 283–293.

Chao, A. 1984. Nonparametric-estimation of the number of classes in a population. Scandinavian Journal of Statistics 11: 265–270.

Chazdon, R.L., Colwell, R.K., Denslow, J.S. & Guariguata, M.R. 1998. Species richness of woody regeneration in primary and secondary rainforests of north-eastern Costa Rica. In: Dallmeier F, Comiskey JA (eds) Forest biodiversity research, monitoring and modeling: conceptual background and old world case studies. Pathenon, Paris, 285–309.

Chen, X. & He, F. 2009. Speciation and endemism under the model of island biogeography. Ecology 90: 39–45.

Clarke, K. & Gorley, R. 2006. PRIMER v6: User manual/tutorial. Primer-E, Plymouth.

Coetsee, C. & Wigley, B.J. 2013, *Virgilia divaricata* may facilitate forest expansion in the afrotemperate forests of the southern Cape, South Africa. Koedoe 55: 1–8.

Colautti, R., Ricciardi, A., Grigorovich, I. & MacIsaac, H. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters 7: 721–733.

Colwell, R.K. 2006. Estimates: Statistical Estimation of Species Richness and Shared Species from Samples. Version 8.0. User's Guide and Application Published at http://purl.oclc.org/estimates.

Colwell, R.K. & Coddington, J.A. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 345: 101–118.

Crawley, M.J. 1997. Plant ecology, 2nd edn. Blackwell Science Ltd., Oxford.

Darwin, E. 1800. Phytologia. Published., London.

Department of Agriculture, Forestry and Fisheries. 2009. State of the forest report.

Drake, J.A., Mooney, H.A., di Castri, F., Groves, R. H., Kruger, F. J., Rejmánek, M. & Williamson, M. (eds). (1989). Biological invasions: a global perspective. Wiley, Chichester.

Dye, P., Moses, G., Vilakazi, P., Ndlela, R. & Royappen, M. 2001. Comparative water use of wattle thickets and indigenous plant communities at riparian sites in the Western Cape and KwaZulu-Natal. Water South Africa 27: 529–538.

Elton, C.S. 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

Enright, W.D. 2000. The effect of terrestrial invasive alien plants on water scarcity in South Africa. Physics and Chemistry of the Earth (B) 25: 237–242.

Franceschi, V., Krokene, P., Christiansen, E. & Krekling, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist 167: 353–375.

Frenzel, M. & Brandl, R. 2003. Diversity and abundance patterns of phytophagous insect communities on alien and native host plants in the Brassicaceae. Ecography 26: 723–730.

Futuyma, D.J., Keese, M.C. & Funk, D.J. 1995. Genetic constrains on macroevolution: the evolution of host affiliation in the leaf beetle genus Ophraella. Evolution 49: 797–809.

Geldenhuys, C.J. 1986. Nature's valley: a refugium for rare southern Cape forest species. Palaeoecology of Africa 17: 173–181.

Geldenhuys, C.J. 1994. Bergwind fires and the location pattern of forest patches in the southern Cape landscape, South Africa. Journal of Biogeography 21: 49–62.

Geldenhuys, C.J. 2000. Classification and distribution of forests and woodlands in South Africa. In: Owen, D.L. & Vermeulen, W.J. (eds.). South African Forestry Handbook 2000, Vol 2, The Southern African Institute of Forestry, Pretoria. 591–599.

Ghazoul, J. 2002. Seed predators and the enemy release hypothesis. Trends Ecology Evolution 17: 308.

Gibson, M.R., Pauw, A. & Richardson, D.M. 2012. Decreased insect visitation to a native species caused by an invasive tree in the Cape Floristic Region. Biological Conservation 157: 196–203.

Gioria, M., Pysek, P. & Moravcova, L. 2012. Soil seed banks in plant invasions: promoting species invasiveness and long-term impact on plant community dynamics. Preslia 84: 327–350.

Goldblatt, P. & Manning, J. 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria : National Botanical Institute, Pretoria.

Govender, P. 2007. Status of seedling establishment pest of Acacia mearnsii De Wild. (Mimosaceae) in South Africa. South African Journal of Science 103: 141-147.

Han, X., Dendy, S.P., Garrett, K.A., Fang, L. & Smith, M.D. 2008. Comparison of damage to native and exotic tallgrass prairie plants by natural enemies. Plant Ecology 198: 197–210.

Harris, R.J., Toft, R.J., Dugdale, J.S.,Williams, P.A. & Rees, J.S. 2004. Insect assemblages in a native (kanuka – Kunzea ericoides) and an invasive (gorse – Ulex europaeus) shrubland. New Zealand Journal of Ecology. 28: 35–47.

Henderson, L. 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37: 215– 248.

Holmes, P.M. 1989. Decay rates in buried alien Acacia seed populations of different density. – South African Journal of Botany 55: 299–303.

Holmes, P.M. & Cowling, R.M. 1997. The effects of invasion by Acacia saligna on the guild structure and regeneration capabilities of South African fynbos shrublands. Journal of Applied Ecology 34: 317–332.

Hortal, J., Borges, P.A.V. & Gaspar, C. 2006. Evaluating the performance of species richness estimators: sensitivity to sample grain size. Journal of Animal Ecology 75: 274–287.

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. Tree 17: 164–170.

Khanna, P.K. 1997. Comparison of growth and nutrition of young monoculture and mixed stands of *Eucalyptus globules* and *Acacia mearnsii*. Forest Ecology and Management 94: 105–113.

Kleinjan, C.A. & Hoffmann, J.H. 2013. Advances in clarifying the phylogenetic relationship of acacias: Relevence for biological control. Acta Oecologica 48: 21–29.

Labandeira, C.C. 1997. Insect mouthparts: Ascertaining the paleobiology of insect feeding strategies. Annual Review of Ecology and Systematics 28: 153–193.

Liu, H. & Stiling, P. 2006. Testing the enemy release hypothesis: a review and meta analysis. Biological Invasions 8: 1535–1545.

Lombardero, M.J., Alonso-Rodríguez, M. & Roca-Posada, E.P. 2012. Tree insects and pathogens display opposite tendencies to attack native vs. non-native pines. Forest Ecology and Management 281: 121–129.

Low, A.B. & Rebelo, A.G. 1996. Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs , Pretoria. 85.

Marchante, H., Marchante, E. & Freitas, H. 2003. Invasion of the Portuguese dune ecosystem by the exotic species Acacia longifolia (Andrews) Willd: effects at community level. Plant invasions: ecological threats and management solutions (ed. By L.E. Child, J.H. Brock, G. Brundu, K. Prach, P. Pysek, P.M. Wade and M. Williamson), 75–85. Backhuys Publishers,Leiden, The Netherlands.

Maron, J.L. & Vilà, M. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. Oikos 95: 361–373.

Mbambezeli, G., & Notten, A. 2003. Virgilia genus. http://www.plantzafrica.com/planttuv/virgilia.htm

McEvoy, P.B. 2002. Insect–plant interactions on a planet of weeds. Entomologia Experimentalis et Applicata 104: 165–79.

Milton, S.J. 1980. Studies on Australian acacias in the south-western Cape, South Africa . MSc thesis, University of Cape Town.

Milton, S.J. & Siegfried, W.R. 1981. Above-ground biomass of Australian acacias in the southern Cape, South Africa. South African Journal of Botany 47: 701–716.

Mooney, H.A. & Hobbs, R.J. EDS (2000). Invasive Species in a Changing World. Island Press

Mucina, L. & Rutherford, M.C. 2006. The Vegetation of South Africa, Lesotho and Swaziland Pretoria: South African National Biodiversity Institute, Pretoria.

Novotny, V. & Basset, Y. 2000. Rare species in communities of tropical insect herbivores: pondering the mystery of singletons. Oikos 89: 564–572.

O'Hara, R.B. 2009. How to make models add up-a primer on GLMMs 46: 124–137.

Ohmart, C.P. 1989. Why are there so few tree killing bark beetles associated with angiosperms? Oikos 54: 242–245.

Olckers, T. & Hulley, P. E. 1991. Impoverished insect herbivore faunas on the exotic bugweed Solanum mauritianum Scop. relative to indigenous Solanum species in KwaZulu-Natal and the Transkei. Journal of the Entomological Society of South Africa 54: 39–50.

Oliver, I. & Beattie, A. J. 1996. Invertebrate morpho - species as a surrogate for species: a case study. Conservation Biology 10: 99–109.

Palgrave, K.C. 1983. Trees of Southern Africa. Edition 2. Struik, Cape Town.

Palgrave, C.M. 2002. Trees of Southern Africa. Edition 3. Struik, Cape Town.

Palmer, E. & Pitman, N. 1972. Trees of South Africa Vol. 2. Balkema. Cape Town.

Parker, J. & Hay, M. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. Ecology Letters 8: 959–967.

Phillips, E.P. 1928. *Virgilia capensis*. The flowering plants of South Africa 8: 305.

Phillips, J.F.V. 1926. *Virgilia capensis* Lam. (‘Keurboom’): A contribution to its ecology and silviculture. South African Journal of Science 23: 435–454.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. 2000. Environmental and economic costs associated with non-indigenous species in the United States. BioScience 50: 53–65.

Poynton, R.J. 2009. Tree planting in southern Africa. Vol. 3 Other genera. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa.

Proches, S., Wilson, J.R.U., Richardson, D.M. & Chown, S.L. 2008. Herbivores, but not other insects, are scarce on alien plants. Austral Ecology 33: 691–700.

Pryke, J.S., Roets, F. & Samways, M.J. 2013. Importance of habitat heterogeneity in remnant patches for conserving dung beetles. Biodiversity and Conservation 22: 2857–2873.

Rejmánek, M., Richardson, D. M., Higgins, S. I., Pitcairn, M. & Grotkopp, E. 2005. Plant invasion ecology: state of the art. In: Invasive Alien Species: A New Synthesis (eds H. A. Mooney, R. N. Mack, J. A. McNeely, L. Neville, P. J. Schei & J. Waage). 104–61. Island Press, Washington, D.C.

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

Richardson, D.M., Macdonald, I.A.W. & Forsyth, G.G. 1989. Reductions in plant species richness under stands of alien trees and shrubs in the fynbos biome. South African Forestry Journal 149: 1–8.

Richmond, C.A. & Graham, H.M. 1969. Two methods for operating a vacuum sampler to sample populations of the cotton leafhopper on wild hosts. Journal of Economic Entomology 62: 525–526.

Ridenour, W.M., Vivanco, J.M., Feng, Y., Horiuchi. J. & Callaway, R.M. 2008. No evidence for tradeoffs: Centaurea plants from America are better competitors and defenders than plants from the native range. Ecological Monographs 78: 369–386.

Riley, C. V. 1893. Parasitic and predaceous insects in applied entomology. Insect Life 6: 130–41.

Riley, W. A. 1931. Erasmus Darwin and the biologic control of insects. Science 73: 475–6.

Roets, F. & Pryke, J.S. 2013. The rehabilitation value of a small culturally significant island based on the arthropod natural capital. Journal of Insect Conservation 17: 53–65.

Roques, A., Auger-Rozenberg, M.A. & Boivin, S. 2006. A lack of native congeners may limit colonization of introduced conifers by indigenous insects in Europe. Canadian Journal of Forest Research 36: 299–313.

Samways, M.J. 1990. Species temporal variability: epigaeic ant assemblages and management for abundance and scarcity. Oecologia 84: 482–490.

SAS 9.1 (SAS Institute Inc. Cary, USA).

Searle, S. 1997. *Acacia mearnsii* De Wild. (black wattle) in Australia. Black wattle and its utilizations.Barton, ACT: Rural Industries Research and Development Corporation: 1-10.

Secretariat of the Convention on Biological Diversity. 2001. The Value of Forest Ecosystems. Montreal, SCBD Technical Series No. 4.

Sherry, S.P. 1971. The black wattle (Acaciamearnsii De Wild.). Pietermaritzburg, University of Natal Press.

Siemann, E. & Rogers, W.E. 2003. Herbivory, disease, recruitment limitation, and success of alien and native tree species. Ecology 84: 1489–505.

Simao, M.C.M., Flory, S.L. & Rudgers, J.A. 2010. Experimental plant invasion reduces arthropod abundance and richness across multiple feeding guilds. Oikos 119: 1553–1562.

Southwood, T.R.E., Moran, V.C., & Kennedy, C.E.J. 1982. The richness, abundance and biomass of the arthropod communities on trees. Journal of Animal Ecology 51: 635–649.

Stewart, A.J.A., & Wright, A.F. 1995. A new inexpensive suction apparatus for sampling arthropods in grassland. *Ecological Entomology*. 20: 98–102.

Stirton, C.H1978. Plant invaders. (ed.). Beautiful but dangerous. ABC Press, Cape Town.

Strong, D.R., Lawton, J.H. & Southwood, R. 1984. Insects on plants: Community patterns and mechanisms. Harward University Press, Harward.

Tallamy, D.W. 2004. Do alien plants reduce insect biomass? Conservation Biology 18: 1689–1692.

Tallamy, D.W., Ballard, M. & D'Amico, V. 2010. Can alien plants support generalist insect herbivores? Biological Invasions 12: 2285–2292

Traveset, A. & Richardson, D.M. 2006. Biological invasion as disruptors of plant reproductive mutualisms. Trends in Ecology and Evolution 21: 208–216.

Van der Bank, H., Van wyk, B. & Van der Bank, M. 1996. Genetic variation within and geographical relations beteen four natural populations of Virgilia oroboides (Tribe Podalyrieae: Fabaceae). Biochemical Systematic sans Ecology 24: 135–143.

Van der Colff, D., Roets, F., Dreyer, L.L. Valentine, A. 2013. Plant stress and the prevalence of pests and pathogens associated with a native and an invasive alien legume tree in the Cape Floristic Region, South Africa. Msc Thesis. Stellenbosch University. Stellenbosch.

Van Wilgen, B.W. & Richardson, D.M. 1985. The effects of alien shrub invasion on vegetation structure and fire behaviour in South African fynbos shrublands: a simulation study. Journal of Applied Ecology 22: 955–966.

Williamson, M. 1996. Biological invasions. Chapman & Hall, London.

Wolfe, L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. American Naturalist 160: 705–11.

Yelenik, S.G., Stock, W.D. & Richardson, D.M. 2004. Ecosystem level impacts of invasive Acacia saligna in the South African fynbos. Restoration Ecology 12: 44–51.

Yoshioka, P. 2008. Misidentification of the Bray-Curtis similarity index. Marine Ecology Progress Series 368: 309–310.

Zas, R., Moreira, X. & Sampedro, L. 2011. Tolerance and induced resistance in a native and an exotic pine species: relevant traits for invasion ecology. Journal of Ecology 99: 1316–1326.

Zuur, A.F., Leno, E.N. & Elphick, C.S. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1: 3–14.

Chapter 3

Drier climatic conditions may lead to increased herbivore pressure on a native tree, but not on an invasive competitor

Abstract

The way in which pests and pathogens interact with their host plants under possible stress conditions is important against the backdrop of predicted global climate change. Here we assess how an invasive plant (*Acacia mearnsii*) interacts with pests and pathogens in its invaded ranges that it sympatrically shares with a related native species (*Virgilia divaricata*) in the Cape Floristic region of South Africa. Specifically, we determine how herbivore abundance and disease development (as measures of tree health) differ between the two species across a moisture gradient and whether observed differences can be explained by moisture availability and/or plant nutrient levels. The two host plants had similar foliar nutrient content, but measurements of $\delta^{13}C$ isotope ratios in leaves indicated that only the native plant experienced drought stress at the drier sites. *A. mearnsii* therefore seems to be better drought adapted than the native plant. The degree of disease development after tree wounding was similar in both plants, but herbivore numbers were significantly higher on the native plant. Disease development was not correlated to soil moisture content for either tree species. In contrast, herbivore numbers on *V. divaricata* increased at drier sites, indicating that it would be more vulnerable to attack if climatic conditions become drier. Herbivore numbers on *A. mearnsii* were unaffected by moisture availability. Therefore, under conditions of increased drought, *V. divaricata* may experience higher levels of drought stress than the invasive *A. mearnsii* and may suffer from increased herbivory. Interestingly, herbivore abundance and disease development was significantly influenced by plant nutrient content for *A. mearnsii* and not for *V. divaricata*. Relatively nutrient poor *A. mearnsii* trees experienced higher herbivore loads and slower disease development than nutrient rich trees. Therefore, the susceptibility of *A. mearnsii* seems to be determined by plant nutrient levels, a factor that varies independently from water availability.

Key words: *Ceratocystis*, *Ophiostoma*, plant health, invasive species

1.Introduction

In the natural environment plants have many enemies that influence their health and survival (Agios, 2005). The ability of a plant to defend against, and overcome, these enemies depends on both its genotype and its interaction with the environment (McMahon, 2012; Huber & Jones, 2013). The interaction of such enemies (pests and pathogens) with both the host plant and the environment are very important in the face of global climatic change (Hogg *et al.*; 2000; IPCC, 2001; Bjorn, 2013) and increased globalization (Ayres & Lombardero, 2000; Wingfield *et al.*, 2001). The increased movement of non-native/invasive alien species causes an increase in the number of encounters with new pests and pathogens for both native and non-native/invasive species (Ayers & Lombardero, 2000).

The interaction of pests and pathogens with their host plants has been the focus of agricultural and forestry research aimed at securing crops (Murdock *et al.*, 2013; Oliveira *et al.*, 2014; Yang *et al.*, 2014a; Yang *et al.*, 2014). Huber & Hanekleus (2007) proposed a disease triangle model to use in such studies, which includes three main factors affecting pest and pathogen attack in plants. These are the host plant, the pathogen/pest and the environment. If any connections are broken between these factors, disease development and/or pest attack can be prevented (Huber & Hanekleus, 2007), but these interactions are very complex (Huber & Jones, 2013). Some studies have shown that plants that show signs of nutrient stress are less vigorous and also more susceptible to disease and/or herbivore attack (Entry 1986; Huber& Hanekleus, 2007; McMahon, 2012). Therefore, even though resistance to a specific infection is genetically controlled, in order to express this genetic ability, adequate resources are required (Huber & Jones, 2013). For this reason resistance to disease and/or herbivores is usually expressed along a continuous scale (highly resistant, resistant, tolerant, susceptible & extremely susceptible) that varies between different plant individuals and species.

Plants with some level of resistance against pathogens and herbivores produce defense molecules when the defense system is activated by attack (Agios, 2005; Pamela *et al.*, 2008). Nutrient shortage may reduce the production of these key anti-fungal or anti-herbivory compounds. Potatoes, for example, are more susceptible to early blight (*Alternaria solani* Sorauer) when they are deficient in N or P (Spectrum, 2013). Inversely, it has also been shown that when N levels are increased beyond the required levels or out of balance with other nutrients, defense

compound production may also decrease (Spectrum, 2013). The severity of fungal and herbivore attack on plants increased when excess levels of N was available in the form of amino acids (McMahon, 2012; Spectrum, 2013). An increase in N may also promote morphological changes in the plant, favoring disease development (Agios, 2005). In addition, excess N has also been associated with a delay in plant maturation, thereby increasing the time available for fungal disease development (Spectrum, 2013).

Similar to plant nutrients, water-stress conditions and fungal infections are intimately linked. Water stressed plants are more vulnerable to attack by pests and pathogens (Schoeneweiss, 1975; Ayres, 1991, Agios, 2005). For example, water-stress is known to enhance fungal infection, but some fungal species are dependent on moisture for successful infection (Agios, 2005). Herbivory by insects is also believed to be enhanced by water-stress as pest outbreaks are commonly associated with water-stressed plants (White 1969, Brodbeck and Strong 1987, Mattson and Haack 1987 a, b). This prediction is based on the plant stress hypothesis (PSH) as proposed by White (1969). It states that physiological changes in the plant due to water stress can make more nitrogen available to herbivorous insects. This can be due to the impairment of protein metabolism and amino acid synthesis (Hsiao, 1973; Brodbeck & Strong, 1987).

The PSH is challenged by Huberty & Denno (2004) who proposed that herbivorous insects may be negatively affected during continuous water-stress conditions. This is true in cases such as when a reduction in plant turgor and water content can interfere with the herbivores ability to access the N (Huberty & Denno, 2004). Furthermore, intermediate water-stress events, along with recovery periods, can make increased N available to the herbivores, which is not the case with continued water-stress conditions. Intermediate water stress-events, which are common in the natural environment, may therefore also benefit and enhance herbivore usage of their host plants (Herberty & Denno, 2004).

In South Africa, the Australian *Acacia mearnsii* De Wild is an important forestry tree (DEA, 2009), but has also become a notorious invasive weed (Henderson, 2007). Within the Knysna-Tsitsikamma forest complex, the largest forest complex in South Africa, it has invaded forest margins (Geldenhuys, 2004) where it grows sympatrically with the native legume tree *Virgilia divaricata* Adamson (Goldblatt and Manning, 2000). Both species belong to the Fabaceae and

they are ecologically similar (Van der Bank *et al.*, 1996, Searle, 1997, Mbambezeli & Notten, 2003). They are fast-growing woody perennial trees, forest pioneer species and both make use of biological nitrogen fixation through rhizobial associates (Joubert *et al.*, 2002; Beukes *et al.*, 2011). These similarities have lead to extensive exchange in their associated organisms, especially arthropods (Van Der Colff *et al.*, 2013; Chapter 1). Although not tested yet, we suspect that they may also share many pathogenic fungi. How these two plant species interact with their pest and pathogens in their shared environment have not been studied. This is an important question, as *A. mearnsii* is both a valuable plantation species (DEA, 2009) and a notorious invader (Dye & Jarmain, 2004; Henderson, 2007; Rodriquez-Echeverria *et al.*, 2011). In turn, *V. divaricata* is important as the most widespread forest margin tree in South Africa (Van der Bank *et al.*, 1996; Mbambezeli & Notten, 2003). An assessment of the interaction between these trees and their environments may provide insight into future reactions to climate change and globalization, which will be future drivers of ecosystem dynamics (Ayres & Lombardero, 2000).

Within plantations, pathogens of *A. mearnsii* have been well-studied (Roux & Wingfield, 1997; Roux *et al.*, 1999; Govender, 2007), but little is known about these organisms in their invaded range. The native ophiostomatoid fungus *Ceratocystis albifundus* M.J. Wingf., De Beer & M.J. Morris is prevalent in plantations in South Africa, where it causes tree wilting and death (Barnes *et al.*, 2005; Roux *et al.*, 2005; Wingfield *et al.*, 1996). This wound-infecting species has recently also been isolated from individuals in its invaded range. Although *C. albifundus* is not known to be associated with *V. divaricata* yet, numerous other ophiostomatoid fungi has been collected from it (Machingambi *et al.*, 2013). These may contain many important pathogenic species, as the ophiostomatoid fungi are known to lead to significant worldwide losses in the agricultural and forestry sectors. It is therefore likely that these fungi may play a significant role in the ecology and population dynamics of both *V. divaricata* and *A. mearnsii*.

In the present study we first compare herbivore numbers and disease development after wounding of individuals of *A. mearnsii* and *V. divaricata* over a moisture gradient. We determine whether any observed differences can be explained by water availability and/or varying plant nutrient levels. We predict that these tree species may be differentially influenced by environmental factors and that this may influence their susceptibility to pathogens and

herbivorous arthropods. We hypothesize that trees with limited resources (e.g. water availability) will be more vulnerable to herbivorous insects and pathogen infection than trees with adequate resources (i.e. water stress can increase susceptibility towards herbivores and pathogens). Individual plants and species with high nutrient levels are further expected to be better protected against herbivores and pathogens. Such trees are thus expected to have lower herbivore numbers and may be less affected by pathogens.

2.Materials and methods

2.1 Site selection

This study was conducted in the Knysna-Tsitsikamma forest complex in the Western and Eastern Cape Provinces of South Africa (Figure 3-1). These forests form part of the Afromontane forest belt situated along the African escarpment from the southern part of South Africa to Tanzania in the northeast (White, 1983). These forests are fragmented across their distribution range and, in the experimental area, are interspersed with fynbos vegetation (Mucina & Rutherford, 2006). Six localities were identified within the natural distribution of *Virgilia divaricata* where *Acacia mearnsii* has invaded (Figure 3-1). These localities provided sites where these species occurred sympatrically from Gouna Pass, near Knysna in the west to Stormsriver in the east (Figure 3-1), presumably following a moisture gradient (drier to wetter) (Goldblatt & Manning, 2000). This area receives between 500 mm to 1200 mm precipitation annually, with peaks during autumn and early summer (Bond, 1981). More detailed site descriptions are provided in Table 2-1 (Van der Colff *et al.*, 2013, Chapter 2).

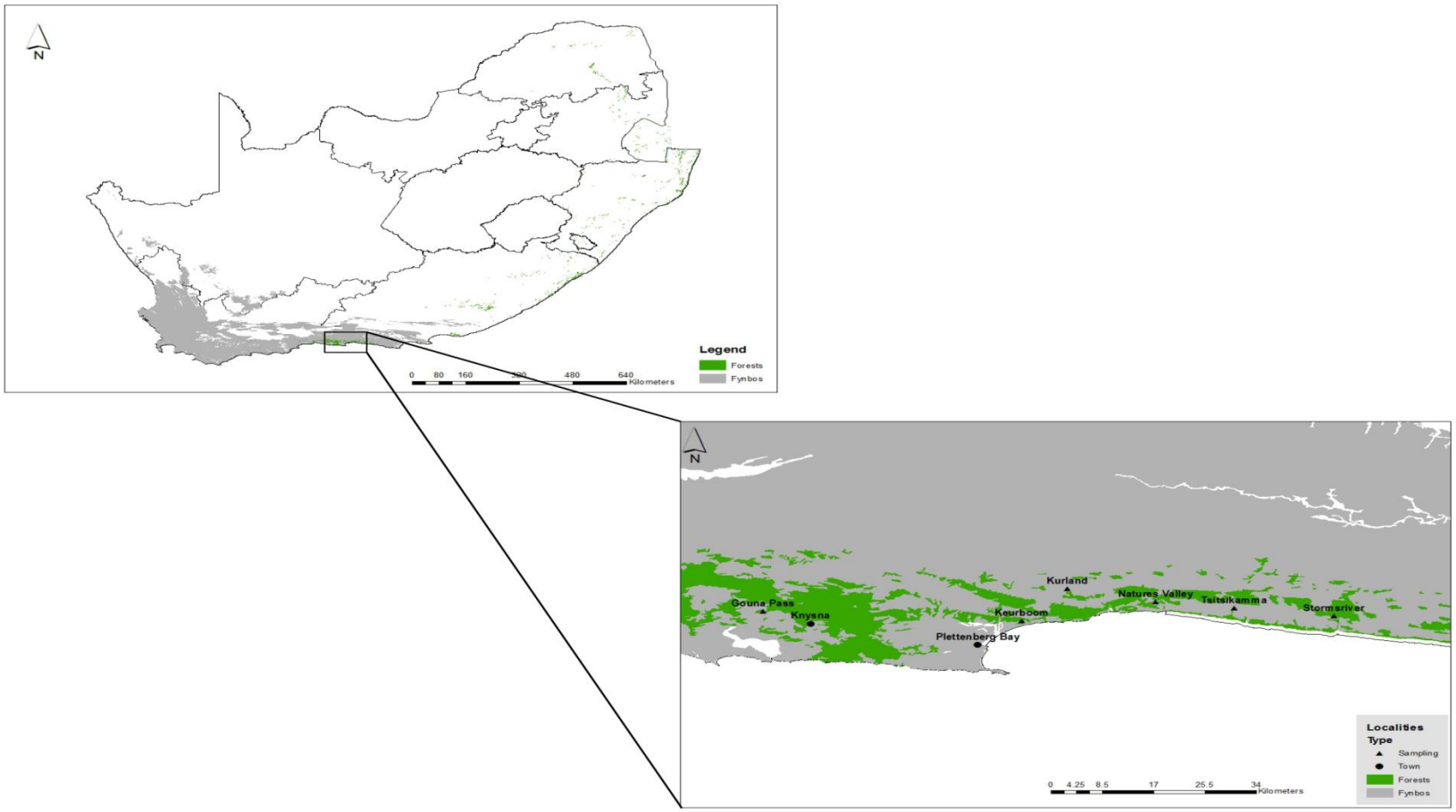

Figure 3-1. Map illustrating the Fynbos biome of the Cape Floristic Regions (grey) of South Africa and its Forest biome (green). Sites sampled are indicated by triangles with the nearest two towns indicated by dot.

2.2 Percentage soil water-content and plant stress

Five soil samples (196.43 cm^3 each), *ca*. 8-10 m apart, were collected at each of the sites (n=6) using a soil auger at 0-10 cm depth, avoiding leaf litter. These samples were collected during December 2012 in mid-summer when conditions were at their driest. Each sample (n=30) was placed separately in moisture proof bags and taken back to the laboratory, where they were weighed, dried for 24 hours in a fan oven at 80 ˚C and weighed again. The drying process was repeated until there was no change in soil weight between drying events. The final dry weight was recorded per sample, the percentage water content calculated and the mean of the five samples per site was used for statistical analyses. The percentage water content was calculated following methods of Hignett & Evett (2005):

$$\text{Water (\%) by mass} = \frac{(X2-X1)}{X2} \times 100$$

Where X_1 = Wet soil mass (g) and X_2 = Final dry mass (g)

Water content data was tested for homogeneity of variances using a Levene's test and based on variances being homogenous. Sites were compared using a One-way Analysis of Variance (ANOVA). Significant differences between means were separated using a Turkey post hoc test. All analyses were conducted using the statistical software package STATISTICA 11 (Statsoft, USA, 2012).

In order to determine if plant individuals experienced drought stress at the drier sites we determined the relationship between leaf $\delta^{12}C/\ \delta^{13}C$ isotope ratio and percentage soil water content for *A. mearnsii* and *V. divaricata* across the sampling range. Water-use efficiency (WUE) can be estimated using carbon isotope discrimination and is therefore a good measure of drought stress. This methodology is based on a higher affinity of the carbon-fixing enzyme (Rubisco) for the more common $\delta^{12}C$ isotope over the less common $\delta^{13}C$ isotope. As the internal CO_2 concentration diminishes in a leaf, the $\delta^{12}C/\ \delta^{13}C$ ratio decreases, which permits less discrimination in favor of $\delta^{12}C$. This lowered internal CO_2 concentration is normally associated with reduced stomatal conductance, which would increase WUE, assuming CO_2 fixation is not primarily limited by other factors (e.g. thermal deactivation of photosynthesis or other metabolic processes). A lower discrimination value would be associated with higher WUE (Richards, 1996) and is indicative of drought. While a higher discrimination value would be indicative of normal plant functioning without drought stress (Farquhar & Richards, 1984).

Leaf carbon stable isotope analyses were performed at the Archeometry Department at the University of Cape Town, South Africa. These values were expressed relative to Pee-Dee Belemnite (PDB) standard for $\delta^{13}C$ and relative to atmospheric nitrogen for $\delta^{15}N$, as (%), according to the following equation:

$$\delta Z=(R_{sample}/R_{standard} - 1) \times 1000$$

where Z is the heavy isotope of either nitrogen or carbon, and R is the ratio of heavier to lighter isotope for the sample and standard ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Oven-dried plant components were milled in a Wiley mill using a 0.5 mm mesh (Arthur H Thomas). Between 2.10 and 2.20 mg of each sample was weighed into 8 mm x 5 mm tin capsules (Elemental Microanalysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany). Samples were weighed to an accuracy of 1 microgram. The sample cups were then enclose and combusted in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit. Three in-house standards (Merck Gel, Lentil, *Acacia saligna* (Labill.) H.L.Wendl) were used and calibrated against IAEA (International Atomic Energy Agency) standards. Leaf material used for this analysis were collected as outlined below. Leaf $\delta^{12}C/\ \delta^{13}C$ ratio of ca. 2g leaf material was measured per tree individual and the mean $\delta^{12}C/\ \delta^{13}C$ per site (n= 6) was correlated with mean site percentage soil water content (n= 6) using Pearson product-moment correlation in the software program STATISTICA 11 (Statsoft, USA, 2012).

2.3 Leaf nutrient content

Fully expanded fresh leaves were collected from five randomly chosen trees of each species at each site and placed in brown paper bags (three branches per tree). These leaf samples were oven dried at 72˚C for 2 days, where after they were milled and sealed into plastic tubes for later analysis of %P, %N and %C content. Phosphorous concentration was determined by an external laboratory (Elsenburg Laboratory services, Stellenbosch) using inductive coupled mass spectrometery (ICP-MS). The $\delta^{15}N$ and N concentration analyses were carried out at the Archeometry Department, University of Cape Town. The isotopic ratio of $\delta^{15}N$ was calculated as δ=1000‰ (Rsample/Rstandard), where R is the molar ratio of the heavier to the lighter isotope of the samples and standards were as defined by Farquhar *et al.* (1989). Between 2.1 and 2.2 mg of each milled sample were weighed into 8 mm x 5 mm tin capsules (Elemental Micro-analysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen,

Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyzer (Fisons instruments SpA, Milan, Italy). The $\delta^{15}N$ values for the nitrogen gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift, and were two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO4$. This analysis provided both $\delta^{15}N$ and N concentration values.

Leaf nutrient content for the two host plants were compared by using a Mann-Whitney U test for P (non-parametric datum) and a t-test for %N and %C (parametric data). These analyses were done following a distribution fitting in the statistical software STATISTICA 11 (Statsoft, USA, 2012).

2.4 Disease development

Trees of similar stem diameter of each species were selected at random (n=10) and wounded during November 2012, when temperatures were between 15˚ C and 26 ˚C (Bond, 1981). Five of each species representing the same individuals used for determining leaf nutrient content and $\delta^{12}C$/ $\delta^{13}C$ isotope ratios were sampled. Wounds (*ca*. 4cm by 7 cm) were created on the trunk of these trees at breast height following methods of Kamgan *et al.* (2008). This wounding method creates a wound by lifting bark to expose the cambium of the tree and leaves a bark flap over the wound to retain moisture within the wound to enhance fungal infection.

In order to determine the identity of the most commonly encountered wound-associated fungi on these individuals, bark and wood samples were taken from these wounds six weeks later, placed in brown paper bags and fungi were isolated in the laboratory. Bark and wood samples were studied using a light microscope (Leica Microsystems, Schweiz) AG, Taiwan) to identify any fungal structures and mycelial growth. Samples without structures were placed in moisture chambers for a few days, whereafter they were inspected for fungi. Most samples were covered with structures that resembled ophiostomatoid fungi. A single spore drop was collected from the apices of the ascomata using a sterile needle and plated on Petri dishes containing 2% malt extract agar (MEA: 20g malt extract, 15g agar/ 50g, Biolab, Midrand, South Africa and 1 Lt deionised water) with 0.05g/l streptomycin (SIGMA-ALDRICH, Steinheim, Germany). Plates were incubated at room temperature (25˚C) for seven days and

sub-cultured to obtain pure cultures. Cultures were grouped into morpho-species according to cultural characteristics and micro-morphology as determined by the use of a Leica EZ4 microscope (Leica Microsystems (Schweiz) AG, Taiwan). Three representative isolates were chosen from each morpho-species and used for molecular identification. All representative cultures were submitted to the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

The selected isolates were grown on 2% MEA for 7-10 days. Mycelium was collected using a sterile scalpel and placed in a 1.5ml Eppendorf tube per isolate. DNA was extracted using a Sigma-Aldrich™ plant PCR kit (USA) using the manufacturer's protocol. The nuclear ribosomal internal transcribed spacer region (ITS1, ITS2), including the 5.8S gene of the rDNA, were amplified using primers ITS1-f (Gardes and Bruns 1993) and ITS4 (White *et al.*, 1990). The 20 µL PCR reaction volumes used contained 10 µL ddH_2O, 5 µL REDExtract-N-Amp PCR ready mix (Sigma-Aldrich™, USA), 4 µL extracted fungal DNA and 0.5 µL (10mM) of each primer. DNA was amplified using a Gene AmpR, PCR System 2700 thermal cycler (Applied Biosystems, Foster City, U.S.A.). PCR reaction conditions were: 2 min of initial denaturing at 95°C, followed by 35 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 55°C and 1 min 30 seconds elongation at 72°C and a final elongation step at 72°C for 8 min. PCR products were separated by agarose gel electrophoresis (1.5 % agarose gel containing ethidium bromide) and visualized under ultraviolet light. The amplified PCR products were purified and sequenced at the Stellenbosch University Central Analytical Facility, Stellenbosch, South Africa. Sequences obtained during this study were compared to published sequences using the BLAST (Basic Logical Alignment Search Tool) algorithm in GenBank (http://www.ncbi.nlm.nih.gov/genbank).

After 9 months (during August 2013) we returned to the wounded trees, removed the bark from the diseased areas around the wound and measured the length of the lesions that resulted from infection by measuring the length of the initial wound, the length of the lesion (dark stained wood) and calculating the difference in length (Matusick *et al.*, 2010). Bark and wound samples were taken from the expansion front of the lesion. Fungi were isolated from this material and identified following methods outlined above.

In order to test for the possible effect of plant age on disease development we determined the diameter of wounded individuals of each species (n= 53) and correlated this to change in

lesion length. Changes in lesion length using individual trees were compared between the two tree species using a Mann-Whitney U test following distributions fitting.

2.5 Herbivore collection

The same individual trees that were used for the wounding experiment were used to sample foliage-associated arthropods using a vacuum sampler. The vacuum sampler was constructed from a Stihl SH 86 leaf blower/vacuum (Stihl, Germany) with a 15 cm diameter nozzle fitted with a collection net as described by Stewart & Wright (1995) (Van der Colff *et al.*, 2013, Chapter 2). Herbivore sampling was conducted during November prior to wounding of the trees. Fifty branches on each tree were sampled by placing each branch within the vacuum nozzle for 3 seconds (Richmond & Graham, 1969). The contents collected per tree was emptied into a reusable plastic bag and frozen until further analyses. Herbivorous arthropods, identified based on their family identity and mouth parts (Labandeira, 1997), were removed from these samples and their abundances were determined following methods of Van der Colff *et al.* (2013, Chapter 2).

To compare arthropod abundance between the two host plants we used Generalized Linear Models (GLZ) with Poisson distribution (with log-link functions as this were count data) (O'Hara, 2009; Zuur *et al.*, 2010) in STATISTICA 11 (Statsoft, USA, 2012). The Wald x^2 (Z) tatistic was calculated using the quasi-likelihood technique, given that the analysis showed no over-dispersion of variances compared to the models (Bolker *et al*, 2009).

2.6 Influence of nutrient levels and soil moisture content on herbivore abundance and lesion development

To test the influence of each of the factors (%N, %P, %C and % soil water content) on herbivore abundance and change (Δ) in lesion length, respectively, GLZ analyses were performed in STATISTICA 11 (Statsoft, USA, 2012). Before analysis, all predictor variables (%N, %P, %C and soil water content) were assessed for any correlations. A strong correlation was found between %N and %P for *A. mearnsii*. Therefore %P was removed as a predictor variable and only %N and %C were used in the model (Bolker *et al.*, 2009). Correlation analysis for nutrient levels in *V. divaricata* found a strong correlation between %P and %C, thus %C was removed from the analysis and only %P and %N was used in this model (Bolker *et al.*, 2009).

Soil water content data (%) was only available as an average per site, while leaf nutrient, lesion length and herbivore abundance data were available for each individual tree. Therefore a separate model was prepared for this predictor variable using mean herbivore abundance and lesion length data for each plant taxon at each site. The following six GLZs were build: **1**) to assess the effect of leaf nutrient levels on changes in lesion length within each individual tree of *A. mearnsii* (Δ lesion length ~ %N + %C), **2**) to assess the effect of % soil water content on lesion lengths across sampled sites of *A. mearnsii* (Δ lesion length ~ % soil water content), **3**) to assess the effect of nutrient levels on herbivore abundances within each individual tree of *A. mearnsii* (herbivore abundance ~ %N + %C), **4**) to assess the effect of leaf nutrient levels on change in lesion lengths within each individual tree of *V. divaricata* (Δ lesion length ~ %N + % P + % N*% P), **5**) to assess the effect of % soil water content on lesion lengths across sampled sites of *V. divaricata* (Δ lesion length ~ % soil water content) and to **6**) to assess the effect nutrient levels on herbivore abundances within each individual tree of *V. divaricata* (herbivore abundance ~ %N + % P + % N*% P). The Wald statistic was used to model the data and a p-value was calculated (McCulloch *et al.*, 2008). Model selection was done based on the lowest AIC value (Bolker *et al.*, 2009). All analyses were conducted in STATISTICA 11 (Statsoft, USA, 2012).

3.Results

3.1 Percentage soil water-content and plant water stress

The leaf $\delta^{13}C$ ratio of *A. mearnsii* was not significantly correlated with percentage soil water content across the sampling sites, while leaf $\delta^{13}C$ ratios of *V. divaricata* was significantly and positively correlated with percentage soil water content (Figure 3-2). Lower negative $\delta^{13}C$ ratio values were found in the leaves of plants growing at the drier sites. The drier sites included Gouna, Keurboom and Kurland, confirming the existence of a moisture gradient form west to east across the sampling range.

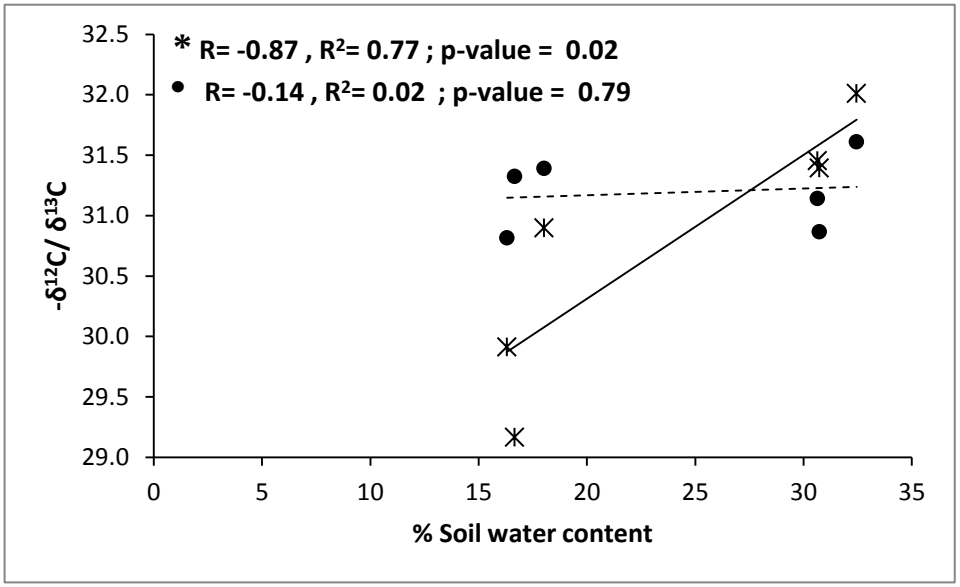

Figure 3-2 The relationship between $\delta^{13}C$ isotope ratio and percentage soil water content for *A. mearnsii* (• & dotted line) and *V. divaricata* (* & solid line) across the sampling range.

3.2 Leaf nutrients

Fewer samples were successfully analyzed for %P content than for other nutrients, resulting in the sample size for %P determination being smaller than that of %N and %C. For analyses where %P was included, we therefore only used data from samples that had the full complement of nutrient data available. Subsequently *A. mearnsii* had 15 full samples and *V. divaricata* 17 full samples that were used in statistical analyses. There was no significant difference in leaf %N (t-value = -0.81; df= 30, p-value = 0.097) and %P (Z-statistic = 0.89; p-value= 0.37) between the two tree species. However, a significant difference in leaf %C (t-value = -2.98, df = 56, p-value = 0.004) was observed, with *A. mearnsii* having higher a concentration than *V. divaricata* (Figure 3-3).

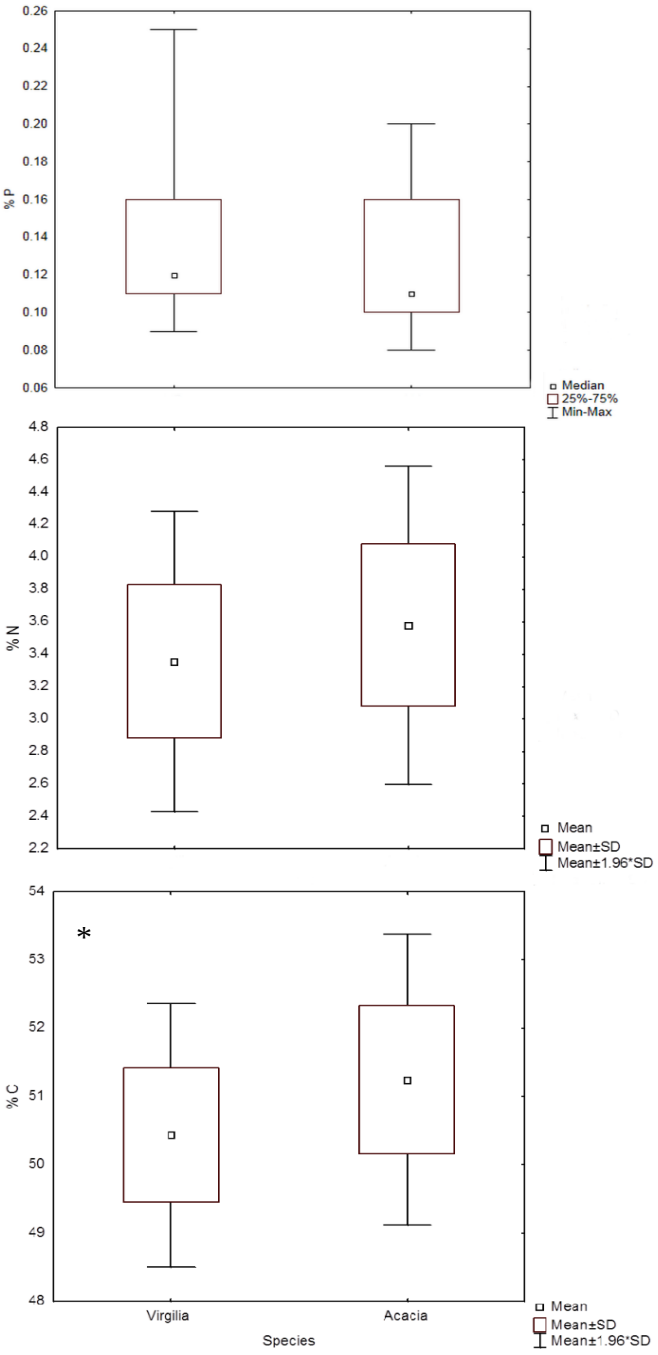

Figure 3-3 Percentage P, C and N for *V. divaricata* and *A. mearnsii* leaves. Plots represent medians or means and whiskers represent min/max or mean ± 2* SD. Asterisks * indicate a significant difference between the tree species.

3.3 Disease development

We isolated a range of fungi from the artificial wounds on the two host trees, but only focused on ophiostomatoid fungi in subsequent analyses as these were most common. The following five species were collected: *Ophiostoma quercus* (Georgevitch) Nannfeldt, *Ophiostoma pluriannulatum* (Hedgcock) H. & P. Sydow, *Ceratocystis fimbriata* Ellis &

Halst, *Ceratocystis savannae* Kamgan & Roux and *Ceratocystis tsitsikammensis* Kamgan & Roux. All species were collected from both host tree species. Samples collected during the second sampling session yielded only *O. pluriannulatum*.

There was no significant relationship between tree diameter and lesion length in either *A. mearnsii* or *V. divaricata* (Figure 3-4). We found no significant difference in lesion lengths between *A. mearnsii* and *V. divaricata* for individual trees (Z-statistic = -0.19, p-value = 0.84) (Figure 3-5).

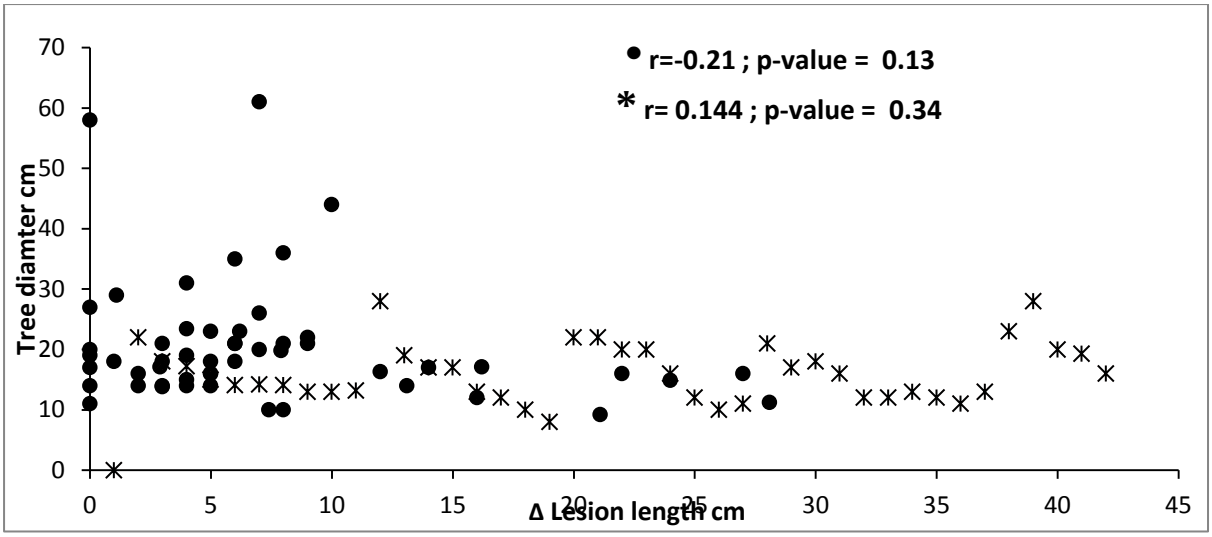

Figure 3-4 Correlation between lesion length resulting from wounds created on bark of *Acacia mearnsii* (•) and *Virgilia divaricata* (*) and tree age (tree diameter at breast height).

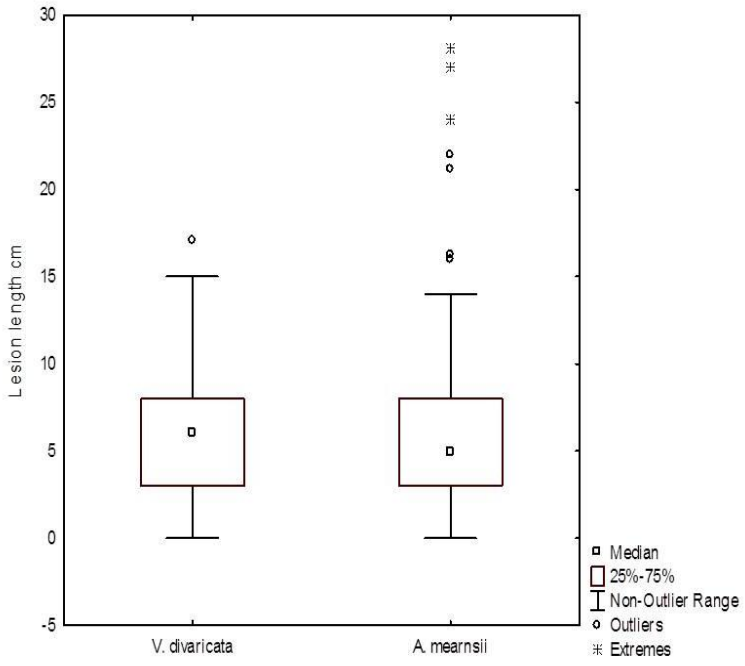

Figure 3-5 Change in lesion length after wounding of *Acacia mearnsii* and *Virgilia divaricata* (n=53 per species).

3.4 Herbivore collection

A significant difference in herbivore abundance was found between the two host plants based on GLZ analyses (Wald statistic = 249.8; p-value = <0.0001). Higher numbers of herbivores were present on the native tree species *V. divaricata* than on the invasive *A. mearnsii* (Figure 6) (n= 53 per species) (Figure 3-6).

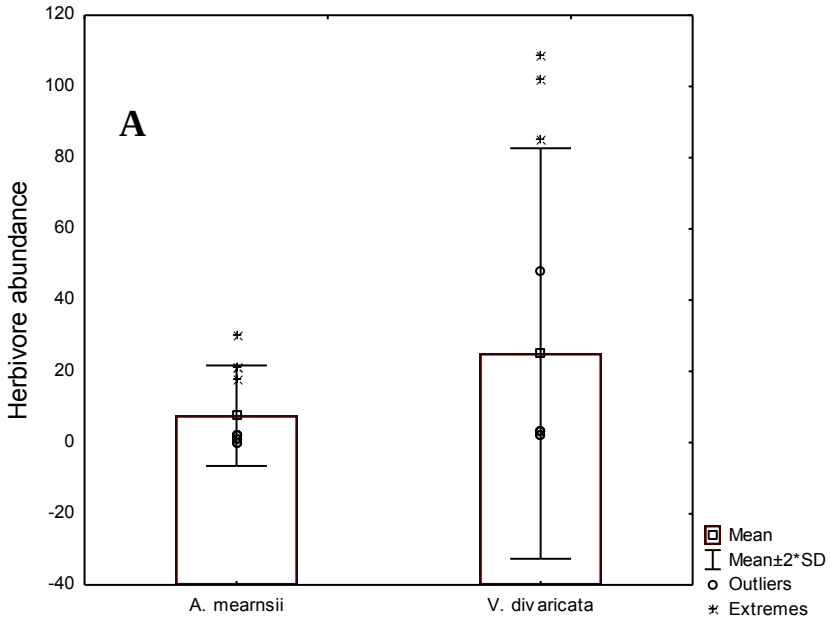

Figure 3-6 Herbivore abundances associated with foliage of V. divaricata and A. mearnsii in the Garden Route National Park. Differences in herbivore abundances are compared using a Generalized Linear Model (GLZ) using a Poisson distribution with a log link function. Means, outliers and extreme values are plotted. Alphabetic letter (**A**) indicates significant difference.

3.5 Influence of water stress and nutrient content on herbivore loads and lesion length

There was no correlation between any of the leaf nutrients and percentage water content in either tree species. Thus these response variables operate independently (data not shown). Changes in lesion length was unaffected by soil water content in both tree species. *A. mearnsii* trees had larger changes in lesion length at high nutrient levels, with a strong positive correlation with %C and %N. In contrast, herbivore abundance was significantly negatively related to %N and %C content of leaves in this species (Table 3-1). No significant effect of leaf nutrient levels on herbivore abundance or lesion length formation was detected for *V. divaricata*. There was, however, a significant negative correlation between herbivore numbers and soil water content in *V. divaricata*.

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Table 3-1 Results of generalized linear models for herbivore abundance using Poisson distribution and log-link function and change in lesion length using normal distribution with log link function, showing the influence of leaf nutrient content (% N, % P and % C) and % soil water content on herbivore abundances and changes in lesion length in the two tree species.

Change in lesion length	***A. mearnsii***			
	df	Wald statistic	p-value	Direction
% N (n=29)	1	**5.41**	**0.02**	**+**
% C (n=29)	1	**23.23**	**<0.05**	**+**
Soil H_2O content (n=6)	1	1.01	0.31	NA
Herbivore abundance				
% N (n= 29)	1	**7.66**	**0.01**	**–**
% C (n=29)	1	**2.44**	**0.12**	**–**
Soil H_2O content (n=6)	1	0.46	0.50	NA
Change in lesion length	***V. divaricata***			
% N (n=17)	1	0.39	0.53	NA
% P (n=17)	1	0.00	0.95	NA
% N * % P (n=17)	1	0.32	0.57	NA
Soil H_2O content (n=6)	1	0.15	0.70	NA
Herbivore abundance				
% N (n=15)	1	0.01	0.91	NA
% P (n=15)	1	3.31	0.07	NA
% N * % P (n=15)	1	1.71	0.19	NA
Soil H_2O content (n=6)	1	**8.39**	**<0.05**	**–**

4.Discussion

Results of this study indicate that the native *V. divaricata* experienced water stress at the drier sites, while the invasive species did not. *A. mearnsii* therefore seems to be much more drought adapted than the native species. This is not surprising, as this species is well-known to be well-adapted to drought conditions (Morris *et al.*, 2011; Crous *et al.*, 2012). However, both tree species appear to have strategies to alleviate drought symptoms, as vascular infecting fungi (e.g. ophiostomatoid fungi) are particularly effective when infecting water-stressed plants (Pegg, 1985, Desprez-Loustau *et al.*, 2006), but plants at drier sampling sites did not show greater symptomatic response to such organisms. Drier conditions expected under current climate change models (IPCC, 1996; 2001; Taylor & Kumar, 2013) may therefore not influence the susceptibility of these plants to wound infecting fungi.

In contrast to disease development, herbivore numbers on *V. divaricata* were significantly and negatively correlated to soil moisture content. Therefore, herbivores seemed to prefer plants that experienced drought. We therefore find support for the PSH (White, 1990) in *V. divaricata* as herbivores were abundant where plants were stressed. This was not the case for *A. mearnsii* that experienced similar herbivore pressure at all sites. This indicates that *V. divaricata* may become more vulnerable to herbivores if climatic conditions became drier than the invasive species (Taylor & Kumar, 2013). The two host trees were remarkably similar in terms of leaf nutrient levels and their response to infection by the same fungal taxa. These plants are also known to share many arthropods and, in this study, we have also shown that they share similar fungal communities. Therefore, the ecological similarities between these two plant species are striking. If *V. divaricata* would experience more stress than *A. mearnsii* under drier conditions, it would give the invasive species a competitive advantage over the ecologically similar native species and effectively outcompete it. This would likely have great ecological consequences as, for example, seedlings of native trees cannot establish in *A. mearnsii* stands (Stinson *et al.*, 2006; Van der Waal, 2009; Richardson & Rejmánek, 2011; Coetsee & Wigley, 2013), which may lead to a net decrease in forest patch size.

Interestingly, herbivore abundance and disease development were significantly influenced by plant nutrient content in *A. mearnsii* and not in *V. divaricata*. The pests and pathogens on *V. divaricata* seems to be well-adapted to it (McMahon, 2012) as their presence and usage of this tree lies beyond the nutritional value and may rather be related to historical associations (Keane & Crawley, 2002). For *A. mearnsii*, nutrient poor trees experienced higher herbivore

loads and slower disease development than nutrient rich trees. Therefore, the susceptibility of *A. mearnsii* seems to be determined by plant nutrient levels. Nutrient content was not correlated to water availability in this study and is therefore determined by other factors not measured here. Factors that may account for this could include differences in soil nutrient levels, differences in micro-climatic conditions and/or plant genotypic variation (McMahon, 2006).

The positive relationship found between lesion length development and plant nutrient contents in *A. mearnsii* is in line with previous studies that show that some fungal species prefer plants with higher nutrient content, as they receive higher quality nutrition (Desaeger *et al.*, 2004). Within the xylem and phloem of their host plants, these fungi have access to nutrient-rich sap, enabling them to spread through the plant (Pegg, 1985). An excess of N in plant tissue has been shown to cause imbalances in other nutrients, decreasing the ability of the plant to produce defense molecules (McMahon, 2012). The negative association between arthropod abundance and plant nutrient contents found in *A. mearnsii* may be linked to weakened herbivore defenses (McMahon, 2012).

Five ophiostomatoid fungi were isolated in this study. *O. quercus* has been isolated from a range of native hardwood and softwood tree species in South Africa (De Beer *et al.*, 2003) as well as from non-native trees, causing sap stain (De Beer *et al.*, 1995; Kamgan *et al.*, 2008). *O. pluriannulatum s.l.* forms part of a large species complex (Kamgan *et al.*, 2008) and was originally collected from *Quercus borealis* Michx. f. & Q in the United States of America (Hedgcock, 1906). In South Africa *O. pluriannulatum* is known from a range of hardwood trees (Farrell *et al.*, 1997; Zhou *et al.*, 2001; Zhou *et al.*, 2004; Kamgam *et al.*, 2008; Machingambi, 2013). The *C. fimbriata s.l.* species complex constitutes one of the two large phylogenetic groups within the genus *Ceratocystis*, forming a distinct monophyletic group (Wingfield *et al.*, 2006; Kamgan *et al.*, 2008). A previous study found *C. fimbriata* isolated in South Africa to group separately from isolates collected in the Northern Hemisphere (Wingfield *et al.*, 1996). *C. tsitsikammensis* closely related to *C. fimbriata*, was originally isolated from native trees in South Africa (Kamgan *et al.*, 2008), and has been identified as a potential pathogen on *Rapaneae melanophloeos* (L) Mez. based on controlled greenhouse experiments (Kamgan *et al.*, 2008). Trees that had been infected with this fungus had large lesions and the infected trees developed epicormic shoots below the inoculation site (Kamgan *et al.*, 2008). *C. savannae* was originally isolated from native South African trees (*Acacia*

nigrescens Oliv., *Combretum zeyheri* Sond., *Terminalia sericea* Burch. ex.DC, *Sclerocarya birrea* (A.Rich.) Hochst. subsp. caffra (Sond.) Kokwaro, *Burkea africana*Hook.) (Kamgam *et al.*, 2008). This species caused small lesions on *A. nigrescens* and *S. birrea* trees, displaying some level of pathogenicity. All ophiostomatoid species isolated in this study were found on both tree species within the sampled range. This illustrates the potential for movement of native pathogens onto invasive / non-native plant species. This is important to both the forestry sector and conservation of native plant species. Foresters should be informed about the pest and pathogens associated with the adjacent native tree species, to take preventative actions to infection and attack. Since a large number of plantation species has become invasive in South Africa, we need to know the pests and pathogens that they can host and spread within natural habitats, especially considering that they transverse multiple habitats.

5.Conclusion

The effect that the environment has on disease development and pest attack is important, since environments are predicted to become drier and soil nutrient levels may change with the influx of new sources of nutrients such as invasive legumes. How native and invasive species respond to these changes influences their interaction with their pests and pathogens. *V. divaricata* was affected by drought conditions and with drying climatic conditions, this may render it a weaker competitor against *A. mearnsii* invasion. While *V. divaricata* is well-adapted to fungal infection during stressed conditions, *A. mearnsii* is affected by nutrient content in both disease development and herbivore attack. Where *A. mearnsii* occurs in sites with limited nutrients and are exposed to these pest and pathogens, they could potentially serve as sources of natural biological control organisms.

6.References

Agios, G.N. 2005. Plant Pathology. UK: Elsevier Academic Press Publication.

Amos, G.L. 1954. Radial fissures in the early wood of conifers. Australian Journal of Botany *2:* 22–34.

Ayres, M.P. & Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance form herbivore and pathogens. The Science of the Total Environment 262: 263–286.

Auerbach, M. & Simberloff, D. 1989. Rapid leaf-miner colonization of intreocued trees and shifts in sources of herbivore mortality Oikos 52: 41–50.

Barnes, I., Nakabonge, G., Roux, J., Wingfield, B.D. & Wingfield, M.J. 2005. Blackwell Publishing, Ltd. Comparison of populations of the wilt pathogen Ceratocystis albifundus in South Africa and Uganda. Plant Pathology 54: 189–195.

Beukes, C.W., Law, I.J., Venter & Steenkamp, E.T. 2011. Phylogenetic characterisation of root-nodule bacteria associated with indigenous fynbos legumes. In Villet, M.H. and Crous, K.L. 2011. Proceedings of the Congresses of Southern African Society for Systematic Biology.

Bjorn, S. 2013. Uncertain then, irrelevant now. Nature 47 (503) 7474. Academic One File.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24: 127–135.

Bond, W.J. 1981, Vegetation gradients in the southern Cape mountains. MSc thesis, University of Cape Town.

Brasier, C.M. & Kirk, S.A. 1993. Sibling species within *Ophiostoma piceae*. Mycological Research 97: 811–816.

Coetsee, C. & Wigley, B.J. 2013, *Virgilia divaricata* may facilitate forest expansion in the afrotemperate forests of the southern Cape, South Africa. Koedoe 55: 1–8.

Crous, C.J., Jacobs, S.M. & Esler, K.J. 2012. Drought-tolerance of an invasive alien tree, *A. mearnsii* and two native competitors in fynbos riparian ecotones. Biological Invasions. 14: 619–631.

Desaeger, J., Meka, R. Rao, & Bridge, J. 2004. Nematodes and Other Soilborne Pathogens in Agroforestry. In Below Ground Interactions in Tropical Agroecosystems: Concepts and Models with Multiple Plant Components, edited by Meine van Noordwijk, Georg Cadisch and C.K. Ong: CABI.

De Beer, Z.W., Wingfield, M.J. & Kemp, G.H.J. 1995. First report of *Ophiostoma querci* in South Africa. South African Journal of Science 91:1–6.

De Beer, Z.W., Wingfield, B.D. & Wingfield, M.J. 2003. The *Ophiostoma piceae* complex in the southern hemisphere: a phylogenetic study. Mycological Research 107: 469–476.

Department of Agriculture, Forestry and Fisheries. 2009. State of the forest report.

Desprez-Loustau, M., Marcais, B., Nageleisen, L., Piou, D. & Vannini, A. 2006. Interactive effecrs of drought and pathogens in forest trees. Annual Forest Science 63: 597–612.

Dye, P. & Jarmain, C. 2004. Water use by black wattle (*Acacia mearnsii*): implications for the link between removal of invading trees and catchment stream flow response. South African Journal of Science 100: 40–44.

Entry, J.A., Martin, N.E., Cromack Jr., K. & Stafford, S.G. 1986. Light and nutrient limitation in *Pinus monticola*: Seedling susceptibility to *Armillaria* infection. Forest Ecology and Management 17: 189–198.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. Annual Review Plant Physiology 40: 503–537.

Farrell, R.L., Hadar, E., Kay, S.J., Blanchette, R.A. & Harrington, T.C. 1997. Survey of sapstain organisms in New Zealand and albino anti-sapstain fungi. In Biology and prevention of Sapstain, A conference sponsored by the Department of Forest Products, Oregon State University, 25 May 1997, Delta Whistler Resort, Whistler, British Columbia, Canada: 57–62.

Gardes, M. & Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Geldenhuys, C.J. 2004. Concepts and Process to Control Invader Plants in and around Natural Evergreen Forest in South Africa. Weed Technology 18: 1386–1391.

Goldblatt, P. & Manning, J. 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria : National Botanical Institute, Pretoria.

Govender, P. 2007. Status of seedling establishment pest of Acacia mearnsii De Wild. (Mimosaceae) in South Africa. South African Journal of Science 103: 141–147.

Grobbelaar, J.W., Aghayeva, D.N., De Beer, Z.W., Bloomer, P., Wingfield, M.J. & Wingfield, B.D. 2009. Delimitation of Ophiostoma quercus and its synonyms using multu gene phylogenies. Mycological Progress 8: 221–236.

Harrington, T.C. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. In: Vega FE & Blackwell M (eds.) Ecological and Evolutionary Advances in Insect-Fungal Associations, Oxford University Press. 257–291.

Harrington, T.C., Farrell, R., Hofstra, D., McNew, D. & Steimel, J. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93: 111–136.

Hawksworth, D.L. 2001. The magnitude of fugal diversity: the 1.5 million species estimate revisited. Mycology Research.105: 1422–1432.

Heath, R.N., Van Der Linde, M., Groeneveld, H., Wingfield, B.D., Wingfield, M.J. & Roux, J. 2010. Factors influencing infection of *Acacia mearnsii* by the wilt pathogen Ceratocystis albifundus in South Africa. Forest Pathology 40: 500–509.

Hedgcock, G.G. 1906. Studies upon some chromogenic fungi which discolor wood. Annual Report Missouri Botanical Gardens 17: 59–114.

Hedgcock, G.G. 1933. The prevention of wood-staining in basket veneers. Journal of Forestry 31: 416–420.

Henderson, L. 2007. Invasive, naturalized and casual alien plats in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37: 215–248.

Hignett, C. & Evett, S. 2005. Direct and surrogate measure of soil water content, in Lee Kheng Heng, L.K. (eds). Field estimation of soil water content: A practical guide to methods, instrumentation and sensor technology. Vienna: IAEA. 1–21.

Hogg, E.H., Brandt, J.P. & Kochtubajda, B. 2000. Growth and dieback of aspen forest in northwestern Alberta, Canada, in relation to climate and insects. Canadian Journal of Forestry Research 32: 823–832.

Huber, D.M. & Hanekleus, S. 2007. Managing nutrition to control plant disease. 2007. Landbauforschung Volkenrode, 57: 313–322.

Huber, D.M. & Jones, J.B. 2013. The role of magnesium in plant disease. Plant and Soil 368: 73-85.

Huber, D., Romheld, V. & Weinmann, M. 2012. Relationship between nutrition, plant diseases and pests. In Nutritional Physiology. Marschner.

Hunt, J. 1956. Taxonomy of the genus Ceratocystis. Lloydia 19: 1–59.

IPCC. 1996. Climate change 1996—the science of climate change. *Edited by* J.T. Houghton, L.G. Meira Filho, B.A. Callander, N. Harris, A. Kattenberg, and K. Maskell. Contribution of WG1 to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, U.K.

IPCC, Climate Change 2001, The scientific basis, Contribution of Working Group I to the third assessment report of the IPCC, Houghton J.T., Ding Y., Griggs D.J., Noguer M., Van derLinder P.J., Dai X., Maskell K., Johnson C.A. (Eds.), Cambridge University Press, Cambridge, UK

Joubert, C. 2002. Rhizobia associated with (Australian Acacia mearnsii, Acacia dealbata and Acacia decurrens) in South Africa as determined by Sodium Docecyl-Sulphate Polyacrylamide Gel Electrophoresis. Master's Thesis University of Pretoria: Protoria.

Kamgan, N.G., Jacobs, K., de Beer, Z.W., Wingfield, M.J. & Roux, J. 2008. *Ceratocystis* and *Ophiostoma* species including three new taxa, associated with wounds on native South African trees. Fungal Diversity 29: 37–59.

Kamgan, N.G., Wingfield, M.J., Mohammed, C., Carnegie, A.J., Pegg, G.S. & Roux, J. 2012. Ceratocystis species, including two new species associated with nitidulid beetles, on eucalypts in Australia. Antonie van Leeuwenhoek 101: 217–241.

Kashiwagi, J., Krishnamurthy, L., Singh, S., Gaur, P.M., Upadhyaya, H.D., Panwar, J.D.S., Basu, P.S., Ito, O. & Tobitas, S. 2006. Relationships between transpiration efficiency and Carbon isotope discrimination in Chickpea (C. arietinum L).

Kawakita, K. & Kojima, M. 1983.The isolation and properties of a factor in taro tuber that agglutinates spores of *Ceratocystis fimbriata*, black rot fungus. Pl. Cell PhysioL 24: 41–49

Kawakita, K. & Kojima, M. 1986.Possible involvement of surface substances on germinated spores of *Ceratocystis fimbriata*, black rot fungus, in determination of host-parasite specificity. Agric. BioI. Chern. 50: 431–436

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.

Kile, G.A. 1993. Plant diseases caused by species of *Ceratocystis* sensu stricto and *chalara*. In: *Ceratocystis* and *Ophiostoma*: Taxonomy, Ecology and Pathogenicity: (eds. M.J. Wingfield, K.A. Seifert and J.F. Webber). American Phytopathological Society Press, St. Paul, Minnesota: 173–183.

Kojima, M. & Uritani. 1974. The possible involvement of a spore agglutinating factor in various plants in establishing host specificity by various strains of black rot fungus. PI. Cell Physial. 15: 733–737

Kojima, M., Kawakita, K. & Uritani. 1982. Studies on a factor in sweet potato root which agglutinates spores of *Ceratocystis fimb,iata*, black rot fungus. PI. Physiol. 69: 474–478

Labandeira, C.C. 1997. Insect mouthparts: Ascertaining the paleobiology of insect feeding strategies. Annual Review of Ecology and Systematics 28: 153–193

Lagerberg, T., Lundberg, G. & Melin, E. 1927. Biological and practical researches into blueing in pine and spruce. Svenska Skogsvardsfo¨reningens Tidskrift 25: 145–272.

Machingambi, N.M., Roets, F., Dreyer, L. L. & Roux, J. 2013. An investigation into the death of native Virgilia trees in the Cape Floristic Region of South Africa. Master Thesis. Stellenbosch: University of Stellenbosch.

Marmolejo, J.G. & Garcıa-Ocanas, F. 1993. Contribuciones micologicas en homenaje al biologo Jose Castillo Tovar por su labor en pro de la micologia mexicana. Reporte Cientifico, No. Especial 13: 155–170.

Matusick, G., Eckhardt, L.G. & Somers, G.L. 2010. Susceptibility of longleaf pine roots to infection and damage by four root-inhabiting ophiostomatoid fungi. Forest Ecology and Management 260: 2189–2195.

Mbambezeli, G., & Notten, A. 2003. Virgilia genus. http://www.plantzafrica.com/planttuv/virgilia.htm

McCulloch, C.E., Searle, S.R. & Neuhaus, J.M. 2008. Generalized, linear,and mixed models, 2nd edn. Wiley, USA

McMahon, P. 2012. Effect of nutrition and soil function on pathogens of tropical tree crops, in Cumagun, C.J.R. (eds.) Plant Pathology. Croatia. 241–263

Morris, T.L., Esler, K.J., Barger, N.N., Jacobs, S.M. & Cramer, M.D. 2011. Ecophysiological traits associated with the competitive ability of invasive Australian acacias. Diversity and Distributions. 17: 898–910.

Murdock, T.Q., Taylor, S.T., Flower, A., Mehlenbacher, A, Montenegro, A., Zwiers, F.W., Alfaro, R. & Spittlehouse, D.L. 2013. Pest outbreak distribution and forest management impacts in a changing climate in British Columbia. Environmental Science & Policy 26: 75–89.

Oliveira, C.M., Auad, A.M., Mendes, S.M. & Frizzas, M.R. 2014. Crop losses and the economic impact of insect pests on Brazilian agriculture. Crop Protection 56: 50–54.

Pamela, P., Dubois, T., Coyne,D. & Viljoen,A. 2008. Defense-Related Gene Expression in Susceptible and Tolerant Bananas (*Musa* Spp.) Following Inoculation with Non-Pathogenic *Fusarium Oxysporum* Endophytes and Challenge with *Radopholus Similis*. Physiological and Molecular Plant Pathology 71: 149–57.

Pegg, G.F. 1985. Life in a black hole-The micro-environment of the vascular pathogen. Transactions of the British Mycological Society 85: 1–20.

Prell, H.H. & Day, P.R. 2001. Plant-Fungal Pathogen Interaction: A Classical and Molecular View Berlin: Springer, Berlin.

Richardson, D.M. & Rejmánek, M. 2011. A global review of trees and shrubs as invasive species. Diversity and Distributions, 17: 788–809.

Richmond, C.A. & Graham, H.M. 1969. Two methods for operating a vacuum sampler to sample populations of the cotton leafhopper on wild hosts. Journal of Economic Entomology 62: 525–526.

Rodrìguez-Echeverrıa S. Le Roux J J. Criso´ stomo J A. & Ndlovu J. 2011. Jack-of-all-trades and master of many? How does associated rhizobial diversity influence the colonization success of Australian Acacia species?. Diversity and Distributions. 17: 946–957.

Roux, J., Dunlop, R. & Wingfield, M. J. 1999. Susceptibility of elite Acacia mearnsii families to Ceratocystis wilt in South Africa. Journal of Forestry Research 4: 187–190.

Roux, J., Meke, G., Kanyi, B., Mwangi, L., Mbaga, A., Hunter, G.C., Nakabonge, G., Heath, R.N. & Wingfield, M.J. 2005. Diseases of plantation forestry trees species in Eastern and Southern Africa. South African Journal of Science 101: 409–413.

Roux, J. & Wingfield, M.J. 1997. Survey and virulence of fungi occurring on diseased *A. mearnsii* in South Africa. Forest Ecology and Management 99: 327–336.

Searle, S. 1997. *Acacia mearnsii* De Wild. (black wattle) in Australia. Black wattle and its utilizations.Barton, ACT: Rural Industries Research and Development Corporation 1–10.

Seifert, K.A. 1993. Sapstain of commercial lumber by species of Ophiostoma and Ceratocystis. In: Wingfield MJ, Seifert KA, Webber JF (eds.), Ceratocystis and Ophiostoma: taxonomy, ecology, and pathogenicity. American Phytopathological Society, St. Paul, pp. 141–151.

Spectrum. 2013. The relationship between nutrients and other elements to plant diseases. Agronomic library. Spectrum Analytic excellence testing. www.spectrumanalytic.com

Stewart, A.J.A. & Wright, A.F. 1995. A new inexpensive suction apparatus for sampling arthropods in grassland. Ecological Entomology. 20: 98–102.

Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D., Klironomos, J.N. 2006. Invasive Plant Suppresses the Growth of Native Tree Seedlings by Disrupting Belowground Mutualisms: 1–5.

Tye, D.R.C., Drake, D.C., 2012. An exotic Australian Acacia fixes more N than a coexisting indigenous Acacia in a South African riparian zone. Plant Ecology 213: 251–257.

Uzunovic, A. & Webber, J.F. 1998. Comparison of bluestain fungi grown in vitro and in freshly cut pine billets. European Journal of Forest Pathology 28: 322–334.

Van der Bank, H., Van wyk, B. & Van der Bank, M. 1996. Genetic variation within and geographical relations beteen four natural populations of Virgilia oroboides (Tribe Podalyrieae: Fabaceae). Biochemical Systematic sans Ecology 24: 135–143.

Van der Colff, D., Roets, F., Dreyer, L.L. & Valentine, A. 2013. Plant stress and the prevalence of pest and pathogens associated with a native and an invasive alien legume tree in the Cape Floristic Region, South Africa. MSc Thesis. Stellenbosch University. Stellenbosch.

Van der Waal, B.W. 2009. The influence of Acacia mearnsii invasion on soil properties in the Kouga mountains, Eastern Cape , South Africa. MSc thesis. Rhodes University.

Villari, C., Battisti, A., Chakraborty, S., Michelozzi, M., Bonello, P. & Faccoli, M. 2012. Nutritional and pathogenic fungi associated with the pine engraver beetle trigger comparable defenses in Scots pine. Tree physiology: 1–13.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York: 315–322.

Wingfield, B.D., Roos, H., Van Wyk, M. & Wingfield, M.J. 2006. Species of *Ceratocystis*: Emerging evidence for discrete generic boundaries. In: Ophiostomatoid fungi: Expanding frontiers, 16- 18 August 2006, North Stradbroke Island Brisbane, Australia, Abstract: 19.

Wingfield, M.J., De Beer, C., Visser, C. & Wingfield, B.D. 1996. A new Ceratocystis species defined using morphological and ribosomal DNA sequence comparisons. Systematic and Applied Micology 19: 191–202.

Wingfield, M.J., Slippers, B., Roux, J. & Wingfield, B.D. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the Southern hemisphere. BioScience 51: 134–140.

Witthuhn, R.C., Harrington, T.C., Wingfield, B.D. & Wingfield, M.J. 1999. PCR-based identification and phylogeny of species of *Ceratocystis sensu stricto*. Mycological Research 103: 743–749.

Yang, N., Zang, L., Wang, S., Guo, J., Xu, H., Zhang, F. & Wan, F. 2014a. Biological pest management by predators and parasitoids in the greenhouse vegetables in China. Biological Control 68: 92–102.

Yang, Z., Wang, X. & Zhang, Y. 2014. Recent advances in biological control of important native and invasive forest pests in China. Biological Control 68: 117–128.

Yasuda, K. & Kojima, M. 1986. The role of stress merabolites in establishing host-parasite specificity between sweet potato and *Ceratocystis fimbriata,* black rot fungus. Agricultur and Biological Chemistry 50: 1839-1846.

Zhou, X., De Beer, Z.W., Cibrian, D., Wingfield, B.D. & Wingfield, M.J. 2004. Characterization of Ophiostoma species associated with pine bark beetles from Mexico, including O. pulvinisporum sp. nov. Mycology Research 108: 690–698.

Zhou, X.D., De Beer, Z.W., Wingfield, B.D. & Wingfield, M.J. 2001. Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. Sydowia 53: 290–300.

Chapter 4

Comparison between N and P cycling abilities of invasive Acacia mearnsii and native Virgilia divaricata trees growing sympatrically in forest margins in South Africa.

Abstract

Australian acacias have significant impacts on the habitats that they invade. They increase the nutrient input in the invaded habitat and alter nutrient cycles. Here we assessed how the nutrient economies of two related legumes compared where they co-occur within forest margins, a habitat significantly understudied in South Africa. We assessed how *Acacia mearnsii* and *Virgilia divaricata*, both nodulating legume trees with similar growth forms, compared in terms of N and P nutrient content, nutrient resorption and their Biological Nitrogen Fixation (BNF) capacities. Fresh and senesced leaf samples were collected with leaf traps in sympatric populations, and analyzed for N and P concentrations. We also measured the δ15N isotope ratio, and used it to calculate percentage nitrogen derived from the atmosphere (% NDFA). The two species proved to be very similar in their nutrient content, but they differed in their use of BNF. Our results present a record of nutrient cycling in forest margins in the largest forest complex in South Africa (Knysna Afromontane forest complex). It provides information on nutritional differences between these two ecologically similar species. Our results also provide insight into the nutritional strategies of *A. mearnsii*, a well-studied invasive alien plant in South Africa.

Key words: nutrient cycling, δ15N isotope ratio, Biological Nitrogen Fixation, *Acacia mearnsii*, *Virgilia divaricata*

1.Introduction

Invasion is recognized as the second largest threat to biodiversity (Mooney and Hobbs, 2000; Secretariaton the Convention on Biological Diversity, 2001). Invasive alien plant species have a significant impact on the environments that they invade (Pimentel *et al.*, 2001). They can alter nutrient cycling (Witkowski, 1989; Le Maitre *et al.*, 2011), impact water resources (Dye & Jarmain, 2004; Naude *et al.*, 2011) and can be very costly to control (Pimentel *et al.*, 2001). Australian acacias are among the most devastating groups of invasive species, and have been introduced to many countries. They were mostly planted for commercial purposes, but have escaped from plantations and become invasive in many instances (Searle, 1997; Kull & Rangan, 2008).

Acacia mearnsii De Wild. is classified as an invasive alien plant in South Africa (IAP) (Henderson, 2007), as it has overcome all barriers proposed by the unified framework for biological invasion (Blackburn *et al.*, 2011). It is the fifth most widespread IAP in the country and has a large ecological and economic impact on the natural environment (Pimentel *et al.*, 2001, Dye & Jarmain, 2004, Henderson, 2007; Naude *et al.*, 2011). It has invaded all habitats, except the very arid Karoo (Henderson, 2007) and dense natural forest (Geldenhuys, 2004). Forest margins, an ecotone between forest and fire prone fynbos vegetation (Mucina & Rutherford, 2006), is more vulnerable to invasion (Geldenhuys, 2004).

Virgilia divaricata Adamson is a native forest pioneer tree species associated with forest margins (Goldblatt & Manning, 2000; Mucina & Rutherford, 2006). It is ecologically similar to *A. mearnsii* in being a short-lived, fast growing and woody perennial (Searle, 1997; Mbambezeli & Notten, 2003). Both species belong to the Fabaceae, but belong to different subfamilies (Searle, 1997; Goldblatt & Manning, 2000). *A. mearnsii* has invaded forest margins and now co-occurs with *V. divaricata* over extensive areas of its native range in the Cape Floristic Region (CFR) of South Africa. Both tree species are nodulating legumes that can acquire N via Biological Nitrogen Fixation (BNF) (Joubert, 2003; Beukes *et al.*, 2011; Rodrìguez-Echeverrıa *et al.*, 2011). Each species has its own unique compliment of rhizobial associates (Rodrìguez-Echeverrıa *et al.*, 2011) that enable them to acquire nitrogen (N) from both the soil and the atmosphere. Nitrogen derived from the atmosphere (NDFA) is converted from N_2 gas into mineral NH_4^+ by these nodular rhizobia, and made available to the plant as alternative source of nitrogen (Allen & Allen, 1981). This ability of legumes may be viewed as both beneficial (sustainable N source) and harmful (successful invaders) to plantations

and/or ecosystems (Brockwell *et al.*, 2005). They can utilize BNF, lessening the demand for fertilizers, but invasive legumes alter nutrient cycles of nutrient poor invaded ranges.

Nutrient cycling in natural environments is very complex and influenced by many interacting factors (Magdoff *et al.*, 1997; Huber & Hanekleus, 2007). New methods focused on nutrient economies have been identified and utilized to aid our understanding of how ecosystems function. The δ15N isotope method is widely used within botanical ecology and agriculture (Isaac *et al.*, 2012). Methods that help understand and predict the nature of nutrient limitation to net primary production is important in a changing world. Reed *et al.* (2012) proposed the stoichiometric approach to assess nutrient resorption. The resorption efficiency metric is useful and informative, because nutrients that are reabsorbed before leaf abscission are directly and immediately available to the plant (Clark, 1977; Turner, 1977). This contributes directly to the nutrient use efficiency of the plant (Vitousek, 1982; Aerts & Chapin, 2000; Franklin & Agren, 2002). It is, however, important to remember that the scale at which the assessment is done may influence the patterns observed (Reed *et al.*, 2012). It is currently not known how nutrient reabsorption affects the mineral nutrition of invasive and indigenous legumes in the same ecosystem. This would potentially contribute to the understanding of the success of invasive and indigenous legumes in the CFR.

The aim of this research was therefore to investigate the role of nutrient reabsorption in the mineral nutrition of *A. mearnsii* and *V. divaricata* in the CFR. We investigated their foliar N and P concentrations, nutrient resorption and their biological N_2 fixation. We also assessed soil N and P to determine its influence on the nutrient economies of these plants. We predicted that since the invasive tree is well-adapted to nutrient limiting environments in its native range, it would have a competitive advantage over the native tree in terms of nutrient economy (higher resorption efficiency depending on soil nutrient availability, and in so doing makes use of a cheaper N source).

2.Materials and methods

2.1 Study area

Study sites were located within the Garden Route National Park in the Western and Eastern Cape Provinces in the CFR, South Africa. The sites stretched from Gouna near Knysna (-33.9804 S, 23.04642 E) in the west to Stormsriver near Tsitsikamma in the east (-33.9901 S, 23.8978 E). Sites were selected ±20 km apart to prevent pseudo-replication and were selected based on the shared presence of *A. mearnsii* and *V. divaricata*. The rainfall varies from an average of 500 mm to 1200 mm per year, with the highest rainfall during autumn and early summer and the lowest during December. Temperatures are mild, ranging from of 7˚C to 19˚C during June and 15˚C to 26˚C during January (Bond, 1981). Soils are largely derived from quartzitic sandstone of the Table Mountain Group (Site soil nutrient content – Appendix 1). More detailed site descriptions are provided in Table 2-1 (Van der Colff *et al.*, 2013; Chapter 2). The sites were located within the ecotone between the Fynbos and the Afromontane biomes. Data collection was done during December 2013 (Figure 4-1).

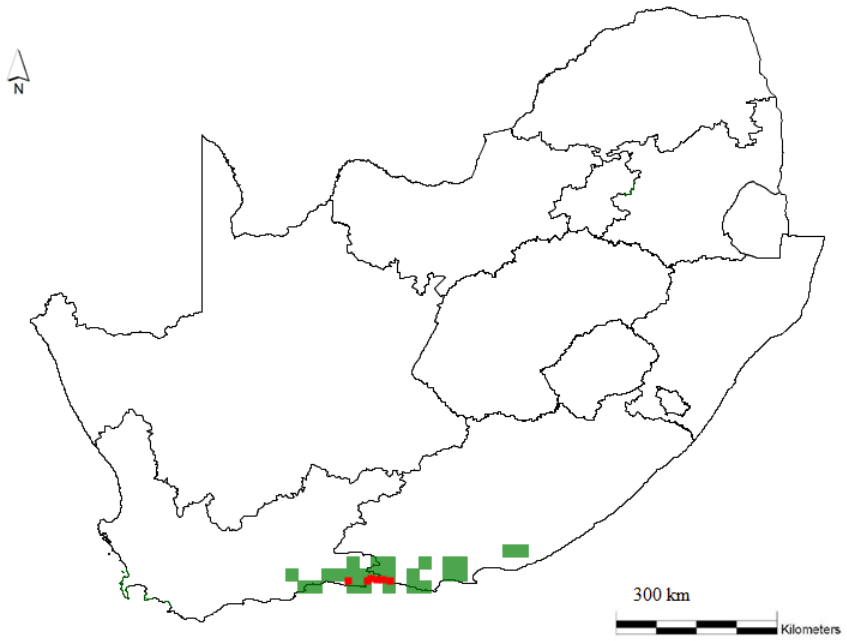

Figure 4-1 Distribution of *Virgilia divaricata* (green) in the southern Cape in forest margins between the Fynbos biome and the Forest biome within the Garden Route National Park. Red squares indicates sampling sites where *V. divaricata* and *A. mearnsii* occurs sympatrically.

2.2 Foliar nutrient content

Within each of the six sites, fully sunlight lit canopy leaves from five trees per species were sampled (Figure 4-1). Four weeks prior to this collection, leaf traps were placed within each of these trees to capture any foliar litter from the selected trees (Reed *et al.*, 2012). These litter traps were made from folded 0.09m^2 mesh netting (2 mm thick), tied below a branch of the selected tree. Fresh and senesced leaves were collected in brown paper bags. Foliar material was first sorted to remove any foreign leaves and reproductive material where applicable. When the sample contained too many leaves from other tree species and if it was not possible to distinguish foliar samples, the sample was discarded. Leaves were oven dried at 80˚C for 48 hours. Foliar samples were milled and analyzed for total N and P content.

Phosphorous concentration was determined by an external laboratory (Elsenburg, Stellenbosch) using inductively coupled mass spectrometry (ICP-MS). N content and N isotope content was determined at the Archeometry Department, University of Cape Town. The isotopic ratio of δ15N and δ14C was calculated as δ = 1000 ‰ (Rsample/Rstandard), where R is the molar ratio of the heavier to the lighter isotope of the samples and standards are as defined by Farquhar *et al.* (1989). Between 2.1 and 2.2 mg of each milled sample were weighed into 8 mm x 5 mm tin capsules (Elemental Micro-analysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany). The samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyzer (Fisons instruments SpA, Milan, Italy). The δ15N values for the nitrogen gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift: two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO4$.

%NDFA was calculated according to Shearer & Kohl (1986):

$$\%NDFA = 100((\delta^{15}N_{reference\ plant} - \delta^{15}N_{legume})/ (\delta^{15}N_{reference\ plant} - B))$$

The reference plant was wheat (*Triticum aestivum* .L) grown under glasshouse conditions. The B-value is the $\delta^{15}N$ natural abundance of the N derived from biological N-fixation of the above-ground tissue of *Virgilia divaricata,* grown in a N-free solution, and was determined as -1.6‰.

N and P resorption efficiency was calculated as described by Killingbeck (1996):

$$\frac{Xfresh - Xsen}{Xfresh} \times 100$$

X fresh, N or P concentration of green foliage, X sen, N or P concentration of senesced leaves.

2.3 Soil Analyses

Soil samples for chemical analyses were collected using a 0-10 cm auger sampler. Five soil samples were collected within the radius of the sampled trees. Soil samples were analysed at an external laboratory (BemLabs). The soil was air dried, sieved through a 2 mm sieve for determination of stone fraction (weight/weight basis) and analysed for pH (1.0 M KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic matter by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990). The extracted solutions were analysed with a Varian ICP-OES optical emission spectrometer. Total P was extracted with a 1:1 mixture of 1N nitric acid and hydrochloric acid at 80^{o}C for 30 minutes. The P concentration in the extract was then determined with a Varian ICP-OES optical emission spectrometer. Total N content of soil were determined through total combustion using a Leco Truspec® CN N analyser.

2.4 Statistical analysis

Complications in the analyses resulted in the sample size for %P in fresh and senesced leaves (n= 7) being smaller than that of %N (n=29). All data were tested for normality using the Shapiro Wilks test. Statistical analyses were conducted using the STATISTICA 11 software package (Statsoft, USA 2012). Distribution fitting revealed a non-normal distribution for of all parameters measured excluding fresh and senesced leaves of *A. mearnsii*. A Mann-Whitney U test was used to test differences between the two tree species for these non-normal data. A t-test was used to test the difference between fresh and senesced leaves of *A. mearnsii*, as data was distributed normally. Pearson product-moment correlation was done between soil %N and P mg/kg soil with leaf %N, %P and resorption efficiency of both N and P, as well as for %NDFA (n= 29) and δ15N/ δ14N (n = 29) with soil N.

3.Results

3.1 Leaf nutrient contents and resorption efficiency

Leaf nutrient content of the two legume species was very similar with no difference between either %N (t-value = -0.81; df= 30, p-value = 0.097 and %P (Z-statistic = 0.89; p-value= 0.37) concentrations. There was a decrease in %N and %P from fresh to senesced leaves in both tree species (Table 4-1). These values were used to calculate the N and P resorption efficiency of each tree species (Figure 4-2). The range of N resorption efficiency in *A. mearnsii* (13.17) was much smaller than in *V. divaricata* (47.04). The range of P resorption efficiency in *A. mearnsii* (30.91) was 50% less than that of *V. divaricata* (63.31). There was no difference between N (Z-statistic = -1.28; p-value = 0.2) and P (Z-statistic= 0.89; p-value = 0.37) resorption between the two tree species.

3.2 %NDFA and δ 15N/ δ 14N

Within each species %NDFA was similar across sampled sites (Appendix 2). *V. divaricata* had higher %NDFA (90.44 ± 4) than *A. mearnsii* (84.42 ± 17.78) (Figure 4-3). There was a significant difference in δ 15N isotope ratio between the tree species, with *A. mearnsii* having a higher δ 15N isotope ratio (-0.97 ± 0.33) than *V. divaricata* (-0.57 ± 1.11) (Figure 4-4).

Soil P was different between sites (F ratio = 4.21; p-value = 0.007), while soil N did not differ across the sampled sites (F ratio= 1.75; p-value = 0.17). Since there was no difference in soil N we could not assess whether soil N had an effect on any of the other measured variables (Table 4-2). We could, however, assess whether soil P had any influence on the leaf P and P resorption efficiency. We found no significant relationship between leaf %P and P resorption with soil P in either *A. mearnsii* or *V. divaricata*.

Table 4-1 The mean (± standard error) %N and median (± standard deviation) %P of *Acacia mearnsii* and *Virgilia divaricata* of fresh and senesced foliar material. Letters denote significant difference between fresh and senesced leaves within a species for %N and %P, respectively.

Species	Mean %N	median %P	Species	median %N	median %P
A. mearnsii			*V. divaricata*		
Fresh (n=7)	3.38(0.26)a	0.16(0.05)a	Fresh (n=7)	3.59(0.45)a	0.16(0.04)a
Senesced (n=7)	1.96(0.22)b	0.05(0.03)b	Senesced (n=7)	1.72(0.0.49)b	0.03(0.05)b

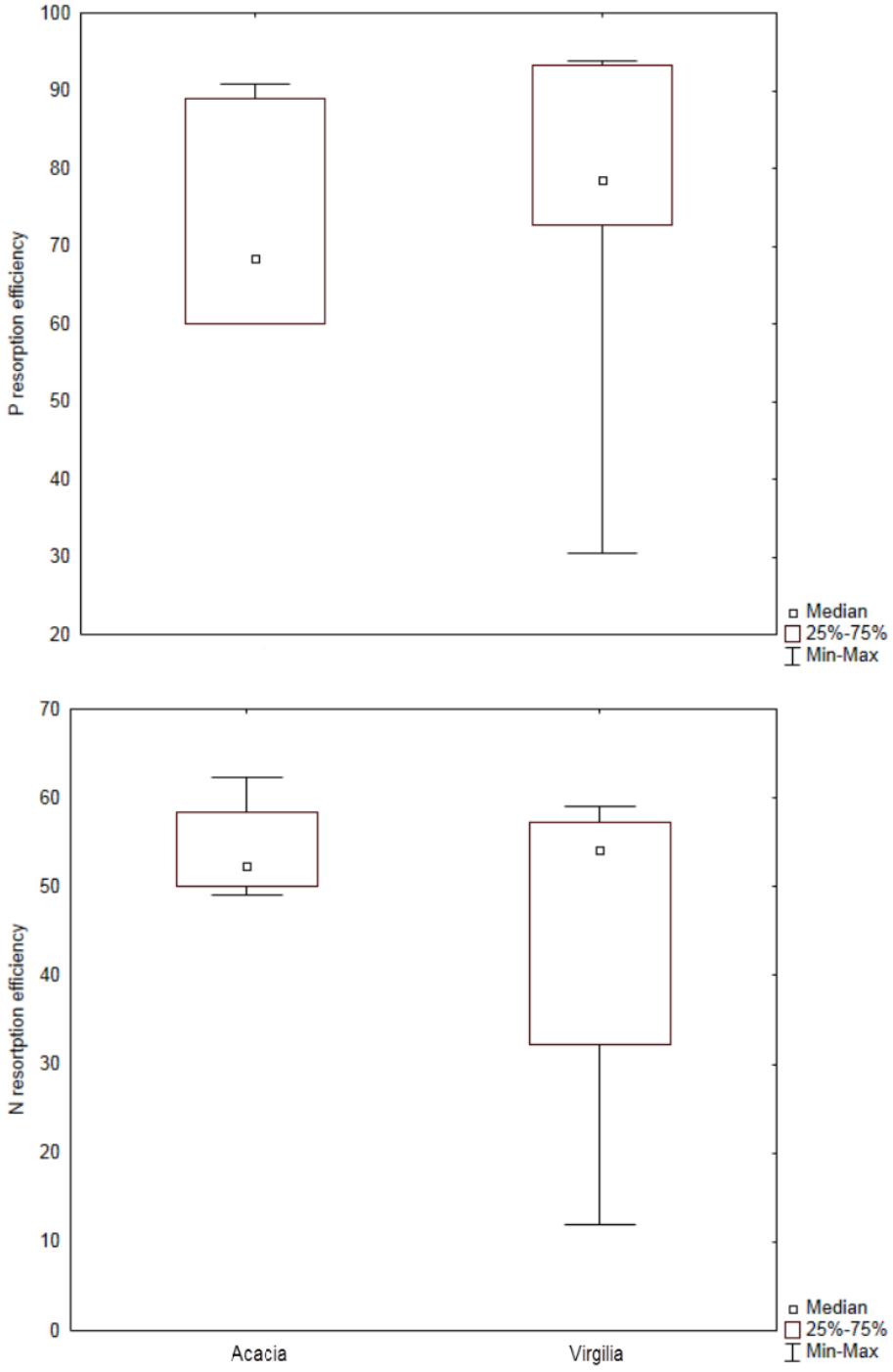

Figure 4-2 Difference in resorption of %P and %N between *A. mearnsii* and *V. divaricata* over the sampled range within the Garden Route National Park. Values represent medians and whiskers indicate the minimum and maximum values. No significant difference was observed between the two tree species.

Table 4-2 Soil samples collected within the sampling sites in close proximity to *Acacia mearnsii* and *Virgilia divaricata* trees from the Garden Route National Park. See Appendix 1 for further soil nutrient content information.

Sampling site	Latitude	Longitude	Soil type	pH	P Bray II mg/kg	%N
Gouna	-33.9804	23.04642	Loam	3.5	1.4	0.1326
Keurboom	-34.0002	23.43232	Sand	4.9	8.0	0.2594
Kurland	-33.9346	23.50062	Loam	4.4	6.2	0.3487
Nature Valley	-33.9614	23.63188	Loam	3.8	1.2	0.5112
Tsitsikamma	-33.9744	23.74923	Loam	3.5	2.8	0.7068
Stormsriver	-33.9901	23.89798	Sand	3.7	5.0	0.5384

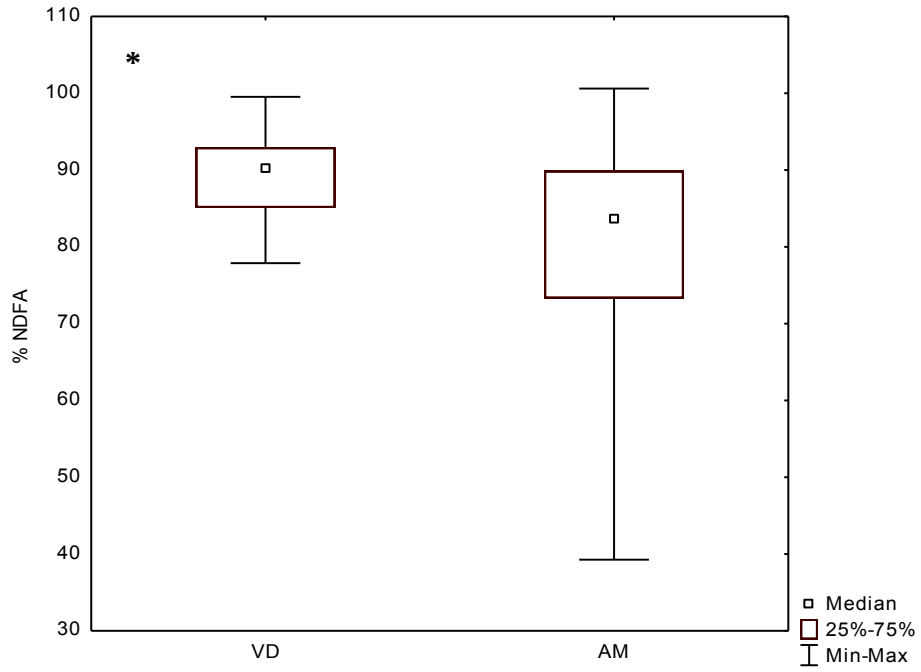

Figure 4-3 Comparison of % NDFA of *A. mearnsii* (AM) and *V. divaricata* (VD) within the sampled range in the Garden Route National Park. Values indicate medians and whiskers the minimum and maximum values. An asterisk indicates significant differences, $p < 0.05$.

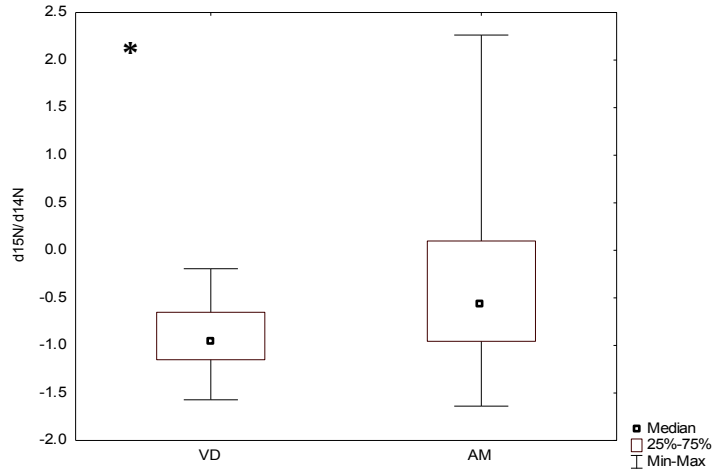

Figure 4-4 Comparison of δ15N/ δ 14N of *A. mearnsii* (AM) and *V. divaricata* (VD) within the sampled range in the Garden Route National Park. Values indicate medians and the whiskers minimum and maximum values. An asterisk indicates significant differences, $p < 0.05$.

4.Discussion

A. mearnsii and *V. divaricata* had similar foliar N and P concentrations. Tye & Drake (2012) compared N and P concentrations of *A. mearnsii* to *Acacia karoo* Hayne, and found these two species also to be similar. This suggests that the foliar nutrient contents of legumes are very similar, regardless the origin of the tree. Interestingly, both Tye & Drake (2012) and Witkowski (1991) found differences in foliar nutrients between legume and non-legume species from the same habitats. We can therefore predict that habitats invaded by legumes will be severely affected by nutrient-input and nutrient cycling (Witkowski, 1991; Yelenik *et al.*, 2004). Both tree species had similar resorption efficiencies, indicating that there was no difference in nutrient use efficiencies between the two. Both plants have high resorption values for both N and P, indicating that they are effectively recycling nutrients before leaf abscission. Within forest margins the habitat consist both of nutrient rich tree litter and nutrient poor fynbos leaf litter (Goldblatt & Manning, 2000; Mucina & Rutherford, 2006), creating a heterogeneous nutrient environment (Witkowski & Mitchell, 1987). This is evident in the large range in *V. divaricata* N and P resorption values, showing that this tree may change its resorption efficiency depending on nutrient availability. We tested this prediction by correlating soil P with P resorption efficiency and leaf %P, but found no support. However, it should be noted that the soil P was measured as available P, and not total soil P. This is a rather important distinction, since available soil P is an expression of the P bound to Fe and Al chemical species in the soil, whilst the total P is an indication of the inorganic and organic pools of P (Gerke *et al.*, 1994).

Utilizing N and P resorption efficiency is a stoichiometric method that has been shown to be quite accurate in predicting nutrient limitations in the environment (Reed *et al.*, 2012). However, the scale at which the relationship is assessed has an effect on the results, as local-scale species or temporal variation can mask the broader scale patterns (Townsend *et al.*, 2007). In this study the sample size of particularly P leaf samples were significantly reduced and we might have lost potential informative data. *A. mearnsii* has a small range of N and P resorption values. This could imply that the species has consistent resorption efficiency, regardless of what nutrients are available in the environment. This would be supported if at a larger scale there is a correlation between soil nutrients and plant resorption efficiency.

The input of N and P into the environment was not measured quantitatively using leaf mass over time, thus we are unaware of the actual amounts of nutrients entering the environment

per time period. However, we found no difference in the concentrations of these two nutrients (N & P) in the senesced leaves of *A. mearnsii* and *V. divaricata*. This may infer that these species contribute equally to the input of foliar N and P concentrations into the environment. This is important, as the presence of the invader increases the amount of N entering the environment, because these legumes make use of BNF. Witkowski (1991) found similar results with *Acacia saligna* (Labill.) Wendl. and *Acacia cyclops* A. Cunn. ex G. Don. invading sandier, low nutrient environments in the CFR.

V. divaricata had higher %NDFA values than *A. mearnsii*, displaying a higher usage of BNF than the invasive tree species. This is supported by the lower δ^{15} N/ δ^{14}N ratio in *V. divaricata*. The δ^{15} N isotope is in lower abundance than the δ^{14} N isotope, because it is utilized more by their rhizobia bacteria associates. The inverse is true for *A. mearnsii* in which larger δ^{15} N/ δ^{14}N ratios were measured. The %NDFA of *A. mearnsii* was higher than what recorded by Tye & Drake (2012) and Brockwell *et al.* (2005) in other *Acacia* species. Our measured value was, however, similar to the %NDFA recorded for *Acacia mangium* Willd. (Galiana *et al.*, 1996). The higher %NDFA in *A. mearnsii* measured in this study could be explained by differences in habitat composition and soil nutrition. There is no comparison data for *V. divaricata* within its natural ranges, but these values are similar to measurements obtained in glasshouse experiments by Magadlela *et al.* (2013).

The invasion of *A. mearnsii* into forest margins is contributing to N input into the environment to the same extent as is true for the native legume. Although the two species are related, they belong to different subfamilies of the Fabaceae. This makes the similarity in their ecological interaction with the habitat very interesting, and stresses the importance of invasions by ecologically similar species. This is important, as in phylogenetically related invasive and native species may occupy different niches, limiting direct competition or interaction. In the case of ecologically similar species or species that share some traits and utilize the same niche, they may be in direct competition, which may result in the exclusion of the weaker tree species. It is also possible that the native and the invasive assessed in this study could co-occur, but the presence of another legume tree within these forest margins may increase nutrient levels above natural conditions.

In conclusion, we found some aspects of the plant physiological resource utilization of the environment to be similar between the two tree species, while others differed significantly. Native tree species utilize more %NDFA than the invasive, while the invasive utilizes less,

which means that it acquires more inorganic mineral N from the soil. There could be some stabilization between input and output of N. Nutrient cycling within forest margins are, however, fairly complex as the environment has a heterogeneous distribution of nutrients and is frequently disturbed by fire. The presence of an invader may further complicate things and result in imbalances in the natural nutrient cycles, as has been illustrated in many previous studies in other environments (Witkowski, 1991; Stock *et al.*, 1995; Jefferies & Maron, 1997; Vitousek *et al.*, 1997). If these species can co-exist, the impact on the habitat may be limited. If, however, the native species cannot successfully compete against this invasive species, the effects could be far reaching. *A. mearnsii* may not provide nursery grounds to the secondary succession tree species to the same extent as *V. divaricata* does (Coetzee & Wigley, 2013).

5.References

Aerts, R. & Chapin, F.S. III. 2000. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research 30: 1–67.

Allen, O.N. & Allen, E.K. 1981. The Leguminosae: a source book of characteristics, uses, and nodulation. University of Wisconsin Press, Madison, Wis.

Beukes, C.W., Law, I.J., Venter, X & Steenkamp, E.T. 2011. Phylogenetic characterisation of root nodule bacteria associated with indigenous fynbos legumes. In Villet, M.H. and Crous, K.L. 2011. Proceedings of the Congresses of Southern African Society for Systematic Biology.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U. & Richardson, D.M. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333–339.

Bond, W.J. 1981.Vegetation gradients in the southern Cape mountains, MSc thesis, University of Cape Town, Cape Town.

Brockwell, J., Searle, S.D., Jeavons, A.C. & Waayers, M. 2005. Nitrogen fixation in Acacias: an untapped resource for sustainable plantations, farm fostry and land reclamation. ACIAR Monograph No. 115: 1–132.

Clark, F.E. 1977. Internal cycling of 15 nitrogen in shortgrass prairie. Ecology 58: 1322–1333.

Coetsee, C. & Wigley, B.J. 2013. *Virgilia divaricata* may facilitate forest expansion in the afrotemperate forests of the southern Cape, South Africa, Koedoe 55: 1–8.

Dye, P. & Jarmain, C. 2004. Water use by black wattle (*Acacia mearnsii*): implications for the link between removal of invading trees and catchment streamflow response. South African Journal of Science 100: 40–44.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review Plant Physiology* 40: 503–537.

Franklin, O. & Agren, G.I. 2002. Leaf senescence and resorption as mechanisms of maximizing photosynthetic production during canopy development at N limitation. Functional Ecology 16: 727–733.

Galiana, A., N'Guessan-Kanga, A., Gnahoua, G.M., Balle, P., Dupuy, B., Domenach, A.M. & Mallet, B. 1996. Nitrogen fi xation in *Acacia mangium* plantations. Bois et Forets des Tropiques 250: 51–62.

Geldenhuys, C.J. 2004. Concepts and Process to Control Invader Plants in and around Natural Evergreen Forest in South Africa. Weed Technology 18: 1386–1391.

Gerke, J., Römer, W. & Jungk, A. 1994. The excretion of citric and malic acid by proteoids roots of Lupinus albus L.: effects on soil solution concentrations of phosphate, iron, and aluminium in the proteoid rhizosphere in samples of an oxisol and a luvisol. Z Pflanzenernaehr Bodenkd 157: 289–94.

Goldblatt, P. & Manning, J. 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria : National Botanical Institute, Pretoria.

Henderson, L. 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37: 215–248.

Huber, D.M. & Hanekleus, S. 2007. Managing nutrition to control plant disease. Landbauforschung Volkenrode 57: 313–322.

Isaac, M.E., Hinsinger, P. & Harmand, J.M. 2012. Nitrogen and phosphorus economy of a legume tree-cereal intercropping system under controlled conditions. Science of the Total Environment 434: 71–78.

Jefferies, R.L. & Maron, J.L.1997. The embarrassment of riches: atmospheric deposition of nitrogen and community and ecosystem processes. Trends in Ecology and Evolution 12: 74–78.

Joubert, C. 2003. Rhizobia associated with Australian Acacia species (*Acacia mearnsii*, *Acacia dealbata* & *Acacia decurrents*) in South Africa as determined by Sodium Dodecyl-Sulphate Polyacrylamide Gel Electrophoresis. MSc thesis. University of Pretoria.

Killingbeck, K.T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77: 1716–1727.

Kull, C.A. & Rangan, H. 2008. Acacia exchanges: Wattles, thron trees and the study of plant movements. Geoforum 39: 1258–1272.

Le Maitre, D.C., Gaertner, M., Marchante, E., Ens, E.-J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J. & Richardson, D.M. 2011. Impacts of introduced Australian acacias: implications for management and restoration. Diversity and Distributions, 17: 1015–1029.

Magadlela, A. 2013. The effect of phosphorus (P) deficiency on growth and nitrogen fixation of Virgilia trees from the Cape Floristic Region (CFR). MSc Thesis. Cape Town: University of Stellenbosch.

Magdoff, F., Lanyon, L. & Liebhardt, B. 1997. Nutrient cycling, transformations, and flows: implications for a more sustainable agriculture. Advances in Agronomy 60: 1–73.

Mbambezeli, G., & Notten, A. 2003. Virgilia genus. **http://www.plantzafrica.com/planttuv/virgilia**

Mooney, H.A. & Hobbs, R.J. 2000. Invasive Species in a Changing World. Island Press, Washington, DC.

Mucina, L. & Rutherford. M. 2006. The vegetation of South Africa, Lesotho and Swaziland.

Streletzia 19, Pretoria: South African National Biodiversity Institute: 77: 93–182.

Naude, M., Jacobs, S.M & Esler, K.J. 2011. Fynbos riparian ecosystem function and IAPs. South African Association of Botanists – Annual Meeting 2011 Abstracts of papers and posters presented at the 37th Annual Congress of the South African Association of Botanists held at Rhodes University, Grahamstown, 17–19 January 2011South African Journal of Botany 77: 510–580.

Pimentel, D., McNair, S., Janecka, J., Wightman, Simmonds, C, O'Connell, C.,Wong, E, Russel. L, Zern, Aquino, Tsomondo, T. 2001. Agricultue, Ecosystems and Environment 84: 1–20.

Reed, S.C., Townsend, A.R., Davidson, E.A. & Cleveland, C.C. 2012. Stoichiometric patterns in foliar nutrient resorption across multiple scales. New Phytologist. 1–8.

Rodrìguez-Echeverrıa, S., Le Roux, J.J., Criso´ stomo, J.A. & Ndlovu, J. 2011. Jack-of-all-trades and master of many? How does associated rhizobial diversity influence the colonization success of Australian Acacia species?. Diversity and Distributions. 17: 946–957.

Searle, S. 1997. *Acacia mearnsii* De Wild. (black wattle) in Australia. Black wattle and its utilizations. Barton, ACT: Rural Industries Research and Development Corporation: 1–10.

Secretariat of the Convention on Biological Diversity. 2001. The Value of Forest Ecosystems. Montreal, SCBD Technical Series No. 4.

Shearer, G.B. & Kohl, D.M. 1986. N2-fixation in the field settings: estimations based on natural 15N abundance. Australian Journal of Plant Physiology 13: 699–756.

StatSoft, Inc. 2012. STATISTICA (data analysis software system), version 11. www.statsoft.com.

Stock, W.D., Wienand, K.T. & Baker, A.C. 1995. Impacts of invading N_2-fixing Acacia species on patterns of nutrient cycling in two Cape ecosystems: evidence from soil incubation studies and ^{15}N natural abundance values. Oecologia 101: 375–382

The Non-Affiliated Soil Analysis Work Committee. 1990. Handbook of standard soil testing methods for advisory purposes. Soil Science Society. South Africa. Sunnyside, Pretoria.

Townsend, A.R., Cleveland, C.C., Asner, G.P. & Bustamante, M.M.C. 2007. Controls over foliar N:P ratios in tropical rain forests. Ecology 88: 107–118.

Turner, J. 1977. Effects of nitrogen availability on nitrogen cycling in a Douglas fir stand. Forest Science 23: 307–316.

Tye, D.R.C. & Drake, D.C. 2012. An exotic Australian Acacia fixes more N than a coexisting indigenous Acacia in a South African riparian zone. Plant Ecology 213: 251–257.

Van der Colff, D., Roets, F., Dreyer, L.L. & Valentine, A. 2013. Plant stress and the prevalence of pest and pathogens associated with a native and an invasive alien legume tree in the Cape Floristic Region, South Africa. MSc Thesis. Stellenbosch University. Stellenbosch.

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. American Naturalist 119: 553–572.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek. M. & Westbrooks R. 1997. Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology 21: 1–16.

Witkowski, E.T.F. 1989. Response to nutrient additions by the plant growth forms of sand-plain lowland fynbos, South Africa. Vegetatio 79: 89–97.

Witkowski, E.T.F. & Mitchell, D.T. 1987. Variations in Soil Phosphorus in the Fynbos Biome, South Africa. Journal of Ecology 75: 11–59.

Witkowski, E.T.F. 1991. Effects of invasive alien Acacias on nutrient cyclinginthr coastal Lowlands of the Cape Fynbos. Journal of Applied Ecology 28: 1–15.

Yelenik, S.G., Stock, W.D. & Richardson, D.M. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. Restoration Ecology 12: 44–51.

6. Appendix 1. Soil sample nutrient information per samples.

Sampling site	LATITUDE	LONGITUDE	Soil type	pH	P Bray II mg/kg	%N
Gouna	-33.98043333	23.04641667	Loam	4	4	0.07
Gouna			Loam	4	2	0.08
Gouna			Loam	4	**0**	0.13
Gouna			Sand	5	1	0.09
Gouna			Loam	4	0	0.29
Keurboom	-34.00015	23.43231667	Sand	5	7	0.13
Keurboom			Sand	4	8	0.62
Keurboom			Sand	4	5	0.18
Keurboom			Loam	4	100	0.22
Keurboom			Sand	4	12	0.15
Kurland	-33.93463333	23.50061667	Loam	4	4	0.29
Kurland			Loam	5	1	0.31
Kurland			Sand	4	10	*
Kurland			Loam	4	13	*
Kurland			Loam	4	3	0.44
Nature Valley	-33.96136667	23.63188333	Loam	4	1	0.13
Nature Valley			Loam	4	2	0.31
Nature Valley			Loam	4	1	0.16
Nature Valley			Loam	4	**0**	0.19
Nature Valley			Sand	4	2	1.76
Tsitsikamma	-33.97435	23.74923333	Loam	4	2	0.48
Tsitsikamma			Loam	4	2	0.61
Tsitsikamma			Loam	4	6	1.18
Tsitsikamma			Loam	4	1	0.41
Tsitsikamma			Sand	3	3	0.86
Stormsriver	-33.99006667	23.89798	Sand	4	7	0.66
Stormsriver			Loam	3	3	0.41
Stormsriver			Loam	4	9	0.56
Stormsriver			Loam	4	3	0.76
Stormsriver			Loam	4	3	0.3

Chapter 5

Physiological responses to infection by the pathogenic fungus Ceratocystis tsitsikammensis in Acacia mearnsii and Virgilia divaricata

Abstract

Ceratocystis tsitsikammensis has recently been described as a native pathogen to *Rapaneae melanophloeos* in South Africa. It has also been associated with dead and dying trees of *Virgilia divaricata*, a native legume whose habitat (forest margins) is invaded by the invasive legume tree *Acacia mearnsii*. This invasive alien plant is also an important forestry species within South Africa. Here we explored the physiological effects of infection by this fungal species on each of these two nodulating legume tree species in a controlled environment. The effect of infection in the two hosts was evaluated against the Biotic Resistance Hypothesis, which predicts the absence of a co-evolutionary relationship between the native fungus (*C. tsitsikammensis*) and the invasive legume tree (*A. mearnsii*). Such a relationship is assumed to be present between the native legume and the native fungus. The effect of fungal infection on the physiological parameters and resource capture parameters was measured by determining effects on photosynthesis, dark respiration, water use efficiency, biomass accumulation and mineral nutrition. Higher photosynthetic rates were recorded in infected plants than in control plants of both tree species. In terms of nutrition, the infected *V. divaricata* trees changed their N economy by relying more on soil derived N and less on biological nitrogen fixation. For biomass accumulation, infected *A. mearnsii* trees altered their root mass allocation in below ground investments. The pathogen elicited a greater functional response from the invasive tree species than from the native legume, thereby providing some support for the BRH.

Key words: *Acacia mearnsii*, plant physiology, *Ceratocystis tsitsikammensis*, plant pathology

1.Introduction

Ceratocystis Ellis & Halst is an ophiostomatiod (Ascomycota: Ophiostomatales and Microascales) fungal genus known to infect and kill various tree species around the world (Brasier, 1990; Juzwik *et al.*, 2008; Jankowiak *et al*., 2012). Despite being a small genus, it contains many plant pathogens (Upadhyay, 1981; Paulin *et al.*, 2002). Many of these pathogens have been moved to new continents, where some have caused severe economic losses (Valarini & Tokeshi, 1980; Teviotdsle & Harper, 1991). Some members of this genus, however, co-exist with their host plants without causing any visible negative effects (Kamgan *et al.*, 2008; 2012). Spores of ophiostomatoid fungi are generally vectored by arthropod taxa such as beetles (Appel *et al.*, 1999; Six, 2003; Kirisits, 2004) and mites (Bridges & Moser, 1983; Moser, 1997; Roets *et al.*, 2006; 2012). These organisms may help transport these fungi to potential new host species (Wingfield *et al.*, 2001; Jackson, 2004). Closely related host plants have a higher probability to be infected than distantly related species (Prell, 2001), but some *Ceratocystis* species have low host specificity and can infect many distantly related hosts (Zhou & Hyde, 2001).

A large body of research has focused on the effects of ophiostomatoid fungi on economically valuable trees, specifically those used in plantations (Roux *et al.*, 2008). In southern Africa these studies focused mainly on three important plantation genera, namely *Eucalyptus*, *Pinus* and *Acacia* (especially *A. mearnsii* De Wild) (DEA, 2009). These are often associated with *Ceratocystis* species, for example *Ceratocystis fimbriata* Ellis & Halst has been shown to severely affect *Eucalyptus* species (Kamgan *et al.*, 2008), while *Ceratocystis albifundus* M.J. Wingf., De Beer & M.J. Morris is detrimental to *A. mearnsii* (Roux & Wingfield, 1997).

Unfortunately many of these plantation taxa are also invasive in South Africa. *Acacia mearnsii*, for example, is the fifth most widespread invasive alien species in South Africa (Anderson, 2007). In the Cape Floristic Region (Goldblatt & Manning, 2000) it often co-occurs with the native *Virgilia divaricata* Adamson along forest margins. Both of these taxa belong to the Fabaceae and share ecological characters such as being fast-growing, forest pioneer species. Both species fix nitrogen through symbiotic relationships with *Rhizobia* species in root nodules (Turk *et al.*, 1992; Mbambezeli & Notten, 2003). These ecological similarities can lead to a significant overlap in the organisms associated with them (e.g. arthropods; Van der Colff *et al.*, 2013; Chapter 2).

Van der Colff *et al.* (2013, Chapter 3) showed that *V. divaricata* and *A. mearnsii* share five ophiostomatoid fungal species (*Ophiostoma quercus* (Georgevitch) Nannfeldt, *Ophiostoma pluriannulatum* (Hedgcock) H. & P. Sydow, *Ceratocystis fimbriata* Ellis & Halst, *Ceratocystis savannae* Kamgan & Roux and *Ceratocystis tsitsikammensis* Kamgan & Roux). If fungi can move from native plants to exotic plantation species and /or invasive trees, they may cause disease in these new hosts. This threatens future forestry activities, but also provides an excellent opportunity to test the Biotic Resistance Hypothesis (BRH). This hypothesis states that native pests and pathogens preferentially target non-native/invasive species as they have fewer defenses against such pathogens (Keane & Crawley, 2002; Lombardero *et al.*, 2012). The non-native host plant is predicted to be more physiologically vulnerable to infection than the native plant due to the historical absence of co-evolution (Lombardero *et al.*, 2012). Although results of some studies have supported the BRH, others have yielded mixed results in terms of the effects of pathogens on native and non-native hosts (Blaney & Kotanen, 2001; Keane & Crawley, 2002; Agrawal *et al.*, 2005; Lombardero *et al.*, 2012).

Plant defenses against a potential pathogen depend on the recognition of that threat (Jones & Dangl, 2006; Parker & Gilbert, 2007) and the virulence of the pathogen. The site or tissue in which the pathogen flourishes determines the extent to which it weakens the host (Huber & Hanekleus, 2007). There are many possible mechanisms by which pathogen infection can affect plant photosynthesis. For example, a typical physiological response to leaf infecting pathogens is a decrease in photosynthesis (Agios, 2005), which may lead to reduced plant vigor and death (Omari *et al.*, 2001). However, it should be noted that this decrease in photosynthesis may be related to leaf wilt (Agios, 2005), which would mechanistically reduce CO_2 assimilation via an effect on stomatal opening. Vascular tissue infecting fungi disturb the translocation of water and nutrients in their host plants (Pegg, 1985), resulting in wilting within days of infection by virulent fungal species (Roux & Wingfield, 1997). Plants infected by these pathogens have shown a 96-98% reduction in water flow through their xylem (Beckman *et al.*, 1953; Henderson *et al.*, 2000; Agios, 2005). Once again, the primary effect on photosynthesis appears to operate via a mechanism which affects water relations. The genera *Ceratocystis, Ophiostoma, Fuserium* and *Verticillium* include members known to infect vascular tissues (Agios, 2005).

Ceratocystis albifundus De Beer, Wingfield & Morris is the most important native pathogen of *A. mearnsii* in plantations in South Africa (Roux & Wingfield, 1997; Roux *et al*., 1999), Uganda (Roux *et al.*, 2001), Kenya (Roux *et al.*, 2005) and Tanzania (Roux *et al.*, 2005). It causes rapid wilting in susceptible trees (Roux *et al.*, 1999), while stem cankers that exude gum from swollen blisters under the bark that develop in tolerant trees. The vascular tissue of infected trees is also discoloured (Morris *et al.*, 1993; Roux *et al*., 1999). Although vascular infecting fungi are the causative agents of disease, plant responses that lead to wilting originate both from the pathogen (Freeman & Beattie, 2008) and the defense system of the plant (Agios, 2005). Disease caused by fungal infection is expected to cause changes in plant size and reproductive output (Marr & Marshal, 2006). The plant size alterations may affect only certain plant parts or the entire plant and are enforced by regulators that affect cell division and cell enlargement in the host. However, the precise mechanisms, compounds and genes involved in these changes are unknown (Agios, 2005). The nutrient contents of infected plants are also affected by infection. Entry (1986) showed, for example, that seedlings of *Pinus monticola* Doulg. that received sufficient nutrients and sunlight were much more resistant to *Armillaria* infection than seedlings grown under conditions of inadequate nutrients and light. These plants also had higher overall plant biomass and shoot:root ratios, although the nutrient content of plants exposed to different treatments did not differ. Both *A. mearnsii* and *V. divaricata* are nitrogen fixing plant species, and biological nitrogen fixation is known to be affected by conditions of stress (Magadlela, 2013). The impact of a vascular infecting fungus on the nutrient budgets of these two species is therefore expected to be different to what is expected in non-nitrogen fixing plants. Nitrogen fixing plants are less dependent on nitrogen capture from the soil, which may be compromised by infection in their roots. In such instances, nitrogen fixing plants can capture nitrogen via Biological Nitrogen Fixation (BNF).

In this study we first confirmed if *C. tsitsikammensis* is pathogenic to *A. mearnsii*. We then assessed and compared the physiological responses to infection by *C. tsitsikammensis* in the native *V. divaricata* and the invasive *A. mearnsii* without identifying the source of the response (plant or pathogen). We hypothesize that the two plant species will respond differently to infection, as their historical relationship to the native fungus *C. tsitstikammensis* differs. The native host has been able to co-evolve with this fungus in the native habitat, while this was not

possible for the invasive tree species. We therefore expect the native plant to be less affected by infection with this fungal pathogen. We measure differences in physiological responses to infection by these plant species based on resource capture parameters such as gas exchange parameters, biomass changes and nutrient content of plant organs.

2.Materials and methods

2.1 Seed germination

Virgilia divaricata seeds were obtained from Silverhills Seeds (Kenilworth, Cape Town, South Africa), while *Acacia mearnsii* seeds were collected from an invasive population near Stellenbosch (33˚54'34.84" S; 18˚ 56'58.45"E). *V. divaricata* seeds were soaked in smoke water (Primer Smoke discs Kirstenbosch) at 25 ˚C for 24 hours, where after they were rinsed once in distilled water. *A. mearnsii* seeds were surface sterilized, scarified by submerging them in 95-99% sulfuric acid (H_2SO_4) for 15 minutes and then rinsed 10 times in distilled water. Following the acid scarification, seeds were also soaked in smoke water as described above. Treated seeds were planted in seedling trays containing sterile autoclaved sand (grain size 2 mm). Once the first true leaves formed, seedlings were transplanted to 15 cm diameter pots in sterilized sand. The experiment was initiated with 16 individual plants, 8 of *A.mearnsii* and 8 of *V. divaricata*. Before fungal inoculation, 5 month old plants (two per species) were harvested to measure initial biomass of plants. After infection, three months later, all plants were harvested. A replication number of three plants per treatment was used, thus n = 3 infected and n = 3 control plants per species.

2.2 Rhizobium inoculation

As both plant species are nodulating legumes, they were root inoculated with their optimum nodulating bacteria (Thrall *et al.*, 2005). *V. divaricata* was inoculated with a cocktail of *Burkholderia* isolates, isolated from *Virgilia* species by Beukes *et al*. (2011). These *Burkholderia* isolates were obtained from the Forestry and Biotechnology Institute at the University of Pretoria (FABI). *Burkholderia* isolates (Kb 2, Kb 13; Kb 15 & Kb 16) were maintained on Triptone Yeast Extract (TYE) agar (6g/L Triptone, 3 g/L yeast extract and 10 g/L agar) at pH 7 made. Plates were placed in an incubator (28˚C) and monitored daily until single colonies were observed. Inoculum was prepared using the same constituents as above-mentioned media, but excluding agar. A loop full of each of the *Burkholderia* isolates was added to separate TYE solutions (250 ml) and placed in a shaking incubator for 3-4 days at 28˚C. Inoculum was ready when the colour of the liquid turned a luxuriant cream. The inoculum obtained from the different isolates were pooled and poured at the base of the *V. divaricata* seedlings (30 ml).

A. mearnsii seedlings were inoculated with *Bradyrhizobium japonicum* (isolate JN2) isolated from *A. mearnsii* in a previous study (Rodriquez-Echeverria *et al.*, 2011). *B. japonicum* was grown on plates of Yeast Mannitol Agar (YMA) with Congo red (1g/L Yeast extract, 10 g/L Mannitol, 0.5 g/L Dipotassium phosphate, 0.2 g/L Magnesium sulphate, 0.1 g/L Sodium chloride, 10 g/L Agar and 0.025 g/L Congo red) at pH 6.8 at 25 ˚C (Himedia M716 Technical Data). The *B. japonicum* isolate was streaked out onto these plates, placed in an incubator (28˚C) and monitored daily for any growth. Colonies of *B. japonicum* bacteria had a low absorption of Congo red, which serves as an indicator for the presence of *Bradyrhizobium* species (Somasegaran & Hoben, 1985). Inoculum was prepared following the methods outlined above, but using Yeast Mannitol growth medium instead of TYE growth medium. Inoculum was ready when the color of the liquid had a greyish color. Inoculum was then poured at the base of *A. mearnsii* seedlings (30 ml).

One week after inoculum application, plants were fed with a Long Ashton nutrient solution (Hewitt, 1966) with a modified P level of 0.05 mM. Ward *et al*. (2011) used similar P levels in their study working on legumous plants. The P level selected for this study was within the range of native soils in which these tree species grow (Wilkowski & Mitchell, 1987; Power *et al.*, 2010; Maistry *et al.*, 2012) to ensure optimal growth. Plants were watered and then fed with 100 ml of nutrient solution weekly and grown from July 2012 to March 2013 in an east-facing glasshouse at the University of Stellenbosch, South Africa. The range of mid-day irradiances was between 630-680 $mol.m^2.s^{-1}$ and the average day/night temperature and humidity were 23/15˚C and 35/75%, respectively.

2.3 Fungal inoculation and pathogenicity

Seedlings were inoculated with *C. tsitsikammensis* after 5 months of growth. The *C. tsitsikammensis* isolate (NM 56) was obtained from a previous study (Machingambi, 2013) and was grown on 2% Malt Extract Agar plates (MEA) (20gL malt extract and 15gL agar, Biolab, Midrand, South Africa and 1L deionised water) incubated at 24ºC. Fungal plugs (9 mm^2) were prepared from these plates and inserted into incisions made on the lower region of the stem (n=3 per tree species), while sterile agar served as controls (n=3 per tree species). Wounds were sealed using Paraflim (Parafilm M, Sigma, Germany). After 3 months, the experiment was terminated and plants were harvested. The inoculation experiment was repeated on mature *A. mearnsii* trees

from the source population from which seeds had been obtained (n = 8). Six weeks after inoculation, lesion lengths were measured of both control and infected plants. Data was non-parametric and was subsequently analyzed with a Mann-Whitney U test (data not shown) in STATISTICA 11 (Statsoft, USA, 2012).

2.4 Gas exchange measurements

Before harvesting at 8 months, the youngest fully expanded leaves were used for photosynthetic measurements (N=3). Saturation light levels of 1800 $mol.m^{-2}s^{-1}$ PAR were used to measure CO_2 assimilation rate, dark respiration rate and to calculate water use efficiency (WUE) (assimilation rate divided by transpiration rate). Parameters were recorded from 8 am–14 pm using a portable infrared gas analyzer (LI-COR Inc., IRGA, Lincolin, NE, USA). Conditions in the leaf chamber were: chamber size 6 cm^2, light intensity 1800 $mol.m^{-2}s^{-1}$ PAR, relative humidity 45%, leaf vapor pressure deficit 1.83 kPa, flow rate 400 $\mu mol.s^{-1}$, reference CO_2 400ppm and leaf temperature 25°C. Data were analyzed using a t-test for normally distributed data (*V. divaricata* – assimilation rate, dark respiration rate and WUE and *A. mearnsii* dark respiration and WUE), while non-parametric data were log transformed and analyzed using a one-way ANOVA (*A. mearnsii* – assimilation rate) in STATISTICA 11 (Statsoft, USA, 2012).

2.5 Biomass measurements

Initial harvesting to compare biomass of the two tree species was done before inoculation, 5 months after the start of the experiment and changes in biomass allocation was calculate as, follows:

$$Organ\ X\ biomass\ allocation = \left(A \times \left(\frac{B - C}{D}\right)\right)$$

"Organ X" was the root or shoot biomass calculated, "A" the relative growth rate of the organ, "B" the fraction of new biomass of the organ, "C" the final biomass of the organ and "D" the final biomass of the whole plant. The final harvest took place 7 days after the gas exchange readings were recorded (8 months). Plants were separated into roots and shoots and dried at 80˚C for 2 days, where after dry weights were recorded (this was during the initial harvest of the two individuals per species). Data were analyzed using a t-test for normally distributed data (*V.*

divaricata – all measured biomass parameters except root:shoot ratio and *A. mearnsii* all measured biomass parameters except root:shoot ratio and root allocation). Non-parametric data were log transformed and analyzed using a Mann Whitney U test (*V. divaricata* - root:shoot ratio and *A. mearnsii* – root:shoot ratio and root allocation).

2.6 Phosphorous and Calcium content

Dried shoots and roots were milled for chemical analysis using a tissue homogenizer (TissueLyser II, Qiagen, Germany) and surface sterilized steel balls. P and C concentration were determined at Elsenburg, Stellenbosch, using an inductively coupled mass spectrometer (ICP-MS). All nutrient data were log transformed and analyzed using a one-way ANOVA.

2.7 Nitrogen stable isotope

$\delta^{15}N$:$\delta^{14}N$ ratio analyses were carried out at the Archeometry Department, University of Cape Town. The isotopic ratio of δ15N was calculated as δ=1000‰ (Rsample/Rstandard), where R is the molar ratio of the heavier to the lighter isotope. Standards used were those defined by Farquhar *et al.* (1989). Between 2.10 and 2.20 mg of each milled sample were weighed into 8 mm x 5 mm tin capsules (Elemental Micro-analysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany). Samples were then combusted in a Fisons NA 1500 (Series 2) CHN analyser (Fisons instruments SpA, Milan, Italy). The δ15N values for the nitrogen gas released were determined on a Finnigan Matt 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), which was connected to a CHN analyzer by a Finnigan MAT Conflo control unit. Three standards were used to correct the samples for machine drift: two in-house standards (Merck Gel and Nasturtium) and the IAEA (International Atomic Energy Agency) standard $(NH_4)_2SO4$.

2.8 Statistical analyses

The effect of infection on the two plant species were assessed separately, as the species specific response would contribute largely to the observed differences. Normality was tested with a Shapiro Wilk's test. Response to infection was tested with a t-test per response variable (N=3), if data was parametric and if data was non-parametric it was transformed using the natural logarithm and analyzed using a one-way ANOVA. Where transformations did not work, a Mann-

Whitney U test was used. The statistical software package STATISTICA 11 (Statsoft, USA, 2012) was used for all data analyses.

3.Results

3.1 Pathogenicity

Infected plants of both *A. mearnsii* (t-value= -15.4; p-value < 0.01) and *V. divaricata* (t-value = -2.9; p-value = 0.04) had longer lesions than control plants (n=3) (Figure 5-1 & 5-2). In order to fulfil Koch's postulate, fungi were re-isolated from the lesions and were subsequently confirmed to be *C. tsitsikammensis*. Since *C. tsitsikammensis* caused significant lesions on seedlings tested, the pathnogenicity of this fungal isolate (NM54) was also tested on mature *A. mearnsii* trees. Inoculated branches formed longer lesions than controls (n= 8, t-value = -3.48; p-value = 0.004) (Figure 5-3). Infected wounds were also characterized by copious amounts of gum oozing from them in most cases.

3.2 Gas exchange

The CO_2 assimilation rates of infected plants were higher on a leaf area basis than in control plants of both species. *A. mearnsii* had much higher average assimilation rates ($mean_{CON}$ = 6.8 µmol $CO_2.m^{-2}.s^{-1}$, $mean_{CT}$ = 17.9 µmol $CO_2.m^{-2}.s^{-1}$) than *V. divaricata* ($mean_{CON}$ = 0.4 µmol $CO_2.m^{-2}.s^{-1}$, $mean_{CT}$ = 1.9 µmol $CO_2.m^{-2}.s^{-1}$). Dark respiration was not different between infected and non-infected plants of (*A. mearnsii* (t-value = 0.07, p-value = 0.94) and *V. divaricata* (t-value = 0.81, p-value=0.11)). WUE, calculated by dividing CO_2 assimilation rate by transpiration rate, was not different between infected and non-infected *A. mearnsii* plants (t-value=0.39; 0.71). The WUE of *V. divaricata* was higher in infected than control plants (t-value= -2.91; p-value = 0.04). *A. mearnsii* had higher overall WUE ($mean_{CON}$= 9.3 , $mean_{CT}$= 8.2) than *V. divaricata* ($mean_{CON}$= 1.2, $mean_{CT}$= 2.2) (Figure 5-4).

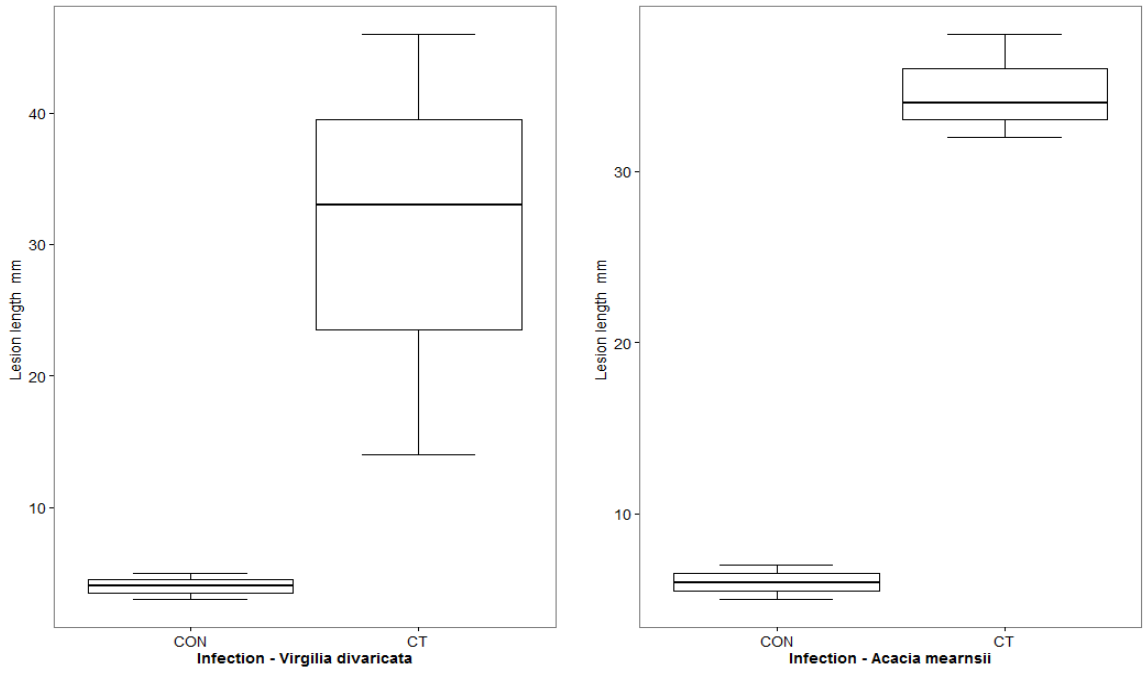

Figure 5-1 Lesion lengths formed 3 months after inoculation with an isolate of *Ceratocystis tsitsikammensis* (NM54) as recorded from 8 month old seedlings of *A. mearnsii* and *V. divaricata*. The values are represented by mean (n =3) for *V. divaricata* and median for *A. mearnsii* with standard deviations. An asterisk indicates significant differences between treatments ($P \leq 0.05$). Infected plants are labelled as CT and control plants as CON.

Figure 5-2 Lesions formed 3 months after inoculation with an isolate of *Ceratocystis tsitsikammensis* (NM54) on 8 month old seedlings of *Acacia mearnsii* **(a-c)** and *Virgilia divaricata* **(d-f)**. (**a & f) =** Control plants. (**b, c & d, e) =** Infected plants.

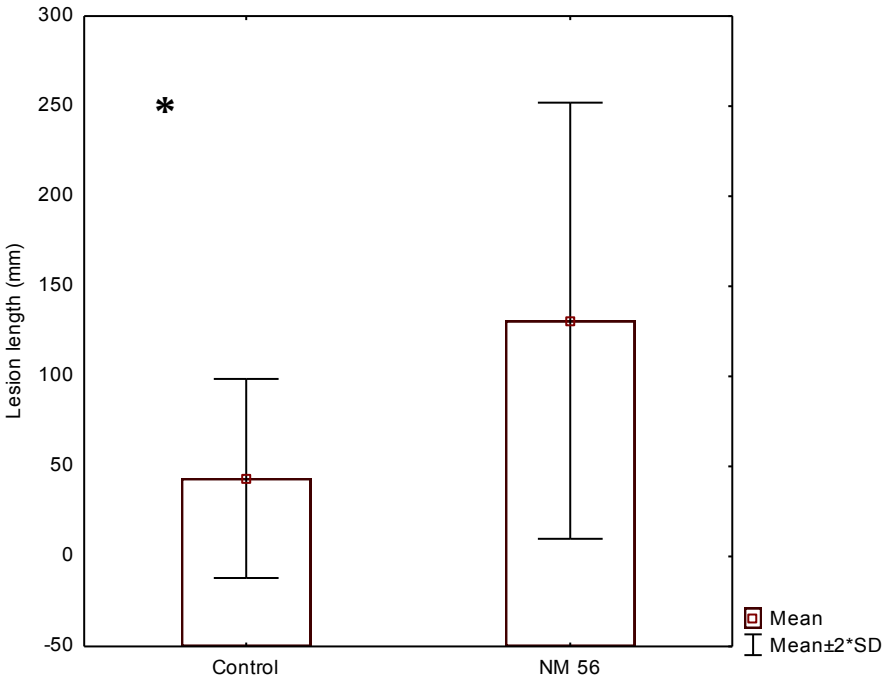

Figure 5-3 Lesion lengths formed 6 weeks after field inoculation with an isolate of *Ceratocystis tsitsikammensis* (NM56) in *A. mearnsii*. The values are represented by means (n =8) with standard deviations. An asterisk indicates significant differences between control and infected plants ($P \leq 0.05$).

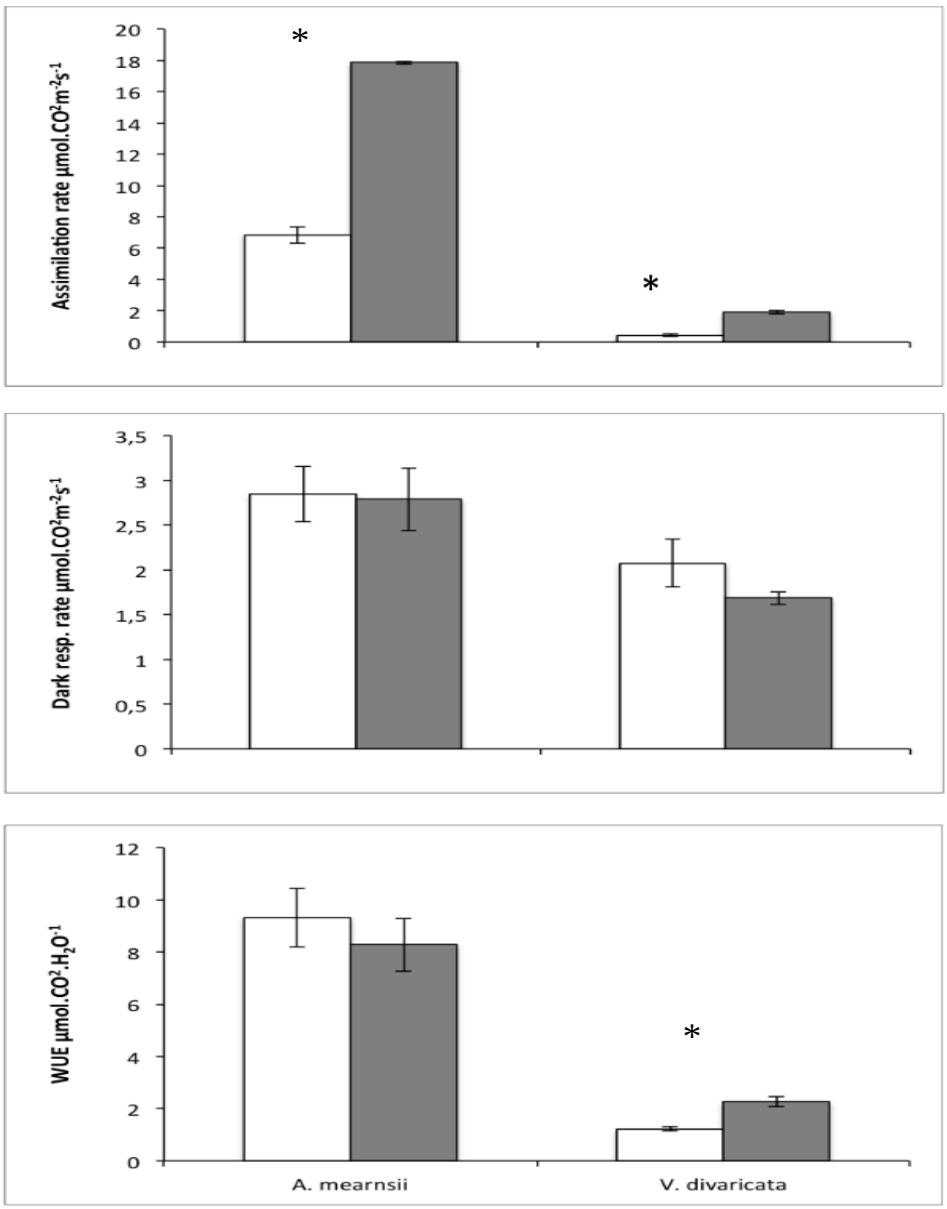

Figure 5-4 The photosynthetic gas exchange of 8 month old *Acacia mearnsii* and *Virgilia divaricata* plants grown in sand culture. Plants were treated with 0.05 mM P and stem-infected with the vascular infecting pathogen *Ceratocystis tsitsikammensis* after 5 months of growth. The values are represented by means (N =3) with standard deviations. An asterisk indicates a significant difference between control and infected plants within each species, respectively ($P \leq 0.05$). Grey columns indicate infected plants and white columns indicate control plants.

3.3 Biomass

The growth rate and overall mass (data not shown) was unaffected by infection status in the two species (Figure 5-5). *A. mearnsii* had a marked change in biomass allocation, with higher root allocation and root mass in infected than control plants (Figure 5-5). Inversely, this species displayed lower shoot mass and shoot allocation in infected plants, although these differences were not significant. The lower stem mass of infected plants was different from that of control plants (data not shown), responding to the higher root allocation and mass in infected plants. The root:shoot ratio was therefore also higher in infected plants. These differences in allocation were not observed in *V. divaricata* (Figure 5-5). This species showed no differences in these biomass response variables. Although a non-significantly larger root mass was observed, there were no signs of a trade-off between allocations to roots, stems or shoots as was seen in *A. mearnsii*.

3.4 Nutrient content

Percentage N, C, P and Ca was similar in all plant parts in both species (Table 5-1). There was no significant difference between infected and control plants, thus infection did not influence the distribution of these minerals between plant parts. Higher $\delta^{15}N$ isotope ratios were recorded in shoots of infected *V. divaricata* plants. There was no difference in $\delta^{15}N$ isotope ratios within *A. mearnsii*.

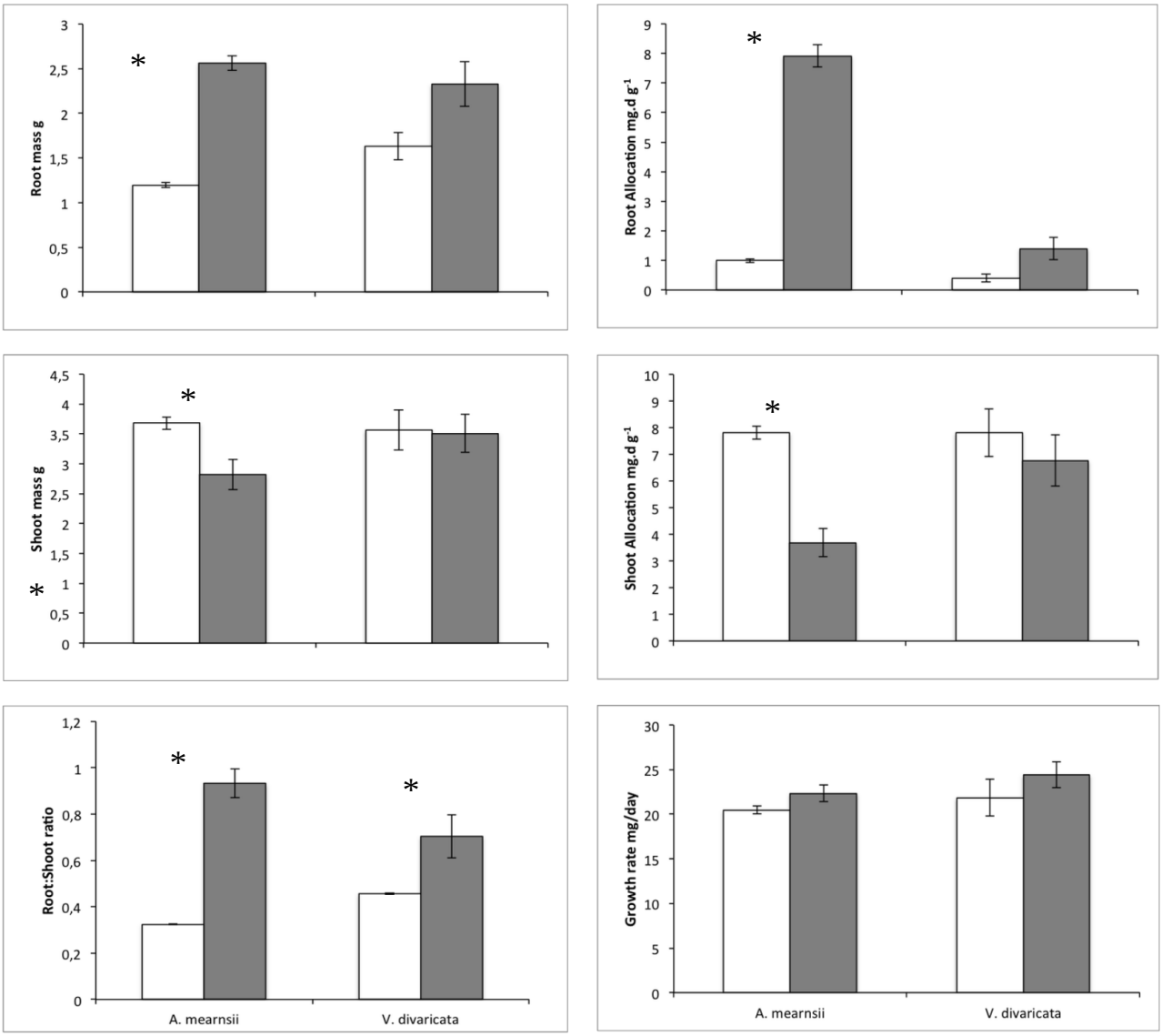

Figure 5-5 Biomass and biomass allocation in 8 month old infected and control plants of *Virgilia divaricata* and *Acacia mearnsii* grown in sand culture. The values are represented by means (N =3) with standard deviations. An asterisk indicates a significant difference between control and infected plants within each species ($P \leq 0.05$). Grey columns indicate infected plants and white columns indicate control plants.

Table 5-1 N, $\delta^{15}N:\delta^{14}N$, C, N, P and Ca concentration of plant organs of 8 month old *A. mearnsii* and *V. divaricata* plants, after infection with the vascular infecting pathogen *Ceratocystis tsitsikammensis*. The values are represented by means (N = 3). Bold asterisks indicate significant differences between treatments ($P \leq 0.05$). Infected plants are labeled as CT and control plants as CON.

	A. mearnsii				*V. divaricata*			
	Roots		Shoots		Roots		Shoots	
	CT	CON	CT	CON	CT	CON	CT	CON
%N	1.37	0.88	1.13	1.27	1.22	1.50	1.25	1.36
$\delta^{15}N:\delta^{14}N$	5.43	8.06	5.70	7.61	6.12	5.70	**6.17***	**4.83***
mmol C/g	33.87	35.07	24.12	31.79	36.99	38.04	31.24	35.70
mmol N/g	0.98	0.63	0.80	0.90	0.87	1.07	0.89	0.97
mmol P/g	0.27	0.37	0.41	0.35	0.02	0.02	0.02	0.01
mmol Ca/g	0.11	0.11	0.27	0.20	0.04	0.04	0.11	0.11

4.Discussion

A. mearnsii and *V. divaricata* plants responded differently to infection by the native fungal species *C. tsitsikammensis*. *V. divaricata* responded to infection by increased photosynthesis, while *A. mearnsii* responded both through increased photosynthesis and in altered biomass allocations. Both species had limited nutritional response to infection, but *V. divaricata* had a change in $\delta^{15}N$ ratio in its shoots. Results could not fully support the BRH, as the response costs were not quantified between the two tree species. We did find partial support for more resistance in the native tree species, as it showed less response to infection and no reallocation of biomass. This delay in the growth response of the native legumes suggests that the local plants may be adapted to this pathogen, and may even respond at a different functional level (Oliveira *et al.*, 2012).

Infection caused higher photosynthetic rates in both plants, which contrasts with results of previous studies (Agios, 2005; Oliveira *et al.*, 2012). Wilt-causing fungal pathogens generally cause a decrease in photosynthetic rate (Agios, 2005) for two possible reasons. Firstly the fungi secrete toxins that cause an increase in lipid peroxidation and membrane degradation (Oliveira *et al.*, 2012). The extent of reduction in photosynthesis caused by stress-infection is dependent on how much the total non-structural carbohydrate pool of the plant relies on immediate photosynthesis. In seedlings (used in this study) this carbohydrate pool is quite small, resulting in depletion during infection or other stress conditions (Niinemts, 2010). Secondly the wilting of leaves would result from a lack of leaf turgor, as mediated by water stress. Therefore a loss of turgor would result in a stomatal closure, and thereby also limit photosynthesis. There are also other examples, by which pathogens affect the water relations of host plants. In the case of Oak wilt, xylem blockage inhibits water flow (Beckman *et al.*, 1953; Anderson *et al.*, 2000), leading to stomatal closure, thereby reducing photosynthesis and eventually causing wilt (TeBeest *et al.*, 1976). Based on this evidence we expected a decrease in photosynthetic rate in infected plants, but this was not found. Instead, as in the case of some endophyte-infected plants, there was an increase in photosynthesis. The precise mechanisms driving this increase is not yet understood (Swarthout *et al.*, 2009), but may be related to sink stimulation of photosynthesis in order to provide C to the host, as an energy source to mount a response.

A possible explanation could be that the photosynthetic machinery of the plant has not been infected. Previous studies have shown photosynthetic increases in the asymptomatic tissues

of infected plants (Horst *et al.*, 2010). This agrees with our results, as in our case the stems (and not leaves) were infected. However, the pathogen is a vascular infecting pathogen and movement to other localities in the plant would be possible. Previous studies found that *Ophiostoma ulmi* (Buimann) Nannf. could colonize remote plant tissue from the point of infection such as the leaf midrib and secondary veins (Pomerleau & Mehran, 1966, Nasmith *et al.*, 2008). As such movement would require time, it may be that movement to the leaf tissue may still have been in progress in our study. We could therefore still identify the leaves as asymptomatic tissue. As found in mildew infection, uninfected leaves or leaf tissue with photosynthesis rates sustained infected leaves/tissue (Horst *et al.*, 2010). Anderson *et al.* (2000) found higher rates of photosynthesis in healthy leaves on recovering trees and asymptomatic leaves on infected trees, while the symptomatic leaves turned into a photosynthate sinks, potentially driving the higher photosynthesis rates. Similarly Horst *et al.* (2010) suggested that healthy maize (*Zea mays L.*) leaves in close proximity to leaves infected by the smut fungus *Ustilage maydis* , may have provided infected tissues with photosynthate. Leaves in our study may have been stimulated in this same sink effect manner to produce more photosynthate in order to sustain the pathogen (Horst *et al.*, 2010).

As the infection progresses over time, the response of the plant may change. Oliveira *et al.* (2012) experienced an initial difference in basal fluorescence between control and infected plants and later an increase to levels similar to the control plants. Anderson *et al.* (2000) found much higher rates of photosynthesis in recovering plants than control plants, implying that plants that are recovering from infection may increase their photosynthesis to provide enough photosynthate to repair damage. The production costs of defense compounds are high (Villari *et al.*, 2012) and may also result in an increase in photosynthesis to replenish the carbohydrate pool (Niinemts, 2010). We measured photosynthetic rates after five months of inoculation. If the pathogen was not very virulent or non-pathogenic, the plants would be in a state of recovery. If, however, the pathogen was highly virulent, the plant would be actively fighting the pathogen (Zangerl *et al.*, 1997). Both responses have C costs associated with them and these may be the drivers of higher photosynthesis in infected plants. As *C. tsitsikammensis* is known to be a very virulent pathogen, and formed significant lesions on plants inoculated in the field, the later scenario is more likely.

Higher photosynthesis in infected plants of both host species did not translate into larger plants or faster growth rates. This was surprising, as a typical response to infection is a

decrease in plant biomass (Atkin *et al.*, 1999; Taiz & Zeiger, 2006; Oliveira *et al*., 2012). Despite the lack of change in net plant biomass and growth rate, *A. mearnsii* demonstrated a trade-off between roots and shoots, with larger roots developing in infected plants. This was not found in *V. divaricata*. The formation of larger roots is a typical response to drought or nutrient shortages (Agios, 2005; Taiz & Zeiger, 2006; McDowell *et al.*, 2008). Stressed plants have been shown to re-allocate their resources to their storage organs (Chapin III 1980; Joern & Mole, 2005; Ward *et al*., 2011) under unfavorable conditions. In this study plants received adequate nutrient sources and water (Wilkowski & Mitchell, 1987; Power *et al.*, 2010; Maistry *et al.*, 2012), therefore the supply of these resources cannot be furnished to account for the increased root mass allocation. Vascular infecting fungi, specifically *Ceratocystis* species, are known to elicit drought stress response symptoms (Pegg, 1985; Agios, 2005). Entry *et al.* (1986), for example, found an increase in root size in infected plants with the root pathogen *Armillaria*. It is therefore possible, that the observed alterations in root allocation could be related to a fungal-induced drought stress.

Vascular infection causes blockage in the xylem tissue by mycelium growth, tyloses or gum accumulation (Berryman *et al.*, 1991; Paine *et al.*, 1997; Croise *et al.*, 2001; Agios, 2005; Kobayashi, 2005 & Villari *et al*., 2012), preventing translocation of water and nutrients. (Ploetz, 2006). Thicker roots would contain more xylem tissue, increasing the probability that some vessels may remain unblocked, despite the infection (Agios, 2005). Larger roots in infected plants could thus be viewed as an attempt to increase the root area for both water and nutrient capture (Forrester *et al*., 2010; Ryan, 2011). An increase in water capture may overcome water stress (Taiz & Zeiger, 2005) and nutrient capture may sustain the increased root growth along with higher assimilation rates as were observed.

The higher photosyntate produced in infected *A. mearnsii* plants could potentially be allocated to increase root growth, while *V. divaricata* may be allocating this nutrition to other mechanisms of defense or plant resistance. While *A. mearnsii* had no change in nutrient accumulation in different plant organs, *V. divaricata* had a significantly higher reliance on NDFA based on $\delta^{15}N{:}\delta^{14}N$ ratio in infected shoots. This species make use of soil derived N more than atmospheric N, indicating that during times of infection-stress these plants utilized N from a cheaper source than the energetic expensive process of BNF (Pate & Layzell, 1990). This provides *V. divaricata* with some competitive advantage over *A. mearnsii*, as this species continues to use BNF irrespective of stress conditions. Tye & Drake (2012)

demonstrated how the native legume species *Acacia karoo* had the ability to switch between the proportions of BNF and N derived from the soil under normal field conditions. It is thus a facultative BNF species, depending on the soil N availability. They also included *A. mearnsii* in their study, but could not determine if it was an obligate or facultative BNF species. Here we find some evidence that *A. mearnsii* maintains its N acquisition strategy even during stressful conditions.

There was a general absence of typical above-ground disease symptoms in both host plants. This could imply that neither of the tree species are hosts to this pathogen (Freeman & Beattie, 2008), and may be viewed as non-host plants. This is, however, unlikely for two reasons. When inoculated in both the laboratory and in the field, *C. tsitsikammesis* formed significant long lesions on infected plants. Secondly, this fungus was isolated from *V. divaricata* in its native ranges by Machingambi *et al.* (2013). In that study, the fungus was associated with the larvae tunnels made by the ghost moth (*Leto venus* Cramer) (Machingambi *et al.*, 2013). These moths use of this tree as a breeding site (Nielsen *et al.*, 2000) and their larvae feed exclusively on *V. divaricata*. The associated fungi may thus also have an established relationship with this plant species. *V. divaricata* cannot be classified as a non-host plant of *C. tsitsikamma* and the lack of visible response to infection may be explained by the plant making use of other mechanisms such as changes in N source to withstand the effects of infection (Agios, 2005).

5.Conclusion

Since plant physiological pathology is very complex in the natural environment, we attempted to remove some of this complexity by using a controlled environment. This preliminary study explored the physiological effects of the native fungus *C. tsitsikammensis* on *A. mearnsii* and *V.divaricata*. Results provided some insight into the use of physiological responses and resource capture, along with lesion lengths, as a measure of the effect of a pathogen on its host plant. The reduction in shoot biomass and enlargement of roots in *A. mearnsii* is not ideal for forestry. From a conservation perspective, this is potentially promising, as the invasion may be slowed down if the plant transfers its resources to below ground parts. This leaves less available resources to other energy expenses such as reproductive output. Although significant lesions were formed after infection of *V. divaricata* with this pathogen, this host tree was able to maintain its biomass distribution and switch to soil derived N. This could be a risky strategy if there is low soil N availability. The difference

in response to the pathogen may be ascribed to a well-established ancient relationship between *V. divaricata* and *C. tsitsikammensis*, which is absent between *A. mearnsii* and this fungus. This study provides the first account of the effects of *C. tsitsikammensis* on photosynthetic rates, plant biomass and nutrient content of these two host plants. Future studies need to include higher replication numbers, repeated harvesting and different isolates of this fungus in order to understand how fungal virulence affects disease development. The collection and analysis of defense chemicals could also provide much more information.

6.References

Agios, G.N. 2005. *Plant Pathology*. UK: Elsevier Academic Press Publication.

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86: 2979–2989.

Anderson, L.J., Harley, P.C., Monson, R.K. & Jackson, R.B. 2000. Reduction of isoprene emissions from live oaks (*Quercus fusiformis*) with aok wilt. Tree Physiology 20: 1199–1203.

Appel, D.N., Kurdyla, T. & Lewis, J., R. 1990. Nitidulids as vectors of the oak wilt fungus and other *Ceratocystis* spp. in Texas. European Journal of Forest Pathology 20: 412–417.

Atkin, O.K., Schortemeyer, M., McFarlene, N. & Evan, J.E. 1999. The response of fast-and slow-growing Acacia species to elevated atmospheric CO_2: an analysis of the underlying components of relative growth rate. Oecologia 120: 544–554.

Beckman, C.H., Kuntz, J.E., River, A.J. & Berbee, J.G. 1953. Host responses associated with the development of oak wilt. Phytopathology 4: 448–454.

Berryman, C.A., Eamus, D. & Farrar, J.F. 1991. The hydraulic conductivity of roots of rust-infected barley seedlings. Physiological and Molecular Plant Pathology 38: 407–415.

Beukes, C.W., Law, I.J., Venter, S.N. & Steenkamp, E.T. 2011. Phylogenetic characterisation of root-nodule bacteria associated with indigenous fynbos legumes. In Villet, M.H. and Crous, K.L. 2011. Proceedings of the Congresses of Southern African Society for Systematic Biology.

Blaney, C.S. & Kotanen, P.M. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. Journal of Applied Ecology 38: 1104–1113.

Brasier, C.M. 1990. China and the origins of Dutch elm disease: an appraisal. Plant Pathology 39: 5–16.

Bridges, J.R. & Moser, J.C.1983. Role of two phoreticmites intransmission of bluestain fungus, Ceratocystis minor. Ecological Entomology 8: 9–12.

Chapin III, F.S., Diaz, S., Eviner, V.T., Hobbie, S.E., Hooper, D.U., Lavorel, S., Mack, M.C., Naylor, R.L., Reynolds, H.L., Sala, O.E., Vitousek, P.M. & Zavaleta, E.S., 2000. Consequences of changing biodiversity. Nature 405: 234

Croise, L., Lieutier, F., Cochard, H. & Dreyer, E. 2001. Effects of drought stress and high density stem inoculations with *Leptographium wingfieldii* on hydraulic properties of young Scots pine trees. Tree Physiology 21: 427–437.

Department of Agriculture, Forestry and Fisheries. 2009. State of the forest report.

Entry, J.A., Martin, N.E., Cromack Jr. & K., Stafford, S.G. 1986. Light and nutrient limitation in *Pinus monticola*: Seedling susceptibility to *Armillaria* infection. Forest Ecology and Management 17: 189–198.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. Annual Review Plant Physiology 40: 503–537.

Forrester, D.I., Theiveyanathan, S., Collopy, J.J. & Marcar, N.E. 2010. Enhanced water use efficiency in a mixed Eucalyptus globulus and *Acacia mearnsii* plantation. Forest Ecology and Management 259: 1761–1770.

Freeman, B.C. & Beattie, G.A. 2008. An overview of plant defenses against pathogens and herbivores. *The Plant Health Instructor.* DOI: 10.1094/PHI-I-2008-0226-01.

Goldblatt, P. & Manning, J. 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria : National Botanical Institute, Pretoria.

Henderson, L. 2007. Invasive, naturalized and casual alien plats in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37: 215–248.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. Technical Communication: Nr. 22. Commonwealth Bureau of Horticulture and Plantation Crops, East Malling.

Horst, R.J., Doehlemann, G., Wahl, R., Hofmann, J, Schmiedl, A., Kahmann, R., Kamper, J., Sonnewals, U. & Voll, L.M. 2010. *Ustilago maydis* Infection strongly alters organic Nitrogen allocation in maize and stimulates productivity of systematic source leaves. Plant Physiology 152: 293–308.

Huber. D.M. & Hanekleus, S. 2007. Managing nutrition to control plant disease. 2007. Landbauforschung Volkenrode, 57: 313–322.

Jackson, A.P. 2004. A reconciliation analysis of host switching in Plant-fungal symbioses. Evolution 58: 1909–1923.

Jankowiak, R., Bilanski, P., Kolarik, M. & Wasiuta, R. 2012. Root-colonizing ophiostomatoid fungi associated with dying and dead young Scots pine in Poland. Forest Pathology 1-9.

Joern, A. & Mole, S. 2005.The plant stress hypothesis and variable responses by blue grama grass (*Bouteloua gracilis*) to water, mineral nitrogen and insect herbivory. Journal of Chemical Ecology 31: 2069–2090.

Jones, J.D. & Dangl, J.L. 2006. The plant immune system. Nature 444: 323–329.

Juzwik, J., Harrington, T.C., MacDonald, W.L. & Appel, D.N. 2008. The origin of Ceratocystis fagacearum, the Oak Wilt Fungus. Annual Review Phytopathology 46: 13–26

Kamgan, G.N., Wingfield, M.J., Mohammed, C., Carnegie, A.J., Pegg, G.S. & Roux, J. 2012. *Ceratocystis* species, including two new species associated with nitidulid beetles, on eucalypts in Australia. Antonie van Leeuwenhoek 101: 217–241.

Kamgan, N.G., Jacobs, K., de Beer, Z.W., Wingfield, M.J. & Roux, J. 2008. *Ceratocystis* and *Ophiostoma* species including three new taxa, associated with wounds on native South African trees. Fungal Diversity 29: 37–59.

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.

Kirisits, T. 2004. Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: *Bark and wood boring insects in living trees in Europe, a synthesis*. (Lieutier F, ed.). Kluwer Academic Press, The Netherlands: 1–55.

Kobayashi, M. 2005. Wilt disease of *Fagaceae* trees caused by *Platypus quercivorus* (Murayama) (Coleptera: Platypodidae) and the associated fungus: Aim is to clarify the damage factor. Forest Experiment Station, Wachi.

Lombardero, M.J., Alonso-Rodríguez, M. & Roca-Posada, E.P. 2012. Tree insects and pathogens display opposite tendencies to attack native vs. non-native pines. Forest Ecology and Management 281: 121–129.

Machingambi, N.M. 2013. An investigation into the death of native Virgilia trees in the Cape Floristic Region of South Africa. Master Thesis. Stellenbosch: University of Stellenbosch.

Magadlela, A. 2013. The effect of phosphorus (P) deficiency on growth and nitrogen fixation of Virgilia trees from the Cape Floristic Region (CFR). MSc thesis. Cape Town: University of Stellenbosch.

Maistry, P.M., Cramer, M.D. & Chimphango, S.B.M. 2012. N and P colimitation of N2-fixing and N-supplied fynbos legume from the Cape Floristic Region. Plant Soil.

Marr, D.L. & Marshall, M.L. 2006. The role of fungal pathogens in flower size and seed mass variation in Hydrophyllum (Hydrophyllaceae). American Journal of Botany 93: 389-398.

Mbambezeli, G., & Notten, A. 2003. *Virgilia* genus. http://www.plantzafrica.com/planttuv/virgilia.htm

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Kold, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yepez, E.A. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist - Tansley review 178: 719–739.

Morris, M. J., Wingfield, M. J. & De Beer, C. 1993. Gummosis and wilt of Acacia mearnsii in South Africa caused by *Ceratocystis fimbriat*a. Plant Pathology 42: 814–817.

Moser, J.C. 1997. Phoretic mites and their hyperphoretic fungi associated with flying Ips typographys japonicus Niijima (Coleoptera: Scolytidae) in Japan. Journal of Applied Entomology 121: 425–428

Nasmith, C., Jeng, R. & Hubbes, M. 2008. A comparison of in vivo targeted gene expression during fungal colonization of DED-susceptible *Ulmus americana*. Forest Pathology 38: 104–112.

Nielsen, E.S., Robinson, G.S. & Wagner, D.L. 2000. Ghost-moths of the world; a global inventory and bibliography of the Exoporia (Mnesarchaeoidae and Hepialoidea - Lepidoptera). Journal of Natural History 34: 823–837.

Niinemets, U. 2010. Response of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management: 260: 1623–1639.

Oliveira, H., Sousa, A., Alves, A., Nogueira, A.J.A. & Santos, C. 2012. Inoculation with *Ophiostoma novo-ulmi subsp. americana* affects photosynthesis, nutrition and oxidative stress in in-vitro *Ulmus minor* plants. Environmental and experimental botany 77: 146–155.

Omari, B.E., Fleck, I., Aranda, X., Moret, A. & Nadel, M. 2001. Effect of fungal infection on leaf gas-exchange and chlorophyll fluorescence in *Quercus ilex*. Annual Forest Science 58: 165–173.

Paine, T.D., Raffa, K.F. & Harrington, T.C. 1997. Interactions among scolytid bark beetles, their associated fungi and live host conifers. Annual Review of Entomology 42: 179–206.

Parker, I.M. & Gilbert, G.S. 2007.When there is no escape: the effects of natural enemies on native, invasive and noninvasive plants. Ecology 88: 1210–1224.

Pate, J.S. & Layzell, D.B. 1990. Energetics and biological cost of nitrogen. In: Stumpf, P.K., Conn, E.K., Miflin, B.J. & Lea, P.J. (ed) The biochemistry of plants, a comprehensive treatise: intermediary nitrogen metabolism assimilation, (16). Academic Press: San Diego, 1–42.

Paulin-Mahady AE, Harrington TC, McNew D, 2002. Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia, 94: 62-72.

Pegg, G.F. 1985. Life in a black hole – The micro-environment of the vascular pathogen. Transactions of the British Mycological Society 85: 1–20.

Ploetz, R.C. 2006. Fuserium induced diseases of tropical perennial crops. Phytopathology 96: 648–652.

Pomerleau, R. & Mehran, A.A. 1966. Distribution of spores of *Ceratocystis ulmi* labeled with phosphorus 32 in green shoots and leaves of *Ulmus americana*. Naturaliste Can. 93: 577–582.

Power, S.C., Cramer, M.D., Verboom, G.A. & Chimphango, B.M. 2010. Does phosphate acquisition constrain legume persistence in the fynbos of the Cape Floristic Region? Plant Soil 46: 333–334.

Prell, H.H., 1925; 2001. Plant-Fungal Pathogen Interaction: A Classical and Molecular View Berlin : Springer, Berlin.

Rodrìguez-Echeverrıa, S., Le Roux, J.J., Criso´ stomo, J.A. & Ndlovu J. 2011. Jack-of-all trades and master of many? How does associated rhizobial diversity influence the colonization success of Australian Acacia species?. Diversity and Distributions. 17: 946–957.

Roets, F., De Beer, Z.W., Dreyer, L.L., Crous, P.W., Zipfel, R. & Wingfield, M.J. 2006. Multi-gene phylogeny of *Ophiostoma* spp. associated with *Protea* infructescenses including two new species. Studies in Mycology 55: 199–212

Roets, F., Theron, N., Wingfield, M.J. & Dreyer, L.L., 2012. Biotic and abiotic constraints that facilitate host exclusivity of Gondwanamyces and Ophiostoma on Protea. Fungal Biology 116: 49–61.

Roux, J., Dunlop, R. & Wingfield, M. J. 1999. Susceptibility of elite Acacia mearnsii families to Ceratocystis wilt in South Africa. Journal of Forestry Research 4: 187–190.

Roux, J., Meke, G., Kanyi, B., Mwangi, A., Hunter, G.C., Nakabonge, G., Heath R.N. & Wingfield, M.J. 2005. Diseases of plantation forestry tree species in Eastern and Southern Africa. South African Journal of Science 101: 409–413.

Roux, J. & Wingfield, M. J. 1997. Survey and virulence of fungi occurring on diseased Acacia mearnsii in South Africa. Forest Ecology and Management 99: 327–336.

Roux, J., Wingfield, M. J. & Byabashaija, D. M. 2001. First report of Ceratocystis wilt of Acacia mearnsii in Uganda. Plant Disease. 85: 10–29

Ryan, M.G. 2011.Tree response to drought: Commentary. Tree Physiology 31: 237–239.

Six, D.L. 2003 Bark beetle–fungus symbioses. In Insect symbiosis (eds K. Bourtzis & T. A. Miller), pp. 97–114. New York, NY: CRC Press.

Somasegaran, P. & Hoben, H.J. 1985. Method in legume-rhizobium technology. MSc Thesis. Hawaii: University of Hawaii.

Swarthout, D., Harper, E., Judd, S., Gonthier, D., Shyne, R., Stowe, T. & Bultman, T. 2009. Measures of leaf-level water-use efficiency in drought stressed endophyte infected and non-infected tall fescue grasses. Environmental and experimental botany 66: 88–93.

Taiz, L. & Zeiger, E. 2006. Plant Physiology Sunderland, Mass: Sinauer Associates, Sunderland, Mass.

TeBeest, D.O., Durdin, R.D. & Kuntz, J.E. 1976. Stomatal resistance of red oak seedlings infected by *Ceratocystis fagacearum*. Phytopathology 66: 1295–1297.

Teviotdale, B.L. & Harper, D.H. 1991. Infection of pruning and small bark wounds in almond by *Ceratocystis fimbriata*. Plant Disease 75:1026-1030.

Thrall, P.H., Millsom, D.A., Jeavons, A.C., Waayers, M., Harvey, G.R., Bagnall, D.J. & Brockwell, J. 2005. Seed inoculation with effective root-nodule bacteria enhances revegetation success. Journal of Applied Ecology 42: 740–751.

Turk, D. & Keyser, H.H. 1992. Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. Canadian journal of microbiology 38: 451–460.

Tye, D.R.C. & Drake, D.C. 2012. An exotic Australian Acacia fixes more N than a coexisting indigenous *Acacia* in a South African riparian zone. Plant Ecology 213: 251–257.

Upadhyay, H.P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*, 176.

Valarini, P.J. & Tokeshi, H. 1980. *Ceratocystis fimbriata*, causal agent of fig dieback, and its control. Summa Phytopathologica.

Van der Colff, D. 2013. Plant stress and the prevalence of pests and pathogens associated with a native and an invasive alien legume tree in the Cape Floristic Region, South Africa. MSc thesis. Univeristy of Stellenbosch, Stellenbosch.

Villari, C., Battisti, A., Chakraborty, S., Michelozzi, M., Bonello, P. & Faccoli, M. 2012. Nutritional and pathogenic fungi associated with the pine engraver beetle trigger comparable defenses in Scots pine. Tree physiology: 1–13.

Ward, C.L., Kleinert, A., Scortecci, K.C., Benedito, V.A. & Valentine, A.J. 2011. Phosphorus-deficiency reduces aluminium toxicity by altering uptake and metabolism of root zone carbon dioxide. Journal of Plant Physiology 168: 459–465.

Wilkowski, E.T.F. & Mitchell, D.T. 1987. Variations in Soil Phosphorus in the Fynbos Biome, South Africa. Journal of Ecology 75: 1159.

Wingfield, M.J., Slippers, B., Roux, J. & Wingfield, B.D. 2001. Worldwide movement of exotic forest fungi, especially in the tropics and the Southern hemisphere. BioScience 51: 134–140.

Zangerl, A.R., Arntz, A.M. & Berenbaum, M.R. 1997. Physiological price of an induced chemical defense: Photosynthesis, Respiration, Biosynthesis & Growth. Oecologia 109: 433–441.

Zhou, D. & Hyde, K. 2001. Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycological Research 105: 1449–1457.

Chapter 6 **Conclusion**

1.Thesis summary

The CFR is a biodiversity hotspot, but invasive alien plants are threatening this diversity (Goldblatt & Manning, 2000; Mucina & Rutherford, 2006; Le Maitre *et al.*, 2011; Richardson & Rejmanek, 2011). Forests within this region are highly fragmented, making them one of the most threatened biomes in South Africa (Mucina & Rutherford, 2006; Van der Merwe, 2011). Within forest margins where *A. mearnsii* invades, it co-occurs with the ecologically similar species *V. divaricata* (Ge1denhuys, 1986; Mbambezeli & Notten, 2003; Henderson, 2007; Geldenhuys, 2004). This is important, as there are not many legume trees present in the CFR (Goldblatt & Manning, 2000; Maistry *et al.*, 2013). The co-occurrence of these ecologically similar species has resulted in an interesting interaction in terms of biotic and abiotic factors. We found that even though these species are not as closely related as congeneric species, they are very similar in their interactions with the natural environment and their associated pest and pathogens.

We assessed how these two species interact with their pest and pathogens using the Enemy Release Hypothesis (Wolfe 2002; Siemann & Rogers 2003) and the Biotic Resistance Hypothesis (Blaney & Kotanen, 2001; Keane & Crawley, 2002; Agrawal *et al.*, 2005; Lombardero *et al.*, 2012) as reference scenarios. Pests and pathogens were found to respond differently to the two host plants as predicted. A high number of arthropods were found to be shared between these two host plants, but the native host had much higher levels of abundance in overall arthropods and herbivores. This supported predictions of the ERH. Herbivores preferred the native host and recognized it as a food source (Keane & Crawley, 2002). As the invasive species hosts shared similar arthropod species with the native host, difference in abundances may decline in time. If, however, *A. mearnsii* possess inherent unique protections, this may not be the case (Callaway & Ridenour, 2004). Our results showed no difference in the nutrients present in the leaves of the native and invasive species. Insect associates were found to preferentially utilize *V. divaricata*, supporting the notion that they share an evolutionary relationship with this plant. These arthropods appear not to be adapted to utilizing *A. mearnsii* as extensively yet.

A. mearnsii herbivore abundances were unaffected by water stress conditions, while herbivore abundances of *V. divaricata* had a negative relationship with water stress (more

herbivores on drought stressed plants). Climatic changes are predicted to cause drier weather conditions (IPCC, 1996; 2001), which could make *V. divaricata* more vulnerable to future herbivorous attack. Within these habitats *A. mearnsii* are thus predicted to be unaffected by the drier conditions in future, and may incur a competitive advantage.

The distribution of the two tree species are contrasting (Goldblatt & Manning, 2000; Mucina & Rutherford, 2006; Henderson, 2007; DEA, 2009), with *A. mearnsii* being spread within and between forest margins, potentially increasing the connectivity of these forest fragments. As pests are shared between these two tree species where they co-occur, such pests may utilize *A. mearnsii* as a corridor to access previously isolated forest margins and other *V. divaricat*a populations. We also found that ophiostomatoid fungal pathogens are associated with both of these tree species. The spread of these pathogens into the forestry areas of South Africa are therefore possible if the connectivity we observed for arthropod communities are held true for fungal associates of *A. mearnsii* as well. Five different ophiostomatoid fungi were collected in association with both tree species, and these were collected for the first time within the invaded ranges of *A. mearnsii*. This emphasises the need for further research focussed on the pathogens associated with both native and invasive flora.

We assessed the influence of *Ceratocystis tsitsikammensis*, isolated from *V. divaricata*, on both these trees in a controlled environment and found some support for the BRH. *A. mearnsii* responded by both changes in photosynthetic rate and biomass re-allocation in infected plants. *V. divaricata* also changed its photosynthesis rate and made use of a cheaper source of N during these infection-caused stress conditions. These findings support the BRH as *A. mearnsii* showed a markedly more severe response to infection. Within the natural environment, uninfected plants of *V. divaricata* utilized more BNF, while *A. mearnsii* switched between the use of BNF and soil nitrogen capture. This showed that the native tree has the capacity to switch to a cheaper source of N under stressed conditions such as fungal infection.

The similar nutrient economies of these two legumous species was interesting, as they have different leaf morphologies (Sherry, 1971; Searle, 1997; Mbambezeli & Notten, 2003) and *A. mearnsii* is pre-adapted to a nutrient impoverish habitat in its native ranges (Orchard & Wilson, 2001). We expected the invasive species to have some competitive advantage in terms of nutrient resorption and capture, but the two species were found to be very similar in terms of these traits. The two species had similar nutrient contents, but the way in which pest

and pathogens responded to these nutrient contents differed between the two host plants. *V. divaricata* herbivore loads were unaffected by plant nutrition, while *A. mearnsii* had higher herbivore loads on nutrient poor trees and larger lesions developed after infection of nutrient rich trees. This supports results of previous studies that showed that herbivores and pathogens respond differently to the nutrition of their host plant (Entry 1986; Huber& Hanekleus, 2007; Ayres & Lombardero, 2000; McMahon, 2012; Huber & Jones, 2013; Oliveira *et al.*, 2014). It has implications for foresters, as nutrient enhancement within plantations may benefit pest control, but it may also have the inverse effects on pathogens. This is less of a threat in the invaded ranges of *A. mearnsii* in South Africa, as wounds on *A. mearnsii* are not as common in these areas.

2.Conservation implication

Although *A. mearnsii* and *V. divaricata* have different spatial distributions, pests and pathogens are shared between these two tree species where they occur sympatrically. This has consequences for *A. mearnsii* clearing activities, as this tree may provide a corridor between previously isolated forest fragments, which from an evolutionary perspective could be seen as biological units. It is thus advised that when clearing is done that the spatial distribution of *A. mearnsii* is considered, especially where it transverse different habitats.

3.Limitations to the study

Chapter 2: This chapter assessed the overlap in arthropods associated with the two tree species. A limitation was the lack of identification of arthropods to species level, as this level of detail could provide information on possible biological control agents and host specialist species.

Chapter 3: We assessed the interaction of the two host plants with the biotic and abiotic environment in which they co-occur. The fact that only a few samples could be analyzed for P concentration, while all the trees were assessed for N and C concentration may have skewed results regarding this nutrient.

Chapter 4: This chapter suffered from the same limitations as Chapter 3. Here senesced leaf material caused a further reduction in the sample size. Better methodology needs to be developed to collect senesced foliage material of especially bi-pinnate and pinnate leaves; because when these leaves drop they drop as single leaflets rather than entire leaves.

Chapter 5: We assessed the effect of a fungal pathogen on the two tree species in a controlled environment. In the context of a plant physiological study a replication number of three is used commonly as the experimental conditions are well controlled. However, for the assessment from a plant pathology perspective a higher replication number would have been preferred.

4.Questions for further study

Aspects of the dynamics of the interaction and exchanges between a native and an invasive legume tree species co-occurring in forest margins were here assessed for the first time. This habitat still holds lots of potential to explore natural process. Future studies should focus on questions such as:

- How do pest and pathogen associates of invasive species compare to their plantation counterparts?
- Which arthropod species are the most important drivers of differences between native and invasive trees within forest margins?
- How do other environmental factors influence the prevalence of pests and pathogens on other tree species within this habitat and how will these interactions change with changing climatic conditions?
- What is the influence of drought stress on *V. divaricata* across its entire distribution range in relation to its pests and pathogens?

5.References

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E., Power, A.G., Godsoe, W. & Klironomos, J. 2005. Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86: 2979–2989.

Ayres, M.P. & Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance form herbivore and pathogens. The Science of the Total Environment 262: 263–286.

Blaney, C.S. & Kotanen, P.M. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. Journal of Applied Ecology 38: 1104–1113.

Callaway, R.M. & Ridenour, W.M. 2004. Novel weapons: invasive success and evolution of increased competitive ability. Frontiers in Ecology and the Environment 2: 436–443.

Department of Agriculture, Forestry and Fisheries. 2009. State of the forest report.

Entry, J.A., Martin, N.E., Cromack Jr., K. & Stafford, S.G. 1986. Light and nutrient limitation in *Pinus monticola*: Seedling susceptibility to *Armillaria* infection. Forest Ecology and Management 17: 189–198.

Geldenhuys, C.J. 1986. Nature's valley: a refugium for rare southern Cape forest species. Palaeoecology of Africa 17: 173–181.

Geldenhuys, C.J. 2004. Concepts and Process to Control Invader Plants in and around Natural Evergreen Forest in South Africa. Weed Technology 18: 1386–1391.

Goldblatt, P. & Manning, C. 1943 - 2000. Cape Plants: A Conspectus of the Cape Flora of South Africa Pretoria: National Botanical Institute, Pretoria.

Henderson, L. 2007. Invasive, naturalized and casual alien plats in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37: 215–248.

Huber. D.M. & Hanekleus, S. 2007. Managing nutrition to control plant disease. 2007. Landbauforschung Volkenrode, 57: 313–322.

Huber, D.M. & Jones, J.B. 2013. The role of magnesium in plant disease. Plant and Soil 368: 73–85.

IPCC, Climate Change. 1996. Climate change 1995—the science of climate change. *Edited by* J.T. Houghton, L.G. Meira Filho, B.A. Callander, N. Harris, A. Kattenberg, and K. Maskell. Contribution of WG1 to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, U.K.

IPCC, Climate Change. 2001. The scientific basis, Contribution of Working Group I to the third assessment report of the IPCC, Houghton J.T., Ding Y., Griggs D.J., Noguer M., Van derLinder P.J., Dai X., Maskell K., Johnson C.A. (Eds.), Cambridge University Press, Cambridge, UK.

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17: 164–170.

Le Maitre, D.C., Richardson, D.M. & Chapman, R.A. 2004. Biological invasions in South Africa: driving forces and the human dimension. South African Journal of Science 100: 103–112.

Lombardero, M.J., Alonso-Rodríguez, M. & Roca-Posada, E.P. 2012. Tree insects and pathogens display opposite tendencies to attack native vs. non-native pines. Forest Ecology and Management 281: 121–129.

Maistry, P.M., Cramer, M. & Chimphango, B.M. 2013. N and P colimitation of N2-fixing and N-supplied fynbos legumes from the Cape Floristic Region. Plant Soil. DOI 10.1007/s11104-013-1793-y.

Mbambezeli, G. & Notten, A. 2003. http://www.plantzafrica.com/planttuv/virgilia.htm.

Mucina, L. & Rutherford. M. 2006. The vegetation of South Africa, Lesotho and Swaziland.

McMahon, P. 2012. Effect of nutrition and soil function on pathogens of tropical tree crops,in Cumagun, C.J.R. (eds.) Plant Pathology. Croatia. 241–263

Oliveira, H., Sousa, A., Alves, A., Nogueira, A.J.A. & Santos, C., 2012. Inoculation with *Ophiostoma novo-ulmi subsp. americana* affects photosynthesis, nutrition and oxidative stress in in-vitro *Ulmus minor* plants. Environmental and experimental botany 77: 146-155.

Orchard, A.E. & Wilson, A.J.G. 2001. Flora of Australia. Vol. 11 A, Mimosaceae, *Acacia*. Part 1. (ABRS/CSIRO Publishing: Melbourne).

Siemann, E. & Rogers, W.E. 2003. Herbivory, disease, recruitment limitation, and success of alien and native tree species. Ecology 84: 1489–1505.

Sherry, S.P. 1971. The black wattle (*Acacia mearnsii* de Wild.). University of Natal Press. Pietermatitzburg.

Searle, S. 1997. *A. mearnsii* De Wild.(black wattle) in Australia. Black wattle and its utilizations. Barton, ACT: Rural Industries Research and Development Corporation: 1-10.

Van der Merwe, I. 2011. Green heritage: Trees, forests and woodlands of South Africa, Department of Agriculture, Forestry and Fisheries, Pretoria.

Wolfe, L. M. 2002. Why alien invaders succeed: support for the escape-from-enemy hypothesis. American Naturalist 160: 705–11.