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 $\delta^{12}\text{C}/\delta^{13}\text{C}$ 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 gebruikte, 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, die grense van die Afromontane woudkompleks in Suid Afrika, is hoog 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 uitruiing van geassosieerde peste en patogene in die vorm van geleedpotiges en fungi spesies. Ons stel die hipotese dat hierdie twee spesies verkillinge 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 assosiasie 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 associate 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. mearnsii* is nie geaffekteer deur waterbeperkings langs hierdie gradient nie, terwyl δ¹²C/δ¹³C analises aangedui het dat *V. divaricata* bome drooggte stres in droër lokaliteite ervaar het. *V. divaricata* het ook hoër herbivoorladings gehad in droër lokaliteite. *A. mearnsii* 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 plantfeitlik glad nie op infeksie gereageer het nie. Beide plante het beduidend langer wondmerke in geïnfekteerde 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 uitrusting 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 Mimosoideae 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,
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 afrotropical 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). $\delta^{15}$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 $\delta^{13}$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 δ15N/δ14N 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 a 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.
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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 afro-montane forests in South Africa (Geldenhuys, 1994). Afro-montane 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 hectares) (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 Chao2 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 taxonomic 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 \(A. \text{mearnsii}\) or \(V. \text{divaricata}\), 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 \(A. \text{mearnsii}\) against all species associated with \(V. \text{divaricata}\)). 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.*

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

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

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. * 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*).

<table>
<thead>
<tr>
<th>Species richness</th>
<th>Mean comparisons</th>
<th>Wald statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>Native = Invasive</td>
<td>1.99</td>
<td>0.1584</td>
</tr>
<tr>
<td>Feeding guilds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivore</td>
<td>Native = Invasive</td>
<td>3.33</td>
<td>0.068</td>
</tr>
<tr>
<td>Detritivore</td>
<td>Invasive = Native</td>
<td>1.93</td>
<td>0.1649</td>
</tr>
<tr>
<td>Nectar feeder</td>
<td>Native = Invasive</td>
<td>0.93</td>
<td>0.3355</td>
</tr>
<tr>
<td>Parasite</td>
<td>Native = Invasive</td>
<td>0.03</td>
<td>0.8628</td>
</tr>
<tr>
<td>Predator</td>
<td>Native = Invasive</td>
<td>0.07</td>
<td>0.7977</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taxa</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneae</td>
<td>Native &gt; Invasive</td>
<td>5.94</td>
<td>0.0148</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Native = Invasive</td>
<td>0.41</td>
<td>0.5213</td>
</tr>
<tr>
<td>Diptera</td>
<td>Invasive = Native</td>
<td>0.11</td>
<td>0.7389</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Native = Invasive</td>
<td>0.65</td>
<td>0.4196</td>
</tr>
<tr>
<td>Hymenoptera&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Native = Invasive</td>
<td>1.24</td>
<td>0.2651</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>Native = Invasive</td>
<td>0.24</td>
<td>0.6223</td>
</tr>
<tr>
<td>Formicidae</td>
<td>Native &gt; Invasive</td>
<td>3.96</td>
<td>0.0467</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Abundance</th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>Native &gt; Invasive</td>
<td>332.85</td>
<td>&lt;0.0001</td>
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<tr>
<td>Feeding guilds</td>
<td></td>
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</tr>
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<td>Herbivore</td>
<td>Native &gt; Invasive</td>
<td>495.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Detritivore</td>
<td>Invasive &gt; Native</td>
<td>17.44</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nectar feeder</td>
<td>Invasive = Native</td>
<td>0.61</td>
<td>0.4334</td>
</tr>
<tr>
<td>Parasite</td>
<td>Native = Invasive</td>
<td>0.6</td>
<td>0.4373</td>
</tr>
<tr>
<td>Predator</td>
<td>Native = Invasive</td>
<td>1.57</td>
<td>0.2098</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taxa</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneae</td>
<td>Native &gt; Invasive</td>
<td>22.12</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Invasive = Native</td>
<td>0.01</td>
<td>9312</td>
</tr>
<tr>
<td>Diptera</td>
<td>Invasive = Native</td>
<td>5.26</td>
<td>0.219</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Native = Invasive</td>
<td>1.42</td>
<td>0.2342</td>
</tr>
<tr>
<td>Hymenoptera&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Invasive = Native</td>
<td>0.11</td>
<td>0.7389</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>Native &gt; Invasive</td>
<td>6.34</td>
<td>0.0118</td>
</tr>
<tr>
<td>Formicidae</td>
<td>Native &gt; Invasive</td>
<td>114.46</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Host plants are ordered with the highest mean on the left and the lowest on the right. "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.

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

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

### 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 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.

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

Figures represent F-values, number of permutations for each analysis = 9,999. 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.

<table>
<thead>
<tr>
<th></th>
<th>Virgilia divaricata</th>
<th>Acacia mearnsii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3.02*</td>
<td>0.68</td>
</tr>
<tr>
<td>Feeding guilds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbivores</td>
<td>0.47</td>
<td>1.92</td>
</tr>
<tr>
<td>Detritivores</td>
<td>4.56</td>
<td>0.88</td>
</tr>
<tr>
<td>Nectar feeder</td>
<td>0.83</td>
<td>0.84</td>
</tr>
<tr>
<td>Parasite</td>
<td>2.44</td>
<td>0.81</td>
</tr>
<tr>
<td>Predator</td>
<td>2.48</td>
<td>2.31</td>
</tr>
<tr>
<td>Taxonomic orders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Araneae</td>
<td>4.77*</td>
<td>1.36</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>9.73**</td>
<td>2.21</td>
</tr>
<tr>
<td>Diptera</td>
<td>1.48</td>
<td>0.40</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>1.33</td>
<td>1.38</td>
</tr>
<tr>
<td>Hymenoptera*</td>
<td>3.10</td>
<td>2.24</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td>1.94</td>
<td>6.61</td>
</tr>
<tr>
<td>Formicidae</td>
<td>2.43</td>
<td>5.25</td>
</tr>
</tbody>
</table>

Figures represent F-values, number of permutations for each analysis = 9,999. * 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


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


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 (Ayres & 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 led 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):

$$\text{Water (\% by mass) = } \frac{(X_2 - X_1)}{X_2} \times 100$$

Where $X_1 = \text{Wet soil mass (g)}$ and $X_2 = \text{Final dry mass (g)}$

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:

$$\delta Z = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

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 enclosed 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 spectrometry (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 $\delta = 1000 \times (R_{\text{sample}}/R_{\text{standard}})$, 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 δ\textsuperscript{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 (\text{NH}_4\textsubscript{2}\text{SO}_4). This analysis provided both δ\textsuperscript{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 δ\textsuperscript{12}C/ δ\textsuperscript{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 ddH₂O, 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 $x^2 (Z)$ 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).

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 δ\(^{13}\)C ratio of *A. mearnsii* was not significantly correlated with percentage soil water content across the sampling sites, while leaf δ\(^{13}\)C ratios of *V. divaricata* was significantly and positively correlated with percentage soil water content (Figure 3-2). Lower negative δ\(^{13}\)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 from west to east across the sampling range.

![Figure 3-2](image-url) The relationship between δ\(^{13}\)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).

![Graph showing correlation between lesion length and tree age for Acacia mearnsii and Virgilia divaricata](image)

* $r = -0.21; p\text{-value} = 0.13$
* $r = 0.144; p\text{-value} = 0.34$

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

![Box plot showing change in lesion length](image)

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.
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.

<table>
<thead>
<tr>
<th>Change in lesion length</th>
<th>A. mearnsii</th>
<th>V. divaricata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Wald statistic</td>
</tr>
<tr>
<td>% N (n=29)</td>
<td>1</td>
<td>5.41</td>
</tr>
<tr>
<td>% C (n=29)</td>
<td>1</td>
<td>23.23</td>
</tr>
<tr>
<td>Soil H$_2$O content (n=6)</td>
<td>1</td>
<td>1.01</td>
</tr>
</tbody>
</table>

**Herbivore abundance**

| % N (n=29) | 1 | 7.66 | 0.01 | − | % N (n=17) | 1 | 0.32 | 0.57 | NA |
| % C (n=29) | 1 | 2.44 | 0.12 | − | % P (n=17) | 1 | 0.00 | 0.95 | NA |
| Soil H$_2$O content (n=6) | 1 | 0.46 | 0.50 | NA | % N * % P (n=17) | 1 | 0.32 | 0.57 | NA |
| Soil H$_2$O content (n=6) | 1 | 8.39 | <0.05 | − | % N * % P (n=15) | 1 | 1.71 | 0.19 | NA |
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
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
A. 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.
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   http://www.plantzafrica.com/planttuv/virgilia.htm


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; Secretariat on 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; Mbmbezeli & 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; Rodríguez-Echeverría et al., 2011). Each species has its own unique compliment of rhizobial associates (Rodríguez-Echeverría 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 N₂ 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 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.](image-url)
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 0.09m² 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 (NH₄)₂SO₄.

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

\[
\text{%NDFA} = 100(\delta^{15}\text{N}_\text{reference plant} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_\text{reference plant} - B)
\]

The reference plant was wheat (*Triticum aestivum* L) grown under glasshouse conditions. The B-value is the δ15N 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):
\[ \frac{X_{fresh} - X_{sen}}{X_{fresh}} \times 100 \]

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°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*.  

95
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean %N</th>
<th>median %P</th>
<th>Species</th>
<th>median %N</th>
<th>median %P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. mearnsii</em></td>
<td></td>
<td></td>
<td><em>V. divaricata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh (n=7)</td>
<td>3.38(0.26)a</td>
<td>0.16(0.05)a</td>
<td>Fresh (n=7)</td>
<td>3.59(0.45)a</td>
<td>0.16(0.04)a</td>
</tr>
<tr>
<td>Senesced (n=7)</td>
<td>1.96(0.22)b</td>
<td>0.05(0.03)b</td>
<td>Senesced (n=7)</td>
<td>1.72(0.049)b</td>
<td>0.03(0.05)b</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Soil type</th>
<th>pH</th>
<th>P Bray II mg/kg</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gouna</td>
<td>-33.9804</td>
<td>23.04642</td>
<td>Loam</td>
<td>3.5</td>
<td>1.4</td>
<td>0.1326</td>
</tr>
<tr>
<td>Keurboom</td>
<td>-34.0002</td>
<td>23.43232</td>
<td>Sand</td>
<td>4.9</td>
<td>8.0</td>
<td>0.2594</td>
</tr>
<tr>
<td>Kurland</td>
<td>-33.9346</td>
<td>23.50062</td>
<td>Loam</td>
<td>4.4</td>
<td>6.2</td>
<td>0.3487</td>
</tr>
<tr>
<td>Nature Valley</td>
<td>-33.9614</td>
<td>23.63188</td>
<td>Loam</td>
<td>3.8</td>
<td>1.2</td>
<td>0.5112</td>
</tr>
<tr>
<td>Tsitsikamma</td>
<td>-33.9744</td>
<td>23.74923</td>
<td>Loam</td>
<td>3.5</td>
<td>2.8</td>
<td>0.7068</td>
</tr>
<tr>
<td>Stormsriver</td>
<td>-33.9901</td>
<td>23.89798</td>
<td>Sand</td>
<td>3.7</td>
<td>5.0</td>
<td>0.5384</td>
</tr>
</tbody>
</table>
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/δ14N 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

*Acacia 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 $\delta^{15}N/\delta^{14}N$ ratio in V. divaricata. The $\delta^{15}N$ isotope is in lower abundance than the $\delta^{14}N$ isotope, because it is utilized more by their rhizobia bacteria associates. The inverse is true for A. mearnsii in which larger $\delta^{15}N/\delta^{14}N$ 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


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

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>LATITUDE</th>
<th>LONGITUDE</th>
<th>Soil type</th>
<th>pH</th>
<th>P Bray II mg/kg</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gouna</td>
<td>-33.98043333</td>
<td>23.04641667</td>
<td>Loam</td>
<td>4</td>
<td>4</td>
<td>0.07</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Loam</td>
<td>4</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Gouna</td>
<td></td>
<td></td>
<td>Loam</td>
<td>4</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>Gouna</td>
<td></td>
<td></td>
<td>Sand</td>
<td>5</td>
<td>1</td>
<td>0.09</td>
</tr>
<tr>
<td>Gouna</td>
<td></td>
<td></td>
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| 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; Teviotsdle & 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 fimбриata 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 CO₂ 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. tsitsikammensis 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$_2$SO$_4$) 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 leguminous 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) (20g/L malt extract and 15g/L agar, Biolab, Midrand, South Africa and 1L deionised water) incubated at 24°C. 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 Parafilm (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, Lincoln, 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 μmol.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.
meamsii – 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:

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

“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

δ^{15}N:δ^{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})_{2}SO_{4}.

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 fulfill 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 pathogenicity 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 ≤ 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 ≤ 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 ≤ 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 ≤ 0.05). Grey columns indicate infected plants and white columns indicate control plants.
Table 5-1 N, δ¹⁵N;δ¹⁴N, 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 ≤ 0.05). Infected plants are labeled as CT and control plants as CON.

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<td>mmol P/g</td>
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<td>0.37</td>
<td>0.41</td>
<td>0.35</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>mmol Ca/g</td>
<td>0.11</td>
<td>0.11</td>
<td>0.27</td>
<td>0.20</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>
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 δ¹⁵N 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 (Niinemets, 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. tsitsikammas* 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. tsitsikammas* 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


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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. divaricata 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 leguminous 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 & Haneklaus, 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


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


