Potential suppressive effects of alien *Acacia melanoxylon* on Afrotemperate Forest tree recruitment

by

Bayleigh Miles

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Supervisor: Dr Tineke Kraaij

Co-Supervisor: Mr Johan Baard

Declaration

I, Bayleigh Miles (216676703), hereby indicate that the dissertation for the degree Master of Science in the Faculty of Science is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another university or for another qualification.

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Abstract

Acacia melanoxylon R.Br. is a prominent alien and invasive species in many parts of the world and evidence exists of its adverse effects on indigenous forest community composition through allelopathy and alteration of light regimes. The species also occurs extensively in Southern Cape Afrotemperate Forest (South Africa) and is thought to suppress indigenous Afrotemperate Forest tree seedlings through various mechanisms such as the alteration of natural light regimes or allelopathy, although this has not been rigorously verified. This study aimed to investigate whether *Acacia melanoxylon* has a suppressive effect on Afrotemperate Forest tree recruitment. Firstly, we assessed the potential allelopathic effect of *Acacia melanoxylon* and an indigenous Afrotemperate Forest species *Olea capensis macrocarpa* (C.H.Wright) I.Verd. on the germination of *Acacia melanoxylon* and three prominent indigenous tree species' seedlings in a nursery trial. Germination of the indigenous species failed; however, we were able to compare the germination of *Acacia melanoxylon* among the three treatments, namely *Acacia melanoxylon* leachate, *Olea capensis* leachate, and no leachate. The average germination of *Acacia melanoxylon* was 67% and germination was marginally higher under *Acacia melanoxylon* leachate and *Olea capensis* leachate, respectively, than under the control. This suggested that germination of the species is improved, or at the least, unaffected, by leachates of itself or that of a common indigenous canopy species. We then critically evaluated the germination requirements of the indigenous test species to determine probable reasons why their germination failed in our trial. The most plausible explanation for the germination failure could be that the trial period did not encompass an entire winter season and may not have provided adequate cold stratification. We concluded that the lack of sensitivity of *Acacia melanoxylon* to leachates of itself or a common indigenous overstorey species likely contribute to its success as an invasive species in Southern Cape Afrotemperate Forest. Secondly, we investigated, through field surveys, whether *Acacia melanoxylon* affects indigenous Afrotemperate Forest tree sapling composition and light regimes underneath its canopy. Using a paired plot design, we compared light intensity, tree sapling species richness, diversity, and density underneath 30 overstorey *Acacia melanoxylon* trees and 30 indigenous counterparts. We recorded 2506 indigenous tree saplings from 29 species in the 60 plots and found that there were no significant differences in richness, diversity, or density of saplings underneath *Acacia melanoxylon* compared to that under indigenous counterparts. Light intensity did not differ significantly underneath *Acacia melanoxylon* and indigenous canopies, however light intensity varied significantly more underneath the canopies of *Acacia melanoxylon.* Canonical correspondence analysis of the abundance of sapling species confirmed that sapling composition was not largely determined by the overstorey species. We concluded that *Acacia melanoxylon* does not significantly alter indigenous tree species composition underneath its canopy at the typical densities (<3 trees per hectare) at which it occurred in the forests of the Garden Route National Park that we surveyed. Nevertheless, populations of *Acacia melanoxylon* in the forest interior still act as a source of propagules for invasion in forest margins, riparian areas, and neighbouring fynbos shrubland. These invasive attributes need to be considered in the management of the species in the region at large.

Keywords: Invasive alien plant species; germination; light regime; forest sapling species diversity; richness; Garden Route National Park; temperate forest.

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Dissertation structure, research publications and conference contributions

This study aimed to investigate the potential suppressive effects that the alien *Acacia melanoxylon* has on the recruitment of indigenous Afrotemperate Forest tree species in the southern Cape of South Africa. Data chapters (2 and 3) were presented in scientific paper format, which accounted for the use of the plural first person (‘we’ instead of ‘I’) and some replication of content throughout the dissertation. The reference lists were provided separately for each chapter to facilitate readability. We adopted the editorial style of the journal *South African Journal of Botany* throughout the dissertation for consistency purposes. The dissertation is structured as follows:

Chapter 1 provided a rationale for this study. We started with a literature review of the effects of invasive alien species in forest ecosystems, more specifically covering the relevant aspects of allelopathy in forest ecosystems. Furthermore, we described the study system and study species with emphasis on disturbance and recruitment dynamics.

Chapter 2 titled ‘Potential allelopathic effects of alien *Acacia melanoxylon* and indigenous *Olea capensis macrocarpa* on germination of *Acacia melanoxylon* and Afrotemperate Forest trees' aimed to investigate whether *Acacia melanoxylon* inhibits its own seedlings through allelopathy or whether it is affected by indigenous species leachates. This chapter has been submitted for publication as a research note to a peer reviewed journal, the *South African Journal of Botany*, on 27 October 2021. Contributing authors are Tineke Kraaij and Johan Baard.

Chapter 3 titled ‘Effects of alien *Acacia melanoxylon* on indigenous Afrotemperate Forest tree sapling composition under its canopy’ provided an assessment of the tree sapling composition, diversity, richness, density, and light intensity underneath *Acacia melanoxylon* canopies compared to that underneath analogous indigenous canopies. This chapter has been submitted for publication as a research paper to an international peer reviewed journal, *Austral Ecology*, on 17 November 2021. Contributing authors are Tineke Kraaij and Johan Baard. The findings of this chapter were presented at the 43rd Fynbos Forum online event, 7- 9 September 2021.

Chapter 4 presented a synthesis of the key findings of Chapters 2 and 3 and indicated the novelty, strengths and shortcomings of these studies and provided recommendations for management and future research.

CHAPTER 1: General introduction

Effects of invasive alien plant species in forests

While there are an increasing number of studies reporting the impacts of invasive alien plant species, there remains a lack of understanding of how the effects vary depending on the recipient ecosystems and of the invaders themselves (Levine *et al.,* 2003). Some alien plant invasions can cause changes on a community level, while others can fundamentally modify the structure and functioning of ecosystems (Gaertner *et al.,* 2014). Invaders will have significant effects on ecosystem processes if they differ from the native species in attributes that are involved in resource acquisition, those that influence resource efficiency, or those that change disturbance regimes (Gaertner *et al.,* 2014).

Alteration of light regimes

Competition for light may have the most impact on tree seedling establishment in forest ecosystems (Langmaier and Lapin, 2020). Some invasive plant species create high volumes of litter which can inhibit the growth of native species through reduced light availability (Gaertner *et al.,* 2014). In pine forest ecosystems in Portugal, *Acacia longifolia* (Andrews) Willd. intercepts light due to its rapid growth rate and thereby reduces light intensity in the understorey, consequently decreasing indigenous species diversity (Rascher *et al.,* 2011). Similarly, in forest ecosystems in New England, reduction in light caused by an alien shrub *Lonicera tatarica* L. reduced indigenous species diversity and cover (Levine *et al.,* 1999). Similar evidence exists for invaders of forest ecosystems in the USA, Europe and, several tropical island ecosystems (Levine *et al.,* 1999). Another example is the dry deciduous forests of India's Vindhyan highlands that are invaded by a prolific alien shrub species *Lantana camara* L. (Raghubanshi and Tripathi, 2009). Dense cover formed by horizontal stratification of *Lantana camara* reduced the intensity and duration of the natural light regime, thus preventing the establishment of tree species seedlings (Raghubanshi and Tripathi, 2009). Consequently, species diversity and richness decreased with increasing cover of *Lantana camara* (Raghubanshi and Tripathi, 2009).

Alteration of soil properties

Invasive alien plants may have chemical impacts on forest ecosystems (Langmaier and Lapin, 2020) through modifying chemical and biochemical soil characteristics, thereby causing changes in the native species richness above and below the ground (Langmaier and Lapin, 2020). Legume species, such as of the genus *Acacia*, are nitrogen fixing and can have chemical effects in the areas where they invade which may lead to structural ecosystem changes and the formation of altered plant communities (Langmaier and Lapin, 2020). Invasive alien plant species are known to cause changes in soil pH in the ecosystems in which they invade, rendering the soil unsuitable for indigenous species (Collins and Jose, 2008). For instance, two alien plant species (*Berberis thunbergii* DC. and *Microstegium vimineum* (Trin.) A.Camus) found in hardwood forests of New Jersey significantly increased the soil pH in invaded areas, causing species compositional changes in indigenous communities and potentially facilitating invasion by other exotic species (Kourtev *et al.,* 1999). A study in longleaf pine forest in the USA showed that the invasive grass *Imperata cylindrica* (L.) Raeusch. was able to lower the nutrient levels of the soil and thereby impede survival of the indigenous species. Furthermore, invasive alien plants can leave legacy effects in the soil that may persist after clearing and thus prevent re-establishment of indigenous species. For example, *Acacia saligna* (Labill.) Wendl. altered the overall soil characteristics of sand fynbos shrublands in South Africa and the suppressive effects persisted up to 10 years after removal (Nsikani *et al.,* 2017). Invasive alien plants may further alter soil humidity (Langmaier and Lapin, 2020). For instance, *Acacia melanoxylon* R.Br. was thought to reduce regeneration of indigenous Southern Cape Afrotemperate Forest trees potentially through soil drying effects (Phillips, 1928). Allelopathy is another prominent mechanism by which invasive alien plants can alter soil chemistry and thus native ecosystems, which is discussed in more detail below.

Alteration of disturbance regimes

Invasive alien plant species can alter existing disturbance regimes as well as creating novel disturbance regimes (Mack and De ’Antonio, 1998). Disturbance regimes that may be modified by invasive plant species include geomorphological disturbance regimes, forest gap formation, flooding regimes, and fire regimes (Mack and De ’Antonio, 1998). High densities of *Acacia mearnsii* De Wild. and *Acacia melanoxylon* that occur in indigenous fynbos shrublands, and Afrotemperate Forest in South Africa are known to change natural disturbance regimes such as fire, floods, and forest gap formation, which may exacerbate their invasiveness (Midgley *et al.,* 1990; Kraaij *et al.,* 2013; Moolman and Rikhotso, 2014). Furthermore, a previous study found that in forest ecosystems where alien species such as *Pteridium aquilinum* (L.) Kuhn form a dense and persistent understorey, tree regeneration was reduced (Royo and Carson, 2006). Competition and allelopathy were stated as the mechanisms behind decreasing tree regeneration, and the main impacts on the ecosystem were increased canopy disturbances and altered fire regimes (Royo and Carson, 2006). Many invasive species modify the natural fire regime of the area that they invade (Mandle *et al.,* 2011). Whilst the effects of grass invasion have been studied extensively, the impact of woody invaders on fire regimes is poorly understood (Mandle *et al.,* 2011). Woody invaders can increase or decrease aspects of the fire regime such as fire intensity, frequency, and extent (Mandle *et al.,* 2011). In forest ecosystems in Chile, the invasive shrub *Genista monspessulana* (L.) L.A.S.Johnson forms dense stands and generally increases aspects of the fire regime (Pauchard *et al.,* 2008). This species is highly flammable and increases fuel load, consequently increasing intensity and frequency of fire (Pauchard *et al.,* 2008). In riparian forests in Australia, *Cryptostegia grandiflora* Roxb. ex R.Br. is a vine species that alters the natural fire regime by reducing grass, smothering trees, and promoting crown fires (Grice, 1997). An invasive tree species, *Mimosa pigra* L., in these same forests reduces herbaceous species richness and indigenous tree regeneration, thereby decreasing understorey fires (Braithwaite *et al.,* 1989).

Most invasive alien plant species exhibit a combination of suppressive mechanisms impacting the regeneration of native plant species (Langmaier and Lapin, 2020). *Robinia pseudoacacia* L. originating from North America is an example of an invading plant species that uses various suppressive mechanisms at different stages of invasion (Langmaier and Lapin, 2020). It increases nitrogen availability, changes light regimes, and is also associated with allelopathy (Langmaier and Lapin, 2020). Furthermore, a previous study reported that a *Tamarix* species suppressed several indigenous riparian tree species by a combination of light and soil water reduction (Levine *et al.,* 1999). It is often difficult to distinguish between the different suppressive mechanisms employed by invasive alien plants and their respective importance as they can operate interactively and concurrently (Mallik, 2008).

Allelopathy in forest ecosystems

Allelopathy has been extensively researched in forest ecosystems (Souto *et al.,* 1994; Gonzalez *et al.,* 1995; Gallet and Pellissier, 1997; Reigosa *et al.,* 2000; Souto *et al.,* 2001; Harris *et al.,* 2003). The understanding of allelopathy in forest ecosystems has evolved from a localized plant chemical interference concept to a more complex, ecosystem-level phenomenon that is largely influenced by ecological factors, such as invasive alien plants and disturbance (Mallik, 2008). Allelopathy in forest ecosystems is still poorly understood due to forest ecosystems being both temporally and spatially complex and having high habitat heterogeneity and species diversity (Mallik, 2008). However, forests are notably good examples of ecosystems in which communities are greatly influenced by the dominant species (Pellissier and Souto, 1999). Allelopathy should therefore be considered as a prominent factor influencing the dynamics of these ecosystems (Pellissier and Souto, 1999).

Allelopathy is a form of interference competition among plants that occurs through the production of chemicals that are released into the environment and that are toxic or stimulating to other species or other individuals (Rice, 1984; Begon *et al.*, 2006; Sunmonu and Van Staden, 2014). This process differs from competition in that it does not involve the depletion of a vital factor but depends on the addition of a negative or positive factor (Muller, 1969). Allelopathy generally involves interspecific (antibiotic) and intraspecific (auto toxic) biochemical coactions (Holzner and Numata, 1984). The most common allelochemicals are phenolics, flavonoids, alkaloids, terpenoids and cyanogenic glycosides (Chou, 2006). Some of the ways in which allelochemicals are released from the plant is through volatiles, root exudates, leaf-, flower- and root leachates or from the decomposing litter (Reigosa *et al.*, 2000; Qasem, 2012). The effects of allelopathy are often subtle and may affect species and their life stages very differently (Willis, 2007).

There are two prominent types of allelopathic interactions in forest (Mallik, 2008). The first allelopathic interaction in forest is among trees, where tree species can impact tree seedlings of conspecifics (auto-toxicity), or tree seedlings of different species (Mallik, 2008). The second allelopathic interaction in forest is between trees and the understorey vegetation (Mallik, 2008). These interactions cause changes occurring at temporal and spatial scales that may cause local and ecosystem-level impacts (Mallik, 2008). However, the relative significance of these chemical interactions in forest depends on, and directly interacts with, the forest type and local environmental factors, such as alien invasive plant species, disturbances, and tree recruitment mechanisms (Hierro and Callaway, 2003; Blanco, 2007).

Allelopathy has been investigated in many forest ecosystems worldwide and generally has a major impact on their biodiversity and dynamics (Blanco, 2007; Hashoum *et al.*, 2017). Allelopathy plays an important part in forest ecosystems for the subsequent reasons; (i) trees can release allelochemicals for extended periods, which may accumulate in the soil to toxic levels, (ii) allelochemicals can impact the understorey, the undergrowth, and the natural regeneration of trees, and (iii) highly productive alien tree species may have the ability to increase the accumulation of allelochemicals in the soil due to the local microbiota being incapable of degrading them (Lebedev *et al.*, 2019). In forest ecosystems, allelopathy may affect several aspects of plant ecology, namely occurrence, growth, succession, community structure, species dominance, diversity, and plant productivity. However, the two most significant impacts of allelopathy in forest are those on forest structure and the dominance of tree species (Chou, 2010; Caboun and Jacob, 2015).

Allelopathy is commonly measured using laboratory or pot experiments. However, these methods may provide results that are not representative of field settings due to the use of artificial substrates and other conditions that fail to take interacting biotic and abiotic factors into account (Inderjit, 1996, Parepa and Bossdorf, 2016) (Table 1). Furthermore, artificial leachates can influence results due to parameters such as pH and electrical conductivity of extracts which are different to those occurring in nature (Reigosa *et al.,* 2006). There are complex interactions in the environment that affect the actions of allelochemicals. Biotic factors such as soil microorganisms and root exudates, and abiotic factors such as shading and soil characteristics, interact with the allelochemicals and the target species. In the field, allelopathy may therefore not be the dominant plant interaction but can be one of many interacting factors (Inderjit and Callaway, 2003).

Allelopathic effects of tree species on tree seedlings and understorey plants

Allelopathy is an aspect involved in the coexistence of tree species and may also be an important factor in the succession of tree species (Fox, 1977). Tree species can affect other tree seedlings and the understorey allelopathically (Table 1). Some of the commonly studied examples of allelopathic tree species are from the genera *Ailanthus, Acacia, Eucalyptus* and *Pinus* (Davis, 1928; Muller, 1969; Reigosa and Gonzalez, 2006; Jose and Holzmueller, 2008). The main impact of allelochemicals from overstorey trees is on the natural regeneration of other tree species which manifests in the inhibition of seed germination, establishment, and growth (Mallik, 2008) (various examples listed in Table 1). This enables the allelopathic tree species to become dominant in their environment (Vandermast *et al.,* 2002). Many conifer species exhibit allelopathy and natural regeneration in conifer forests is purportedly poor (Singh *et al.,* 1999). Overstorey trees may also inhibit the recruitment of their own seedlings through allelopathy (Mallik, 2008). Autotoxicity occurs in many conifer species such as *Picea abies* (L.) H.Karst. (Pellissier, 1994). Tree species can also have strong allelopathic effects on understorey species other than the seedlings of overstorey trees (Mallik, 2008). Allelopathic overstorey tree species can reduce species diversity and alter the composition of understorey plant communities by suppressing germination and seedling growth, and causing seedling mortality (Lee and Monsi, 1963; Kil and Yim, 1983). However, most examples of allelopathy in trees pertain to invasive alien species (Reigosa and Gonzalez, 2006). Hence allelopathic effects of alien trees on indigenous forest trees are considered in detail in a subsection dedicated to that topic.

The allelopathic effects of indigenous tree species on the understorey in forest ecosystems in South Africa are poorly understood (Sunmonu and Van Staden, 2014). Indigenous tree species that are known to have such effects are *Albizia adianthifolia* (Schum.) W.Wight, *Buddleja saligna* Willd., *Combretum kraussii* Hochst., *Halleria lucida* L. and *Rapanea melanophloeos* (L.) Mez (Sunmonu and Van Staden, 2014). These species demonstrated varying degrees of allelopathy, including inhibition of germination and reduction in radicle and plumule length of lettuce seedlings (Sunmonu and Van Staden, 2014), although extrapolation of such findings to field conditions may not be simple.

Table 1: Prominent examples of allelopathic interactions of alien and indigenous species in forest ecosystems globally, differentiating between overstorey effects on the understorey, and *vice versa*. Nomenclature follows the plantlist.org.

Allelopathic species	**Target species**	**Effect**	**Forest type**	**Region**	**Study setting**	**Reference**
			Overstorey on understorey			
Ailanthus altissima, (Mill.) Swingle (alien tree)	Dominant indigenous tree species such as *Acer saccharum* Marshall and *Quercus rubra* L.	Suppression of seedling growth	Temperate forests	United States	Field	Gomez-Aparicio and Canham (2008)
Castanea dentata (Marsh.) Borkh (indigenous tree)	Co-occurring tree species	Dominates by suppressing the germination and growth of seedlings	Eastern deciduous forests	United States	Laboratory	Vandermast *et al.* (2002)
Duroia hirsuta (Poepp.) K.Schum. (indigenous tree)	Herbaceous understorey species, such as *Cordia nodosa* Lam. and *Miconia* species	Reduction of germination and radical elongation	Moist tropical forest	South America, Brazilian Amazon	Laboratory	Campbell *et al.* (1989)
Picea abies (L.) H.Karst. (indigenous tree)	*Picea abies*	Inhibition of own germination	Boreal forest	Europe	Field	Pellissier (1994)
Pinus densiflora Siebold & Zucc. (alien tree)	Understorey herbaceous species such as *Miscanthus sinensis* Andersson; Tree species such as *Bidens bipinnata* L. and *Achyranthes bidentata* Blume	Reduction of vitality and germination of seedlings	Coniferous forest	Korea and Japan	Laboratory	Lee and Monsi (1963); Kil and Yim (1983)
Pinus ponderosa Douglas ex C. Lawson (indigenous tree)	Herbaceous understorey, such as *Andropogon gerardii* Vitman and *Schizachyrium scoparium* (Michx.) Nash	Reduction in growth and germination of seedlings	Coniferous forest	North America	Laboratory	Lodhi and Killingbeck (1982)

Allelopathic species	**Target species**	**Effect**	**Forest type**	**Region**	**Study setting**	**Reference**
			Understorey on overstorey			
Empetrum nigrum hermaphroditum (Hagerup) Böcher (indigenous shrub)	Tree species such as *Pinus sylvestris* L. and *Populus tremula* L.	Suppressed germination and seedling growth	Boreal and conifer forests	Sweden	Laboratory	Nilsson (1994); Zackrisson and Nilsson (1992)
Kalmia angustifolia L. (alien shrub)	Tree species such as *Picea mariana,* (Mill.) Britton, Sterns & Poggenb. *Abies balsamea,* (L.) Mill.	Inhibition of primary root development and root and shoot growth of seedlings	Boreal forest	Canada	Laboratory	Mallik (1987); Thompson and Mallik (1989); Mallik and Roberts (1994)
Lantana camara L. (alien shrub)	Indigenous tree species such as *Alectryon subcinereus* A.Gray and *Cryptocarya rigida* Meisn.	Increases mortality of seedlings and decreases germination	Dry rainforest, warm temperate rainforest, wet sclerophyll forest	Eastern Australia	Field	Gentle and Duggin (1997)
Pteridium aquilinum, L. Kuhn. (alien fern)	*Pinus sylvestris, Populus tremula*	Inhibition of germination and seedling growth	Boreal forest	Europe	Laboratory	Dolling *et al.* (1994)
Rhododendron maximum L. (indigenous shrub)	Canopy tree species such as *Quercus rubra* and *Prunus virginiana* L.	Inhibition of seedling establishment and survival	Mixed hardwood forest	North America, Appalachian Mountains	Laboratory and nursery	Nilsen *et al.* (1999)
Vaccinium myrtillus L. (alien shrub)	Indigenous trees such as *Pinus sylvestris, Picea abies*	Inhibition of germination and seedling growth	Boreal forest and subalpine spruce forest	Europe	Field and laboratory	Jäderlund *et al.* (1996); Pellissier (1994)

Allelopathic effects of understorey species on trees

The allelopathic effects of the forest understorey on tree species has also been extensively researched (Pellissier, 1993; Mallik, 1987, 2001, 2003; Table 1). Multiple studies have demonstrated the allelopathic effects of understorey plants on the regeneration capacity of canopy trees, and this is especially apparent in temperate forest (Wardle *et al.,* 1998; Pellissier *et al.,* 2002; Mallik, 2003, 2008). Shrub species often quickly invade disturbed forest (Duncan and Chapman, 2003) and form a dense understorey that can alter the regeneration of trees (Mallik and Prescott, 2001). Allelopathy is one factor that may contribute to the formation of dense understorey stands (Mallik, 2003). Understorey species largely have allelopathic effects on canopy species during the sapling stage, as allelochemicals inhibit germination and early growth (Pellissier *et al.*, 2002; Mallik, 2008), hence allelopathy could be a cause of regeneration failure of canopy species.

Accordingly, it was shown in conifer and deciduous forests that allelopathic effects of the understorey can directly inhibit tree regeneration (Pellissier and Souto, 1999). This interaction has been widely investigated in coniferous forests, but less so in deciduous forests (Pellissier and Souto, 1999). Several shrubs of the families Ericaceae and Empetraceae have allelopathic potential (Rice, 1979; Putnam and Tang, 1986; Fischer *et al.,* 1994; Nilsson, 1994) and many ericaceous species contain a range of allelochemicals in their leaf litter, which are known to have inhibitory effects on conifer species germination (Facelli and Pickett, 1991; Pellissier, 1993, 1994; Gallet and Lebreton, 1995; Mallik, 1987, 1992; Zhu and Mallik, 1994; Mallik and Zhu, 1995; Mallik *et al.,* 1998; Mallik and Pellissier, 2000). In coastal oceanic temperate rainforests and high elevation forest in Canada and the Pacific Northwest of the USA, ericaceous shrubs such as *Gaultheria shallon* Pursh and many *Vaccinium* species have been shown to reduce the growth of several conifer species such as *Thuja plicata* Donn ex D.Don*, Picea sitchensis* (Bong.) Carrière and *Abies amabilis* (Douglas ex Loudon) J.Forbes (Bunnell, 1990; Messier, 1993; Fraser, 1993; Prescott *et al.*, 1996; Fraser *et al.*, 1993, 1995).

The suppressive allelopathic effects of the understorey on tree species can be extensive. Inhibition of canopy tree seedlings by the ericaceous shrub *Rhododendron maximum* L. in the subcanopy is an example that represents a pattern in many other forests around the world *(*Nilsen *et al.,* 1999). *Rhododendron maximum* is the dominant subcanopy evergreen shrub in the mixed hardwood forests of the Appalachian Mountains which inhibits the recruitment of most dominant canopy tree species (Nilsen *et al.,* 1999). *Rhododendron maximum* inhibits the establishment and survival of canopy tree seedlings through allelopathy in addition to a reduction in light intensity, and has thus become dominant in its community, occupying approximately 30 million hectares of the Appalachian Mountains (Nilsen *et al.,* 1999). Similarly, *Empetrum nigrum hermaphroditum* (Hagerup) Böcher is dominant in boreal forests and has the ability to monopolize the ground surface through chemically inhibiting indigenous tree species such as *Pinus sylvestris* L. (Nilsson, 1994).

One of the most significant examples of an allelopathic understorey species in forests is bracken fern *Pteridium aquilinum.* It is a common understorey species worldwide and multiple studies have shown that it can affect forest regeneration through allelopathy (Gliessman *et al.,* 1975; Ferguson and Boyd, 1988; Ooka and Owens, 2018.). *Pteridium aquilinum* invades aggressively due to its ability to produce profuse amounts of leaf litter from which large quantities of allelochemicals accumulate in the soil (Ooka and Owens, 2018). Allelopathy plays a substantial role in regulating species diversity in bracken communities globally (Ooka and Owens, 2018). Communities where *Pteridium aquilinum* dominates, have fewer herbaceous and woody species compared to adjacent communities without this species (Chou, 2010). In Southern Cape Afrotemperate Forest in South Africa, *Pteridium aquilinum* and *Helichrysum petiolare* Hilliard & B.L.Burtt create dense understories that persist for long periods and may have a potential for strong allelopathic effects on indigenous tree recruitment (Geldenhuys, 1975; Royo and Carson, 2006).

Studies of allelopathy in tropical forests are considerably more limited than studies in temperate forest (Mallik, 2008). Examples from Australian tropical forest includes *Lantana camara* which is an invasive woody species that suppresses germination and increases mortality in indigenous tree species (Gentle and Duggin, 1997). Furthermore, *Psychotria leiocarpa* Cham. & Schltdl. is a native woody species that is dominant in the understorey of Brazilian subtropical forest communities. The leaves of *Psychotria leiocarpa* contain allelochemicals that inhibit the initial development of some tree species and could be a factor contributing to the formation of high-density stands (Correa *et al.,* 2008).

Allelopathy and alien species in forest ecosystems

Plant biological invasions are one of the most significant causes of ecosystem degradation and biodiversity loss and due to this, extensive research has been done on the mechanisms behind invasions. Recent findings present compelling evidence that allelopathic interactions between invasive and native species can be one of the major processes behind the success of some of the most prominent plant invaders (Bais *et al.,* 2003; Callaway and Ridenour 2004; Prati and Bossdorf 2004; Stinson *et al.,* 2006). The role of allelopathy in the success of alien plant invasion is supported by two premises (Hierro and Callaway, 2003). Invasive alien plant species often establish as vast monocultures that completely exclude indigenous species in areas that were previously diverse, a phenomenon which does not commonly occur in natural communities (Hierro and Callaway, 2003). Additionally, the novel weapon hypothesis suggests that allelopathy may be more significant in recipient communities (non-native range) than in origin communities (native range) because the former is more likely to be un-adapted to the novel chemicals released by invader species (Hierro and Callaway, 2003; Donnelly *et al.*, 2008). For example, *Eucalyptus globulus* Labill. has greater allelopathic inhibitory effects on understorey plant growth in non-native regions than in its native range (Becerra Osses *et al.*, 2018). Furthermore, invader species may modify the soil microbial community through allelopathy, which may change the plant-microbe relationships of indigenous species (Hierro and Callaway, 2003).

Most examples of allelopathy in trees are associated with alien species that become invasive or dominant in the ecosystem (Reigosa and Gonzalez, 2006). The germination and sapling growth of forest species are *inter alia* adapted to the allelochemicals released by dominant indigenous species and each may release a different compound, thus creating variation in species composition (Reigosa, *et al.* 1999). In contrast, the monospecific stands created by invasive alien plants allow for the accumulation of only certain allelochemicals which thereby affect species composition (Reigosa *et al.*, 1999). For example, in the fire-driven Boreal forests in Sweden, an invasive shrub species, *Empetrum nigrum hermaphroditum* (Hagerup) Böcher, alters the community composition through allelopathy by suppressing the germination, growth and nutrient acquisition of several species of indigenous tree saplings (Wardle *et al.*, 1998). As the community becomes dominated by invasive shrubs instead of the indigenous *Pinus* species, ecological processes are altered, such as the fire regime and decomposition rates (Wardle *et al.*, 1998). Another instance is *Eucalyptus globulus*, an allelopathic forestry species originating from Australia, of which the litter may contribute to lower species richness and plant growth in its understorey (Becerra Osses *et al.*, 2018). Where it was introduced in California and western Europe, plant understoreys beneath *Eucalyptus globulus* were species-poor in contrast to plant communities just beyond the *Eucalyptus globulus* stands (Becerra Osses *et al.*, 2018). Besides *Eucalyptus* species, another Australian genus which is known for its invasiveness and allelopathy is *Acacia* (Gibson *et al.*, 2011). In the coastal zone of the north-western Iberian Peninsula, *Acacia melanoxylon* is known to create dense, homogenous stands by outcompeting indigenous species, such as *Rumex acetosa,* L. through strong allelopathic effects (Hussain *et al.*, 2011). *Acacia dealbata* Link. is another example of an *Acacia* species that alters indigenous species composition in forest communities in Spain through allelopathy (Lorenzo *et al.,* 2011). Overall, the significance of allelopathy in driving the impacts of invasive alien plant species are still inadequately understood (Orr *et al.,* 2005).

The study system and its disturbance and recruitment dynamics

Forest in South Africa is mainly evergreen and is the smallest, most fragmented and widely distributed biome (Geldenhuys and Mucina, 2006). South African forest can be broadly defined as a multi-layered vegetation that is dominated by trees, and whose collective strata have overlapping crowns and where graminoids are rare in the herbaceous layer (Geldenhuys and Mucina, 2006). These forests occur in scattered patches that are embedded within other biomes such as fynbos shrubland or grassland (Mucina and Geldenhuys, 2006). South African forests have been classified into seven main forest groups, comprising 24 floristic-biogeographic forest types (Geldenhuys and Mucina, 2006). This study focuses on the Southern Cape Afrotemperate Forest type (hereafter, ‘Afrotemperate Forest’), which occurs in scattered patches in the Western and Eastern Cape provinces of South Africa (Mucina and Geldenhuys, 2006). Here they occupy sheltered seaward slopes, plateau, and coastal scarps at altitudes ranging from 10 m to 600 m above sea level (Mucina and Geldenhuys, 2006). The climate of this region is warm temperate and humid with precipitation falling throughout the year (Seydack *et al.*, 2012). Afrotemperate Forest is generally confined to regions of high water availability (Mucina and Geldenhuys, 2006). Mean annual precipitation varies between 700 mm and 1200 mm according to orographic factors and increases from west to east (Seydack *et al.*, 2012). Temperatures within the southern Cape are moderated by proximity to the coast (Mucina and Geldenhuys, 2006) with daily minimum and maximum air temperatures at 15 °C – 25 °C in summer and 7 °C – 19 °C in winter (Tyson and Preston-White, 2000; Mucina and Geldenhuys, 2006; Russell *et al.*, 2012). South-easterly winds are common in the summer whilst south-westerly winds and hot, desiccating, north-westerly bergwinds are common in winter and autumn (Baard and Kraaij, 2014). Afrotemperate Forest grows on soils derived from the Table Mountain Group sandstones, shales of the Cape Supergroup and Cape Granite (Geldenhuys, 1991, 1993a; Mucina and Geldenhuys, 2006). The soils vary from Mispah, Glenrosa and Houwhoek forms to sandy, humic Fernwood form (Geldenhuys, 1991, 1993a; Mucina and Geldenhuys, 2006).

The closed-canopy Afrotemperate Forests are multi-layered (Mucina and Geldenhuys, 2006) with an average canopy height of 20 m to 25 m (Geldenhuys and Van Laar 1980). Species composition and the disturbance regime can be used to further describe three distinct forest types, namely coastal, plateau and mountain forest (Geldenhuys, 1996a; Mucina and Geldenhuys, 2006). Species such as *Afrocarpus falcatus* (Thunb.) C.N.Page*, Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. and *Olea capensis macrocarpa* (C.H.Wright) I.Verd. dominate the canopy of coastal forests (Lawes *et al.,* 2004). Plateau forests comprise of species such as *Olea capensis macrocarpa, Podocarpus latifolius, Pterocelastrus tricuspidatus* Walp.*, Gonioma kamassi* E.Mey.*, Curtisia dentata* (Burm. f.) C.A. Smith. and *Apodytes dimidiata* E.Mey. ex Arn. (Geldenhuys, 1980). Mountain forest is dominated by *Ocotea bullata* (Burch.) E. Meyer in Drege and *Cunonia capensis* L. (Geldenhuys 1982, 1996a), and *Virgilia divaricata* Adamson commonly occurs along the forest margin (Coetsee and Wigley, 2013). Three main factors, combined or separate, may determine the species composition of forest, namely the site requirements of particular species, past disturbances of a site, and the regenerative processes of a species (Geldenhuys, 1993a).

In moist and mesic localities, the shrub understorey and herbaceous layer are well established (Mucina and Geldenhuys, 2006). Fire does not generally play a major role in forest function and dynamics, except at the forest margin (Geldenhuys and Mucina, 2006), and in exceptional weather conditions where adjacent vegetation affects fire penetrability (Giddey *et al.*, 2021). Afrotemperate Forest persists in fire refugia and is surrounded by fire-prone fynbos shrubland, however, commercial timber plantations, largely of *Pinus* species, abut the forest in many areas today (Geldenhuys and Mucina, 2006).

Afrotemperate Forests are dominated by species that regenerate from seedlings, and therefore these tall forests display a monopodial growth form (Kruger *et al.,* 2015). The ecology of the indigenous Afrotemperate Forest is notably affected by disturbance and gap dynamics (Mucina and Geldenhuys, 2006). Afrotemperate Forests are not generally exposed to major disturbances and canopy gaps are mostly created by trees that die standing or by windfalls (Geldenhuys and Maliepaard, 1983). In the coastal and plateau forests and river valley slopes, the main disturbances are trees that die standing from infrequent lightning strikes and small windfalls (Geldenhuys, 1996a). On steep slopes, disturbances can be created by lightning fires, landslides and large windfalls, where large, open gaps are created (Geldenhuys, 1996a). In mountain forests, fire acts as a disturbance that limits forest to wind-shadow and thus fire-refuge areas (Geldenhuys, 1996a). The frequency and the extent of the disturbances can influence the forest structure through the reseeding or resprouting response of species (Kruger *et al.,* 2015). The disturbance dynamics of Afrotemperate Forests are typically fine-grained (Midgley *et al.*, 1990) and regeneration occurs over small spatial scales (Van Wyk *et al.*, 1996; Lawes *et al.*, 2007). Grain refers to the relationship between the species composition of the canopy and the species composition of the regeneration stand (Geldenhuys, 1996a). More specifically in the Afrotemperate Forest types, plateau and coastal forests tend toward fine-grained forest, while mountain forests tend toward coarse-grained forest (Geldenhuys, 1996a). Most of the species in fine-grained forest are shade-tolerant and dominate the canopy in mature forest (Lawes *et al.*, 2007). Shade-tolerant species can have a short seed dormancy period and germination, therefore, occurs shortly after the seed is released (Lawes *et al.,* 2004). *Podocarpus latifolius* and *Curtisia dentata* are examples of prominent shade-tolerant canopy species, and their sapling banks require intermittent exposure to light to grow to the canopy (Lawes *et al.,* 2004).

The study species and their recruitment and germination

Indigenous species

The study focussed on three of the most common canopy tree species in Afrotemperate Forest, namely *Podocarpus latifolius* (real yellowwood)*, Afrocarpus falcatus* (Outeniqua yellowwood) and *Olea capensis macrocarpa* (ironwood). The two yellowwood species (family Podocarpaceae) are important timber species, of which *Podocarpus latifolius* is the more common and more popular timber species (Geldenhuys, 1993b). These species have very different population structures which are largely attributed to their recruitment rates (Geldenhuys, 1993b). *Podocarpus latifolius* occurs at a high frequency and density and has a high recruitment rate (Geldenhuys, 1993b). It is long-lived, shade-tolerant and grows to 20 m – 30 m in height (Adie and Lawes, 2009). In comparison, *Afrocarpus falcatus* is widely scattered at a low density and has a low recruitment rate (Geldenhuys, 1993b). This species is the more light-demanding, is fast growing and grows up to 45 m tall. In Afrotemperate Forest both species occur in high and scrub forest types and on the same associated geological formations, slope and aspect (Geldenhuys, 1993b). Both species show maximum growth (height, bole diameter, and crown size) in moist forest types (Geldenhuys, 1993b). *Afrocarpus falcatus* has large, fleshy, yellow drupe-like 'fruit' with stony seeds with a rough surface which are primarily dispersed by fruit bats (Geldenhuys, 1996b). Seeds can be clumped close to the parent tree, but some seeds can be dispersed further away (Geldenhuys, 1993b). The seeds have a long germination period and hence have high post dispersal predation (Geldenhuys, 1993b). Seedlings only establish after mast seed years so this species has a low recruitment rate (Geldenhuys, 1993b). *Podocarpus latifolius* have large spherical seeds with a leathery coat on a fleshy, purplish-black receptacle (Geldenhuys, 1996b) and are generally dispersed by birds and mammals such as the Knysna Turaco and Chacma baboon. The seeds can be concentrated close to the parent tree, or dispersed further away (Geldenhuys, 1993b). This species has a short germination period and there is regular establishment of seedlings both close and far from the parent trees, thus leading to a higher recruitment rate (Geldenhuys, 1993b).

Olea capensis macrocarpa (family Oleaceae) is the most abundant canopy species in moist to dry Afrotemperate Forest (Geldenhuys, 1996b). It has an important ecological role in maintaining a closed canopy and canopy height and is an important food source for the Rameron pigeon (Geldenhuys, 1980). It is a mostly shade-tolerant and relatively slow growing species (Geldenhuys, 1975). Trees of this species have a high mortality rate and have shallow and poorly developed root systems that make them prone to windfalls (van Daalen, 1993). Despite this, they show good regeneration, and they occur at a high frequency and density (Geldenhuys, 1980). However, the reason behind their dominance in the forest is poorly understood (van Daalen, 1993). *Olea capensis macrocarpa* has a large, fleshy purple drupe with a large stony seed. The fruit is eaten by birds which act as their main dispersal agents (Geldenhuys, 1996b). *Olea capensis macrocarpa* contributes largely to gap formation in the forest and is also one of the main gap filler species (Midgley *et al.*, 1995). They establish well in both disturbed and undisturbed areas (Lübbe and Geldenhuys, 1991).

Acacia melanoxylon

Acacia melanoxylon (Australian-, Tasmanian- or Swamp Blackwood; family Fabaceae) is a prominent alien species in Afrotemperate Forest which was introduced into the southern Cape in 1856 for its valuable timber and planted into forest gaps resulting from harvesting of indigenous trees and in gaps resulting from localised burns (Geldenhuys, 1996c). Perspectives of the value and potential impacts of this species in these systems vary. Early in the 20th century it was already noted that the species may suppress the regeneration of indigenous forest species (Phillips, 1928). However, *Acacia melanoxylon* is a valuable timber species and is also thought to act as a nurse plant which can facilitate indigenous tree regeneration (Geldenhuys *et al.*, 2017). Controversy thus exists about appropriate management of the species – over the last century the approach in state forests of the region switched between attempts to promote or manage the species to produce sustainable timber harvests and attempts to control the species when and where it was deemed to be invasive (Seydack, 2002).

Acacia melanoxylon is a medium to tall tree that can reach a lifespan of up to 100 years (Louppe *et al.,* 2008). It is a wide-ranging leguminous tree species native to

Australia (Searle, 2000). The native range covers the cool, humid areas of southern and eastern Australia and Tasmania at altitudes of 1000 m to 1500 m (Jennings, 2004). In the subtropical areas of its native range, *Acacia melanoxylon* is considered an early successional species, as it is absent from mature rainforest and is shade-intolerant (Geldenhuys, 1996a). It has a wide ecological range, and occurs as an understorey species in wet eucalypt forests, a co-dominant species in riverine forest, and a dominant species in swamps within its native range (Jennings, 2004). It is a versatile and exceedingly adaptive species that has become invasive in many parts of the world, including South Africa, Kenya, Argentina and the USA (Hussain *et al.*, 2011; CABI, 2018). The invasive potential of *Acacia melanoxylon* is facilitated by its wide ecological tolerance, being adapted to a variety of edaphic and climatic conditions (GISD, 2005).

An invader species in the closed forest environment is defined as one that will increase in number and consequently degrade the forest ecosystem through several processes such as the suppression and prevention of regeneration of indigenous species and altering the natural disturbance regime (Geldenhuys, 1996a). *Acacia melanoxylon* behaves like an invader in habitat types such as forest margins, riparian areas and in fynbos shrublands of South Africa, although there is contention about the species' invasiveness in mature forest interior (Geldenhuys, 2004; Goets *et al.*, 2018). Its seed ecological traits contribute to its invasion potential because the species produces abundant and persistent soil seed banks (Le Maitre *et al.*, 2011) that have a high germination rate (90–100%) (Phillips, 1928; Geldenhuys, 1996a). In South Africa, *Acacia melanoxylon* seed bank density can reach a high of 48 739 seeds per m^2 (Milton and Hall, 1981) and the seed viability is 70–87% (Milton and Hall, 1981; Gibson *et al.*, 2011; Goets *et al.*, 2018). Furthermore, the seeds are bird and water dispersed (Richardson and Kluge, 2008) which facilitates the extensive spread of the species (Geldenhuys, 1996a). The seeds are stimulated to germinate after scarification which is provided by ingestion by birds or by disturbances such as fire or logging (Phillips, 1928; Geldenhuys, 1996a; Jennings, 2004). *Acacia melanoxylon* can also regenerate vigorously after disturbance by means of re-sprouting from the root system which makes it particularly difficult to control (Phillips, 1928; Gibson *et al.*, 2011; Arán *et al.*, 2017). *Acacia melanoxylon* is under biological control of *Melanterius acaciae* Lea to limit seed production in South Africa. Following its release in 1986, *Melanterius* *acaciae* has caused extensive seed damage to *Acacia melanoxylon*, particularly in the southern Cape (Zachariades *et al.,* 2017).

In South Africa, *Acacia melanoxylon* is listed as a Category 2 invader species under the National Environmental Management Act: Invasive Species Regulations and is also categorized as a Category 2 species under the Conservation of Agricultural Resources Act (Act 43 of the Republic of South Africa, 1983) (Baard and Kraaij, 2014). This legislation stipulates that if *Acacia melanoxylon* is grown for a purpose, a demarcation permit is required, that control must take place outside of demarcated areas, and that it may not be allowed to spread (National Environmental Management: Biodiversity Act, 2016). *Acacia melanoxylon* has also been given the status of invasive elsewhere in Africa, Europe, New Zealand, the Pacific Islands, Atlantic Islands and South America (Richardson and Rejmánek, 2011). *Acacia melanoxylon* in the southern Cape of South Africa meets the criteria for the status of 'transformer' (Baard and Kraaij, 2014), meaning that the species is capable of altering ecosystem functioning, structure and condition over a considerable area relative to the extent of that ecosystem (Richardson *et al.*, 2000). *Acacia melanoxylon* is nitrogen-fixing, and outside of its native range has a rapid growth rate and accumulates biomass rapidly which is linked to superior resource acquisition rates, further contributing to its competitive dominance over indigenous species (Le Maitre *et al.*, 2011; Goets *et al.*, 2018). Many studies suggest that allelopathy may assist in particular invasive alien plants becoming dominant in the invaded habitat (Hierro and Callaway, 2003). The possible reasons why invasive alien plants competitively exclude and eliminate indigenous vegetation but do not have this effect in their native range are not well understood (Reigosa and González, 2006). If allelopathy is involved, efforts to control invasive alien plants may be much less effective (Hierro and Callaway, 2003). *Acacia melanoxylon* has been shown to suppress the seed germination and seedling growth of native species potentially through allelopathy in Spain (Hussain *et al.,* 2011), and although it is thought that the same may happen in Afromontane Forest (Philips, 1928), the species' allelopathic effects have not been rigorously tested in the context of Afromontane Forest.

Acacia melanoxylon has established in all the main Southern Cape Afrotemperate Forest patches (Geldenhuys, 1996a) and has become particularly troublesome in disturbed forests and ecotones, fynbos shrublands and riparian habitat in the region (Geldenhuys, 2004). In disturbed sites, *Acacia melanoxylon* can act as a pioneer, creating a nurse stand for shade-tolerant forest species to become established, a seemingly natural forest succession process (Geldenhuys *et al.*, 2017). The indigenous *Virgilia oroboides* (P.J.Bergius) T.M.Salter plays a similar pioneer and forest recruitment facilitation role along forest margins and after substantial disturbances such as fire (Geldenhuys, 1982). However, if *Acacia melanoxylon* has allelopathic effects on indigenous forest species, these nurse stands may not necessarily facilitate natural forest successional processes. Another aspect to consider with nurse stands of *Acacia melanoxylon* is that they are a source of invasion into neighbouring fynbos shrublands and riparian habitat. In these areas, the impacts of *Acacia melanoxylon* are more detrimental (Baard and Kraaij, 2014).

The recruitment dynamics of *Acacia melanoxylon* differ from that of indigenous Afrotemperate Forest canopy species (Lawes *et al.*, 2004). *Acacia melanoxylon* is a light-demanding pioneer species which recruits in large forest gaps whilst indigenous shade-tolerant species recruit under the canopy or in very small forest gaps (150 m^2) (Lawes *et al.*, 2004). *Acacia melanoxylon* has a large canopy compared to the dominant indigenous canopy species, such as *Olea capensis macrocarpa,* shallow root systems, and emerges above the forest canopy (Geldenhuys, 1996a) which make the species more susceptible to windfalls, which in turn create large gaps and more extensive damage to other trees (Geldenhuys, 2004). *Acacia melanoxylon* has soil-stored seed banks that can lie dormant for 50 years and more (Holmes*,* 1989; Seydack, 2000) and while the seeds do not require sunlight to germinate, the saplings need large gaps with enough sunlight to grow well (Geldenhuys, 1996a; Goets *et al.*, 2018). By comparison, shade-tolerant indigenous tree species have a short seed dormancy period and the saplings do not require full sunlight to grow to the canopy (Lawes *et al.*, 2004). Due to the divergent gap-creation and recruitment dynamics, an increase in *Acacia melanoxylon* populations within the forest interior may potentially alter the disturbance dynamics of Southern Cape Afrotemperate Forests (Moolman and Rikhotso, 2014). *Acacia melanoxylon*, by virtue of its large and dense canopies, may furthermore alter the light regime underneath its canopy so significantly that indigenous saplings may be incapable of establishing, or, if able to establish themselves, may be incapable of normal development (Phillips, 1928).

Study objectives

The conservation and restoration of forest ecosystems require a good understanding of complex biological processes, including the biochemical interactions between invasive alien plant species and indigenous plant species (Reigosa and González, 2006). There is a lack of rigorous evidence that supports the claim that *Acacia melanoxylon* is a threat to the conservation of Southern Cape Afrotemperate Forests (Geldenhuys, 1996a). The management of this species in state forests, including those of the Garden Route National Park, has in the past switched between attempts to promote or manage the species to produce sustainable timber harvests and attempts to control the species when and where it was deemed to be invasive (Seydack, 2002). Controversy persists over whether this species is invasive in Afrotemperate Forest interior and whether it has detrimental effects on the regeneration and composition of indigenous forest communities (Geldenhuys, 1996a) The overall aim of this study was therefore to determine whether *Acacia melanoxylon* has suppressive effects on indigenous Southern Cape Afrotemperate Forest tree recruitment. More specifically, the study objectives were to:

1. Investigate the potential allelopathic effect of alien *Acacia melanoxylon* and indigenous *Olea capensis* on the germination of *Acacia melanoxylon* seedlings and three common indigenous forest tree canopy species' seedlings in a nursery study.
2. Determine whether large, mature *Acacia melanoxylon* trees affect indigenous tree sapling composition and light intensity under their canopies compared to the understories of three common indigenous forest canopy species.

Our results should establish whether *Acacia melanoxylon* suppresses Southern Cape Afrotemperate Forest tree recruitment and thereby potentially alter forest structure and composition. Improved knowledge of this aspect is imperative to guide conservation management actions in future.

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CHAPTER 2: Potential allelopathic effects of alien *Acacia melanoxylon* and indigenous *Olea capensis macrocarpa* on germination of *Acacia melanoxylon* and Afrotemperate Forest trees

Abstract

Acacia melanoxylon R.Br., has been known to invade some forest ecosystems in Europe and there is evidence that allelopathy may be an important process favouring invasion. *Acacia melanoxylon* is widespread in Southern Cape Afrotemperate Forest and may pose a significant threat because of its potential to suppress or facilitate the regeneration of indigenous canopy species. In a nursery trial we compared the allelopathic potential of phyllode or leaf leachates of *Acacia melanoxylon* and a common overstorey indigenous species *Olea capensis macrocarpa* (C.H.Wright) I.Verd. on the germination of seeds of *Acacia melanoxylon* and seeds of three prominent indigenous forest canopy species. Although germination of the three indigenous test species failed, we could compare the germination of *Acacia melanoxylon* seeds among the three treatments, namely *Acacia melanoxylon* leachate, *Olea capensis* leachate, and no leachate (control). The average germination of *Acacia melanoxylon* was 67% (range 58% – 72%). There was a significant difference in the number of *Acacia melanoxylon* germinants between the three treatments, with pairwise multiple comparisons of ranks revealing that germination was marginally higher under *Acacia melanoxylon* leachate treatment than the control and marginally higher under *Olea capensis* leachate than the control. This suggests that germination of the species is improved, or at the least, unaffected, by potential allelopathic substances of itself or of a common indigenous overstorey species. We critically considered the germination requirements of the indigenous test species to determine possible reasons why their germination failed in our trial. Poor seed viability could not have accounted for germination failure as tetrazolium-chloride stain testing showed high viability in all species (*Acacia melanoxylon* 90%*, Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. 80%, *Pterocelastrus tricuspidatus* Walp. 72%, and *Curtisia dentata* (Burm. f.) C.A. Smith. 70%). The only plausible explanation for the germination failure could be that the trial period did not encompass an entire winter season and may not have provided adequate cold stratification. We concluded that the successful germination of *Acacia melanoxylon* and its lack of sensitivity to potential allelopathic substances of itself or a common indigenous overstorey species likely contribute to its success as an invasive species in Southern Cape Afrotemperate Forest.

Keywords: germination requirements, germination trial, invasive alien plant species, leachates, temperate forest, southern Cape of South Africa.

Introduction

Allelopathy is a form of interference competition among plants that occurs through the production of chemicals that are released into the environment and that are toxic to other species but not to the producer (Begon *et al.,* 2006). Allelopathy generally involves interspecific (antibiotic) and intraspecific (auto toxic) biochemical coactions that may have positive or negative effects (Holzner and Numata, 1984). Plants release allelochemicals through volatiles, root exudates, leaf and root leachates or from the decomposing litter (Reigosa *et al.*, 2000; Qasem, 2012). These metabolites may be phenolics, flavonoids, alkaloids, terpenoids or cyanogenic glycosides (Chou, 2006). Many of these compounds have important biological functions, such as the inhibition of seed germination and plant growth (Chou, 2006). Therefore, allelopathy can be a major factor in regulating plant community dynamics (Lotina-Hennsen *et al.*, 2006). Allelopathic tree species release allelochemicals into their environment over long periods which accumulate in the soil, and which may ultimately create a sterile environment (Reigosa *et al.*, 2000). Generally, the effects of allelopathy are subtle and may affect species or their life stages very differently (Willis, 2007).

Allelopathy has been well studied in forest ecosystems, especially regarding the suppressive allelopathic effects of understorey species on tree species (Reigosa and Gonzalez, 2006). The two most significant impacts of allelopathy in forest are changes in the structure of forest stands and the dominance of tree species (Chou, 2010; Caboun and John, 2015). Generally, allelopathic interactions may occur between trees of the same or different species, or between trees and the understorey vegetation (Mallik, 2008). These interactions cause changes at varying temporal and spatial scales and may result in local and ecosystem-level impacts (Mallik, 2008). The relative significance of these chemical interactions in forest ecosystems depends on and directly interacts with the forest type and local environmental factors, such as alien invasive plant species, disturbances, and tree recruitment dynamics (Hierro and Callaway, 2003; Blanco, 2007).

Compared to native species, invasive alien plant species may release allelochemicals at higher concentrations or during different stages of the year and this may disrupt co-evolved interactions in the indigenous community (Reigosa and González, 2006). Fast-growing species used in commercial forestry operations are generally more allelopathic than native species occurring within the context of native communities (Reigosa *et al.*, 2000). The role of allelopathy in the success of alien plant invasion is supported by two premises (Hierro and Callaway, 2003). Invasive alien plant species can establish as vast monocultures facilitated by allelopathy and thereby completely exclude indigenous species in areas that were previously diverse, a phenomenon which does not commonly occur in natural communities (Hierro and Callaway, 2003). Additionally, the novel weapon hypothesis suggests that allelopathy may be more significant in recipient communities (non-native range) than in origin communities (native range) because the former is more likely to be un-adapted to the novel chemicals released by invader species (Hierro and Callaway, 2003; Donnelly *et al.*, 2008). For example, *Eucalyptus globulus* Labill. has greater allelopathic inhibitory effects on understorey plant growth in non-native regions than in its native range (Becerra *et al.*, 2018). Furthermore, invader species may modify the soil microbial community through allelopathy, which may change the plant-microbe relationships of indigenous species (Hierro and Callaway, 2003).

Accordingly, most examples of allelopathy in trees are associated with invasive alien plant species (Reigosa and Gonzalez, 2006). Prominent genera that exhibit strong allelopathic effects include *Acacia, Ailanthus, Eucalyptus, Juglans, Leucaena* and some *Quercus* species (Reigosa and Gonzalez, 2006). Many species from the genus *Acacia* (Fabaceae) are known to have allelopathic potential (Hussain *et al.,* 2011), including *Acacia dealbata* Link (Lorenzo *et al.*, 2008), *Acacia confusa* Merr. (Chou *et al.*, 1998), *Acacia auriculiformis* Benth. (Oyun, 2006), and *Acacia* (*Vachellia) nilotica* L. (Al-Wakeel *et al.*, 2007). *Acacia dealbata* is an example of a highly invasive species in forest communities of Spain where it alters indigenous species composition through allelopathy (Lorenzo *et al.,* 2011). Another prominent species which is known to rapidly colonise space and resources in invaded areas through allelopathy is *Acacia melanoxylon* R. Br (Arán *et al.*, 2017). A previous study found that *Acacia melanoxylon* suppressed the root growth and seedling growth rate of indigenous perennial species of forest systems on the Iberian Peninsula (Hussain *et al.,* 2011). Both the phyllodes and flowers produce allelochemicals that inhibit other species and allelopathic competition generally increases with the age of the individual (Arán *et al.*, 2017). Ultimately, invasions of *Acacia melanoxylon* may thereby alter the structure and dynamics of an ecosystem (Hussain *et al.*, 2011).

Acacia melanoxylon occurs extensively as an alien tree species in Southern Cape Afrotemperate Forest (hereafter 'Afrotemperate Forest') of South Africa, and is thought to threaten ecosystem dynamics through suppression of the regeneration of indigenous canopy species (Phillips, 1928). The mechanisms through which *Acacia melanoxylon* may suppress the regeneration of Afrotemperate Forest canopy species are poorly understood (Geldenhuys, 1996a), but allelopathy has been suggested as a potential mechanism (Philips, 1928) and substantial evidence exists of this species' potential allelopathic effects in other forest systems in Europe (Aran *et al.,* 2017; Hussain *et al.*, 2011). It is therefore important to investigate rigorously whether *Acacia melanoxylon* has allelopathic effects on the recruitment of Afrotemperate Forest canopy species. Recruitment is a key process in determining the species composition and thus structure of these mixed-species Afrotemperate Forests (Lübbe and Geldenhuys, 1991). By means of a nursery trial we aimed to compare the allelopathic potential of phyllode or leaf extracts of *Acacia melanoxylon* and an indigenous canopy species, *Olea capensis macrocarpa* (C.H.Wright) I.Verd. (hereafter '*Olea capensis*'), on the germination of seeds of *Acacia melanoxylon* and three common indigenous canopy species, namely *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb., *Curtisia dentata* (Burm. f.) C.A. Smith*.,* and *Pterocelastrus tricuspidatus* Walp.

Methods

Study area

This study was conducted on the southern Cape coast of South Africa near the town of Plettenberg Bay (34.0575° S, 23.3645° E). Due to the maritime influence, the climate of the area is warm temperate and humid (Schulze, 1965). Rainfall occurs throughout the year, peaking in autumn and spring (Seydack *et al.,* 2012). Mean annual precipitation varies between 700 mm and 1 200 mm according to orographic factors and increases from west to east (Seydack *et al.,* 2012). Temperatures within the southern Cape are moderated by proximity to the coast (Mucina and Geldenhuys, 2006) with daily minimum and maximum air temperatures at 15 °C – 25 °C (summer) and 7 °C – 19 °C (winter) (Tyson and Preston-White, 2000; Mucina and Geldenhuys, 2006; Russell *et al.*, 2012). The soils of the area are generally nutrient poor and are derived from the Table Mountain Group sandstones, shales of the Cape Supergroup and Cape Granite (Geldenhuys, 1991; Geldenhuys, 1993a; Mucina and Geldenhuys, 2006). Southern Cape Afrotemperate Forest is a tall, multi-layered evergreen forest, dominated by species such as *Podocarpus latifolius* and *Olea capensis*

(Mucina and Geldenhuys, 2006). The understorey layers are well established, particularly in mesic locales (Mucina and Geldenhuys, 2006). *Acacia melanoxylon* was introduced into the southern Cape in 1856 for its valuable timber and planted into forest gaps resulting from harvesting of indigenous trees and in gaps resulting from localised burns (Geldenhuys, 1996a). It has since become widespread in Afrotemperate forest, and is invasive in areas such as the forest margin and fynbos shrublands (Geldenhuys, 1996a). The management of this species has periodically shifted between promoting the species for sustainable timber harvesting and controlling the species where it is considered invasive (Philips, 1928; Geldenhuys, 1996a; Seydack 2002; Baard and Vermeulen, 2003; Moolman and Rikhotso, 2014).

Test species

The three indigenous species selected in this study are prominent canopy species in Afrotemperate Forest (Geldenhuys, 1993b; Mucina and Geldenhuys, 2006) with comparable stature (crown size and tree height) to that of *Acacia melanoxylon.* Their seeds are readily available, their germination requirements are well known (Phillips, 1926; Geldenhuys, 1993b; Geldenhuys, 1996b), and seeds are considered to germinate readily (Geldenhuys, 1996b; Silverhill Seeds, personal communication). Many Afrotemperate Forest species produce fleshy fruit or seed with a fleshy aril which are adaptions for dispersal by forest birds and mammals and their germination requirements are directly linked to the structure of the fruits and seeds (Geldenhuys, 1996b). The indigenous test species all have fleshy fruits, and their seeds germinate better when the fleshy coverings are removed, however when the seeds are very ripe, the presence of the fleshy covering makes little difference to germination (Geldenhuys, 1996b). Generally, the seed of many Afrotemperate Forest species germinate well regardless of long delays between the time of fruit ripening and the time of seed germination (Geldenhuys, 1996b). The indigenous test species require semi-shaded conditions and moist soil for good germination (Geldenhuys, 1993b, 1996b).

Podocarpus latifolius is a common canopy species of moist to dry forest (Geldenhuys, 1993b). Seeds germinate within 8 to 16 weeks, have a high recruitment rate and the seeds of *Podocarpus latifolius* have a high viability (Geldenhuys, 1993b). The seeds have a thin sclerotesta enabling rapid germination which is an adaptive strategy that allows the seed to be exposed to predation for only a short period (Geldenhuys, 1993b). The seeds of *Podocarpus latifolius* germinate well irrespective of treatment however, a cold-water treatment can be used to improve germination (Geldenhuys, 1996b). Seeds should be sown as soon as possible after treatment (Geldenhuys, 1996b). *Curtisia dentata* is a canopy tree in moist to dry forest (Geldenhuys, 1996b). Pre-treatment of the seed is vital to stimulate germination as the presence of the hard pericarp and fleshy covering delays and reduces germination (Geldenhuys, 1996b; Shaik, 2012). A previous germination trial showed that the viability of *Curtisia dentata* seed was 98% and seeds germinated within 10 to 16 weeks (Geldenhuys, 1996b). *Pterocelastrus tricuspidatus* is a canopy tree of moist to dry forest and scrub forest (Geldenhuys, 1996b). The capsule and aril should be removed and pre-treating with cold or warm water can improve germination (Geldenhuys, 1996b). A previous germination trial reported that the seed viability of *Pterocelastrus tricuspidatus* was 78% and that seeds germinated within 18 to 19 weeks (Geldenhuys, 1996b). *Acacia melanoxylon* seeds do not require light to germinate but the seedlings are shade intolerant (Goets *et al.,* 2017). The seeds of *Acacia melanoxylon* require scarification to stimulate germination and therefore should be pre-treated with boiling water and left to soak overnight (Geldenhuys, 1996b). Previous germination trials reported that the seeds of *Acacia melanoxylon* may germinate within 2 weeks (Geldenhuys, 1996b) and that the viability of seeds was 87% (Goets *et al.,* 2018).

Nursery trial

Seed germination trials are useful to determine allelopathic potential of species (Hussain *et al.,* 2011; Cummings *et al.*, 2012; Qasem, 2012), and we therefore undertook a germination trial in a nursery context. *Acacia melanoxylon* seeds were harvested from natural populations in the area approximately 60 km north-west of Plettenberg Bay (33.5331° S, 22.5800° E). The seeds were collected from the soil and litter underneath the canopies of several mature *Acacia melanoxylon* individuals. Seeds were extracted from the soil and litter by sieving out fine material and sorting through the remaining material by hand. *Acacia melanoxylon* has high soil-stored seed bank densities with high viability, hence this method was suitable for seed collection (Aran *et al.,* 2017; Goets *et al.,* 2018). Harvesting seed of the indigenous test species from natural populations ourselves, would pose significant challenges in terms of timing and accessibility in obtaining sufficient quantities of viable seeds. We thus purchased seeds of these species from Silverhill Seeds, an online seed store that supplies fresh seed harvested from natural populations (Silverhillseeds.co.za, 2020).

A tetrazolium-chloride stain test (Lakon, 1949) was used to determine the proportional viability of seeds from the test species. Sample sets of 30 seeds of each species were cut longitudinally, soaked overnight in a 2% 2,3,5-triphenyl-tetrazolium chloride solution, and the visibly stained seeds enumerated. The seeds of all test species were pre-treated as done in previous studies (Geldenhuys, 1993b, 1996b) to improve germinability. The fruit cover and other external attachments were removed from all four species. The seeds of *Podocarpus latifolius* were soaked in cold water for 36 hours, and those of *Pterocelastrus tricuspidatus* for 72 hours. The seeds of *Acacia melanoxylon* and *Curtisia dentata* were placed in boiling water and left to soak overnight.

The experimental treatments comprised of *Acacia melanoxylon* leachate, *Olea capensis* leachate and a control (no leachate). *Olea capensis* was selected as the species for indigenous leachate because it is a dominant overstorey species (Mucina and Geldenhuys, 2006) which would ease the regular collection of leaves to produce leachates. Fresh phyllodes or leaves were harvested from populations of *Acacia melanoxylon* and *Olea capensis* trees in the study area. The leachates were prepared by cutting and soaking 125 g of *Acacia melanoxylon* phyllodes and 125 g of *Olea capensis* leaves (respectively) in one litre of rain water each (Van den Bosch *et al.*, 2004) at room temperature for 24 hours (Hussain *et al.*, 2011). The solution was then subsequently filtered and stored at room temperature in sealed plastic containers. New batches of leachates were prepared every two weeks. Rain water without leachate was used as the control (Van den Bosch *et al.,* 2004; Hussain *et al.*, 2011).

We filled standard nursery germination trays (540 mm x 275 mm; 70 ml) with a mixture of equal parts of sterile sand and humic compost (Silverhill Seeds, personal communication, 2020). For each of the three treatments (*Acacia melanoxylon* leachate, *Olea capensis* leachate, and control), 50 seeds of each of the four test species (*Acacia melanoxylon, Podocarpus latifolius, Curtisia dentata*, and *Pterocelastrus tricuspidatus*) were planted, with ten seeds of a species per planting tray to create five replicates per species. The trial thus entailed three treatments applied to four test species in a completely crossed design, with five replicates per species × treatment combination, resulting in 60 trays (3 × 4 × 5). The seeds were planted on the 1st of September 2020 in the trays at a depth of 2 cm and covered with a shallow layer of soil mixture (Silverhill Seeds, personal communication, 2020). The trays were kept in a nursery facility covered by 50% shade cloth to represent the conditions underneath the forest canopy (Geldenhuys, 1993a; Silverhill Seeds, personal communication, 2020). Leachate solution or rain water was applied in equal amounts to the trays every four to five days to keep the soil moist. The number of germinated seeds and the date of germination were recorded once per week. The trial was run for 9 months until June 2021.

Data analysis

Given that the three indigenous test species completely failed to germinate we could only assess the effects of leachate treatments on the germination of *Acacia melanoxylon* seeds. For this purpose, a Kruskal Wallis test followed by multiple comparison of mean ranks were used as the data did not conform to normality. The response variable was the number of germinants per tray, while the grouping variable was the treatments with three levels, namely *Acacia melanoxylon* leachate, *Olea capensis* leachate, and control. Statistical analysis was done with Statistica v. 10 (StatSoft, Inc. 1984-2011).

Results

Viability of the sample sets of seeds subjected to stain testing was 90% in *Acacia melanoxylon,* 80% in *Podocarpus latifolius*, 72% in *Pterocelastrus tricuspidatus* and 70% in *Curtisia dentata*. In the nursery trial, none of the indigenous species germinated, while 67% of the *Acacia melanoxylon* seeds germinated. There was a significant difference in the number of *Acacia melanoxylon* germinants between the three treatments ($H = 6.16$; $df = 2$; $P = 0.04$; Fig. 1). Pairwise multiple comparisons of ranks revealed that germination was marginally higher under *Acacia melanoxylon* leachate treatment than the control ($Z = 1.97$; $df = 2$; $P = 0.14$) and marginally higher between *Olea capensis* leachate and the control ($Z = 0.12$; $df = 2$; $P = 0.12$).

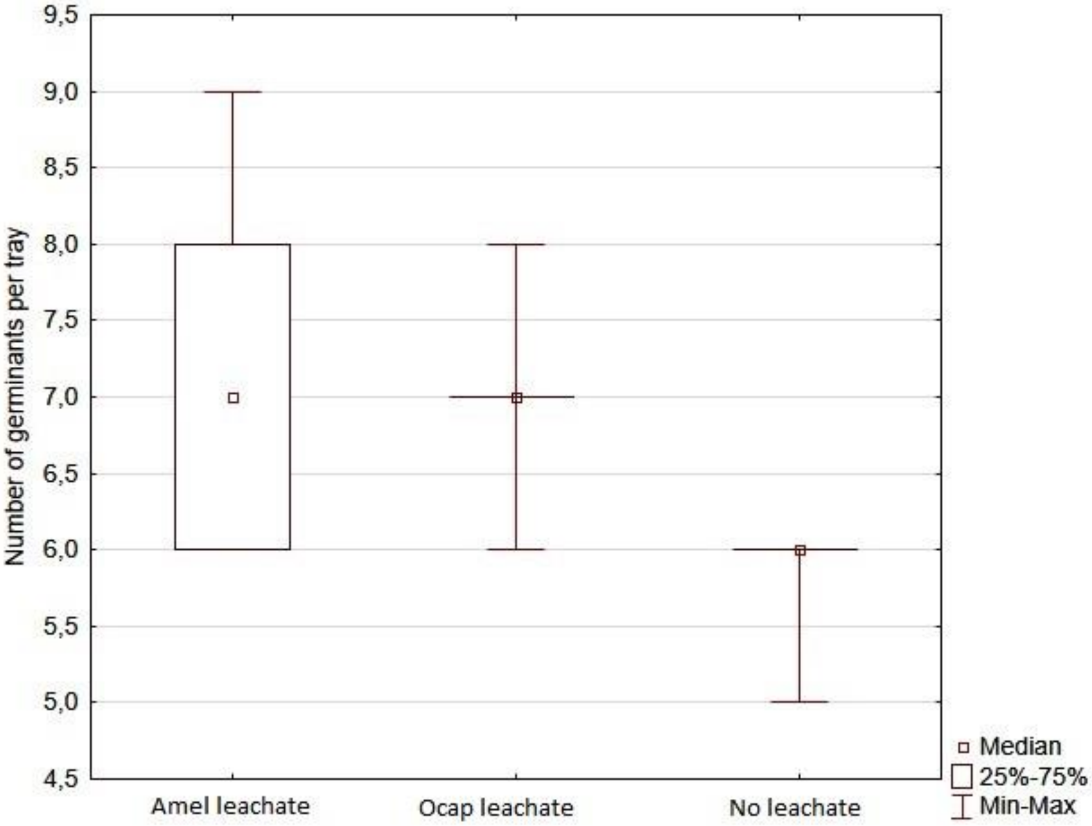

Figure 1: The number of germinants out of 10 seeds planted per tray under the three treatments, namely *Acacia melanoxylon* (Amel) phyllode leachate, *Olea capensis* (Ocap) leaf leachate, and no leachate.

Discussion

Acacia melanoxylon germination

The germination performance of *Acacia melanoxylon* was consistently superior over that of indigenous species in terms of all aspects considered in this study. The viability of seeds of this species was the highest; germination ranged between 60% and 90% under conditions wherein indigenous species failed to germinate; and germination seemed improved or unaffected by treatment with potentially allelopathic substances. Germination of *Acacia melanoxylon* seeds was not inhibited by its own leachates. Other related species that are known to inhibit their own germination are *Acacia dealbata* (Aguilera *et al.,* 2017) and *Vachellia tortilis raddiana* (Savi) Brenan (Noumi *et al.*, 2010; Noumi and Chaieb, 2011). However, in forest ecosystems, most examples of autotoxicity are found in coniferous forest in species such as *Abies balsamea* (L.) Mill., *Picea abies* (L.) H.Karst., *Picea mariana* (Mill.) Britton, Sterns & Poggenb. and *Pinus radiata* D. Don (Singh *et al.,* 1999) but has also been observed in other tree species such as *Eucalyptus globulus* and *Quercus ilex* L. (Singh *et al.,* 1999). *Acacia melanoxylon* was furthermore not inhibited by a common indigenous species' leachate. Previous studies found that *Acacia confusa* was inhibited by the allelopathic tree species, *Leucaena leucocephala* (Lam.) de Wit and *Eucalyptus urophylla* S.T.Blake (Chou and Kuo, 1986; Qui *et al.,* 2007). Overall, *Acacia melanoxylon* germination appeared to be insensitive to potential allelopathic substances which may contribute to the persistence and potential invasion of *Acacia melanoxylon* in Afrotemperate Forest.

It needs to be noted that laboratory or nursery studies may be an over simplified method to assess allelopathy as biotic and abiotic variation in the environment can create conditionality in the effects of chemicals, by either increasing or decreasing their effects (Inderjit and Callaway, 2003). Artificial substrates can exaggerate allelopathy which may overestimate the allelopathic effect of a species (Parepa and Bossdorf, 2016). Furthermore, artificial leachates can influence the effect of allelochemicals due to differences in pH and electrical conductivity (Reigosa *et al.,* 2006). The environment also plays a role in plant-plant interactions as many biotic (micro-organisms), and abiotic (shading) factors can influence and interact with allelochemicals and the target species (Inderjit and Callaway, 2003). This suggests that the findings of controlled nursery studies may not simply be extrapolated to field situations generally.

Indigenous species' germination failure

Given that all three indigenous test species failed to germinate in our trial, we considered potential reasons for this result by critically re-evaluating our methods against what is known from the literature about these species' seed ecology and germination requirements (summarised in Supplementary 1). For optimal germination of forest species, it is important to collect and plant the fruit when ripe. As we purchased the seeds commercially, this aspect was out of our control, but the supplier assured us that ripe seeds were collected and that these were fresh. The seeds of most Afrotemperate Forest species quickly lose viability through desiccation if not immediately exposed to favourable germination conditions (Geldenhuys, 1996b). The seeds of the test species were bought fresh from the supplier and planted within three weeks of ordering. The seeds did not visibly appear to be dry at the time of planting. However, the germination potential of Afrotemperate Forest species can vary from year to year (Geldenhuys, 1996b). For instance, in a multi-year germination trial, *Podocarpus falcatus*, seeds germinated well in one year and poorly in other years which may be associated with conditions during flowering, pollination or fruit development varying among years (Geldenhuys, 1975). To account for potential bias due to such influences, we tested sample sets of seeds for viability using a stain test, and although viability seemed to differ among species, all species showed viability greater than 70%. Poor viability can therefore not account for the complete failure in germination that was observed for indigenous species. Future studies may however consider applying the floating method to remove most of the non-viable seeds (Geldenhuys, 1996b) prior to conducting stain-testing to aim to obtain start-up sets of seeds with more similar viability. All seeds were pre-treated appropriately, as well as the fleshy coverings removed. This was particularly important for *Curtisia dentata* seeds as the fleshy covering is known to reduce germination (Shaik, 2012). Our trial thus complied, in terms of the collection, viability, and pre-treatment of seeds, with all known requirements to ensure germination.

Standard seed bed requirements pertaining to nursery trials were adhered to in our study; the seeds were planted 2 cm deep and were covered with a layer of soil when planted. The depth of planting is important as regular rain can cause the soil, sand, or litter cover to be splashed out of the planting trays, along with smaller seeds (Geldenhuys, 1996b). Heavy rain was experienced throughout the trial period; however, no seeds were observed to have been splashed out of the trays and is, therefore, unlikely to have contributed to complete germination failure.

In proceeding with the trial, the nursery environment should replicate as far as possible the conditions prevailing underneath the forest canopy. Germination of most Afrotemperate Forest species is shade tolerant and we accordingly covered the nursery area with 50% shade cloth to provide semi-shaded conditions akin to that underneath the forest canopy. For seeds to germinate, they furthermore require adequate moisture, temperature, and oxygen (Geldenhuys, 1996b). Given that our trial was undertaken within the confines of where Afrotemperate Forest occurs, the temperature regime would have been representative of Afrotemperate Forest. The maintenance of adequate soil moisture in the summer months may have been an issue. Although watering took place every four days, the time lapse between watering sessions may have been too long to prevent desiccation during summer. It is thus important to adjust the watering schedule according to the season, to prevent desiccation in summer, and waterlogging in winter, or to install soil moisture meters to inform optimal watering. However, inadequate soil moisture for a limited period in summer is unlikely to have accounted for complete failure in germination. Seed predation may also have affected germination in our trial as various insects were observed in the nursery area and in the planting trays. However, no seeds were visibly damaged or predated upon and this factor is unlikely to have been critical.

Lastly, the period required for germination of our test species according to the literature is 19 weeks, i.e., 4.5 months (Supplementary 1). In our study, seeds were sown in spring, which is the optimum planting period (Silverhill seeds, 2020) and the trial was terminated after 9 months in the following winter which should have allowed sufficient time to allow for germination. However, in exceptional cases, germination of Afrotemperate Forest species may take up to a year or longer (Geldenhuys, 1996b). Some Afrotemperate Forest species also require a cold moist period (stratification) to break seed dormancy (Geldenhuys, 1996b). These seeds are generally larger seeds with hard coverings enveloped in fleshy fruit, oily seeds of species occurring in mountain and coastal forest, or species which shed their seed in late summer or autumn (Geldenhuys, 1996b). Nursery conditions do not always allow for stratification as a germination trigger (Geldenhuys, 1993b) and seeds that are sown in summer generally only germinate early in the following summer, and therefore require a longer trial period to accommodate this (Geldenhuys, 1996b). Our trial did not run through an entire winter season, and it is therefore possible that stratification may not have occurred sufficiently and that seeds would only have germinated in the following summer.

Conclusion

Although the germination of the indigenous test species failed, we showed that *Acacia melanoxylon* does not chemically inhibit the germination of its own seedlings and neither is it inhibited by potential allelopathic substances from a common indigenous Afrotemperate Forest species. Further studies of the allelopathic potential of *Acacia melanoxylon* on the recruitment of Afrotemperate Forest species would be required to better understand this phenomenon. Our critical evaluation of potential reasons why germination of indigenous species failed is useful to inform future germination trials in terms of optimal practices and potential issues to consider.

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Supplementary Material

Supplementary 1: The seed ecology and germination requirements of selected indigenous Afrotemperate Forest tree species.

Species	Fruit and seed characteristics	Seed bed require-ments	Treatments	Removal of fruit from seed	Period required for germination	Light require-ments	Climatic conditions	Seed predators	Seed viability	References
Curtisia dentata	Fleshy drupe with small four locular stony seed. Ripens August to November	Plant 2 cm deep in a layer of sand or soil	Germinates well irrespective of treatment but responds well to hot water treatment and 5% HCl	Presence of the fleshy cover of the seed delayed and reduced germination. Remove fleshy covering	10–16 weeks	Semi-shade (50% shade cloth)	Conditions associated with forest interior. Keep soil moist but not waterlogged until germination	Dipterous and Lepidonterous larvae destroy embryos	98%	Phillips (1926); Geldenhuys (1996b); Shaik (2012)
Pterocelastrus tricuspidatus	A semi-fleshy yellow-orange capsule with small, orange-arillated brown, leathery-coated seed. Fruit ripens October to December	Plant 2 cm deep and cover with a layer of sand or soil	Germinates well irrespective of treatment but responds well to weak HCl or warm water for one day or cold water for three days	Remove capsule and aril	18–19 weeks	Semi-shade (50% shade cloth)	Conditions associated with forest interior. Keep soil moist but not waterlogged until germination	Dipterous larvae destroy seeds	78%	Phillips (1926); Geldenhuys (1996b)
Podocarpus latifolius	A large spherical seed with leathery coat on a fleshy, purplish-black receptacle. Ripens throughout year	Plant 2 cm deep and cover with a layer of sand or soil	Soak in cold water for two days	Remove fleshy receptacle	8–16 weeks	Semi-shade (50% shade cloth)	Conditions associated with forest interior. Keep soil moist but not waterlogged until germination	Lepidoptera and *Ceratitis* larvae cause damage to the seed	High viability	Phillips (1926); Phillips (1931); Laughton (1938); van Daalen (1981); Geldenhuys (1993b); Geldenhuys (1996b); Adie and Lawes (2009)

CHAPTER 3: Effects of alien *Acacia melanoxylon* on indigenous Afrotemperate Forest tree sapling composition under its canopy

Abstract

The alien tree species *Acacia melanoxylon* R.Br. occurs extensively in Southern Cape Afrotemperate Forest of South Africa, and concern exists about its potential negative effects on indigenous tree saplings, by virtue of altered light- or disturbance regimes or allelopathy. We investigated whether *Acacia melanoxylon* affects indigenous Afrotemperate Forest tree sapling composition underneath its canopy in the Garden Route National Park. Using a paired plot design, we compared light intensity, sapling species richness, diversity, and density underneath 30 overstorey *Acacia melanoxylon* trees and 30 indigenous counterparts of the species *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb.*, Afrocarpus falcatus* (Thunb.) C.N.Page*,* or *Olea capensis macrocarpa* (C.H.Wright) I.Verd. We recorded 2506 indigenous tree saplings from 29 species and only three *Acacia melanoxylon* saplings in the 60 plots. There were no significant differences in the richness, diversity, or density of saplings underneath *Acacia melanoxylon* trees versus their indigenous counterparts. Neither did average light intensity differ significantly underneath *Acacia melanoxylon* and indigenous canopies. However, light intensity varied significantly more underneath the canopies of *Acacia melanoxylon* than under the canopies of indigenous species. A canonical correspondence analysis accordingly showed that sapling composition was not largely determined by the overstory species. We concluded that *Acacia melanoxylon* does not significantly alter indigenous tree species composition underneath its canopy at the typical densities (<3 trees per hectare) at which it occurs in the areas of Afrotemperate Forest that we surveyed. However, populations of *Acacia melanoxylon* in the forest interior still act as a source of propagules for invasion in forest margins, riparian areas, and neighbouring fynbos shrubland, while at high density the species may alter forest gap-forming dynamics. These invasive attributes need to be considered in the management of the species in the region at large.

Keywords: forest sapling species diversity; Garden Route National Park; invasive alien plant species; southern Cape of South Africa; understorey light regime; understorey sapling density

Introduction

The introduction of invasive alien plant species has had a significant impact on the structure and ecological functioning of forest ecosystems on a global scale (Kohli *et al.*, 2008). Invader plant species in forest ecosystems often cause the loss of several species due to the impediment of indigenous tree regeneration (Kohli *et al.*, 2008). Australian Blackwood (*Acacia melanoxylon* R.Br.*,* Fabaceae) is one prominent example of an alien species in Southern Cape Afrotemperate Forest (hereafter Afrotemperate Forest) of South Africa that invades forest margins, riparian areas, and adjacent fynbos shrublands (Geldenhuys, 1996a). However, *Acacia melanoxylon* is a valuable timber species and has been managed to produce sustainable harvests in some interior parts of Afrotemperate Forest (Geldenhuys, 1996a). Controversy exists over whether this species is invasive in Afrotemperate Forest interior and whether it has detrimental effects on the regeneration and composition of indigenous forest species (Geldenhuys, 1996a; Baard and Vermeulen, 2003). Phillips (1928) noted that *Acacia melanoxylon* suppresses the regeneration of indigenous Afrotemperate Forest tree species, whereas Geldenhuys *et al.* (2017) suggested that the species acts as a nurse plant which can facilitate indigenous tree regeneration. This occurs under certain conditions where dense stands of *Acacia melanoxylon* thin out as they age, thus creating gaps that facilitate recruitment of indigenous forest tree species (Geldenhuys *et al.,* 2017).

There are various factors that could be responsible for the suppression or facilitation of forest regeneration (Kohli *et al.*, 2008). Generally, invasive alien plant species may be successful due to traits that afford them an ecological advantage over the indigenous vegetation and they may have the potential to alter the successional processes of a community over time through displacing the indigenous vegetation (Orr *et al.,* 2005). Competition for light may have the most impact on tree seedling establishment in forest ecosystems (Langmaier and Lapin, 2020). Accordingly, it has been suggested in the context of Afrotemperate Forest that indigenous species' regeneration may be suppressed by a significant reduction in light-intensity under alien *Acacia melanoxylon* canopies in addition to this species' potential allelopathic and soil drying effects (Phillips, 1928). *Acacia melanoxylon*, by virtue of its large and dense canopies, is thought to reduce the light-intensity underneath the forest canopy so significantly that indigenous saplings may be incapable of establishing, or, if able to establish themselves, may be incapable of normal development (Phillips, 1928). Under low light intensity conditions, competition for light becomes even more important for the establishment of tree seedlings (Langmaier and Lapin, 2020). In a previous study, the light intensity underneath *Acacia melanoxylon* stands was measured using a photometer and values as low as 1/50 – 1/700 of full sunlight were recorded (Phillips, 1928). There were very few herbaceous species observed under these stands and few poorly developed saplings of *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb.*,* *Afrocarpus falcatus* (Thunb.) C.N.Page*, Olea capensis macrocarpa* (C.H.Wright) I.Verd. (hereafter *Olea capensis*) and *Curtisia dentata* (Burm. f.) C.A. Smith (Phillips, 1928). Furthermore, a previous study found that the presence of another *Acacia* species, *Acacia longifolia* (Andrews) Willd. in pine forests in Portugal was associated with a reduction in light intensity in the understorey (Rascher *et al.,* 2011). *Acacia longifolia* intercepts light due to its rapid growth rate and small tree life form and thereby reduces light intensity, ultimately leading to a 50% decrease in indigenous species abundance and diversity (Rascher *et al.,* 2011).

Another prominent suppressive mechanism is allelopathy, whereby allelochemicals leach into the soil from the understorey litter (Reigosa and González, 2006) and commonly causing reduced seed germination and seedling growth (Ferguson and Rathinasabapathi, 2013). Consequently, plant diversity and species richness can be significantly reduced underneath allelopathic tree species' canopies (Reigosa and González, 2006). Evidence for allelopathic effects of alien trees or shrubs exists for many forests and shrublands of the world. Invasive alien species from the genera *Eucalyptus* and *Acacia* commonly alter the community composition and diversity of understorey vegetation potentially through allelopathic effects (Gibson *et al.*, 2011) and alteration of light regimes, e.g., *Eucalyptus globulus* Labill. in California and western Europe (Becerra Osses *et al.*, 2018), *Acacia saligna* (Labill.) Wendl., in the Cape of South Africa (Mehta, 2000), and *Acacia dealbata* Link (Lorenzo *et al.,* 2011) and *Acacia melanoxylon* on the Iberian Peninsula (Hussain *et al.,* 2011).

Allelopathy can be more effective when plants endure other environmental stresses, such as increased competition for light (Reigosa *et al.,* 1999).

Afrotemperate Forests are dominated by species that regenerate from seedlings, and therefore these tall forests display a monopodial growth form (Kruger *et al.,* 2015). The ecology of the indigenous Afrotemperate Forest is notably affected by disturbance- and gap dynamics (Mucina and Geldenhuys, 2006). Afrotemperate Forests are not generally exposed to major disturbances and canopy gaps are mostly created by trees that die standing or by windfalls (Geldenhuys and Maliepaard, 1983). In the forests on the coastal platform and scarp, and the river valley slopes, the main disturbances are trees that die standing from infrequent lightning strikes, and small windfalls (Geldenhuys, 1996b). The frequency and the extent of the disturbances can influence the forest structure through the reseeding or resprouting responses of species in relation to the availability of light and other resources (Kruger *et al.,* 2015). The disturbance dynamics of Afrotemperate Forests are typically fine-grained (Midgley *et al.*, 1990) and regeneration occurs over small spatial scales (Van Wyk *et al.*, 1996; Lawes *et al.*, 2007). Grain refers to the relationship between the species composition of the canopy and the species composition of the regeneration stand (Geldenhuys, 1996b). Most of the fine-grained species are shade-tolerant and dominate the canopy in mature forest (Lawes *et al.*, 2007). Shade-tolerant species often have a short seed dormancy period and germination occurs shortly after the seed is released (Lawes *et al.,* 2004). *Podocarpus latifolius, Curtisia dentata* and *Gonioma kamassi* E.Mey. are examples of prominent shade-tolerant canopy species of which the saplings require intermittent exposure to light to grow to the canopy (Lawes *et al.,* 2004).

As outlined above, *Acacia melanoxylon* may alter the disturbance dynamics and light regime, and may have allelopathic effects in Afrotemperate Forest, thereby affecting the recruitment and composition of indigenous forest tree saplings. However, the potential effects of these factors have not been rigorously verified and are subject to contention which makes decisions about the management of this alien species in the region challenging (Geldenhuys *et al.,* 2017; Moolman and Rikhotso, 2014; Baard and Vermeulen, 2003). The aim of our study was therefore to assess whether *Acacia melanoxylon* trees alter the tree sapling composition underneath their canopies. Specifically, we compared the indigenous sapling species richness, diversity, and density under *Acacia melanoxylon* and indigenous overstorey trees in mature interior forest. Additionally, we investigated whether *Acacia melanoxylon* trees alter the light intensity under their canopies compared to that under indigenous canopies.

Methods

Study area

Afrotemperate Forest occurs in scattered patches among fynbos shrublands in the Western and Eastern Cape provinces of South Africa (Mucina and Geldenhuys, 2006). This study focused on the largest complex of patches, which occurs in the Southern Cape-Tsitsikamma region, and most of which is included in the Garden Route National Park (Mucina and Geldenhuys, 2006; Kraaij *et al.*, 2011). These forests occupy altitudes ranging from 10 m to 600 m above sea level (Mucina and Geldenhuys, 2006). The climate of this region is warm temperate and humid, with precipitation throughout the year (Seydack *et al.,* 2012). Afrotemperate Forests are generally confined to regions of high water availability (Mucina and Geldenhuys, 2006). Mean annual precipitation varies between 700 mm and 1 200 mm according to orographic factors and increases from west to east (Seydack *et al.,* 2012). Temperatures are moderated by proximity to the coast (Mucina and Geldenhuys, 2006) with daily minimum and maximum air temperatures at 15 °C – 25 °C (summer) and 7 °C – 19 °C (winter) (Tyson and Preston-White, 2000; Mucina and Geldenhuys, 2006; Russell *et al.*, 2012). South-easterly winds are common in the summer whilst south-westerly winds and hot, desiccating bergwinds commonly occur in winter and autumn (Baard and Kraaij, 2014). Afrotemperate Forest grows on soils derived from the Table Mountain Group sandstones, shales of the Cape Supergroup and Cape Granite (Geldenhuys, 1991; Geldenhuys, 1993a; Mucina and Geldenhuys, 2006). The soils vary from Mispah, Glenrosa and Houwhoek forms to sandy, humic Fernwood form (Geldenhuys, 1991; Geldenhuys, 1993a; Mucina and Geldenhuys, 2006).

Closed-canopy Afrotemperate Forest is tall and multi-layered (Mucina and Geldenhuys, 2006). There are three distinct forest types, namely river valley and scarp forest, platform forest, and mountain forest (Geldenhuys, 1996b) with species such as *Afrocarpus falcatus, Podocarpus latifolius* and *Olea capensis* dominating the canopy of the platform forests (Mucina and Geldenhuys, 2006). In moist and mesic localities, the shrub understorey and herbaceous layer are well established (Mucina and Geldenhuys, 2006). Fire does not generally play a major role in forest function and dynamics, except at the forest margin (Geldenhuys and Mucina, 2006), and in exceptional weather conditions where adjacent vegetation affects fire penetrability (Kraaij *et al.*, 2018; Giddey *et al.*, 2021). Afrotemperate Forests persist in fire refugia and are surrounded by fire-prone fynbos shrublands, however, commercial plantations of invasive alien *Pinus* trees abut the forest in many areas today (Geldenhuys and Mucina, 2006; Kraaij *et al.*, 2011).

Acacia melanoxylon was introduced into the southern Cape in 1856 for its valuable timber and planted into forest gaps resulting from harvesting of indigenous trees, and in gaps resulting from localised burns (Geldenhuys, 1996a). Over the last century, the approach to the species' management in state forests of the region switched between attempts to promote or manage the species to produce sustainable timber harvests, and attempts to control the species when and where it was deemed to be invasive (Philips, 1928; Geldenhuys, 1996a; Seydack 2002; Baard and Vermeulen, 2003; Moolman and Rikhotso, 2014). Periodic harvesting operations, and more recently, control of saplings in forest margins, riparian areas, and adjacent fynbos shrublands, contributed to the maintenance of relatively low densities of the species within state forests that form part of the Garden Route National Park (Seydack, 2002; Baard and Vermeulen, 2003).

Field surveys

We undertook one-off surveys of tree sapling composition in platform forest in areas that were level or with gentle slopes underneath the canopies of large (with a diameter at breast height (DBH) of ≥ 40 cm and a canopy radius of ≥ 5 m), mature *Acacia melanoxylon* trees and three species of indigenous trees. These large overstorey trees served as focal trees positioned in the centre of our circular survey plots with 5 m radius. For each *Acacia melanoxylon* focal tree, an indigenous counterpart of the species *Afrocarpus falcatus, Podocarpus latifolius* or *Olea capensis* was selected that (i) was at least one canopy diameter distance away from the *Acacia melanoxylon* focal tree, (ii) had a comparable canopy size, and (iii) occurred in similar habitat with similar aspect and slope. Sixty circular plots were surveyed, 30 of which had *Acacia melanoxylon* as focal tree, paired with 30 plots having indigenous focal trees (10 each of the three indigenous species), with the focal trees having an average DBH of 75 cm (range 40 cm – 120 cm) and an average canopy radius of 7 m (range 5 m – 12 m). Underneath the focal tree canopies, all *Acacia melanoxylon* saplings and indigenous forest tree saplings with a stem diameter of between 5 mm and 30 mm were identified. All the study species were trees (Geldenhuys, 1993a), although, some of these species may take on other growth forms such as *Halleria lucida* L. which may take on a shrub growth form, and *Rhoicissus tomentosa* (Lam.) Wild & R.B. Drumm. which commonly grow as a liana. For each of these saplings, its stem diameter was measured in centimetres at 15 cm above ground level (using a caliper) and its height (using a measuring tape). The light intensity underneath the canopies of focal trees was measured at ground level at eight locations radiating out from the focal tree's stem in the eight cardinal and intercardinal directions, 2 m away from the stem of the focal tree. For this we used a photometer application (Lux Light Meter Pro) on a mobile phone (iPhone 6). We surveyed areas that are representative of the densities at which *Acacia melanoxylon* occurs in Afrotemperate Forest in the Garden Route National Park. The survey areas had an average density of mature *Acacia melanoxylon* trees of 1.6 trees per hectare (range 0.6 – 2.6 trees per hectare) and no recent history of harvesting of mature trees or control of seedlings and saplings (South African National Parks, unpublished data). Areas of very high densities of *Acacia melanoxylon* in the forest interior are scarce and not representative of the prevailing situation in the Garden Route National Park and most other state forests. Neither would it have been feasible to find nearby analogous indigenous trees when surveying high density *Acacia melanoxylon* stands.

Statistical analysis

A direct gradient analysis technique, canonical correspondence analysis (CCA) (Hotelling,1936; ter Braak, 1985), was used to examine the relationship between tree sapling composition (expressed as the abundance per species) as the response variable and the species of overstorey tree (*Acacia melanoxylon, Afrocarpus falcatus, Podocarpus latifolius* or *Olea capensis*) as predictor or 'environmental' variable in the 60 plots. The CCA was performed using the Multivariate Statistical Package (MVSP v. 3.12d, 1985-2001, Kovach Computing Services) with its default settings.

The paired design of our survey plots permitted the use of paired t-tests to compare sapling composition and light intensity underneath *Acacia melanoxylon* trees and their indigenous counterparts. We compared the following variables between the 30 pairs of plots: (i) indigenous sapling species richness (count of the number of species); (ii) indigenous sapling diversity (calculated by means of the Shannon-Wiener diversity index, H' = $\sum_{i=l}^{s} pi\, ln\, pi$; Spellerberg and Fedor, 2003); (iii) indigenous sapling density (number of individuals per square meter); and (iv) light intensity (mean of eight readings per plot). The average light intensity was square root transformed prior to statistical analysis to correct a right-skewed distribution. To compare the variation in light intensity (calculated as the standard error of the mean of eight transformed readings per plot) under *Acacia melanoxylon* canopies versus indigenous tree canopies, a Wilcoxon matched pairs signed rank test was performed, as the data did not conform to normality as per the Shapiro-Wilks test.

Results

A total of 2503 indigenous tree saplings from 28 species and three *Acacia melanoxylon* saplings were recorded (Table 1). The CCA produced small eigenvalues for all axes (< 0.35) indicating "short" gradients, i.e., suggesting that most species occurred throughout the gradients (ter Braak, 1988, 1995). The first two axes jointly explained only 6.9% of the variance in the data (Table 2). The species-environment correlations (i.e., the sapling species-overstorey species relationship) for the first two axes were also weak (0.59 and 0.59 respectively). The first canonical axis was best correlated with the overstorey species *Olea capensis* (Fig. 1). The sapling species that had the strongest association with the overstorey species *Olea capensis* were *Maytenus acuminata* (L.f.) Loes., *Canthium inerme* (L.f.) Kuntze and *Halleria lucida.* The second canonical axis was best correlated with the overstorey species *Podocarpus latifolius,* and to a lesser extent *Afrocarpus falcatus.* The sapling species that had the strongest association with the overstorey species *Podocarpus latifolius* was *Ochna serrulata* Walp. The overstorey species *Acacia melanoxylon* was associated with the sapling species *Rhoicissus tomentosa, Ilex mitis* (L.) Radlk., *Virgilia oroboides* (P.J.Bergius) T.M.Salter and *Acacia melanoxylon.* However, these associations were very weak given that the first two axes explained <10% of the variation.

A total of only three *Acacia melanoxylon* saplings occurred in our survey plots, all of which were underneath *Acacia melanoxylon* canopies (Table 1). The indigenous sapling species that were exclusively associated with *Acacia melanoxylon* plots were *Ilex mitis, Rhoicissus tomentosa,* and *Virgilia oroboides* (Table 1; Fig.1). Relatively common indigenous sapling species (i.e., species for which we recorded at least 20 individuals) that were notably more abundant underneath *Acacia melanoxylon* canopies than underneath indigenous canopies, were *Podocarpus latifolius* (70% of individuals recorded underneath *Acacia melanoxylon*)*, Rapanea melanophloeos* (75%) and *Gonioma kamassi* (65%) (Table 1). Conversely, four indigenous sapling species were exclusively associated with the indigenous overstorey species, namely *Canthium inerme, Halleria lucida, Maytenus acuminata* and *Ochna serrulata*, although very few individuals were recorded of these species (Table 1; Fig. 1).

Table 1: List of 29 sapling species surveyed in Afrotemperate Forest, with their abbreviations, the number of individuals recorded, the percentage of the total number of saplings of a species recorded under indigenous canopies, and the number of plots underneath *Acacia melanoxylon* (Amel) and indigenous trees (respectively) in which sapling species occurred. Species with a sample size of ≥20 individuals are indicated in bold text. Nomenclature follows www.theplantlist.org.

Species	Abbre-viation	No. individuals (% under indigenous)	No. of Amel plots	No. of indigenous plots
Acacia melanoxylon R.Br.	Amel	3 (0%)	3	0
Afrocanthium mundianum **(Cham. & Schltdl.) Lantz**	**Amun**	**816 (43%)**	**3**	**3**
Afrocarpus falcatus **(Thunb.) C.N.Page**	**Afal**	**47 (11%)**	**11**	**3**
*Burchellia bubalina (*L.f.) Sims	Bbub	9 (33%)	2	2
Canthium inerme (L.f.) Kuntze	Cine	3 (100%)	0	1
Carissa macrocarpa **(Eckl.) A.DC.**	**Cmac**	**36 (75%)**	**7**	**12**
Cassine peragua L.	Cper	9 (44%)	3	2
Curtisia dentata **(Burm. f.) C.A. Smith.**	**Cden**	**40 (40%)**	**4**	**9**
Diospyros dichrophylla **(Gand.) De Winter**	**Ddic**	**22 (55%)**	**6**	**6**
Diospyros whyteana **(Hiern) P.White**	**Dwhy**	**44 (66%)**	**8**	**10**
Elaeodendron croceum **(Thunb.) DC.**	**Ecro**	**119 (62%)**	**17**	**23**
Gonioma kamassi **E.Mey.**	**Gkam**	**120 (35%)**	**18**	**14**
Gymnosporia harveyana Loes.	Ghar	17 (82%)	2	6
Halleria lucida L.	Hluc	1 (100%)	0	1
Ilex mitis (L.) Radlk.	Imit	1 (0%)	1	0
Lauridia tetragona (L. f.) R.H. Archer	Ltet	9 (67%)	1	3
Maytenus acuminata (L.f.) Loes.	Macu	6 (100%)	0	2
Ochna arborea Burch. ex DC.	Oarb	7 (71%)	2	3
Ochna serrulata Walp.	Oser	3 (100%)	0	2
Ocotea bullata **(Burch.) E. Meyer in Drege**	**Obul**	**241 (60%)**	**21**	**20**
Olea capensis macrocarpa **(C.H.Wright) I.Verd.**	**Ocap**	**374 (50%)**	**25**	**24**
Platylophus trifoliatus D. Don.	Pltri	4 (75%)	1	1
Podocarpus latifolius **(Thunb.) R.Br. ex Mirb.**	**Plat**	**442 (30%)**	**27**	**19**
Pterocelastrus tricuspidatus Walp.	Pttri	9 (56%)	2	4
Rapanea melanophloeos **(L.) Mez**	**Rmel**	**126 (25%)**	**13**	**10**
Rhoicissus tomentosa	Rtom	1 (0%)	1	0
Scutia myrtina **(Burm.f.) Kurz**	**Smyr**	**44 (66%)**	**5**	**8**
Trichocladus crinitus **Pers.**	**Tcrin**	**752 (59%)**	**21**	**26**
Virgilia oroboides (P.J.Bergius) T.M.Salter	Voro	1 (0%)	1	0

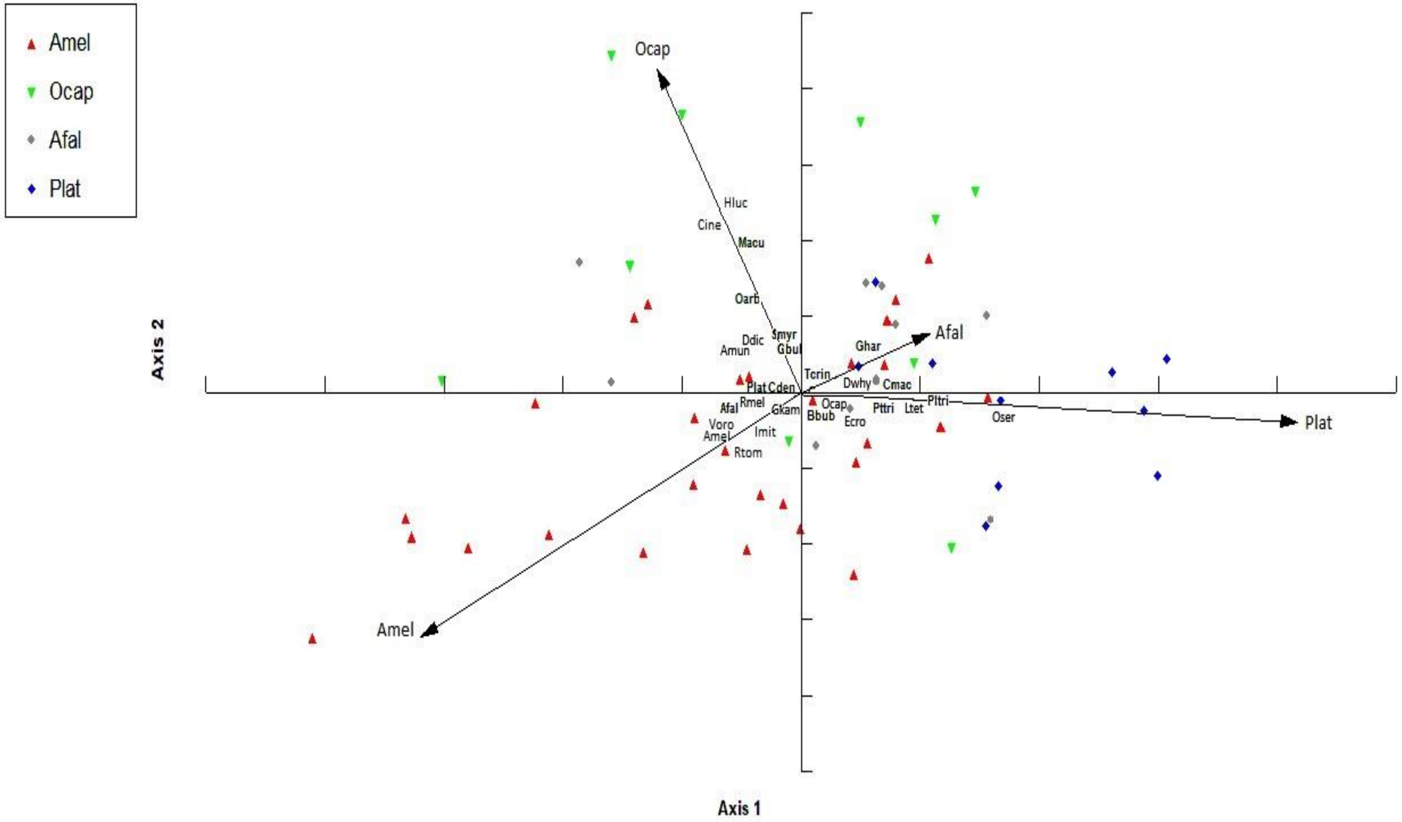

Figure 1: Joint plot of 1st and 2nd canonical correspondence axes from a canonical correspondence analysis (carried out in MVSP v. 3.12d, 1985-2001, Kovach Computing Services) of the abundance of 29 tree sapling species across 60 plots. The arrows represent the environmental variables, being the four overstorey species (Amel, *Acacia melanoxylon;* Plat, *Podocarpus latifolius;* Ocap, *Olea capensis;* and Afal, *Afrocarpus falcatus*). A long arrow located close to an axis indicates a strong relationship with that axis (ter Braak 1986; Palmer 1993). Sapling species situated near to the arrows have a strong association with that overstorey species (ter Braak 1986; Palmer 1993). For sapling species abbreviations, see Table 1

Relatively common indigenous sapling species (i.e., species for which we recorded at least 20 individuals) that were notably more abundant underneath indigenous tree canopies than under *Acacia melanoxylon* canopies were *Carissa macrocarpa* (Eckl.) A.DC. (75% of individuals underneath indigenous), *Diospyros whyteana* (Hiern) P.White (66%), and *Scutia myrtina* (Burm.f.) Kurz (66%) (Table 1).

Table 2: Summary of canonical correspondence analysis (CCA) results for sapling composition-overstorey tree relationships in 60 plots in Southern Cape Afrotemperate Forest.

Axes	***λ* 1**	***λ* 2**
Eigen values	0.117	0.076
Percentage variance	4.2	2.7
Cumulative percentage variance:	4.2	6.9
Species-environment correlations	0.595	0.592

There were no significant differences between the richness, diversity, or density (respectively) of saplings underneath *Acacia melanoxylon* trees and their indigenous counterparts (Table 3). Neither did light intensity differ significantly underneath *Acacia melanoxylon* and indigenous canopies. However, light intensity varied significantly more underneath the canopies of *Acacia melanoxylon* than under the canopies of indigenous species ($Z = 2.335$; $P = 0.019$).

Table 3: The mean (± S.E.) and paired t-test results for comparisons of species richness, Shannon-Wiener diversity, sapling density, and light intensity of indigenous tree sapling communities under *Acacia melanoxylon* and indigenous overstorey trees.

Index	**Mean (± S.E.) *A. melanoxylon***	**Mean (± S.E.) Indigenous**	**t**	**df**	***P***
Indigenous species richness (number of species)	6.93 (± 0.41)	7.20 (± 0.49)	-0.519	29	0.608
Shannon-Wiener diversity index	1.45 (± 0.06)	1.39 (± 0.08)	0.902	29	0.375
Indigenous sapling density (number per square meter)	0.55 (± 0.04)	0.52 (± 0.03)	0.477	29	0.637
SQRT light intensity (luminous flux per unit area)	37.51 (± 1.40)	36.03 (± 1.25)	1.057	29	0.299

Discussion

This study showed that low densities of *Acacia melanoxylon* in Afrotemperate Forest did not significantly alter indigenous tree sapling composition under its canopy relative to that under common indigenous overstorey trees. This was evident from the weak sapling species-overstorey species correlation shown by the CCA suggesting that sapling composition was not largely determined by the overstorey species. The lack of significant differences in richness, diversity, and density of saplings underneath *Acacia melanoxylon* trees and their indigenous counterparts further confirmed this result. Our findings are in contrast with evidence from indigenous forest communities in other parts of the world where invasion by various alien *Acacia* species was associated with reductions in species richness, density, diversity, and total plant cover (Lorenzo *et al.,* 2012; Fuentes-Ramírez *et al.,* 2010; González-Muñoz *et al.,* 2012; Lazarro *et al.,* 2014). More specifically, a study of *Acacia dealbata* (occurring at an average density of 7 trees per square meter) in oak and pine forest communities reported a significant alteration of understorey species composition through a combination of reduced light availability and allelopathic effects (Lorenzo *et al.,* 2012). We postulate that the low densities (<3 trees per hectare) at which *Acacia melanoxylon* occurred in our study area likely account for the lack of effect that we observed.

In our study, the number of recorded *Acacia melanoxylon* saplings was low (three individuals) and none were recorded underneath indigenous tree canopies. *Acacia melanoxylon* seeds are dispersed by birds and water, thus one would expect long dispersal distances, resulting in low-density seedbanks far away from the parent plants but relatively large seedbanks underneath mature trees (Goets *et al.,* 2018). Given that our plots were underneath large, mature *Acacia melanoxylon* trees, factors other than the availability of viable seedbanks are likely to have limited recruitment of this species, although Goets *et al.* (2018) found *Acacia melanoxylon* seedbanks to be particularly variable under mature parent plants in the study region. Evidence obtained from a germination trial suggests that germination of *Acacia melanoxylon* seeds is not impeded by allelopathic effects from parent plants or an indigenous Afrotemperate Forest species (*Olea capensis*) either (Chapter 2). Nevertheless, the observed scarcity of *Acacia melanoxylon* saplings supports the notion that *Acacia melanoxylon* does not recruit in mass in intact Afrotemperate Forest interior like it does in the forest margin, surrounding fynbos and other areas where extensive disturbances (usually in the form of fire) occur which stimulate mass germination (Geldenhuys, 1996a; Seydack, 2002).

Several prominent indigenous canopy species (e.g., *Podocarpus latifolius, Rapanea melanophloeos,* and *Gonioma kamassi*) occurred in abundance under *Acacia melanoxylon* canopies. This suggests that common canopy species did not suffer underneath the canopies of *Acacia melanoxylon*. *Podocarpus latifolius* is one of the most common canopy species and 70% of all saplings of this species recorded in this study occurred underneath *Acacia melanoxylon* canopies. Of the *Rapanea melanophloeos* saplings that were recorded, 75% occurred underneath *Acacia melanoxylon* canopies. *Rapanea melanophloeos* is a common canopy species that also acts as a pioneer species that can facilitate forest recovery (Geldenhuys, 1993a, 1994, 2004a). Typically, pioneer species are light demanding and recruit into large gaps, although the threshold gap size for pioneers vary greatly (Obiri and Lawes, 2004). Other light tolerant canopy species (Seifert *et al.,* 2014) that commonly occurred underneath *Acacia melanoxylon* canopies were *Afrocarpus falcatus* (89% of individuals recorded) and *Olea capensis* (50%). On the other hand*,* several shade-tolerant species (Geldenhuys, 1993b; Seifert *et al.,* 2014) also occurred abundantly underneath *Acacia melanoxylon* canopies, including *Curtisia dentata* (60%)*, Gonioma kamassi* (65%) and *Podocarpus latifolius* (70%). The finding that shade-tolerant and light-demanding species both recruited well underneath *Acacia melanoxylon* is in line with our finding that the light regime under *Acacia melanoxylon* is varied. Although our findings show that average light intensity did not differ between indigenous and *Acacia melanoxylon* canopies, our measurement of light intensity was very basic, and a more comprehensive investigation of understory light regimes would facilitate better understanding of this complex metric.

Species that occurred exclusively under *Acacia melanoxylon* or the indigenous overstorey species, were largely species that are naturally rare in these forests, such as *Virgilia oroboides*, (Phillips, 1926; Geldenhuys, 1993a), *Ilex mitis* (Geldenhuys, 1993a), and *Maytenus acuminata* (Geldenhuys, 1993a). We thus postulate that their occurrences were linked to particular habitat conditions or forest type variants, rather than strong associations with specific overstorey species. For example, *Virgilia oroboides* is exclusively associated with the forest margin (Phillips, 1926), *Ilex mitis* occurs scattered in moist forest and commonly beside streams (Geldenhuys, 1993a), whereas *Maytenus acuminata* occurs in dry and scrub forest and often in the forest margin (Geldenhuys, 1993a). Indigenous sapling species that were notably more abundant underneath indigenous tree canopies than under *Acacia melanoxylon* canopies were *Carissa macrocarpa, Diospyros whyteana* and *Scutia myrtina.* These species are all common in the understorey layer (Geldenhuys, 1996c).

Overall, the species-specific differences noted underneath *Acacia melanoxylon* and indigenous canopies were insubstantial and suggest that *Acacia melanoxylon* is unlikely to cause structural or compositional change to Afrotemperate Forest interiors when occurring at the densities typically found in the study area. However, there is preliminary evidence that *Acacia melanoxylon* at high densities (approx. 17 trees per hectare) may alter the gap forming disturbance regime of forest and thereby affect indigenous forest regeneration (Moolman and Rikhotso, 2014). *Acacia melanoxylon* causes increased incidence of, and larger, windfall gaps than those caused by indigenous large-crowned *Olea capensis* due to its fast growth, large emergent crowns, and poor root development (Geldenhuys, 2013). Larger gaps can be more susceptible to mass regeneration of, and thus dominance by, saplings of this light-demanding alien pioneer species (Lawes *et al.*, 2004) which can prevent indigenous pioneer species from regenerating in the gaps (Geldenhuys, 2013). Conversely, the Geldenhuys *et al.* (2017) nurse plant model postulates that alien plant communities may facilitate natural forest establishment. In fire excluded landscapes, invasive alien plants usually establish as dense stands. As the invader stands mature, they become less dense through natural self-thinning, which creates the gaps that may facilitate regeneration of indigenous shade-tolerant species (Geldenhuys *et al.,* 2017). Stand density additionally has relevance for potential allelopathic effects of species (Reigosa *et al.,* 1999; Hussain *et al.,* 2011).

Although the findings of this study suggest that *Acacia melanoxylon* does not have significant effects on forest interior sapling communities, the highly invasive nature of *Acacia melanoxylon* in neighbouring vegetation types (Geldenhuys, 2004b) should still be considered when managing *Acacia melanoxylon* populations in Afrotemperate Forest. These populations of *Acacia melanoxylon* may act as an important source of propagules and thus invasion in areas such as the forest margin, rivers, ecotones, and disturbed areas. *Acacia melanoxylon* populations can invade over long distances from their source populations; for instance, *Acacia melanoxylon* was found to have spread 32 km along a stream bank within 13 years of the establishment of a plantation upstream (Phillips, 1928), while seedlings and saplings have been documented up to 450 m from a single parent tree (Geldenhuys, 1996a). *Acacia melanoxylon* is furthermore capable of root suckering, as much as 10 m – 20 m away from the parent plant, when the root system is disturbed, which renders this species exceedingly difficult to control via mechanical and chemical means (Seydack, 2002). These suckers can grow into large individuals which in turn produce seeds and act as further sources for invasion (Philips, 1928).

The fact that *Acacia melanoxylon* still occurs at low densities in the state forests of the study area 165 years after being introduced is likely attributed to the history of management of this species (Seydack, 2002). Since its introduction to the southern Cape in 1856, populations in state forests have been managed by periodic harvesting of mature individuals which have contributed to the maintenance of low densities within the survey area (Seydack, 2002). Additionally, from 2003 to 2014, the management approach in the Garden Route National Park was to control *Acacia melanoxylon* in forest margins, riparian areas, and adjacent fynbos shrublands, whereas populations in the forest interior were not controlled and were allowed to grow to maturity for harvesting (Seydack, 2002). This approach was based on the assertion that regeneration of shade-intolerant species (such as *Acacia melanoxylon*) is prevented by the lack of sunlight in the forest interior (Seydack, 2002). It was thought that populations of *Acacia melanoxylon* in the forest interior would ultimately stabilise and have little ecological impact and that the populations from which large individuals were harvested would gradually decrease (Geldenhuys, 1996a). More recently, this management approach has been questioned in light of preliminary results emanating from a long-term monitoring program (Moolman and Rikhotso, 2014), showing that *Acacia melanoxylon* displays demographics characteristic of pioneer species (successful recruitment and population increases), suggesting that the species behaves like an invader, even in forest interior and despite being harvested (Moolman and Rikhotso, 2014). Considering the threat that *Acacia melanoxylon* poses as an aggressive invader and source of further invasion in vegetation types abutting forest, as well as its potential to alter the disturbance dynamics in the forest interior when occurring at high densities, we suggest that populations in the forest interior be maintained at low densities (if economic incentives urge against total control) in addition to controlling the species in adjacent vegetation where it is invasive.

Conclusion

We conclude that *Acacia melanoxylon* trees, at the typically low densities at which it occurs in the state forests that we surveyed, did not significantly alter the richness, diversity, or density of Afrotemperate Forest saplings under their canopies. Furthermore, average light intensity did not differ significantly, but was more variable, under *Acacia melanoxylon* than under indigenous canopies. *Acacia melanoxylon* is unlikely to alter the structure of indigenous tree communities given our findings that most common canopy species, including light demanding and shade tolerant species, were able to recruit under *Acacia melanoxylon* canopies. However, *Acacia melanoxylon* populations in Afrotemperate Forest remain a concern as these comprise a source of propagules for invasion into adjacent vegetation types. We advise that the invasive traits of *Acacia melanoxylon* still be considered when managing the species in the region at large.

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CHAPTER 4: Synthesis

Summary of major findings

The alien tree species, *Acacia melanoxylon* R.Br., occurs extensively in Southern Cape Afrotemperate Forest (South Africa) however little is known of its potential suppressive effects on indigenous tree species. It has been hypothesized that *Acacia melanoxylon* may have a negative effect on indigenous Afrotemperate Forest tree species through suppressive mechanisms such as the alteration of natural light regimes or allelopathy (Phillips, 1928). The overarching aim of this study was therefore to investigate the potential suppressive effects that *Acacia melanoxylon* may have on Southern Cape Afrotemperate Forest tree species recruitment. This was achieved in two parts: (i) by investigating the potential allelopathic effects of alien *Acacia melanoxylon* compared to that of an indigenous canopy tree *Olea capensis* (C.H.Wright) I.Verd. on the germination of *Acacia melanoxylon* and Afrotemperate Forest trees; and (ii) by assessing whether *Acacia melanoxylon* alters the light regime and composition of indigenous Afrotemperate forest tree saplings under its canopy compared to that under indigenous tree canopies.

The assessment of the potential allelopathic effects of *Acacia melanoxylon* and *Olea capensis* (Chapter 2) was undertaken in a nursery trial which was based in the southern Cape of South Africa where Afrotemperate Forest occurs. The germination performance of *Acacia melanoxylon* was superior to that of the indigenous species (for which germination failed altogether) and was improved, or unaffected, by potential allelopathic substances of itself or of the common indigenous overstorey species *Olea capensis*. Though the germination of the indigenous test species failed, we critically considered the germination requirements of the indigenous test species and proposed that the most probable explanation for the germination failure could be that the trial period may not have provided adequate cold stratification. We furthermore concluded that the superior germination of *Acacia melanoxylon* relative to three common indigenous species and its lack of sensitivity to potential allelopathic substances of itself or a common indigenous overstorey species likely contribute to its success as an invasive species in Afrotemperate Forest.

The field studies to assess whether *Acacia melanoxylon* alters Afrotemperate Forest tree sapling composition under its canopy (Chapter 3) were undertaken in the largest complex of Afrotemperate Forest patches, which occurs in the Garden Route National Park. Field surveys of tree sapling composition underneath *Acacia melanoxylon* and indigenous tree species canopies revealed that there were no significant differences in richness, diversity, or density of indigenous tree saplings based on the overstorey species. Furthermore, we found that light intensity did not differ significantly underneath *Acacia melanoxylon* and indigenous canopies but was more varied underneath the alien species' canopy. A canonical correspondence analysis accordingly showed that sapling composition was not largely determined by the overstorey species. We concluded that *Acacia melanoxylon* does not significantly alter indigenous tree species composition underneath its canopy at the typical densities (<3 trees per hectare) at which it occurred in the areas of Afrotemperate Forest that we surveyed.

Implications for management

The management of *Acacia melanoxylon* in Southern Cape Afrotemperate Forest over the last century has been controversial. The approach to the species' management in state forests of the region (including the Garden Route National Park) have periodically switched between attempts to promote or manage the species to produce sustainable timber harvests and attempts to control the species when and where it was deemed to be invasive (Philips, 1928; Geldenhuys, 1996a; Seydack 2002; Baard and Vermeulen, 2003; Moolman and Rikhotso, 2014). Periodic harvesting operations, and more recently, control of saplings in forest margins, riparian areas, and adjacent fynbos shrublands, have contributed to the maintenance of relatively low densities of the species within the Garden Route National Park (Seydack, 2002; Baard and Vermeulen, 2003). However, controversy persisted over whether this species is invasive in Afrotemperate Forest interior and whether it has detrimental effects on the regeneration and composition of indigenous forest communities (Geldenhuys, 1996a; Baard and Vermeulen, 2003). Our findings (Chapter 2) now showed that *Acacia melanoxylon* germination appeared to be insensitive to potential allelopathic substances which may contribute to the persistence and potential invasion of *Acacia* *melanoxylon* in Afrotemperate Forest. On the other hand, we found no evidence for significant alteration of tree sapling composition under *Acacia melanoxylon* trees compared to that under indigenous canopies, suggesting that the species is unlikely to materially alter forest tree community structure and composition at the densities (<3 trees per hectare) representative of the area.

The densities of mature *Acacia melanoxylon* trees (range 0.6 – 2.6 trees per hectare) in the Garden Route National Park that we surveyed are comparable to the prevailing densities of some dominant indigenous emergent species in Afrotemperate Forest, such as *Afrocarpus falcatus* (Thunb.) C.N.Page (2.9 trees per hectare) and *Olinia ventosa* (L.) Cufod. (2.8 trees per hectare) (Seydack *et al.*, 2012). Our finding that *Acacia melanoxylon* does not materially alter tree sapling composition in its understory thus applies to situations where this alien species occurs at densities similar to that of dominant indigenous canopy species. There is, however, preliminary evidence suggesting that where *Acacia melanoxylon* occurs at very high densities (approximately 17 trees per hectare) in the region, that it may alter the gap forming disturbance regime of forest and thereby affect indigenous forest regeneration (Moolman and Rikhotso, 2014). Fast growth of this species in combination with poor root development and large emergent crowns relative to those of indigenous canopy species make *Acacia melanoxylon* more susceptible to windfall from strong winds than indigenous trees (Geldenhuys, 2013). *Acacia melanoxylon* thus causes increased incidence of, and larger, windfall gaps than those caused by indigenous large-crowned *Olea capensis* (Geldenhuys, 2013). Larger gaps can be more susceptible to mass regeneration of, and thus dominance by, saplings of this light-demanding alien pioneer species (Lawes *et al.*, 2004) which can prevent indigenous pioneer species from regenerating in the gaps (Geldenhuys, 2013). *Acacia melanoxylon* can also cause more prolific damage by falling on, and felling, indigenous tree species at a greater rate than when indigenous species are wind-felled naturally (Geldenhuys, 2004). At significant densities, *Acacia melanoxylon* may therefore impact the ecology of the forest interior by altering the natural disturbance patterns (Moolman and Rikhotso, 2014). Conversely, *Acacia melanoxylon* may also facilitate indigenous forest regeneration according to the nurse plant model which postulates that as dense shade-intolerant invader stands age, natural self-thinning creates the gaps that facilitate recruitment of shade-tolerant indigenous tree species (Geldenhuys *et al.,* 2017). However, if *Acacia melanoxylon* has allelopathic effects on indigenous forest species, these nurse stands may not necessarily facilitate natural forest successional processes.

Stand density additionally has relevance for potential allelopathic effects of species. Low concentrations of allelochemicals may promote or have no effect on the germination of some species, while high concentrations reduce germination, which suggests that the effect of allelochemicals is a function of the concentration (Reigosa *et al.,* 1999; Hussain *et al.,* 2011). Accordingly in Europe, the presence of large amounts of allelochemicals in the soil, leached from large quantities of leaf litter in dense *Acacia melanoxylon* stands, caused allelopathic effects on indigenous species (González *et al.,* 1995). In this study (Chapter 3) we investigated potential effects of *Acacia melanoxylon* on indigenous tree sapling composition at the densities at which it typically occurs in the Garden Route National Park; it is conceivable that in our survey plots allelopathy may have been too weakly developed under single, scattered individuals. Whether the effects of allelopathy may become more apparent in high density stands of *Acacia melanoxylon* requires further investigation. Furthermore, allelopathy may increase with the size and age of the individual (Arán *et al.,* 2017). It was observed while undertaking the field surveys (Chapter 3) that very large *Acacia melanoxylon* trees (> 100 cm DBH) with very large canopies (> 10 m radius) had very few indigenous species recruiting under their canopy. However, such large individuals were not included in our surveys as we were not able to find comparable indigenous counterparts to survey in our paired-plot study design. It needs to be noted that the current study tested for potential negative effects of *Acacia melanoxylon* on indigenous sapling composition generally, rather than for allelopathic effects specifically. Chapter 2, however, subjected indigenous tree seeds to *Acacia melanoxylon* leachates and indigenous leaf leachates and was undertaken to specifically investigate potential allelopathic effects of *Acacia melanoxylon*, but unfortunately the germination of indigenous species failed, which left this pertinent question unanswered.

We concluded that *Acacia melanoxylon* does not significantly alter indigenous tree species composition underneath its canopy at the typical densities at which it occurs in the forests of the Garden Route National Park that we surveyed (Chapter 3). However, light intensity varied significantly more underneath the canopies of *Acacia melanoxylon* than under the canopies of indigenous species which may have implications for Afrotemperate Forest. Considering the threat that *Acacia melanoxylon* poses as an aggressive invader and source of further invasion in vegetation types abutting forest (Geldenhuys, 2004), we suggest that it is critically important to maintain populations in the forest interior at low densities (aim to keep densities as low as practical by focusing on newly formed gaps in the canopy and by preventing young trees from maturing), in addition to controlling the species in adjacent vegetation, such as riparian areas and fynbos shrublands, where it is invasive.

Study limitations

A limitation pertaining to our study in Chapter 2 was the failure of germination of the indigenous species. This resulted in limited data obtained, and we therefore could not assess the potential allelopathic effects of *Acacia melanoxylon* on indigenous species recruitment or early growth. Our analyses were therefore limited to the germination of *Acacia melanoxylon* seeds under the three treatments. The most probable reason as to why germination of the indigenous study species failed was due to the length of the study period, which may not have included a sufficiently long cold period to accommodate stratification as a germination trigger (Geldenhuys, 1996b), despite the duration of the germination trial (approx. 40 weeks) having exceeded the recommended threshold of 19 weeks (see Supplementary 1 of Chapter 2). In retrospect, the length of the study period could therefore be considered as a limitation of Chapter 2 as germination might have occurred during the following summer (Geldenhuys, 1996b); however, our trial design met the criteria proposed by others that apply to germination trials involving Afrotemperate forest tree species (detailed in Supplementary 1 of Chapter 2). The inability to collect seeds ourselves from natural populations was also a limitation of Chapter 2, as this would have enabled us to plant the seeds as fresh as possible as well as allowing us to collect more seeds for a greater sample size for the nursery trial. Another limitation pertaining to Chapter 2 is that nursery studies may be an overly simplistic method if assessing allelopathy which fail to account for interactions and soil conditions that are representative of real field conditions (Inderjit and Callaway, 2003).

A limitation pertaining to Chapter 3 was the simple method and equipment used to measure light intensity underneath *Acacia melanoxylon* and indigenous tree canopies. The method employed provided a relatively superficial rather than a comprehensive measure of how the light regime may have been affected. More focused methods of measuring light intensity, which were not within the scope of this study, may have given more conclusive data and insight into this complex metric. In hindsight, it would have been informative to have done a comparative assessment of sapling communities underneath *Acacia melanoxylon* and indigenous canopies in areas of low and high densities of *Acacia melanoxylon*. However, areas of very high densities of *Acacia melanoxylon* in the forest interior are scarce and not representative of the prevailing situation in the Garden Route National Park and most other state forests. Neither would it have been feasible to find nearby analogous indigenous trees when surveying high density *Acacia melanoxylon* stands.

Study contributions and recommendations for future research

This study contributed important information about the alien tree species *Acacia melanoxylon* in Afrotemperate Forest interior in the southern Cape. The information contributed by this study may assist in the management of populations of *Acacia melanoxylon* present in the forest interior in the Garden Route National Park and surrounds. Chapter 2 represented the first study of the potential allelopathic effects of *Acacia melanoxylon* and *Olea capensis* on the germination of *Acacia melanoxylon* seeds. Furthermore, this study provided a critical evaluation of the germination requirements of three indigenous forest species and potential issues to consider in future germination studies. The findings may assist future germination studies of indigenous Afrotemperate Forest species in terms of best practices to ensure germination as well as highlighting the importance of accommodating an extended cold stratification period when germinating Afrotemperate Forest tree species (Geldenhuys, 1996b).

Chapter 3 investigated whether *Acacia melanoxylon* alters the indigenous species sapling composition and light regime under its canopy compared to indigenous overstorey species and provided valuable results to inform management of the species in Afrotemperate Forest interior. The study highlighted that *Acacia melanoxylon* may cause a more varied light regime underneath its canopy compared to that under indigenous tree canopies, which may have implications for Afrotemperate

Forest tree species. Collectively, the results of this study concluded that relatively low density (<3 trees per hectare) populations of *Acacia melanoxylon* that prevail in the forest interior do not have a suppressive effect on the indigenous tree sapling composition under their canopies, which is a positive conclusion for the conservation of Afrotemperate Forest.

Little is known of the allelopathic potential of *Acacia melanoxylon* in Afrotemperate Forest or how stand density may interact with the potential suppressive effects of *Acacia melanoxylon*. Further studies of the allelopathic potential of *Acacia melanoxylon* on the recruitment of Afrotemperate Forest species would be required to better understand this phenomenon. Furthermore, the effect of *Acacia melanoxylon* on sapling composition and light intensity can be compared among a range of densities of *Acacia melanoxylon* in Afrotemperate Forest. This study provided a preliminary understanding of how *Acacia melanoxylon* impacts the light regime under its canopy compared to that under three indigenous species, but a more comprehensive assessment will facilitate better understanding of this complex metric.

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