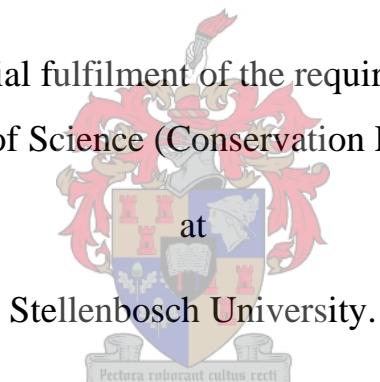


Current and possible future impacts of the tree-killing Polyphagous Shot Hole Borer beetle in a Southern Afrotropical forest complex in South Africa

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science (Conservation Ecology)



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December 2021

Declaration

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Summary

The Polyphagous Shot Hole Borer beetle (PSHB) (*Euwallacea fornicatus*) is a recently introduced pest in South Africa that can kill susceptible host trees rapidly. Since its first detection in 2012, PSHB has spread to all but one province in the country. Its ecological and economic impact is most profound in urban areas, but it has been found infesting important forestry, agricultural and native forest species. Many native trees in urban settings are susceptible to infestation, but its presence in natural ecosystems is unstudied. In this thesis the presence and determinants for PSHB colonization were determined for the very early stages of its invasion in a native Southern Afrotropical forest complex. More than 1600 individual trees representing 68 species was monitored over a one-year period. Breeding populations of PSHB was detected in six native species and these may be especially vulnerable to population reductions. The tree-pathogenic fungus *Fusarium euwallaceae* (a PSHB mutualist) was isolated from an additional eleven tree species, without signs of beetle breeding. These species may still be vulnerable to fusarium dieback disease. PSHB colonization was more apparent at monitoring sites in close proximity to highly infested urban areas, especially when containing dense stands of reproductive breeding hosts. However, PSHB was also detected at distant sites where tourists are allowed to use firewood procured from areas outside of these protected forests. Larger trees were generally infested first and by more individuals than smaller trees and when surrounded by other infested trees. This is of particular concern given that these forests contain some of the largest native trees in South Africa. The beetle had a preference for eight tree species, most of which were identified as reproductive hosts. Infestation severity increased more rapidly for individuals in pioneer forest than in mature forest. Direct anthropogenic impact had no discernible effect on levels of infestation but did aid colonization of distant sites. Projections based on the severity of infestations indicated that PSHB will have lasting economic and social impacts such as decreased carbon storage value and decreased value of provisioning, regulating and cultural services of these forests. Total carbon storage in trees across the entire forest complex was calculated as *ca.* 14.48 Mt at a value of *ca.* \$ 241 780 818 (R 3.55Bn). Future infestation of these forests by PSHB may lead to a loss of *ca.* 1.2 Mt of the stored carbon, with economic losses of *ca.* \$ 20 540 841 (R 301 266 352) expected. This study therefore confirms that Southern Afrotropical forests are highly susceptible to invasion by PSHB and that severe impacts on ecosystem function and services can be expected. Mitigation efforts should therefore be a high priority.

Opsomming

Die polifagies Stompneus kewer (PSHB) is ‘n onlangse insekpes in Suid-Afrika wat vinnig hoogs vatbare gasheer bome kan vernietig. Sedert sy ontdekking in 2012, het die kewer na al die provinsies behalwe een in die land versprei. Sy ekologiese en ekonomiese impak is nadelig in stedelike gebiede, maar was ook gevind om belangrike bosbou, landbou en inheemse woud spesies te besmet. Baie inheemse bome in stedelike nedersettings is vatbaar tot infestasie, maar die kewer se teenwoordigheid in natuurlike ekosisteme is nog onbekend. In hierdie tesis was die teenwoordigheid en bepalende faktore vir PSHB kolonisasie vir die vroeë fases van infestasie in ‘n natuurlike Suidelike Afrotemperate woud kompleks bepaal. Meer as 1600 individuele bome wat 68 spesies verteenwoordig was oor ‘n tydperk van een jaar gemonitor. Broeiende bevolkings van PSHB was in ses inheemse boom spesies ontdek en hierdie spesies mag dalk veral vatbaar wees tot bevolking afname. Die boom-patogeen fungus *Fusarium euwallaceae* (‘n PSHB mutualist) was geïsoleer van ‘n addisionele elf boom spesies, sonder tekens van kewer teling. Hierdie spesies mag dalk nog steeds vatbaar wees tot fusarium terugsterf siekte. PSHB kolonisasie was meer prominent by monitor plotte wat naby aan hoogs infesteerde stedelike gebiede was, veral wanneer daar ‘n digte hoeveelheid van besmette gashere was. PSHB was ook ontdek by plotte ver weg van groot infestasies, waar daar egter baie toeriste toegelaat word om aangeskafde braaihout van infestasie areas te verbruik in die beskermde woude. Groter bome teenoor kleiner bome was oor die algemeen eerste geinfesteer en wanneer dit omring was deur ander besmette bome. Dit is ‘n besonderse bekommernis, gegee dat hierdie woude van die grootste inheemse bome in Suid-Afrika bevat. Die kewer het ‘n voorkeur vir agt boom spesies vir infestasie en meestal van die is geïdentifiseer as gasheer plante waarin die kewer broei. Die kewer bevolking het vinnig vermeerder vir gasheer individue in pionier/woudrand woude as in klimaks/volwasse woude. Direkte menslike impak het geen onderskeibare effek op infestasie gehad nie, behalwe vir die bydra tot kolonisasie in woud landskappe ver wag van groot infestasies. Projeksies gebaseer op die geweldigheid van infestasies het aangedui dat PSHB ekonomiese en sosiale impakte veroorsaak wat die koolstof stoor waarde en die waardes van voorsiening, regulering en kulturele dienste in die woude sal verminder. Die berekende totale koolstof stoor in bome wat strek regdeur die hele woud kompleks was *ca.* 14.48 Mt met ‘n waarde van *ca.* \$ 241 780 818 (R 3.55Bn). Toekomstige infestasie van hierdie woude deur PSHB mag dalk lei tot ‘n neerlaag van *ca.* 1.2 Mt van gestoorde koolstof met ‘n verwagte ekonomiese skade van *ca.* \$ 20 540 841 (R 301 266 352). Hierdie studie erken dat Suidelike Afrotemperate woude hoogs vatbaar is tot infestasie deur

PSHB en dat hewige impakte op ekosisteem funksie en dienste gevvolglik verwag kan word. Verligting pogings is daarom ‘n prioriteit.

Acknowledgements

I would firstly like to thank my main supervisor, Professor Francois Roets, for his enduring guidance and patience. During my project you were always very supportive, understanding and allowed me to finish my research, despite several setbacks. Thank you for your input and advice on the research and the life lessons in between some drinks. Many thanks to Prof. Wilhelm de Beer who initially showed me how to identify beetle entries on infested trees. Both, you, and Francois displayed the importance of this project and this encouraged me to progress as far as I have.

I would also like to thank my lab members and friends, Llyle Stevens, Luther van der Mescht, and Heather Nependa for their ongoing support and interest. To Rudi Swart and Joey Hulbert for guidance and giving me a growing interest in forest ecology. To Minette Havenga, for teaching me how to conduct molecular research.

Many thanks to FABI (Forestry and Agricultural Biotechnology Institute), DST-NRF, CTHB (Centre of excellence in tree health biotechnology), and Stellenbosch University for funding me throughout my research project. Thanks to SANparks for allowing me to work in the forests of the Southern Cape and accommodating me at Rondevlei and Stormsrivier while conducting my fieldwork. Big thanks to Nerina Kruger, that always arranged the accommodation with a smile, Graham Durrheim for ongoing support, and all staff members involved for their cooperation, interest, and friendliness. To Dr Trudy Paap and Professor Martin Hill for their contribution and support. Thanks to Gordon, Robyn (and their dogs) that we could use their farm for plots and the warm hospitality we received.

My field partners, Garyn Townsend and Allan Gonzalez, thank you guys for all the help, conversations, and planning of the research. But most of all, thank you for the friendship that we have gained through this research. Without you guys, this project would not have existed. I would never forget the utopia we experienced at Rondevlei and in the “Bos” together.

Dankie aan my Ma en Pa vir hulle gereelde ondersteuning. Dankie dat julle darem probeer verstaan het wat my navorsing behels. En dankie dat ek by julle nog kon huisves en vir kos op die tafel.

Lastly, a big thanks to the forests of the Southern Cape. I have learned and gained so much more than I initially thought I would. I not only gained valuable insight in my research topic, but also about the history, mythologies, and elegance of the poetic Afrotropical forests. The forests made me learn a great deal about myself, which is the biggest reward I gained from this project.

Table of Contents

Declaration	ii
Summary	iii
Opsomming.....	iv
Acknowledgements.....	iv
List of Figures	x
List of Tables	xii
List of Addendum	xv
1) Chapter 1.....	16
1.1) Bark and Ambrosia beetles: a growing global threat	16
1.1.1) Bark and ambrosia beetles	16
1.1.2) Fungal symbionts of bark and ambrosia beetles	18
1.1.3) Invasion by bark and ambrosia beetles	20
1.1.4) Impact of invasive ambrosia and bark beetles in non-native systems	22
1.2) PSHB: current status of a perfect invader in South Africa	24
1.2.1) History of invasion.....	24
1.2.2) Factors driving invasion by PSHB in South Africa	25
1.2.3) Distribution of PSHB in South Africa	28
1.2.4) Impact of PSHB	30
1.2.5) Mitigation.....	33
1.3) Forests in South Africa.....	34
1.4) Aims and objectives of study	35
1.5) Literature cited	36
2) Chapter 2.....	50
2.1) Abstract	50
2.2) Introduction	51
2.3) Materials and Methods	53

2.3.1) Site selection	53
2.3.2) Data Collection	57
2.3.3) Infested Tree confirmation.....	58
2.3.4) Statistical Analysis.....	59
2.4) Results	62
2.4.1) Factors that influence the number of trees infested by PSHB in plots (i.e invasibility of a site)	65
2.4.2) Factors that influence the severity and probability of infestation by PSHB in susceptible hosts	67
2.4.3) Distance to infested host	71
2.4.4) Progress of infestation at the plot level (increase in probability of infestation over time)	71
2.4.5) Progress of infestation at the individual level (increase in severity of infestation over time).....	73
2.4.6) Preferential colonization	76
2.5) Discussion	77
2.5.1) Factors that influence invasibility of Afrotemperate forests.....	78
2.5.2) Factors that increase the severity and probability of infestation by PSHB of susceptible host individuals	79
2.5.3) Progression of infestation	81
2.5.4) Management considerations.....	82
2.6) References	83
3) Chapter 3.....	93
3.1) Abstract	93
3.2) Introduction	94
3.3) Materials and Methods	96
3.3.1) Study area.....	96
3.3.2) Data Collection	97

3.3.3) Calculation of carbon storage	98
3.3.4) Comparison between aboveground biomass indices and choice of model.....	99
3.3.5) Possible impact of PSHB on mature forest vs. edge habitat.....	99
3.3.6) Estimated cost of PSHB invasion.	101
3.4) Results	102
3.4.1) Comparison between aboveground biomass indices and choice of model.....	102
3.4.2) Possible impact of PSHB on mature forest vs. edge habitat.....	105
3.4.3) Short-term carbon mass loss	105
3.4.4) Long-term carbon mass loss	106
3.4.5) Estimated carbon loss across Garden Route Forest Regions	108
3.4.6) Estimated cost of PSHB invasion.	109
3.5) Discussion	110
3.5.1) Possible impact of PSHB on mature forest vs. edge/pioneer habitat.....	111
3.5.2) Estimate carbon loss and social cost across Garden Route Forest Regions	113
3.5.3) Conclusion	114
3.6) References	115
4) Chapter 4.....	127
4.1) References	129
5) Addenda.....	132
Addendum A: Confirmed host plants of PSHB in South Africa.	132
Addendum B: Supporting photographs of study areas of edge and mature habitats	135

List of Figures

Figure 1.1: Adult (A) and Larva (B) of the Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus*. Scale bars = 1 mm.....25

Figure 1.2: Distribution of the Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus* in South Africa. Only those localities from which PSHB or its symbiotic fungus *Fusarium euwallaceae* have been collected, and their identities verified using DNA sequencing, have been depicted.....28

Figure 1.3: Signs of infestations of Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus* on various hosts. (A) 1 mm diameter entrance hole of PSHB on the bark of *Erythrina caffra*. (B) Lesions on the bark of *Acacia mearnsii* around entrance holes of PSHB. (C) Colonization of vascular tissues by *F. euwallaceae* (staining) surrounding the entrance hole of PSHB under the bark of *Platanus x acerifolia*. (D) Wet patch on the bark of *Nuxia floribunda* following infestation by PSHB. (E) Sugar fountain (dried sugary sap) on the bark of *Grewia occidentalis* following infestation by PSHB. (F) Gum exudates (tree resin flow) on the bark of *Podalyria calyptrata* following infestation by PSHB. (G) Noodles (compacted frass) extruded from the galleries of PSHB following infestation of *Harpephyllum caffrum*. (H) *Liquidambar styraciflua* individuals showing dieback of branches and tree death following heavy infestations by PSHB.....31

Figure 2.1: Map of the Southern Cape of South Africa depicting monitoring sites for PSHB. Sites from where evidence of PSHB invasion was found are indicated by a yellow outline. .54

Figure 2.2: The regression estimates of number of infested trees as influenced by host species diversity at a plot, abundance of host species per plot and the distance to infestation border.66

Figure 2.3: Variables that significantly influenced the severity of PSHB infestations (a – c) and the probability of PSHB infestations (d-e) at the George Botanical Gardens ($n = 10$ plots). (a) The effect of breeding host abundance on infestation severity. (b) The effect of tree size on the infestation severity. (c) The effect of the number of infected hosts in a plot on infestation severity. (d) The effect of tree size on infestation probability. (e) The effect of number of infested trees in a plot on infestation probability.....70

Figure 2.4: Mean log distance (m) from an infested breeding hosts to nearest other host (healthy and infested) categorised as either any host (i.e. breeding and non-breeding hosts), breeding hosts only (irrespective of breeding host species identity) and conspecific breeding host species. Different letters above bars indicate significantly different means (5% level). .71

Figure 2.5: Variables that significantly affected the severity of individual trees. (a) – The effect of infestation severity (increase in holes) on the number of breeding hosts found in plot. (b) – The effect of infestation severity (increase in holes) on the diameter at breast height of trees.

.....75

Figure 5.1: Examples of the two habitat types included in the present study. Top: Typical edge/pioneer forest habitat. Bottom: Typical mature/climax forest habitat.135

List of Tables

Table 2.1: Description of sites in the Southern Cape of South Africa that were monitored for PSHB infestations.....	55
Table 2.2: List of tree species found in all 51 monitoring plots. Summaries are presented for average diameter at breast height (cm), the percentage of individuals of each species out of all individuals of all species (dominance), PSHB breeding host or not, percentage of individuals that were infested by PSHB, the percentage increase of infested individuals from time 1 to time 4 and the percentage increase in the number of holes from time 1 to time 4.	63
Table 2.3: Model-averaged estimates of variables predicting invasion by PSHB at a site.....	65
Table 2.4: All tree species found in ten PSHB monitoring plots set out at the George Botanical Garden. Presented is the dominance (% of all tree individuals encountered), number of individual trees infested with PSHB, % of infested individuals of that particular species and the average diameter at breast height (cm) of each particular species encountered.	67
Table 2.5: Model-averaged estimates of environmental variables predicting probability of severity by PSHB in susceptible hosts.....	68
Table 2.6: Model-averaged estimates of environmental variables predicting probability of infestation by PSHB in susceptible hosts.....	69
Table 2.7: Model-averaged estimates of environmental variables predicting progress of infestation by PSHB at plot level.....	72
Table 2.8: Model-averaged estimates of environmental variables predicting progress of severity by PSHB at the individual level without species as explanatory variable.	73
Table 2.9: Model-averaged estimates of environmental variables predicting progress of severity by PSHB at the individual level by including species as explanatory variable.	74
Table 2.10: Chi-square test table showing the sample size (n), the Chi-squared statistic, P-value, Yates correction p-value for the preferential colonization of PSHB between species..	76

Table 3.1: All tree species encountered across all 51 monitoring plots in this study. Represented is the mean carbon mass (kg) (aboveground and belowground biomass) across all individuals measured per species in all the plots, whether it is a breeding host (**BH**); non-breeding host (**NB**); or a non-host (NH), its wood density (WD) and the source of where the wood density data was obtained/compiled from. Also provided is expected percentage carbon lost for each species (and in each habitat type) that is currently infested in these plots, assuming that 25% of breeding hosts and 3% of non-breeding hosts will succumb in the short term..102

Table 3.2: Short-term impact of PSHB on carbon mass loss of the 27 infested plots in mature and edge forest habitats. The following is given in the table: The total carbon mass (kg) of all 27 plots; the mean (\pm SE) carbon mass (kg) per plot; the total carbon mass (kg) loss; the mean (\pm SE) carbon mass (kg) lost per plot; percentage carbon lost per habitat type; total abundance of trees in habitat type; total infested abundance of trees in habitat type and percentage of infested trees per habitat type.105

Table 3.3: Long-term impact of PSHB on carbon mass loss of all the 51 plots in mature and edge forest habitats. The following is given in the table: The total carbon mass (kg) of all 51 plots; the mean (\pm SE) carbon mass (kg) per plot; the total carbon mass (kg) loss; the mean (\pm SE) carbon mass (kg) lost per plot; percentage carbon lost per habitat type; total abundance of trees in habitat type; total infested abundance of trees in habitat type and percentage infested trees per habitat type.106

Table 3.4: All 51 infested plots categorized by Edge and Mature habitat. Table shows plot variables for carbon loss expected at time 1 (Short term) and 2 (Long term). **Abundance** - Total number (n) of trees per plot. **TCM** - Total carbon mass (kg) per plot. **CLT1** – Total carbon mass (kg) loss per plot short term. **CLT2** – Total carbon mass (kg) loss per plot long term. **%Lost1**: percentage carbon mass loss in the short term of the total carbon weight per plot. **%Lost2**: percentage carbon mass loss in the long term of the total carbon weight per plot. The sum and means of each variable are given below each habitat table.....107

Table 3.5: List of most of the forested zones across the Garden Route following Holness et al. (2010). **P** – A rough measurement of the perimeter of these forested zones. **E(ha)** – Hectare calculated for edge habitats using perimeter multiplied by 50 m edge penetration. **M (ha)** – Hectare calculated for mature habitats using perimeter. **ETC** – Total carbon mass Gigagrams

(Gg) for edge habitats. **MTC** – Total carbon mass (Gg) for mature habitats **Edge TCL** – Total carbon mass lost in (Gg) for edge habitat regions using carbon mass lost data from infested plots. **Mature TCL** - Total carbon mass lost (Gg) for mature habitat regions using carbon mass lost data from infested plots.....109

Table 3.6: Economic value and costs of each forested zone described by Holness et al. (2010). Values and costs are given for both habitats edge and mature. Value is the total value of each zone and habitat (US \$) and costs is the total long-term carbon lost cost from PSHB for each habitat (US \$).....110

Table 5.1: List of confirmed host plants of the Polyphagous Shot Hole Borer beetle in South Africa. The list includes only those taxa from which its primary fungal symbiont, *Fusarium euwallaceae*, or a beetle individual was successfully isolated, and identity confirmed using DNA sequence fingerprinting. Reproductive hosts include only those hosts that have been shown to be suitable for PSHB reproduction at least once. Non-reproductive hosts include those from which either PSHB or *F. euwallaceae* was isolated, but no evidence of PSHB reproduction has been verified.132

List of Addendum

Addendum A: Confirmed host plants of PSHB in South Africa.....132

Addendum B: Supporting photographs of study areas of edge and mature habitats135

1) Chapter 1

The Polyphagous Shot Hole Borer (PSHB) beetle: current status of a perfect invader in South Africa

1.1) Bark and Ambrosia beetles: a growing global threat

1.1.1) Bark and ambrosia beetles

Bark beetles comprise *ca.* 3700 of the 7500 weevil (Curculionidae) species in the subfamily Scolytinae (Bright, 1993; Marvaldi *et al.*, 2002; Wood, 1982). Of the remaining species, *ca.* 3400 are ambrosia beetles or pith and seed-feeding beetles (~400 species). In fact, the Scolytinae is exceptionally diverse in their ecology and can be classified into six major groups based on their feeding habits alone. Phloeophagy involves feeding on the phloem of a woody host as is prevalent for most bark beetles (Beaver, 1989). Less common habits include herbiphagy (feeding on soft plant tissues), myelophagy (feeding on twigs and piths), xylophagy (feeding on the xylem of woody hosts) and spermatophagy (feeding on seeds and fruits) (Beaver, 1989). The major feeding habit in ambrosia beetles is xylomycetophagy, which is feeding on wood-inhabiting ectosymbiotic fungi introduced by the beetle itself and that forms a major part of the larval and adult diet (Beaver, 1989). However many variations of these main dietary preferences exists and both ambrosia and bark beetles are associated with fungi to some extent and both make use of host plant substrate (Six, 2012).

Bark beetles construct their galleries in the phloem layer of trees underneath the outer bark where they lay eggs and their brood feeds and develops (Six, 2012). Bark beetles feed on tree tissues and gain some of their nutrients directly from the host (Six, 2012). However, phloem has a low nutritional value to insects (Ayres *et al.*, 2000; Scriber & Slansky, 1981; Slansky & Scriber, 1985) and bark beetles often supplement their diet by including fungal associates (Ayres *et al.*, 2000; Hodges *et al.*, 1968; Hodges & Loria, 1969). The term ‘ambrosia’ refers to the farming of fungal gardens which ambrosia beetles cultivate on their gallery walls and use as an exclusive food source (Beaver, 1989; Kok *et al.*, 1970; Six, 2012). They need the fungi as the wood in which they construct galleries consist mainly of lignin and cellulose which can’t be digested by the gut enzymes found in beetles, but that are more readily broken down by various species of fungi (Six, 2012). In this mutualism, the beetles acquire amino acids, sterols

and vitamins from the fungi which they are obligately dependant on, while the fungi is dispersed to uncanonised substrates by the beetles (Beaver, 1989; Kok *et al.*, 1970; Six, 2012). Female beetles may even control the growth and composition of the fungal garden as, when the female dies, the garden is often overgrown by contaminant fungi and bacteria which results in the death of the brood (Biedermann & Taborsky, 2011; Norris, 1979). Adult beetles often transport their fungal partners to new host trees in structures called mycangia (Francke-Grosmann, 1967; Six, 2003). A mycangium is a specialized structure, often with secretory cells and glands, for the acquisition and transport of fungi (Six & Klepzig, 2004). This mutualism allows the beetles to exploit nutritionally poor wood, while providing the fungi consistent transport to a relatively rare and short-lived resource (Beaver, 1989; Mueller *et al.*, 2005).

Most ambrosia beetles are polyphagous and have a wide variety of plant hosts that they attack while bark beetles are more host specific (Beaver, 1989). This is likely because bark beetles directly feed on plant tissues that contain numerous anti-herbivore compounds to which the beetles need to adapt on different hosts (Beaver, 1989). In contrast, ambrosia beetles feed exclusively on their vectored fungi within metabolically relatively inactive woody tissues and can infest a variety of hosts without much interference from tree anti-herbivore mechanisms once past the metabolically active phloem and sapwood. Ambrosia beetles may therefore lose their evolutionary ability to combat living host defences, which is why they prefer to infest dead or dying individual plant hosts. Even so, ambrosia beetles may be able to select preferred hosts (Beaver, 1989). If the abundance of ambrosia beetles is high, it seems as if host identity may play less of a role in selection of host tree individuals (Hulcr *et al.*, 2007). Tree host specificity may therefore change when ambrosia beetles need an immediate food source (Beaver, 1989).

The Scolytinae arose around 100 million years ago in the late Jurassic period (Berbee & Taylor, 2001; Cognato & Grimaldi, 2009; Kirejtshuk *et al.*, 2009). Therefore, conifers are likely the ancestral hosts of Scolytinae beetles (Farrell *et al.*, 2001; Sequeira *et al.*, 2000). After this, the different fungal feeding habits of bark and ambrosia beetles evolved several times independently and do not form monophyletic groups (Six, 2012). For example, the origins of ambrosia feeding habits shifted to angiosperms and has evolved at least eight times from phloem-feeding beetles (Berbee *et al.*, 2001; Farrell *et al.*, 2001; Hulcr *et al.*, 2007) and arose between 21 to 60 million years ago depending on beetle lineage (Six, 2012). Floristic composition and plant diversity are some of the major factors that influenced ambrosia

diversification (Fagan *et al.*, 2002). Warm temperatures and high humidity are also contributing factors as it will favour fungal growth (Atkinson & Equihua-Martinez, 1986).

1.1.2) Fungal symbionts of bark and ambrosia beetles

All ambrosia beetles and many bark beetles are closely associated with fungi (Beaver, 1989; Kirisits, 2007; Six, 2003; Six & Elser, 2019). The term symbiosis has been defined by Zook (1998) as “the acquisition and maintenance of one or more organisms by another that results in novel structures and (or) metabolism”. Scolytinae beetles represent a rich source in studies in the field of symbiosis (Six *et al.*, 2004). The beetles have complex interactions with fungi, but also with various other organisms including bacteria (Bridges, 1984), mites (Klepzig *et al.*, 2001; Moser *et al.*, 1995) and nematodes (Moser *et al.*, 2005). These different types of interactions may be obligatory, incidental, mutualistic, antagonistic or commensal (Six, 2003; Six & Wingfield, 2011). The mutualistic associations often result in the evolution of novel structures in the beetles specifically for the transport of fungal spores. The mycangium is a specialised structure on bark and ambrosia beetles with the purpose to carry the symbiotic fungi and transporting it between hosts (Six *et al.*, 2004). Any structure, regardless of how it is built that function in a similar fashion to transport fungi is known as an mycangium (Six, 2003). There are three morphological classes of mycangia namely pit mycangia, sac mycangia and setal brush mycangia (Six, 2003). Pit mycangia are shallow depressions in the exoskeleton where the fungal repositories are formed; setal brush mycangia consist of dense brushes of setae that arise from depressions of the exoskeleton and sac mycangia, the most effective and selective mycangia, have complex deep pockets, cavities or tubes in the exoskeleton (Mayers *et al.*, 2020; Six, 2003). Fungi may also produce novel structures to aid in transport. For example, many of these fungi produce sticky spores, and have adhesive coats that disperse well in resin and not in water which enables it to be released from beetles when an appropriate substance is encountered (Six *et al.*, 2004). Among ascomycetes there are characters such as evanescent asci and long-necked perithecia that are selected for arthropod dispersal (Cassar & Blackwell, 1996). The spores of the fungi may sometime be carried by other vectors such as predators or saprophagous woodborers that developed in the beetle-colonized trees (Six *et al.*, 2004), however the reliability of dissemination is unknown.

Ambrosia beetles are often obligatory mutualists associated with anamorphic fungal species in the Ophiostomatales (e.g. *Raffaelea* and *Afroraffaelea*) and Microascales (e.g. *Ambrosiella*, *Meredithiella*, and *Phialophoropsis*). However, associations with fungi in Hypocreales (e.g.

Fusarium and *Geosmithia*), Microascales (*Phialophoropsis*) (Lehenberger *et al.*, 2019) and even with the basidiomycete *Flavodon ambrosius* (Kasson *et al.*, 2016; Simmons *et al.*, 2016) are also found (Hulcr & Stelinski, 2016; Mayers *et al.*, 2015; Ploetz *et al.*, 2013). Bark beetles are typically associated with ascomycetes in four teleomorph genera namely *Ophiostoma*, *Grosmannia*, *Ceratocystis*, and *Ceratocystiopsis* (Harrington, 2005; Kirisits, 2007; Six, 2003; Zipfel *et al.*, 2006) but they may also be strongly associated with fungi in other genera such as *Geosmithia* (Machingambi *et al.*, 2014). These fungal symbionts usually aid in beetle nutrition by concentration of nitrogen or provision of sterols (Six *et al.*, 2004). These sterols are essential for the production of eggs and provide elements for cellular structure (Clayton, 1964; Six *et al.*, 2004). Beetle-associated fungi are protected from desiccation under limited direct UV sunlight while in transit in the mycangium (Six *et al.*, 2004). They may also help overcome tree defences, therefore some may be pathogens of trees such as species in the genera *Leptographium*, *Ophiostoma* and *Raffaelea* (Ploetz *et al.*, 2013; Wingfield *et al.*, 2017). Yeasts are known to be associated with bark and ambrosia beetles relatively early in the colonization period of a tree host (Bridges, 1984) and it seems that the larvae of the beetles probably eat and rely on some yeasts for development (Six *et al.*, 2004). Adams, *et al.* (2008) discovered that both bacteria and yeasts had a substantial effect on the growth of two mycangial fungi of *Dendroctonus ponderosae*. The yield of the fungus grown *in vitro* was significantly greater when grown with the yeast than the yield when growing alone.

In addition to fungi, numerous studies have identified actinomycete bacteria from the gastrointestinal tracts of adult bark beetles and from their galleries (Bridges, 1984; Moore, 1972; Oberle, 1966). These bacteria may be antagonistic to pathogenic fungi commonly associated with the beetles and are therefore important in maintaining mutualism between the beetle and the fungi (Scott *et al.*, 2008). Some bacteria can also capture atmospheric nitrogen and may directly aid in beetle nutrition in galleries (Bridges, 1981). Mites are also very commonly associated with bark and ambrosia beetles. For example there are ca. 100 species of mites associated with *Dendroctonus frontalis* alone. (Moser & Roton, 1971). Some of these mite species are phoretic and only use the beetle for dispersal (Moser *et al.*, 1995, 1971) and are not directly harmful to their host (Six *et al.*, 2004). However, many species may be detrimental to the fungus-beetle interaction (Klepzig *et al.*, 2001; Lombardero *et al.*, 2003, 2000). Other species of mites likely prey on nematodes associated with beetles and have a more mutualistic association (Kinn, 1983). Nematodes that are often associated with bark and ambrosia beetles have largely understudied roles, but some species (e.g. *Neoparasitylenchus*

associated with *S. scolytus*) (Moser *et al.*, 2005) may cause sterility in both sexes of the beetle (Oldham, 1930). In fact, most of the roles of phoretic mites and nematodes on scolytine beetles are poorly known, but there is enough evidence to mention that their biology and ecology is diverse and should not be ignored (Klepzig *et al.*, 2001; Lombardero *et al.*, 2003; Moser *et al.*, 1995).

1.1.3) Invasion by bark and ambrosia beetles

The difference between exotic and invasive is important to understand. Introduced species implies that the species or its propagule was transported by humans across a geographical barrier (Richardson *et al.*, 2000). Exotic species are species that have been introduced from another geographical region to an area outside its natural range, while invasive species are naturalized/established species that produce high numbers of reproductive offspring often far away from parents and have the capability to spread over a vast region or area (Richardson *et al.*, 2000). In most cases invasive species suppress and eradicate native species causing a loss of biodiversity (Richardson *et al.*, 2000). Therefore, exotic species can be invasive, but it does not mean it will have drastic effects on their ecosystems. The term invasive has a connotation to its geographic reference and not to its impact (Richardson *et al.*, 2000). However, species that can change the character, condition and form of the nature of ecosystems is termed habitat transformers or ecosystem engineers (Richardson *et al.*, 2000).

Non-native species are becoming increasingly difficult to manage as populations expand into new areas (Liebhold & Tobin, 2008). There are about 4500 introduced exotic arthropod species in the USA alone and 95% of these have been accidental (Pimentel *et al.*, 2005). Ambrosia and bark beetles are among the most successful invasive species affecting trees worldwide (Haack & Rabaglia, 2013). The United States Department of Agriculture has maintained a database for pests intercepted at ports of entry since 1984 (Haack, 2006, 2001; McCullough *et al.*, 2006). In a 25-year analysis, Haack & Rabaglia (2013) found 8286 interception records for Scolytinae beetles associated with wood. Only 42% of these records could be identified to species level. All of these were intercepted on wood from at least 85 different countries (Haack *et al.*, 2013) with most interceptions were from Europe (48%), while Mexico had the most interceptions from a neighbouring country with 2581 interceptions (Haack *et al.*, 2013).

Key processes of the dispersal of wood-boring insects have been identified as human-assisted dispersal and introductions (Rassati *et al.*, 2016). Wood-boring insects are usually dispersed in

wood-packaging material associated with imported goods and firewood carried by visitors to recreational sites or vacation properties (Jacobi *et al.*, 2012; Koch *et al.*, 2014). The primary entry point of non-native ambrosia and bark beetles will likely be at seaports and airports which bring in imported goods from across the world (Rassati *et al.*, 2016). In a study by Haack *et al.* (2010), from 1045 examined pieces of surveyed firewood, 55 pieces contained live bark beetles and 9 pieces contained living ambrosia beetles. Therefore, it is important to be conscious that all firewood, wherever it comes from, can carry a potential pest or disease (Jacobi *et al.*, 2012).

Of all the interceptions, 58 Scolytine species (many in the tribe Xyloborini) in 27 genera have established in the USA (Haack *et al.*, 2013). This number is likely an underestimate, because not all intercepted species could be identified to species level. This phenomenon is not restricted to the USA. In Europe, Rassati *et al.* (2016) reported 18 exotic Scolytine species that established and were becoming invasive. New Zealand intercepted 1505 cases of Scolytine beetles between 1950 and 2000 that originated from 59 different countries and included 103 species belonging to 38 genera (Brokerhoff *et al.*, 2006). The number of exotic species that have established in developing countries such as South Africa are unknown as these countries generally have insufficient interception and reporting policies and resources.

Most invaders rely on disturbance properties of the invaded system to enhance establishment success (Richardson *et al.*, 2000). For Scolytinae, this would be to colonize damaged trees that was weakened through natural or unnatural disturbance (Six *et al.*, 2011). Once established, many of these taxa can become invasive and some can have divesting ecological and economic impacts. There are multiple variables that affect the spread pattern of ambrosia and bark beetles across invaded environments (Rassati *et al.*, 2016) namely the climate (rainfall, temperature), their feeding habit, human-assisted dispersal, flight ability and environmental landscape. Bark beetles can spread naturally to surrounding areas of infestation due to their ability to fly short distances (Doležal *et al.*, 2016; Rassati *et al.*, 2016). Due to their within-tree lifestyle, they are less dependent on strict climatic conditions than free living arthropods which allows them to colonize larger areas within a country, and therefore are more likely to become invasive (Rassati *et al.*, 2016). However, they typically establish at suitable climatic conditions even though some are polyphagous (Rassati *et al.*, 2016).

1.1.4) Impact of invasive ambrosia and bark beetles in non-native systems

There are numerous invasive ambrosia and bark beetles that have had substantial impacts on the economy, human health, and native habitats around the world. The impact that invasive species can have on nonnative systems is summarized below by focusing at three extents: Natural, Agricultural and Urban environments.

A) Natural

There are only a few exotic scolytine beetles that cause ecological damage in the USA (Haack *et al.*, 2013). Notable species are *Scolytus multistriatus*, a bark beetle, and *Xylosandrus crassiusculus* and *Xyleborus glabratus* that are ambrosia beetles. *S. multistriatus* has caused mortality of Elm trees (*Ulmus*) by vectoring fungi that cause Dutch Elm Disease (Haack *et al.*, 2013). Estimates of the spread are 8 km yr⁻¹ (Gibbs & Wainhouse, 1986), related to the flight distance of *S. multistriatus*. In Montreal and Toronto in Canada the disease had been catastrophic. In the early 1970s the cities had a total of 50 000 individual trees, but between 1970 and 1980, 90% of the trees were removed from the cities due to Dutch Elm disease and only 1400 individual elm trees survived (Rioux, 2003). *X. crassiusculus* has killed many broadleaf trees globally especially those with a small diameter (Kirkendall & Ødegaard, 2007). Many Lauraceae species are at risk from *X. glabratus*, especially in the tropics of Asia and the Americas where the family has its greatest diversity (Chanderbali *et al.*, 2001). The Walnut Twig Borer (WTB) is threatening many urban street trees in Colorado, but also the concern is in the natural setting where WTB can have damaging effects on endemic walnut trees (*J. californica* and *J. hindsii*) in California, USA (Flint, 2009).

Many bark and ambrosia beetles act as habitat transformers where they have eliminated valuable trees from the ecosystem (Richardson *et al.*, 2000). An example is the Kuroshio shot hole borer (*Euwallacea kuroshio*) that had a wide ecosystem impact on willow trees in the Tijuana River Valley, California, USA (Boland, 2017). It caused extensive damage to the trees and also formed a novel form of disturbance in the valley (Boland, 2017). Invaders such as these can affect the disturbance regimes in many ways which can alter the composition and succession of a community (Mack & D'Antonio, 1998). Some can alter fire regimes, increase erosion, increase biotic disturbance and change the susceptibility of communities to forces of disturbance (Mack *et al.*, 1998). When trees are killed by invasive ambrosia and bark beetles, it can affect the woody substrate and alter the fire regime.

Biological homogenization is termed as the change in the community similarity between two time periods or between two sites and is based on records of species presence or absence (Olden, 2006). Bark and ambrosia beetles can be involved in biotic homogenization by substantially altering environments. As invasive bark and ambrosia beetles such as *Xyleborus glabratus* or *Euwallacea fornicatus* infest and kill trees, a part of the community is lost which can change the structure of the environment and give rise to common invasive weeds and plants to establish (Boland, 2016). Taxonomic homogenization therefore follows due to the establishment of cosmopolitan species and extirpation of endemic species (Olden, 2006).

B) Agricultural

The beetle *E. fornicatus* is a major insect pest of tea plants in Sri Lanka (Walgama & Zalucki, 2007) and on avocado plants in Israel (Mendel *et al.*, 2012). The shot hole borer has become an serious threat and attacks the major cultivars of avocado, ‘Haas’ being to most susceptible (Mendel *et al.*, 2012). The coffee berry borer (*Hypothenemus hampei*) is a serious insect pest of coffee plants in the major coffee producing countries around the world (Damon, 2000). It has severe impacts on the berry quality that results in heavy crop losses (Damon, 2000). In the USA, there are a few bark and ambrosia beetles that have had severe impacts on the agricultural sector. The walnut twig borer attacks urban walnut trees, but also some of the walnut orchards in California (Seybold *et al.*, 2019) where 99 percent of USA orchards are located (Beede & Hasey, 1997). *X. glabratus* and its symbiotic fungus *Raffaelea lauricola*, has had a massive impact on especially avocado production in the USA (Haack *et al.*, 2013). When the beetle bores into the wood it inoculates its fungus and this disrupts vascular flow in the tree that results in vascular wilt (Haack *et al.*, 2013). The direct loss to the industry in terms of sales, increase management costs and other damages could be as high as \$356 million in a do-nothing scenario and up to \$181 million in a situation with 50% control success (Evans & Finkral, 2010).

C) Urban

Urban forests in the USA were infested by WTB and had massive effects on *Juglans nigra* street trees in Colorado between 2004 and 2010 (Tisserat *et al.*, 2011) and more recently in Eastern Tennessee (Grant *et al.*, 2011). In that time period, 1300 street trees were removed in Denver and Boulder costing \$675 on average per tree (Seybold *et al.*, 2019). Vector borne tree diseases such as these can have a large impact on human health (NASEM, 2016). Urban trees have impacts on quality of life through the provision of factors that are ecological, social,

aesthetic, and economic in nature (Tyrväinen *et al.*, 2005). Many trees, for example, provide climate stabilization by being a carbon sink and creating microclimates which provides habitat for other plants and animals (Tyrväinen *et al.*, 2005). Urban trees provide educational value for children by helping them learn the natural processes in an artificial environment (Tyrväinen *et al.*, 2005).

All invasive ambrosia and bark beetles that have been discussed can have large impacts on plant species, communities and ecosystems if the infestations continue to expand and spread across many natural systems (Seybold *et al.*, 2019). Discovering the initial stage of invasion of non-native species provides the opportunity to rapidly act and implement mitigating and prevention methods, before the invasion spreads and have damaging effects on trees, their ecosystems and the economic costs involved (Epanchin-Niell & Liebhold, 2015; Poland & Rassati, 2019).

1.2) PSHB: current status of a perfect invader in South Africa

This section has been adapted and submitted for publication as a review paper in the *South African Journal of Science*

1.2.1) History of invasion

Euwallacea (Coleoptera: Scolytinae) contains 45 species including four in the *E. fornicatus* species complex, namely *E. fornicatior*, *E. perbrevis*, *E. kuroshio* and *E. fornicatus* commonly known as the Polyphagous Shot Hole Borer (PSHB) (Fig. 1.1A) (Gomez *et al.*, 2018; Smith *et al.*, 2019; Stouthamer *et al.*, 2017). Diagnostic characters separating these species have not been described and the most reliable means of identification is *via* DNA sequence comparisons (Gomez *et al.*, 2018; Smith *et al.*, 2019) or high-resolution melt assay of the 28S ribosomal subunit (Rugman-Jones *et al.*, 2020). PSHB originates from South-East Asia (Storer *et al.*, 2015; Stouthamer *et al.*, 2017) and like other ambrosia beetles, it has a mutualistic association with fungi, notably its primary symbiont *Fusarium euwallaceae*, the main food source for the beetle and its larvae (Fig. 1.1B) (Freeman *et al.*, 2016, 2013). It was first detected outside of

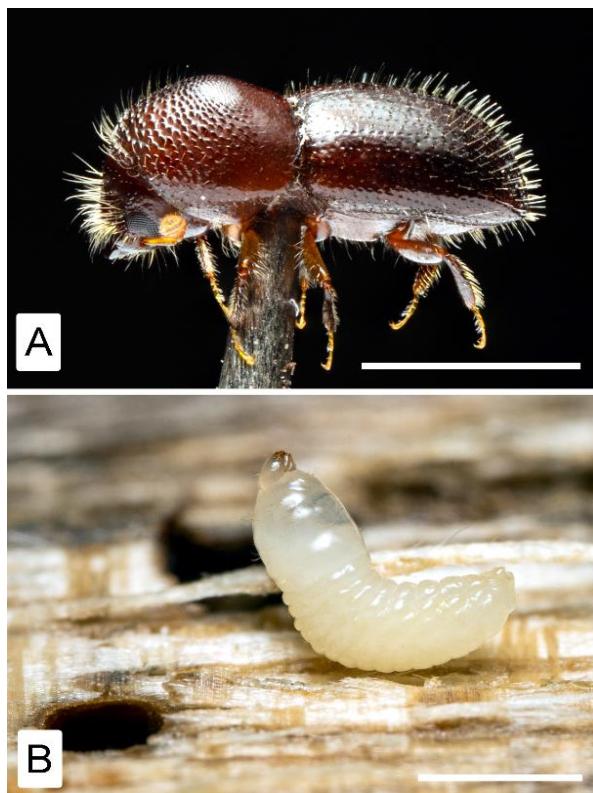


Figure 1.1: Adult (A) and Larva (B) of the Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus*. Scale bars = 1 mm.

its native range in 2003 in urbanised California [reported as the Tea Shot Hole Borer (TSHB)] (Rabaglia *et al.*, 2006). In 2009 it was detected on avocado in Israel (as *Euwallacea* sp. nr *fornicatus*) (Mendel *et al.*, 2012) but anecdotal evidence suggests that it was introduced as early as 2004 (Mendel *et al.*, 2012). It is now known to infest avocado trees and many other woody plants in urban and agricultural areas in these countries (Eskalen *et al.*, 2013; Mendel *et al.*, 2012). Even though exotic *Euwallacea* was known from Hawaii since 1910 (Swezey, 1941), *E. fornicatus* was only confirmed among populations of *E. perbrevis* in 2020 (Mitchell & Maddox, 2010; Rugman-Jones *et al.*, 2020). PSHB was also recently (April 2020) detected in a greenhouse in Italy (EPPO, 2020).

In 2017, as part of a plant sentinel project, PSHB was found infesting plane trees (*Platanus x acerifolia*) in a botanical garden in the KwaZulu-Natal Province, South Africa (Paap *et al.*, 2018). However, a DNA sequence of PSHB was obtained from a specimen in Durban in 2012 as part of the Barcode of Life project (BOLD: ACC9773, ETKC270–13) (Stouthamer *et al.*, 2017). This location is ca. 50 km SE of the 2017 collection site, indicating a much earlier introduction into the country. Since then, the beetle has been found in eight of the nine South African provinces, reaching as far as 1000 km from the first detection locality, making it the largest current outbreak of this pest globally.

1.2.2) Factors driving invasion by PSHB in South Africa

A) Transport pathway

Central to the understanding of the likelihood of establishment of species beyond their native range are the characters that enhance their dispersal i.e. departure, transport, and arrival in non-native environments (Gippet *et al.*, 2019). For PSHB, chances for departure are high due to

their small size (females are 1.8-2.5 mm long while males are only 1.5-1.67 mm long) and cryptic coloration (dark to light brown) (Jones & Paine, 2015). Therefore, they are easily missed during routine inspections when hitchhiking on goods and biological material such as nursery stock, even when not colonizing the material (Liebhold *et al.*, 2012). Their cryptic life cycle adds considerably to their successful departure as contaminants that colonize wood products (Meurisse *et al.*, 2019). Ambrosia beetles typically infest host trees by constructing galleries deep within the vascular tissues (Hulcr & Dunn, 2011). They are therefore easily missed in untreated wooden articles and packaging materials (Allen *et al.*, 2017). Potential transport vectors for PSHB include airplanes, trains, trucks, cars, and boats. PSHB was most likely introduced into South Africa *via* ships as the earliest known record for it is close to two major ports (Stouthamer *et al.*, 2017). Subsequent spread throughout the country was probably aided by the movement of infested wood (e.g. firewood).

As hitchhikers, characters such as resistance to drastic temperatures, starvation, dehydration, or exposure to toxins enhance survival during the transport phase (Gippet *et al.*, 2019; Renault *et al.*, 2018). As there is no evidence that PSHB is particularly resistant to any of these conditions, it likely survives during transport as contaminants due to their colonization of wooden products (Cooperband *et al.*, 2016). Wood acts as a buffer against rapid temperature fluctuations and is therefore a good insulator. Additionally, many wood types are not particularly porous, preventing chemical treatments from penetrating far enough to reach the beetle colony. Finally, moisture content is often fairly high, benefitting fungal growth and beetle survival (Huang *et al.*, 2020), but adults of PSHB are often found in fairly dry wood, where its second nutritional symbiont *Graphium euwallaceae* thrives (Freeman *et al.*, 2016). As contaminants of wood, individuals may be resistant to phytosanitary treatments (Whattam *et al.*, 2014) and are difficult to manage through application of a single phytosanitary measure (Allen *et al.*, 2017). Their small size and cryptic colouration also aid in preventing detection during inspections (Eschen *et al.*, 2015). In South Africa, accidental introductions are possible because phytosanitary inspections are limited due to the lack of trained personnel and funding (Parliament of the Republic of South Africa, 2017).

B) Reproduction and propagule pressure

High propagule pressure can enhance the chances of dispersal at all three levels (departure, transport, and arrival) (Grousset *et al.*, 2020; Haack *et al.*, 2010; Liebhold *et al.*, 2017). In the

case of PSHB, a single foundress female lays an average of 20 eggs and the first adults can appear in as little as 22 days at 24°C (Cooperband *et al.*, 2016). Colonies contain multiple overlapping generations and a colony from a single foundress can produce up to 57 reproductive female adults within 6–7 weeks (Cooperband *et al.*, 2016). Infested wood generally contains multiple colonies from numerous foundress females and population sizes can be tremendously large and self-sustaining over relatively long periods of time. For example, a single piece of infested wood weighing ca. 500 g collected in South Africa produced 44 mature females and five males after drying. These adult females are able to produce ca. 880 offspring within 22 days at 24°C if all were able to successfully construct galleries. It is clear that propagule pressure for PSHB is high, enhancing its chances of dispersal and establishment (Simberloff, 2009).

For PSHB, outbreeding is not required for reproduction. It has a haplodiploid mating system in which haploid males develop from unfertilized eggs (Cooperband *et al.*, 2016). If a mated female initiates a colony, both male and female offspring may be produced (ca. 7% of beetles may be male). These mate within the galleries resulting in mated female progeny that can leave the natal gallery and start new colonies. However, virgin females that construct galleries can lay haploid eggs that will result in all-male offspring (Cooperband *et al.*, 2016). She can then mate with her male offspring to produce diploid eggs (Cooperband *et al.*, 2016). The result is that a single female beetle, whether mated or not, can start new infestations with no indication that the populations will suffer from inbreeding (Peer & Taborsky, 2005; Stouthamer *et al.*, 2017). For PSHB, successful establishment is therefore possible even with low propagule pressure (Gomez *et al.*, 2018).

C) Fungal associations and host range

A close association with *F. euwallaceae* aid in the establishment of PSHB in novel environments as it can infect a multitude of different tree species (Dodge *et al.*, 2017; Gomez *et al.*, 2019). Along with two other fungal symbionts, *G. euwallaceae* and *Paracremonium pembeum*, it is introduced into the galleries from mandibular mycangia – specialised internal organs evolved for fungal transport (Freeman *et al.*, 2019, 2016; Lynch *et al.*, 2016). In South Africa signs of PSHB infestations have been confirmed from 117 tree species of which 38 are suitable for reproduction (i.e. reproductive hosts) and 79 can sustain fungal growth (i.e. non-reproductive hosts) (Addendum A). This wide fungal host range ensures availability of suitable

hosts near the introduction point. When beetle populations are very high, the insects appear to assess newly encountered hosts almost indiscriminately for fungal establishment (Coleman *et al.*, 2019; Eskalen *et al.*, 2013) explaining the high numbers of non-reproductive hosts. Such “attacks” may also lead to severe decline in tree health due to fusarium wilt (Eskalen *et al.*, 2013). *F. euwallaceae* may also help PSHB to overcome tree defences and enhance successful colonization of a host, even those that are only marginally suitable for beetle development (Norris & Baker, 1968).

1.2.3) Distribution of PSHB in South Africa

In South Africa, PSHB is wide-spread, and has a fairly continuous distribution in both KwaZulu-Natal (between Durban and Richards Bay) and the Southern Cape (between George and Plettenberg Bay) (Fig. 1.2). This indicates a relatively long history in these areas where initial populations expanded from the points of introduction. Unlike males, females can fly, but most disperse only short distances (*ca.* 30 m from point of release (Owens *et al.*, 2019)). This

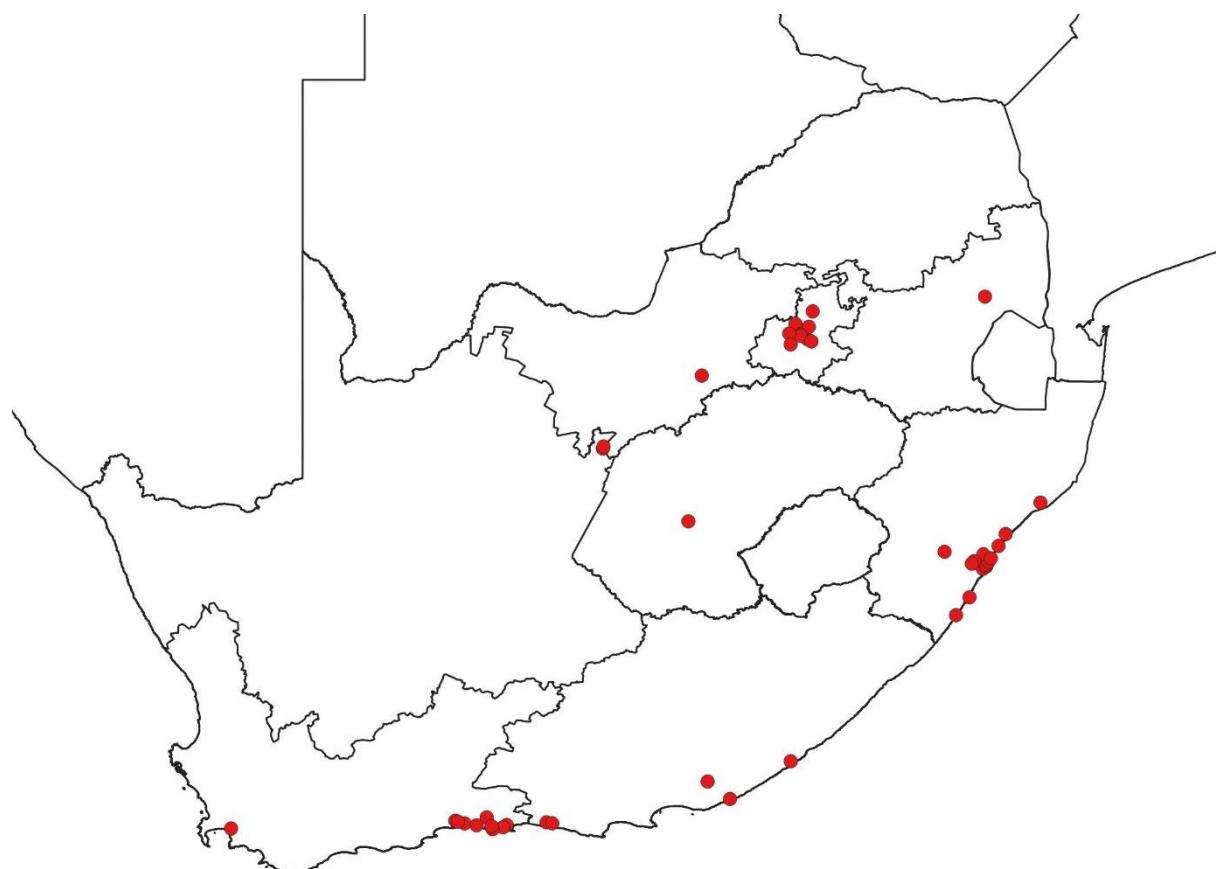


Figure 1.2: Distribution of the Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus* in South Africa. Only those localities from which PSHB or its symbiotic fungus *Fusarium euwallaceae* have been collected, and their identities verified using DNA sequencing, have been depicted.

may seem unremarkable, but factors like wind can have tremendous influence on the number of dispersing individuals, the direction of dispersal and the dispersal distance (Owens *et al.*, 2019). In Somerset-West (Western Cape Province) *E. fornicatus* dispersed at least 3 km from the putative point of introduction in only two months, against prevailing winds. Therefore, when suitable hosts are present, PSHB can expand its range rapidly without direct human assistance. However, distances between closest confirmed PSHB populations in South Africa can be as far as 500 km (Fig. 1.2). This can only be achieved when dispersal is aided by humans and human-mediated dispersal remains the most substantial form of long-distance transport and establishment of new colonies.

PSHB has a wide range of climatic conditions in which it can survive, increasing its chances of finding sites open for establishment. It is native to equatorial climates and has successfully established in temperate (California and Israel) and tropical (Hawaii) regions. Currently occupied climate zones as defined by Köppen-Geiger, updated by Peel *et al.*, (2007) include Af, Am, Aw, BSk, Csa, Csb, Cfa, Cfb and Cwa. Within South Africa, PSHB has established in at least six climactic zones: BSh, BSk, Cfa, Cfb, Csb and Cwb. In addition, Csa and Cwa are also present in South Africa, making much of the country climatically suitable for PSHB. Minimum, maximum, and optimum temperature for PSHB reproduction have been determined to be 13.34°C, 33.08°C and 27.51°C, respectively. Whilst PSHB were unable to reproduce below the minimum temperature threshold, they are able to persist as adults (Umeda & Paine, 2019). Also, microclimates exist in urban and agricultural settings, which provide a conducive climate in an otherwise unsuitable area.

In South Africa, PSHB is currently mostly confined to urban environments, likely due to greater invasibility and anthropogenically-mediated dispersal. Also, urban areas are far less biologically diverse than natural environments and offer less of a biological buffer (Potgieter *et al.*, 2020). Typically, ambrosia beetles favour trees that are stressed in their natural environments (Ploetz *et al.*, 2013). Even in novel environments this is often the case. Anthropogenic activities such as urbanization, gardening, trampling, and pruning can cause tree stress (Paap *et al.*, 2017). As a result, urban areas have high invasibility (Potgieter *et al.*, 2020; Richardson & Pyšek, 2006). Natural disturbances such as drought, storms, fire, and other pests can also produce added stress to trees in both urban and natural settings (Sukopp & Starfinger, 1999). Combined, these factors promote the spread and status of PSHB, but none have yet been comprehensively evaluated. However, unlike many other ambrosia beetle species, PSHB can easily infest healthy hosts (Kühnholz *et al.*, 2003). If tree stress is still a contributing factor to

epidemiology, a natural or anthropogenic disturbance in future could have catastrophic consequences.

1.2.4) Impact of PSHB

The first indication of PSHB in invaded habitats is benign. It tends to infest healthy hosts where one-millimetre diameter holes penetrate through the bark directly into the sapwood (Fig. 1.3A). Lesions may be visible on the outside of the tree (Fig. 1.3B), but more commonly a brown to pinkish stain, resulting from colonization by *F. euwallaceae*, can be seen spreading from the gallery into the vascular tissues below the bark (Fig. 1.3C). Different hosts respond to infestation in different ways, complicating initial diagnosis. External signs of infestations include wet patches and gum exudates (tree resin flows), sugar fountains, and accumulating frass (mixture of sawdust and excreta) or noodles (compacted frass) (Figs. 1.3D – 1.3G). Successful colonization of reproductive hosts often result in wilting and death of the infested branch or the entire tree (Fig. 1.3H) (Dodge *et al.*, 2017; Mendel *et al.*, 2012). By this stage, beetle populations are well-established. Detection in natural systems is very challenging due to a greater tree diversity, fewer people, and larger areas to survey.

Most notable agricultural impact of PSHB invasion has been on the avocado industries of Israel and California. In Israel, it was detected in orchards in 2009 and by 2016 it had spread to nearly all avocado growing areas in the country (Mendel *et al.*, 2017). Symptoms such as stem and branch breakages, wilting, dieback and tree death are common and devastating (Mendel *et al.*, 2017). South Africa currently has around 17 500 ha of avocado orchards, producing around 170 000 tons (or around R 700 million worth) of avocado annually. However, to date, no PSHB infestations have been found in commercial avocado orchards (only in backyard avocado trees). The only agricultural crops thus far affected by PSHB infestation in the country appear to be Pecan and Macadamia, but the effect on these crops appear limited, most likely as they are not reproductive hosts (Rugman-Jones *et al.*, 2020). PSHB has also been found associated with numerous other agricultural crops such as cherry, apple, citrus, peach, guava, olive, grape vine, and prune (Addendum A). Whether any of these can act as reproductive hosts is still unclear as all reports are from urban settings. Even so, when grown in close proximity to reproductive hosts, there is strong possibility of infection by *F. euwallaceae*. Therefore, current research in South Africa is aimed at establishing whether these are susceptible to fusarium wilt. Besides agriculturally important tree species, PSHB have been observed on *Acacia mearnsii*, a commercially grown forestry species in South Africa, but to date, no trees in commercial

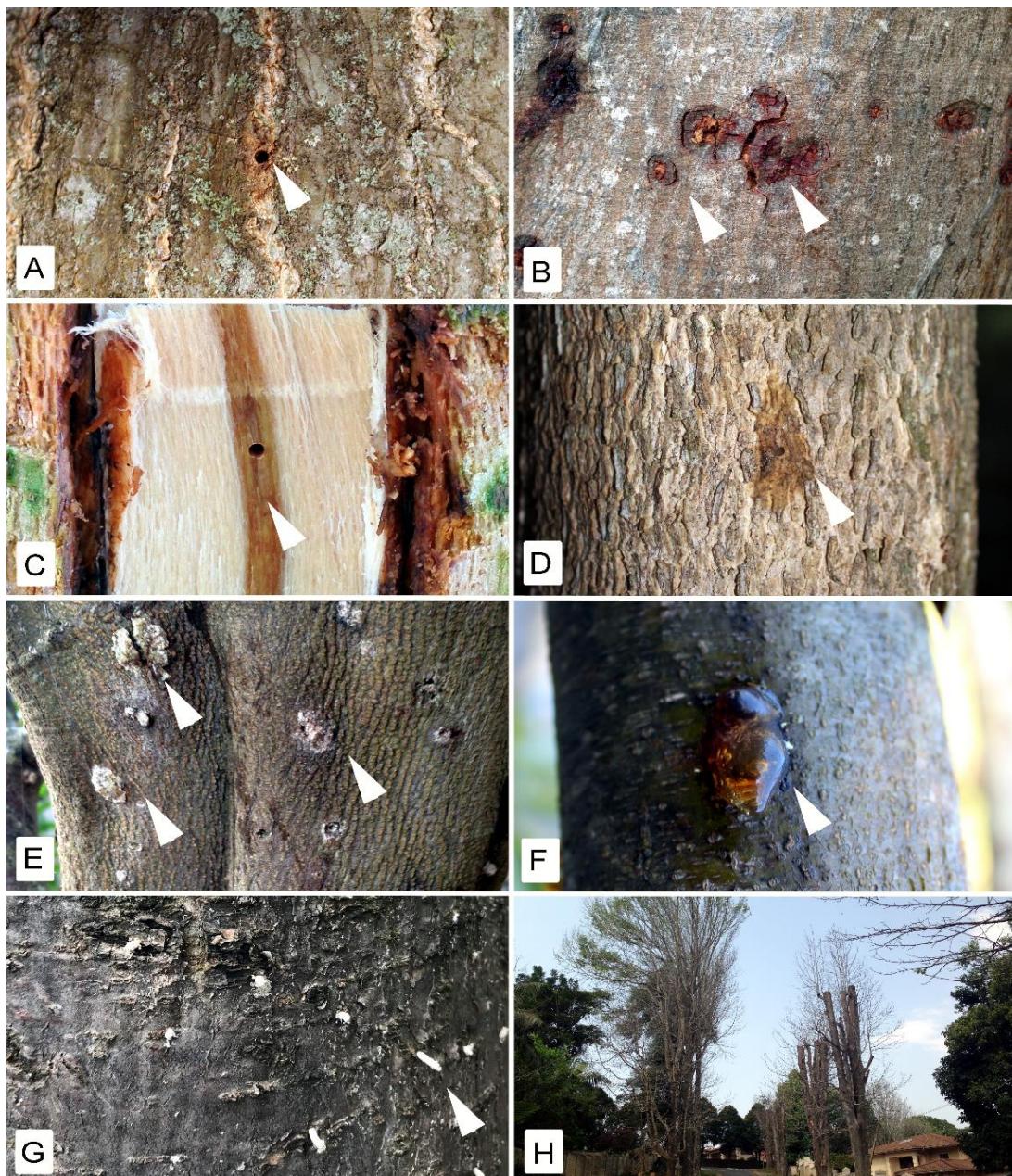


Figure 1.3: Signs of infestations of Polyphagous Shot Hole Borer Beetle (PSHB), *Euwallacea fornicatus* on various hosts. (A) 1 mm diameter entrance hole of PSHB on the bark of *Erythrina caffra*. (B) Lesions on the bark of *Acacia mearnsii* around entrance holes of PSHB. (C) Colonization of vascular tissues by *F. euwallaceae* (staining) surrounding the entrance hole of PSHB under the bark of *Platanus x acerifolia*. (D) Wet patch on the bark of *Nuxia floribunda* following infestation by PSHB. (E) Sugar fountain (dried sugary sap) on the bark of *Grewia occidentalis* following infestation by PSHB. (F) Gum exudates (tree resin flow) on the bark of *Podalyria calyptrata* following infestation by PSHB. (G) Noodles (compacted frass) extruded from the galleries of PSHB following infestation of *Harpephyllum caffrum*. (H) *Liquidambar styraciflua* individuals showing dieback of branches and tree death following heavy infestations by PSHB.

plantations have been reported to be infested. Based on observations on *Acacia* spp. from plantations in SE Asia, there are concerns surrounding the threat posed to the South African forestry industry (Hulcr *et al.*, 2017).

The most notable impacts of PSHB are on urban trees, with hundreds of different species susceptible to beetle infestation and fusarium wilt and thousands of individuals have already been removed [Owens *et al.*, 2019; Addendum A]. In the USA, more than 200 tree species have been reported to be susceptible to infestation by PSHB (Freeman *et al.*, 2013). It is expected that near 100% mortality could occur in urban stands of highly susceptible reproductive hosts (e.g. *Quercus robur*) and highly susceptible trees may die rapidly (12-18 months) following infestation. The resulting impact on urban environments could be substantial, including impacts on storm water runoff, losses to ecosystem services and carbon dioxide sequestration, and reduced shade contributing to remediation of the urban heat island effect and pollution reduction. There is increasing evidence linking widespread death of trees in urban environments following pest and pathogen invasions, and increased mortality in trees related to cardiovascular and respiratory tract illness (Donovan *et al.*, 2013). This may be particularly relevant in large cities with an urban/industrial interface where trees play an important role in pollution remediation. As trees succumb, falling trees and branches may impact human safety directly by causing traffic accidents and damaging vehicles and infrastructure such as buildings, fences, and power lines.

Negative economic impacts are incurred with the removal of infested trees and their replacement. However, tree removal have additional negative effects, such as a reduction in property values. In South Africa this has already resulted in at least one court case where PSHB infestations were not reported to potential buyers. The cost of tree removal in Somerset-West (Western-Cape Province) amounted to just over R 7000/tree in 2019. With more than 600 trees earmarked for removal in this town alone, the City of Cape Town has requested a stipend of R3 million for 2020 to monitor and remove infested trees. Services provided by these infested trees (e.g. carbon sequestering, providing shade, aesthetics, and emotional health) are also severely negatively affected by their removal. The replacement cost for trees that have already been removed in Somerset-West is estimated to be between R4.5 million and R5.8 million depending on tree species and labor costs. An unmitigated baseline social cost of \$27.12bn (R397.51bn) is expected for PSHB invasion in South Africa over ten years and not including replacement costs (de Wit *et al.*, 2021, unpublished data). This is about 1% of the country's

total GDP, or around \$50 per year for each South African citizen. By far the largest proportion of this cost will be incurred in urban areas (de Wit *et al.*, 2021, unpublished data).

Most worrying perhaps is the ability of PSHB to infest and kill native trees in its invaded ranges. Many of the host species in gardens are native to South Africa (Addendum A) and severe ecological consequences may result after invasion of natural environments, as was seen with the destruction of endemic willow forests in the Tijuana River Valley (Boland, 2016). Approximately 120 000 (30%) native willows (*Salix* spp.) were killed following infestation by *E. kuroshio*, a close relative of PSHB (Boland & Woodward, 2019). Loss of shade resulted in increased river temperatures, negatively impacting aquatic organisms. Recovery from the loss of large established trees will take time and encroachment by invasive alien plants has prevented full recovery of some stands (Boland & Uyeda, 2020). In South Africa, the loss of even a single species from an ecosystem can be catastrophic. For example, *Virgilia oroboides* is a native reproductive host for PSHB. It is an important pioneer forest species in the Southern Cape that protects forests from severe climatic fluctuations and fire, and houses a large number of native organisms (Coetsee & Wigley, 2013; Machingambi *et al.*, 2014). Elimination of this single species could have irreversible consequences for native forest integrity.

1.2.5) Mitigation

Biocontrol is generally difficult for ambrosia beetles owing to their cryptic habits and inbreeding success (Mendel *et al.*, 2017). There is no biocontrol agent available against PSHB. However, Californian researchers have been investigating PSHB in its natural range to identify potential natural enemies (Stouthamer pers. comm. 2017). In South Africa, investigations into biocontrol options are being initiated. Control of low levels of PSHB infestation may be achievable by direct injection of insecticides and fungicides. This not only maximizes pesticide impact, but reduces environmental contamination. Control of PSHB in more heavily infested trees seems unattainable even with the direct application of chemicals (Mayorquin *et al.*, 2018). Chemicals that show the most promise include combinations of insecticide and fungicide treatments, like emamectin benzoate or bifenthrin combined with propiconazole (Eatough Jones & Paine, 2018; Grosman *et al.*, 2019; Jones *et al.*, 2017; Mayorquin *et al.*, 2018). These controls are currently being investigated in South Africa, but comes at a high cost (Eatough Jones *et al.*, 2018), and even if they are proven effective, their application in natural settings are unlikely. Additionally, the application of commercial chemicals for prolonged periods of time could result in phytotoxicity (Bonilla-Landa *et al.*, 2018; van der Werf, 1996). In South

Africa, no insecticides or fungicides that have been evaluated for efficacy in PSHB management, have been registered for use. The use of any chemicals for PSHB management in South Africa is not currently recommended.

As there is no effective means to control pre-existing beetle infestations within a tree, the best alternative is to reduce the propagule number by physical removal of highly infested reproductive host trees. This reduces propagule pressure and potential of spread to new localities. Infested material needs to be properly treated to reduce beetle survival. Currently, best protocols dictate chipping of the wood to pieces smaller than 5 cm followed by solarisation under plastic sheeting for 4-6 weeks at 55°C (Chen *et al.*, 2020; Jones *et al.*, 2015). Movement of infected wood and wood chips should be avoided (Grousset *et al.*, 2020; Haack, 2001). Minimizing the spread of invasive reproductive host plant species is crucial, as these may create pathways for autonomous spread (van der Colff *et al.*, 2015).

1.3) Forests in South Africa

Forests in South Africa typically occur as small scattered patches (Cooper, 1985; Geldenhuys, 1991; Midgley *et al.*, 1997) that are imbedded within other biomes such as Savanna, Grassland, Albany thicket and Fynbos (Mucina *et al.*, 2006). There are 26 forest types recognized in South Africa which are grouped into 8 zonal groups and 1 azonal group (Von Maltitz *et al.*, 2003). The largest single forest in South Africa is found surrounding Knysna in the south-western Cape and forms part of the Southern Afrotropical forest complex. The 25 706 ha forest forms part of a larger 60 560 ha forest complex spanning from the Langeberg mountain range above Mossel Bay (22° E) to the Tsitsikamma mountain range close to Clarkson, Eastern Cape (24° 30' E) (Geldenhuys, 1991). The forest distribution is determined by climate, rainfall and substrate, but also importantly by fire patterns (Eeley *et al.*, 1999; Geldenhuys, 1994).

Forests are limited to regions with high water availability. This is mostly determined by rainfall, though riverine environments outside high rainfall areas can support forests due to groundwater, flood water and the shelter phenomenon (deep gorges with low solar radiation) (Mucina *et al.*, 2006). Most Southern Afrotropical forests are linked to orographic precipitation where mountainous areas have steep gradients in precipitation and localised rain shadows (Mucina *et al.*, 2006). Southern Afrotropical forests persist in regions with mean annual rainfall >525 mm with strong summer rainfall (Geldenhuys, 1991; Rutherford & Westfall, 1986). Mean daily maximum temperature ranges between 23.8°C for February and

18.2°C for August, the mean daily minimum between 19.7°C and 8.9°C (Geldenhuys, 1991). There are several vegetation types adjacent to the forests such as Fynbos, Renosterbos and Valley Bushveld (Acocks & Raup, 1955; Phillips, 1931). The substrates that support forests are mainly rocks of the Cape Supergroup (super mature quartzitic sandstones, mudstone, dolerites, dolomite, subordinate shales) (Geldenhuys, 2000, 1992). The soils are generally acidic, low in nutrients and have a poor internal drainage and buffering capacity (Geldenhuys, 2000). Trees have very shallow root systems due to high rainfall causing waterlogged soil conditions (Mucina *et al.*, 2006).

Southern Cape forests has the third largest total flora, the largest herbaceous flora and the fifth largest woody flora compared to other forest complexes in southern Africa (Geldenhuys, 1991). There are several forest endemics of the Southern Cape forest. Of the endemic plant species, 7 are trees and shrubs, 2 are classified as climbers, 2 ferns, 1 epiphyte, 3 geophytes and 1 sedge species (Geldenhuys, 1991). The remaining forest of South Africa is well protected as 73% of the forest complexes in the Southern Cape are being controlled by Government State Forestry, conservation NGO's and local administrative regions (Castley & Kerley, 1996). However, only 20% of these forests are in proclaimed nature reserves (Geldenhuys, 1991). In the past, large trees were harvested for building material such as poles and sleepers and firewood which was a major threat to the forests (McKenzie *et al.*, 1977; Mucina *et al.*, 2006). This timber exploitation has since been halted from 1939 and a sustainable forest management system was put in place (Geldenhuys, 1980; Phillips, 1963). Other threats to the forest biodiversity include poaching (bush meat), florist trade, crafts, traditional medicine and food resources (Geldenhuys, 1992; McKenzie, 1988).

1.4) Aims and objectives of study

PSHB has proven to be one of the most difficult tree pests to manage. This is largely due to its ability to colonize a massive variety of tree species and its ease of antropogenically-mediated long-distance dispersal. In the absence of a rapidly implemented nationally coordinated strategy, and without strong stakeholder and public engagement, PSHB was able to easily spread throughout the country (Paap *et al.*, 2020). Thus far, it has invaded numerous major cities, but, as South Africa is climatically suitable, PSHB is expected to rapidly spread to new areas. Impacts of the invasion are currently only in their early stages and major social and ecological costs are expected in the near future (de Wit *et al.*, 2021, unpublished data). Continued monitoring of the spread of the beetle is crucial if we are to mitigate its impact.

Mitigation will prove difficult, but is of the utmost of importance. Even halting the movement of infested wood and wood products will be problematic. Despite the informal urban firewood trade being widespread and important energy and income security source for many poor urban residents, its prevention is seen as the most effective way to curb long distance spread.

The aim of the present study was to make use of continued monitoring of the spread and impacts of PSHB in the Garden Route of the Southern Cape of South Africa to highlight current drivers and possible future impacts. Since many towns in the area have severe infestations of PSHB, there is concern that the beetle may be rapidly and unbeknown to us be spreading into the adjacent native forests. Specifically, this study set out to determine whether PSHB is currently affecting southern Afrotropical forest ecosystems, by determining which native trees are currently infested and are potential hosts. Objectives included 1) to identify native forest tree species vulnerable to infestations by PSHB, 2) to determine the impact of infestations on the health of infested trees, 3) to determine the current distribution of PSHB in these native forests, 3) to identify factors that increase invisibility of sites and individual trees to PSHB, 4) determine factors that aid in the spread of PSHB and 5) to estimate the possible economic loss and loss to carbon stocks due to unmitigated future PSHB invasion.

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Chapter 2

Invasion of a Southern Afrotropical forest complex by the Polyphagous Shot Hole Borer beetle

2.1) Abstract

The Polyphagous Shot Hole Borer beetle (PSHB) is a significant tree-killing invasive pest recently introduced into South Africa. Many native trees in urban settings are susceptible to infestation, but its presence in natural ecosystems is unstudied. The presence and determinants for PSHB colonization in 1682 individual trees of 68 species was evaluated in 51 monitoring plots across a native Afrotropical forest complex. PSHB could breed in six native species and these are at high mortality risk. An additional eleven species did not contain PSHB breeding colonies, but hosted its mutualistic tree-pathogenic fungus *Fusarium euwallaceae*. Susceptibility of plots to invasion increased when plots were closer to the urban infestation border and with an increase in the abundance of breeding hosts (host density). However, PSHB was also found in climax forest distant to urban areas at sites frequented by tourists. The severity and probability of infestation of individual trees increased with increases in host diameter and the number of surrounding infested individuals. Infested tree individuals were not spatially clumped. Instead, PSHB preferentially selected eight of the 17 native host species and larger tree individuals of these. An additional eight species were infested at random and two were infested seemingly accidentally. Infestation severity increased more rapidly on larger individuals and on those surrounded by a high abundance of breeding hosts. There was a more rapid increase in infestation severity for individuals in pioneer forest than in climax forests. This study confirms that Afrotropical forests are highly susceptible to invasion by PSHB with severe consequences for normal ecosystem processes and services. Direct anthropogenic impact had no discernible effect on infestations, but humans aided spread of PSHB to distant sites. Halting the movement of contaminated wood is therefore fundamentally important. Management of PSHB should focus on highly infested areas and individuals as these increase the likelihood of further infestations. Larger trees are more at risk of infestation which is concerning given that these forests contain some of the largest native trees in South Africa.

2.2) Introduction

The rate of biological invasions is globally increasing, in particular in South Africa (van Wilgen *et al.*, 2020). Exotic pests and pathogens caused world-wide alteration of biological communities and lead to severe degradation of native forests (Cobb *et al.*, 2012; Hughes *et al.*, 2017). The susceptibility of forests to invasion depends on numerous biotic (e.g. tree species identity, tree size, tree distribution and tree species diversity), abiotic (e.g. temperature and moisture) and anthropogenic factors (e.g. tree mechanical injury and forest clearing) (Choudhury *et al.*, 2020; Ghazoul *et al.*, 2015; Holt *et al.*, 2003; Shimwela *et al.*, 2018). Understanding the relative importance of these in explaining the spread and impact of invasive species is critical for their management.

Invasive ambrosia beetles pose one of the most serious threats to native forests (Boland & Uyeda, 2020; Holmes *et al.*, 2009; Hulcr & Dunn, 2011). For example, the Polyphagous Shot Hole Borer beetle, *Euwallacea fornicatus* (PSHB) (Curculionidae: Scolytinae), and its mutualistic tree-killing pathogenic fungus *Fusarium euwallaceae*, originally from South East Asia, has now spread to urban, agricultural and native environments in many parts of the world (Gomez *et al.*, 2019; Li *et al.*, 2016; Mendel *et al.*, 2012). In South Africa it was first reported in an botanical garden in 2016 (Paap *et al.*, 2018), although present in South Africa from at least 2012 (Stouthamer *et al.*, 2017). It has since spread to eight of the nine South African provinces encompassing the largest PSHB outbreak globally (de Wit *et al.*, 2021, unpublished data; Chapter 1). Human-mediated dispersal is the main pathway for its long-distance dispersal (Crowl *et al.*, 2008; Paap *et al.*, 2018, Chapter 1). Once established, it also rapidly expands its influence especially in urban environments (Chapter 1). Like other ambrosia beetles, PSHB spread fungal mutualists to new hosts through vectoring in mycangia (Six, 2003). An extreme infestation leads to dieback and death of a large diversity of trees and shrubs (Gomez *et al.*, 2019, Chapter 1). It has particularly high infestation success on hosts on which it can reproduce (reproductive hosts) which include native trees in urban areas throughout South Africa (Chapter 1). To date, no surveys of PSHB infestations in native South African forests have been undertaken.

South African forests comprise only 0.56% of the land surface and are patchy in distribution (Low & Rebelo, 1996; Mucina *et al.*, 2006), making them particularly vulnerable to disturbance and invasion (Berliner, 2011; King & Lively, 2012; Prospero & Cleary, 2017). Disturbances such as storms and fire are frequent. Combined with anthropogenic disturbances such as plant

harvesting, agriculture and tourism, increased chances for successful colonization of South African forests by PSHB is expected (Boland, 2016; Coleman *et al.*, 2019; Keesing *et al.*, 2006). When these forests are in close proximity to PSHB-colonised urban environments, such as the Afrotropical forest complex in South Africa, predictably they will suffer increased chance of invasion (Haack *et al.*, 2010; Jacobi *et al.*, 2012, Chapter 1). The largest South African forest complex, southern Afrotropical forests, is situated in the Southern Cape close to the PSHB-invaded towns of Knysna and George (Mucina *et al.*, 2006; Phillips, 1931). It is considered comparatively depauperate in tree species diversity (Mucina *et al.*, 2006) increasing its risk to severe impacts by invasive pests and pathogens (Carlsson *et al.*, 1990; Choudhury *et al.*, 2020; Garrett & Mundt, 1999; Gilbert *et al.*, 2002; Haas *et al.*, 2016; Jactel *et al.*, 2021; Keesing *et al.*, 2006; King *et al.*, 2012; Ostfeld & Keesing, 2012; Roy, 1993). In addition, PSHB is polyphagous (Gomez *et al.*, 2019; Lynch *et al.*, 2020, Chapter 1) and will likely encounter suitable hosts more easily than host-specialists, increasing its invasion success of natural systems (Lantschner *et al.*, 2020).

Microclimatic variation in forests are shaped by factors such as canopy cover and may have a significant effect on Scolytinae activity (Hulcr *et al.*, 2007; Rassati *et al.*, 2016). Many taxa prefer a more open tree canopy layer and have high population densities at forest edges (Chen *et al.*, 2010; Igeta *et al.*, 2004), which may hold true for PSHB (Umeda & Paine, 2019). In addition, proximity to surface water has been shown to increase PSHB infestations in the USA (Boland, 2016). The mechanism for this is unknown, but the growth of the symbiotic fungi of ambrosia beetles is usually positively correlated with moisture content in a host tree (Rudinsky, 1962). Therefore, Afrotropical forest edges and pioneer forests, especially those in close proximity to water, may suffer from higher PSHB infestations.

Larger tree individuals may be more susceptible to Scolytinae infestations compared to smaller trees (Haas *et al.*, 2016; Kendra *et al.*, 2013; Smith & Hoffman, 2001). Larger host individuals present a larger surface area and emit comparatively larger volumes of kairomones, making these easier to find (Choudhury *et al.*, 2020; Mayfield & Brownie, 2013). Some Scolytinae may even visually select for larger individuals by comparisons of their silhouettes (Mayfield *et al.*, 2013). A high density of larger diameter hosts therefore tends to have a higher pest and disease incidence (Anderson & May, 1986; Choudhury *et al.*, 2020; Holt *et al.*, 2003; Reynolds & Burke, 2011). Infestations could also increase as a result of increased abundance of infested breeding hosts (infestation density/disease incidence) (Christiansen *et al.*, 1987; Ostfeld *et al.*,

2012) and a decreased distance to nearest infested host or outbreak area (Kautz *et al.*, 2011; Muirhead *et al.*, 2006; Wichmann & Ravn, 2001).

Once established, ecosystem impacts of PSHB invasion into native South African forests are expected to be severe. In 2018, PSHB was detected in the towns of George and Knysna (Southern Cape) causing extensive damage to urban trees. It has likely started to invade native forests adjacent to these towns, but this has not yet been determined. The main aim of this study was therefore to investigate current PSHB infestation levels in these forests and to evaluate its potential impact on specific tree species. Factors that affect the invasibility of these forests and the factors that affect the susceptibility of particular host individuals were also considered.

2.3) Materials and Methods

2.3.1) Site selection

Surveys to assess the level of PSHB infestation in Southern Afrotemperate forests were conducted between George (33.9425 S, 22.4613 E) in the west and Tsitsikamma (33.9641 S, 23.8997 E) to the east (Southern Cape, South Africa), between June 2019 and September 2020 (Figure 2.1). Surveys were conducted at 12 sites that were chosen to represent a diverse array of impacts and ecologies (Table 2.1). For example, sites were chosen where humans frequent for picnics and with fireplaces in mature forest (where PSHB can presumably enter with the importation of firewood), next to roads (routes along which PSHB can presumably spread with human assistance), next to urban areas (that are known to be invaded by PSHB), and next to hiking trails (sites less likely to have PSHB introduced with human activities). A focal site for more in depth study of PSHB infestations was selected at the George Botanical Garden as this site showed substantial invasion by the beetle. Ten permanent monitoring plots were erected at the George Botanical Garden and at the Pledge Nature Reserve. At the other sites, the number of plots varied between 1 and 6 ($n=51$). Plots were 15 m x 10 m, at least 30 m apart, and selected to include a large number of different tree species growing under a large variety of different conditions (e.g. different distances to surface water or nearest human impact).

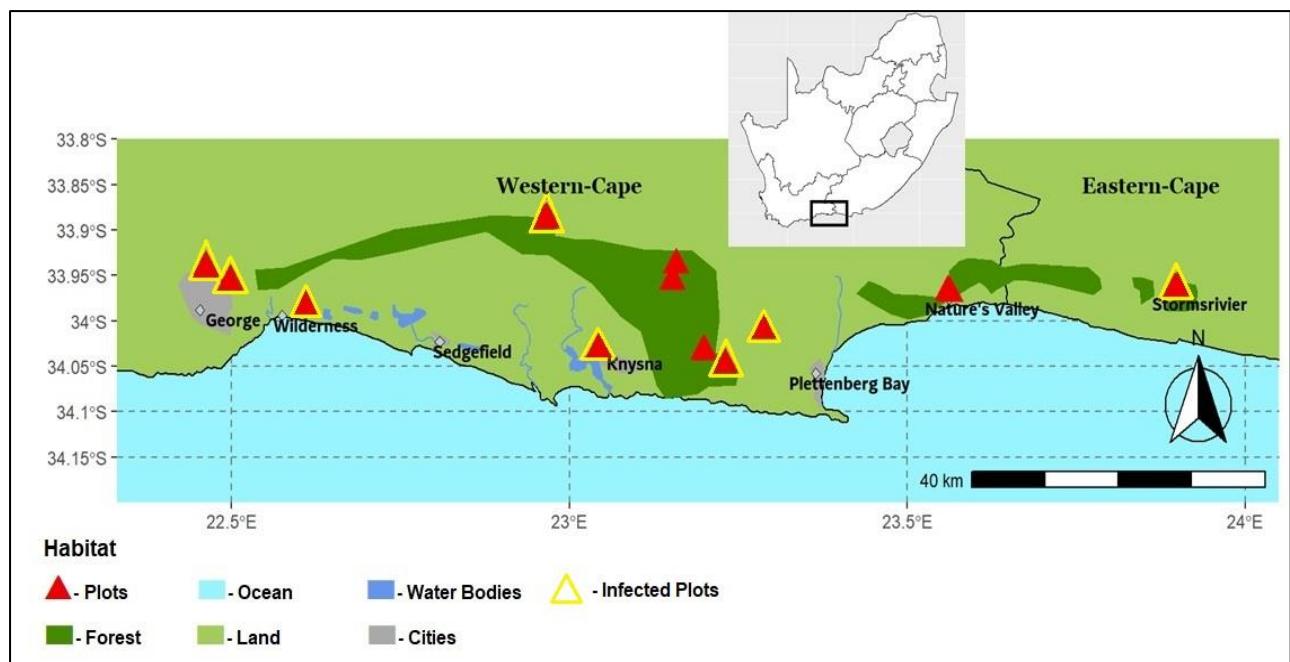


Figure 2.1: Map of the Southern Cape of South Africa depicting monitoring sites for PSHB. Sites from where evidence of PSHB invasion was found are indicated by a yellow outline.

Table 2.1: Description of sites in the Southern Cape of South Africa that were monitored for PSHB infestations.

Site	Plot	Coordinates	Tree richness	Tree abundance	PSHB presence	Site description
George Botanical Garden	1	33°56'31.80"S, 22°27'41.90"E	4	35	Yes	Pioneer forest. Previously a pine plantation but has been rehabilitated for the past 15 years. Close to urban areas that has infestations.
	2		4	61	Yes	
	3		6	50	Yes	
	4		4	45	Yes	
	5		5	36	No	
	6		5	21	Yes	
	7		6	40	Yes	
	8		4	34	Yes	
	9		3	45	Yes	
	10		8	56	Yes	
Jubilee Creek	11	33°53'18.50"S, 22°57'58.50"E	15	55	No	A picnic site in climax forest with fire making facilities next to a creek. Distant to urban areas.
	12		13	34	No	
	13		12	31	Yes	
	14		4	13	No	
	15		13	43	No	
Katrivier	16	33°57'25.80"S, 22°29'55.80"E	9	23	Yes	Small forest patch next to the Katrivier, a small river. Close to urban areas.
	17		18	31	No	
Wilderness Ebb & Flow	18	33°59'2.20"S, 22°36'33.00"E	6	7	Yes	Overnight camping area with fire making facilities, next to large river.
	19		6	17	Yes	
	20		2	14	Yes	
Pledge Nature Reserve	21	34°1'54.60"S, 23° 2'39.50"E	16	33	Yes	Small nature reserve situated in urban area.
	22		20	31	No	Impacted by a severe fire in 2017 that damaged many trees. Contains numerous planted trees.
	23		21	40	Yes	
	24		9	24	No	
	25		11	40	No	

	26		13	54	Yes	
	27		8	20	Yes	
	28		11	26	No	
	29		7	19	Yes	
	30		13	22	Yes	
Diepwalle	31	33°57'22.00"S, 23° 9'9.00"E	12	42	No	Climax forest site with fire making facilities. Distant to urban areas.
	32		18	49	No	
	33		12	43	No	
Garden of Eden	34	34° 2'0.70"S, 23°11'53.70"E	12	43	No	A small hiking trail that loops through climax forest next to a major highway. Experiences high tourist numbers.
	35		9	26	No	
	36		12	23	No	
Harkerville	37	34° 2'54.15"S, 23°13'49.83"E	17	39	Yes	Climax forest at the start of a day-hiking trail. Has few of large, exotic <i>Quercus robur</i> trees in the vicinity (infested with PSHB).
	38		17	40	Yes	
Leermansdrift	39	34° 0'37.10"S, 23°17'11.60"E	12	22	Yes	A farm containing a patch of native forest adjacent to the farmstead and a river. Closest town is Wittedrift. Exotic <i>Q. robur</i> trees are found in the vicinity (infested with PSHB).
	40		9	20	No	
	41		6	25	Yes	
	42		12	37	No	
	43		11	28	No	
Nature's Valley	44	33°58'11.50"S, 23°33'47.40"E	15	27	No	Campsite with fire making facilities on a forest edge.
	45		5	10	No	
Tsitsikamma	46	33°57'53.90"S, 23°53'57.90"E	9	45	Yes	Climax forest situated next to a major highway. Forest receives 80000 tourists annually. A major attraction is the "Big Tree", a very tall <i>Afrocarpus falcatus</i> tree that is one of South Africa's champion trees.
	47		14	29	No	
	48		11	36	No	
	49		9	25	Yes	
	50		10	36	No	
	51		10	37	No	

2.3.2) Data Collection

All permanent monitoring plots were surveyed *c.a.* every 3 months during the study period (n = 4 times). Surveys comprised all trees/shrubs found in the plot that had a stem circumference larger than 30 mm at breast height regardless of health condition. Plot-level data variables that were recorded included: distance to surface water from the middle of the plot, distance to the border of closest known infestations (usually nearest urban border), abundance of individuals of species that were confirmed or suspected as breeding hosts for PSHB at any time throughout this study (i.e. breeding hosts as defined below), canopy cover (%) as average of estimation by two observers), degree of natural impact (e.g. storm damage) scored as the percentage of trees in a plot that had signs of damage by natural causes, degree of physical human impact scored as the percentage of trees in a plot that had signs of damage caused by humans, total tree species richness and abundance, and number of trees showing signs of PSHB colonisation (breeding hosts) or attempted colonization (non-breeding hosts). Host species included all species in which either the beetle or its fungal symbiont *Fusarium euwallaceae* could be isolated in at least one individual across all sampling sites (confirmed host, see later section), or in South Africa (Chapter 1). Breeding hosts included all species that contained at least one individual with more than 10 PSHB entry holes across all sites, or that showed signs of multiple gallery branching when opening up the gallery system within wood (extensive gallery formation). Non-hosts species were those that never showed signs of PSHB infestations or attempted infestations across all sites.

Data recorded from each tree surveyed included tree species, diameter at breast height (DBH), canopy condition scored as percentage of crown dieback/yellowing (average of 2 observers), overall health condition scored as a rank between 1 and 5 (5 = completely healthy, 1 = dead/dying), tree injury scored as the average percentage (between two observers) damage to the entire tree (irrespective whether fungal, insect or human), distance to nearest human impact (hiking trails, road, etc.), and distance to nearest natural impacts (fallen tree, root excavations by wild pigs etc.). If an infested tree was discovered, the number of PSHB holes was counted (standardized from the base to the breast height of the tree (*ca.* 1.5m)). From every individual infested breeding host (focal tree) in the plots, the distance to the nearest healthy and infested conspecific individuals was measured. The distance from every focal tree to the nearest infested and healthy breeding host species (which could include any breeding host and not just conspecifics) was also measured. Lastly, the distance from every focal tree to the nearest

infested and healthy tree individual from any tree species that have been confirmed to be infested by PSHB during the study period (i.e. the nearest confirmed non-reproductive host) was measured.

2.3.3) Infested Tree confirmation

All individual trees were inspected for PSHB colonization or attempted colonization by evaluating trunks for entry holes of the correct size (1.00 - 1.50 mm) or other signs of tree reactions to boring beetles (sap flows, presence of frass, etc. as highlighted in Chapter 1). When present, bark was removed from the affected area using a sterile chisel to reveal any damage to the cambium and deeper wooden tissues. Boring activity was confirmed by the presence of an entry hole of the expected size for PSHB under the bark that may or may not have been accompanied by wood staining around the gallery (indicative of fungal growth). Based on the notion that *F. euwallaceae* is a host species specific symbiont of *E. fornicatus* in South Africa, and that it cannot spread without the help of its symbiotic beetle partner, confirmation of host status was based on the presence of *F. euwallaceae* within these galleries in wood. A sample of wood that contained a part of the gallery and stained wood was removed for further analyses. In the laboratory the wooden sample was surface sterilised by submerging in 70% ethanol for 30 s and then air dried. Small pieces of wood were removed from the infection border and plated onto malt extract agar (MEA; Merck). The plates were incubated at 25°C in the dark for 2 weeks and purified by subsequent series plating. Isolates that morphologically resemble those of *Fusarium* were used for molecular characterization. For these, the total genomic DNA was extracted from mycelium using the modified cetyl trimethylammonium bromide (CTAB) extraction method (Lee *et al.*, 1988; Wu *et al.*, 2001). For DNA fingerprinting the translation elongation factor 1- α (EF1- α) gene fragment was characterised for these isolates following procedures of Paap *et al.* (2018). Each 20 μ l reaction mixture for Polymerase Chain Reaction (PCR) contained 5 μ l PCR-grade water, 10 μ l standard Taq reaction buffer, 1 μ l MgCl₂, 1 μ l forward primer, 1 μ l reverse primer and 2 μ l extracted DNA. Thermocycling conditions were: 94°C for 3 min, followed by 34 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 60 s. The PCR products were visualized under UV light after separation on a 1% agarose gel with 0.5× Tris-boric acid-EDTA buffer (Na *et al.*, 2018; O'Donnell *et al.*, 1998). Amplification products were purified and sequenced by the Central Analytical Facilities (CAF) at Stellenbosch University.

2.3.4) Statistical Analysis

A) Factors that influence the number of trees infested by PSHB in plots (i.e invasibility of a site)

The influence of selected variables on the number of infested trees per plot ($n = 51$ plots) were tested using Generalized Linear Models (GLM's) fitted to a negative binomial distribution using the *lme4* package (Bates *et al.*, 2007) in R (R Development Core Team, 2020). The overall model incorporated the fixed plot effects of distance to infection border, abundance of breeding hosts (host density), percentage of trees with human impact, percentage of trees with natural impact, overall tree species richness, canopy cover and distance to nearest surface water. Second-order Akaike's information criterion (AICc) was used to rank candidate models using the *dredge* function in the '*MuMin*' package in R (Bartoń, 2009). The best model selected was the one with lowest AICc and all models that had a $\Delta\text{AICc} \leq 2$ were included in the subset of best models for further evaluation (Burnham & Anderson, 2002). Values of parameters retained in all included models according to this criterion were estimated by conditional model averaging of the best model subset using the *model.avg* function in the '*MuMin*' package in r (Bartoń, 2009).

B) Factors that influence the severity and probability of infestation by PSHB in susceptible hosts

To reduce biases in tree-colonization due to timing of arrival of PSHB in distant areas, evaluation of tree-level infestation data was focused on a single locality, George Botanical Gardens ($n=10$ plots). As the total forested area is small, there is increased chance that all trees had a fairly similar chance of infestation when only considering spatial extent. The effect of selected variables on the number of holes within tree individuals of breeding host species were tested using GLM's fitted to a negative binomial distribution using the *lme4* package in R. The overall model incorporated the fixed effects of DBH, abundance of breeding hosts in the plot (host density), abundance of infested breeding hosts in the plot (infestation density), distance to nearest human impact, distance to nearest natural impact, canopy cover, overall tree injury level, distance to nearest surface water and distance to nearest infested breeding host. Best candidate models and model averaging procedures followed those previously outlined. These analyses were repeated on a data set that contained only presence/absence data (i.e. whether an individual was infested or not) instead of the numbers of holes in the individual. Therefore, we

were able to test whether the abovementioned variables could lead not only to an increased severity of infestation on a particular individual (number of holes), but also whether these variables could influence the probability of infestation (infested or not).

C) Distance to infested host

To determine patterns of colonization of the PSHB beetle in plots (if any), inter-individual distances between all infested breeding hosts (across all 51 monitoring plots) and nearest healthy and nearest PSHB infested conspecific individuals, individuals of any breeding host species and individuals of any species that can host the beetle or fungus (not a breeding host) were measured. Inter-individual distances were compared using linear modelling (ANOVA) for the log-transformed data (to enforce normality of the data) in R. Post-hoc analysis were performed on the Inter-individual distances using a Tukey post-hoc test in R in the *multcomp* package (Hothorn *et al.*, 2008). Using these data, it was possible to determine whether PSHB infestation of individual trees were clumped i.e., whether attacks spread from reproductive hosts preferentially to any other possible host (breeding or non-breeding) just because of its close proximity to the source tree.

D) Progression of infestation at the plot level (increase in probability of infestation over time)

To determine which factors may aid an increase in the number of trees that become infested over time, data collected during the first survey (June 2019) were compared with that collected during the final survey (September 2020). For these analyses, only plots that had confirmed presence of PSHB during the final survey were considered ($n = 27$ plots). The increase in number of infested trees per plot was used as the response variable and the same plot-level variables were used as described previously as explanatory variables. In addition, the explanatory variables of number of breeding hosts that were colonised by PSHB during the 1st sampling period (i.e. expect to see a larger increase in infestations in plots with higher numbers of infested breeding hosts), and the total number of holes of PSHB on breeding hosts (i.e. expect to see a larger increase in plots that contain breeding hosts that are more severely infested) per site at the 1st sampling period were included. A Generalized Linear Model (GLM's) fitted to a negative binomial distribution using the *lme4* package (Bates *et al.*, 2007) in R (R Development Core Team, 2020) was used following procedures described before.

E) Progression of infestation at the individual level (increase in severity of infestation over time)

To determine which factors may lead to a faster increase in infestation severity over time, data collected during the first survey were compared with that collected during the final survey. For these analyses, all individuals infested with PSHB at any time during the survey period from all plots were considered. Data was analysed using GLM procedures as outlined before. The overall model incorporated all fixed effects as outlined in the section “*Factors that influence the severity and probability of infestation by PSHB in susceptible hosts*”. Variables added to these for this particular analysis included (1) the number of holes recorded during the first sampling season (i.e. expect to see a larger increase in infestations in individuals with higher levels of initial infestations), (2) total number of holes on all breeding hosts in the plot during the first survey (i.e. expect to see a larger increase in infestations in individuals that are surrounded by more severely infested breeding hosts), (3) distance to nearest infested breeding host as of the first sampling period (i.e. expect to see whether close proximity to an infested breeding host can increase chances of increased infestation severity. Best candidate models and model averaging procedures followed those previously outlined. In addition, abovementioned analyses were repeated, but included host identity as an explanatory variable to determine whether certain hosts have potential for faster colonization by PSHB than others. For this analysis only those host species that had at least 3 infested individuals and more than 5 individuals in total were included. Post-hoc analyses were performed on the different species using a Tukey post-hoc test in R in the *multcomp* package (Hothorn *et al.*, 2008).

F) Preferential Colonization

Preferential colonization of particular host species (breeding or not) was tested by comparing the percentage of infested and healthy individuals of each host species that was found infested at least once. Only data from plots that had at least one infested individual ($n = 27$) was included to minimize biases caused when PSHB infestations have not yet reached some plots. The percentage infested and percentage healthy trees observed for each species was compared to that expected by chance (i.e. the % composition of all infested vs. all healthy host individuals (breeding or not)) using χ^2 tests for goodness of fit followed by a Yates correction for multiple testing (Yates, 1934).

2.4) Results

The presence of PSHB was confirmed in eight sites (out of 12 sites) and in a total of 27 plots (out of 51 plots) at the end of the monitoring period (Table 2.1). These were spread across the survey area (Figure 2.1) but most, e.g., those in the George Botanical Garden and in the Pledge Nature Reserve, were in close proximity to urban transition zones. However, PSHB infestations were confirmed in climax forest areas distant from the urban fringes such as at Jubilee Creek and Tsitsikamma. All of these had facilities catering for visitors where they can also make fires with own imported wood.

A total of 1682 trees and 68 species were assessed for possible infestation across the 51 monitoring plots. Six species assessed were exotic, while the rest were all indigenous to the area. *Rapanea melanophloeos* was the dominant species across all plots, comprising 18.91% of the stands (Table 2.2). This was followed by *Trichocladus crinitus* (6.18%) and *Clutia pulchella* (5.95%). Some species were rare in these plots including *Allophylus decipiens*, *Scolopia mundii* and *Dovyalis rhamnoides*. Six native and one exotic host were confirmed as breeding hosts for PSHB including *Afrocarpus falcatus*, *Diospyros glabra*, *Gymnosporia buxifolia*, *Halleria lucida*, *Sparrmannia africana*, *Quercus robur* (exotic), and *Vepris lanceolata* (Table 2.2). Hosts in which only infestation attempts were detected without any PSHB establishment success included *Burchellia bubalina*, *Canthium inerme*, *Diospyros whyteana*, *Ekebergia capensis*, *Nuxia floribunda*, *Olinia ventosa*, *Rapanea melanophloeos*, *Scutia myrtina*, *Searsia chirindensis*, *Searsia lucida* and *Virgilia oroboides* subsp. *ferruginea*. A total of 50 plant species did not have any visible signs of attempted PSHB infestation even though some of these occurred in high numbers in plots that also contained PSHB infested hosts. Only two host individuals (one *D. glabra* and one *V. lanceolata*) died during the monitoring period likely due to PSHB infestations. The crowns of all other infested host individuals did not show any signs of reduction in health at the end of the monitoring period even though some were heavily infested. External symptoms of infestations varied considerably on different tree species with some (e.g. *D. glabra*, *D. whyteana*, *H. lucida* and *Q. robur*) showing dark staining on the bark around the entry holes and others with no external symptoms other than a small entrance hole and extruded frass (e.g. *H. lucida*, *N. floribunda* and *G. buxifolia*). Dried sap was also often observed around entry holes on recently colonised individuals of species such as *R. melanophloeos*, *V. lanceolata* and *V. oroboides* subsp. *ferruginea*. After removal of bark and cambial tissues, fungal colonisation was evident for all

host species, but the colour of the wood staining varied between reddish brown to black depending on species.

Table 2.2: List of tree species found in all 51 monitoring plots. Summaries are presented for average diameter at breast height (cm), the percentage of individuals of each species out of all individuals of all species (dominance), PSHB breeding host or not, percentage of individuals that were infested by PSHB, the percentage increase of infested individuals from time 1 to time 4 and the percentage increase in the number of holes from time 1 to time 4.

*exotic to South Africa; **exotic to research area

Species	Average diameter at breast height (cm ± SE)	Dominance (%)	Breeding host	Number of infested individuals (%)	Increase in infested individuals (%)	Increase in number of holes (%)
<i>Acacia mearnsii</i> *	2.33 (1.73)	0.18	No	0.00	0.00	0.00
<i>Acacia melanoxylon</i> *	18.56 (1.41)	0.12	No	0.00	0.00	0.00
<i>Acokanthera oppositifolia</i>	1.14 (1)	0.06	No	0.00	0.00	0.00
<i>Afrocanthium mundianum</i>	8.78 (7.55)	3.39	No	0.00	0.00	0.00
<i>Afrocarpus falcatus</i>	28.01 (8.89)	4.70	Yes	10.84	22.22	74.78
<i>Allophylus decipiens</i>	7.37 (1)	0.06	No	0.00	0.00	0.00
<i>Apodytes dimidiata</i>	22.35 (3.46)	0.71	No	0.00	0.00	0.00
<i>Buddleja saligna</i>	11.24 (5.57)	1.84	No	0.00	0.00	0.00
<i>Burchellia bubalina</i>	4.86 (9.64)	5.53	No	1.20	100.00	100.00
<i>Canthium inerme</i>	10.16 (4.24)	1.07	No	1.20	0.00	0.00
<i>Carissa bispinosa</i>	2.67 (2.45)	0.36	No	0.00	0.00	0.00
<i>Cassine peragua</i>	8.97 (2.65)	0.42	No	0.00	0.00	0.00
<i>Cassine schinoides</i>	7.95 (1)	0.06	No	0.00	0.00	0.00
<i>Celtis africana</i>	8.95 (1.41)	0.12	No	0.00	0.00	0.00
<i>Chionanthus foveolatus</i>	2.38 (1.41)	0.12	No	0.00	0.00	0.00
<i>Clutia pulchella</i>	3.20 (9.95)	5.89	No	0.00	0.00	0.00
<i>Crassula ovata</i>	5.80 (2.24)	0.30	No	0.00	0.00	0.00
<i>Cunonia capensis</i>	15.27 (2.45)	0.36	No	0.00	0.00	0.00
<i>Curtisia dentata</i>	17.28 (3.61)	0.77	No	0.00	0.00	0.00
<i>Diospyros dichrophylla</i>	11.98 (6.08)	2.20	No	0.00	0.00	0.00
<i>Diospyros glabra</i>	4.02 (8.19)	3.98	Yes	20.48	35.29	44.50
<i>Diospyros whyteana</i>	10.76 (4.24)	1.07	No	1.20	100.00	100.00
<i>Dovyalis rhamnoides</i>	2.54 (1)	0.06	No	0.00	0.00	0.00
<i>Ekebergia capensis</i>	23.37 (3.74)	0.83	No	3.61	0.00	20.00
<i>Elaeodendron croceum</i>	10.63 (4.80)	1.37	No	0.00	0.00	0.00
<i>Ficus burtt-davyi</i>	8.29 (1.73)	0.18	No	0.00	0.00	0.00
<i>Ficus sur</i>	8.12 (2.24)	0.30	No	0.00	0.00	0.00
<i>Euryops virgineus</i>	5.18 (1.41)	0.12	No	0.00	0.00	0.00

<i>Gardenia thunbergia</i>						
**	2.92 (1.00)	0.06	No	0.00	0.00	0.00
<i>Gonioma kamassi</i>	8.34 (4.36)	1.13	No	0.00	0.00	0.00
<i>Gymnosporia buxifolia</i>	6.87 (9.70)	5.59	Yes	8.43	71.43	21.43
<i>Halleria lucida</i>	9.41 (7.00)	2.91	Yes	15.66	23.08	79.22
<i>Harpephyllum caffrum</i>	30.17 (1.41)	0.12	No	0.00	0.00	0.00
<i>Ilex mitis</i>	18.37 (3.87)	0.89	No	0.00	0.00	0.00
<i>Kiggelaria africana</i>	12.70 (2.65)	0.42	No	0.00	0.00	0.00
<i>Maytenus acuminata</i>	1.91 (1.00)	0.06	No	0.00	0.00	0.00
<i>Maytenus peduncularis</i>	1.91 (1.00)	0.06	No	0.00	0.00	0.00
<i>Mystroxylon aethiopicum</i>	2.23 (1.00)	0.06	No	0.00	0.00	0.00
<i>Nuxia floribunda</i>	21.31 (7.21)	3.09	No	6.02	40.00	13.33
<i>Ochna arborea</i>	5.01 (2.83)	0.48	No	0.00	0.00	0.00
<i>Ocotea bullata</i>	24.30 (7.28)	3.15	No	0.00	0.00	0.00
<i>Olea capensis</i> subsp. <i>macrocarpa</i>	10.06 (7.00)	2.91	No	0.00	0.00	0.00
<i>Olinia ventosa</i>	17.60 (3.00)	0.54	No	1.20	0.00	0.00
<i>Osteospermum moniliferum</i>	2.39 (2.00)	0.24	No	0.00	0.00	0.00
<i>Pinus pinaster</i> *	2.16 (1.00)	0.06	No	0.00	0.00	0.00
<i>Pittosporum viridiflorum</i>	8.20 (2.24)	0.30	No	0.00	0.00	0.00
<i>Platylophus trifoliatus</i>	27.77 (3.46)	0.71	No	0.00	0.00	0.00
<i>Podocarpus latifolius</i>	8.00 (8.72)	4.52	No	0.00	0.00	0.00
<i>Polygala myrtifolia</i>	3.23 (3.74)	0.83	No	0.00	0.00	0.00
<i>Pterocelastrus tricuspidatus</i>	13.81 (5.83)	2.02	No	0.00	0.00	0.00
<i>Quercus robur</i> *	61.86 (2.65)	0.42	Yes	4.82	50.00	75.00
<i>Rapanea melanophloeos</i>	10.40 (17.86)	18.97	No	8.43	14.29	26.53
<i>Rhamnus prinoides</i>	3.70 (3.16)	0.59	No	0.00	0.00	0.00
<i>Rhoicissus tomentosa</i>	4.14 (2.00)	0.24	No	0.00	0.00	0.00
<i>Robsonodendron eucleiforme</i>	10.50 (2.45)	0.36	No	0.00	0.00	0.00
<i>Rothmannia capensis</i>	7.65 (3.32)	0.65	No	0.00	0.00	0.00
<i>Scolopia mundii</i>	2.23 (1.00)	0.06	No	0.00	0.00	0.00
<i>Scutia myrtina</i>	7.84 (3.32)	0.65	No	1.20	100.00	100.00
<i>Searsia chirindensis</i>	21.90 (3.32)	0.65	No	2.41	0.00	0.00
<i>Searsia lucida</i>	7.83 (6.00)	2.14	No	2.41	100.00	100.00
<i>Searsia pallens</i>	25.43 (1.00)	0.06	No	0.00	0.00	0.00
<i>Sideroxylon inerme</i>	15.37 (2.00)	0.24	No	0.00	0.00	0.00
<i>Sparrmannia africana</i>	3.60 (2.83)	0.48	Yes	3.61	33.33	6.67
<i>Syzygium cordatum</i> **	6.79 (2.00)	0.24	No	0.00	0.00	0.00
<i>Trichocladus crinitus</i>	3.98 (10.20)	6.18	No	0.00	0.00	0.00
<i>Trimeria grandifolia</i>	9.26 (2.00)	0.24	No	0.00	0.00	0.00

<i>Vepris lanceolata</i>	16.38 (3.00)	0.54	Yes	2.41	50.00	98.08
<i>Virgilia oroboides</i>	12.79 (4.12)	1.01	No	4.82	50.00	61.54

2.4.1) Factors that influence the number of trees infested by PSHB in plots (i.e invasibility of a site)

The best model had an AICc value of 154.53 and a weight of 0.18 (df = 5, LogLink = -71.60). Nine competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, six variables were retained, of which two were significant (Table 2.3). The number of infested trees across all 51 survey plots decreased with an increase from infestation border and increased with an increase in breeding host abundance in a plot (Table 2.3, Figure 2.2). An increase in tree species richness led to a decrease in number of infested trees in a plot, but this effect was only marginal (Table 2.3, Figure 2.2).

Table 2.3: Model-averaged estimates of variables predicting invasion by PSHB at a site.

Variable	Estimate	SE	Relative importance	Number of containing models	Z-value	P-value
Intercept	0.588	0.617	-	-	0.935	0.350
Distance to infestation border	-0.375	0.115	1.00	9	3.180	0.001
Breeding host abundance	0.109	0.032	1.00	9	3.345	0.001
Tree species richness	-0.062	0.032	0.74	6	1.864	0.062
Human impact	3.414	2.248	0.30	3	1.480	0.139
Distance from water	0.016	0.011	0.43	4	1.499	0.134
Tree abundance	-0.022	0.015	0.33	3	1.489	0.136

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

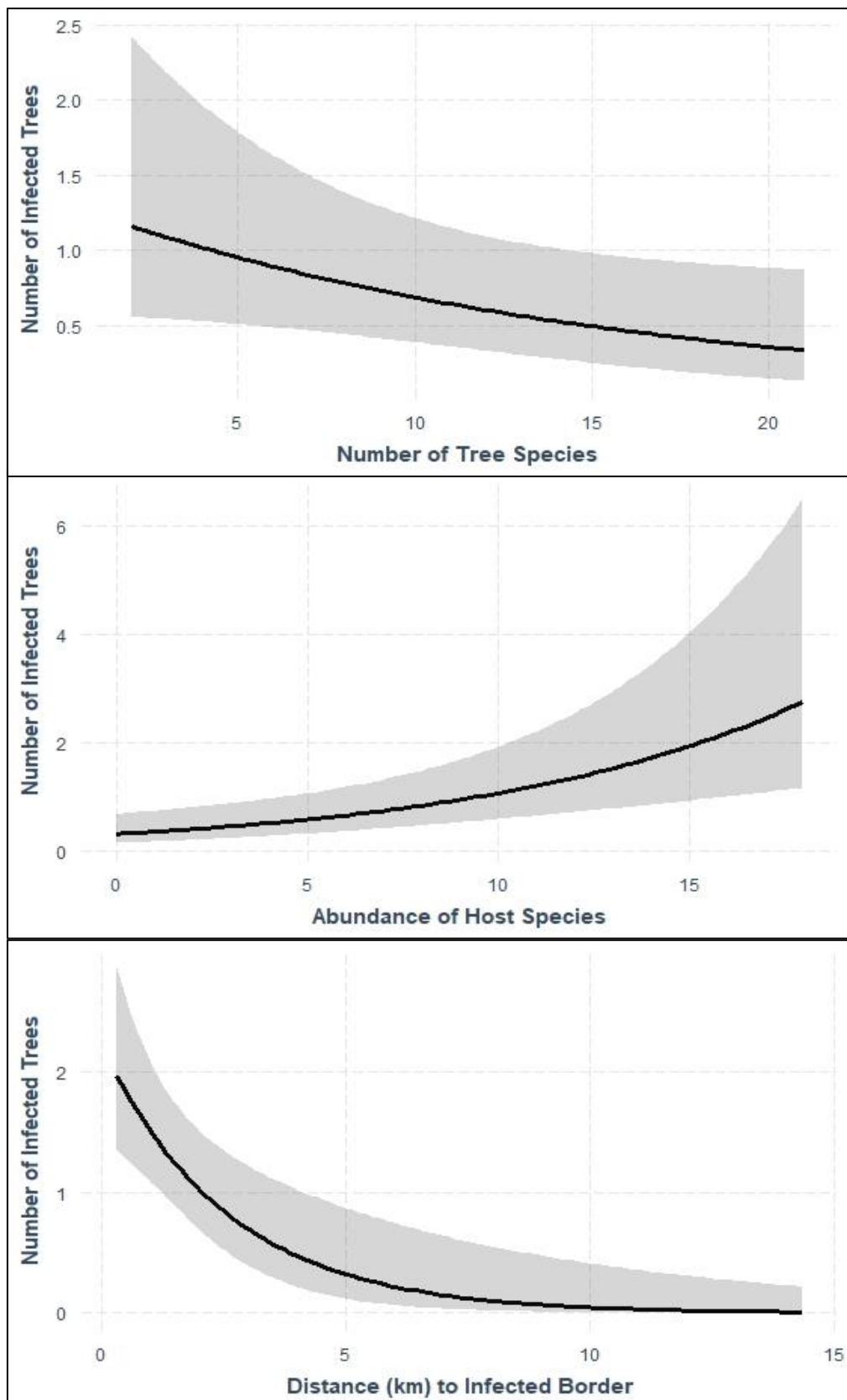


Figure 2.2: The regression estimates of number of infested trees as influenced by host species diversity at a plot, abundance of host species per plot and the distance to infestation border.

2.4.2) Factors that influence the severity and probability of infestation by PSHB in susceptible hosts

These analyses only included the 10 plots from the George Botanical Garden which contained a total of 423 tree individuals. Here a total of 36 trees were infested (8.51% of all) of which 26 individuals were of breeding hosts (5.44% of all). *Diospyros glabra* had the highest number of infested individuals (17 = (25.37% of all individuals)), followed by *Halleria lucida* (10 (= 58.82% of individuals)) and *Rapanea melanophloeos* (6 = (2.68% of individuals)) (Table 2.4). *Diospyros glabra* was the most severely infested in terms of number of holes, with a maximum of 230 holes on a single individual. A dominant tree species that seemed unfavourable for PSHB infestation at the George Botanical Garden was *Clutia pulchella* (Table 2.4). There were also several other tree species that presented no sign of infestation, but these were not dominant in the environment (Table 2.4).

Table 2.4: All tree species found in ten PSHB monitoring plots set out at the George Botanical Garden. Presented is the dominance (% of all tree individuals encountered), number of individual trees infested with PSHB, % of infested individuals of that particular species and the average diameter at breast height (cm) of each particular species encountered.

Species	Dominance	Number of infested trees	Percentage of infested individuals	Average diameter
<i>Burchellia bubalina</i>	1.18	0	0	2.52
<i>Clutia pulchella</i>	20.33	0	0	3.13
<i>Cunonia capensis</i>	0.24	0	0	16.24
<i>Diospyros glabra</i>	15.84	17	25.37	4.02
<i>Gymnosporia buxifolia</i>	1.89	0	0	3.31
<i>Halleria lucida</i>	4.02	10	58.82	6.60
<i>Kiggelaria africana</i>	0.24	0	0	2.01
<i>Nuxia floribunda</i>	0.24	1	100	19.23
<i>Olinia ventosa</i>	0.24	1	100	19.36
<i>Rapanea melanophloeos</i>	52.96	6	2.68	9.09
<i>Rhamnus prinoides</i>	1.18	0	0	3.34
<i>Searsia chirindensis</i>	1.42	1	16.67	11.98
<i>Trimeria grandifolia</i>	0.24	0	0	1.43

When considering the severity of infestations on hosts the best model had an AICc value of 332.55 and a weight of 0.52 (df = 6, LogLink = -159.78). Three competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, five variables were retained, of which three were significant (Table 2.5). Here, trees with a larger diameter tended to present more holes, an increase in the number of breeding hosts at a site increased the severity of infestations on individuals and the severity of infestations also increased with a higher number of infested host individuals (breeding and non-breeding) at a site (Table 2.5' Figure 2.3).

Table 2.5: Model-averaged estimates of environmental variables predicting probability of severity by PSHB in susceptible hosts.

Variable	Estimate	SE	Relative importance	Number of containing models	Z-value	P-value
Intercept	-4.767	1.967	-	-	2.409	0.016
Diameter at breast height	0.021	0.005	1.00	3	4.464	< 0.001
Breeding host abundance	0.182	0.080	0.79	2	2.259	0.024
Infested host abundance	0.287	0.116	1.00	3	2.446	0.014
Overall tree injury level	0.055	0.039	0.73	2	1.386	0.166
Distance nearest infested breeding host	-0.248	0.133	0.21	1	1.835	0.067

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

When considering the probability of infestations on hosts the best model had an AICc value of 85.96 and a weight of 0.28 (df = 4, LogLink = -38.75). Six competing models were retained after model selection procedures. After model averaging procedures, seven variables were retained, of which two were significant (Table 2.6). As with data on severity, the probability of infestation also increased with an increase in tree diameter and with the number of infested individuals (breeding and non-breeding) in a site (Figure 2.3). The number of breeding hosts at a site alone did not significantly affect the probability of infestation.

Table 2.6: Model-averaged estimates of environmental variables predicting probability of infestation by PSHB in susceptible hosts.

Variable	Estimate	SE	Relative importance	Number of models containing	Z-value	P-value
Intercept	-3.601	0.952	-	-	3.740	<0.001
Diameter at breast height	0.008	0.004	1.00	6	2.171	0.030
Infested host abundance	0.304	0.092	1.00	6	3.277	0.001
Overall tree injury level	0.645	0.050	0.82	5	1.285	0.199
Distance nearest infested	-0.120	0.127	0.16	1	0.937	0.349
Breeding host						
Canopy cover	-0.019	0.020	0.15	1	0.932	0.351
Distance from natural impact	0.076	0.098	0.12	1	0.762	0.446
Distance from water	0.013	0.024	0.11	1	0.542	0.588

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

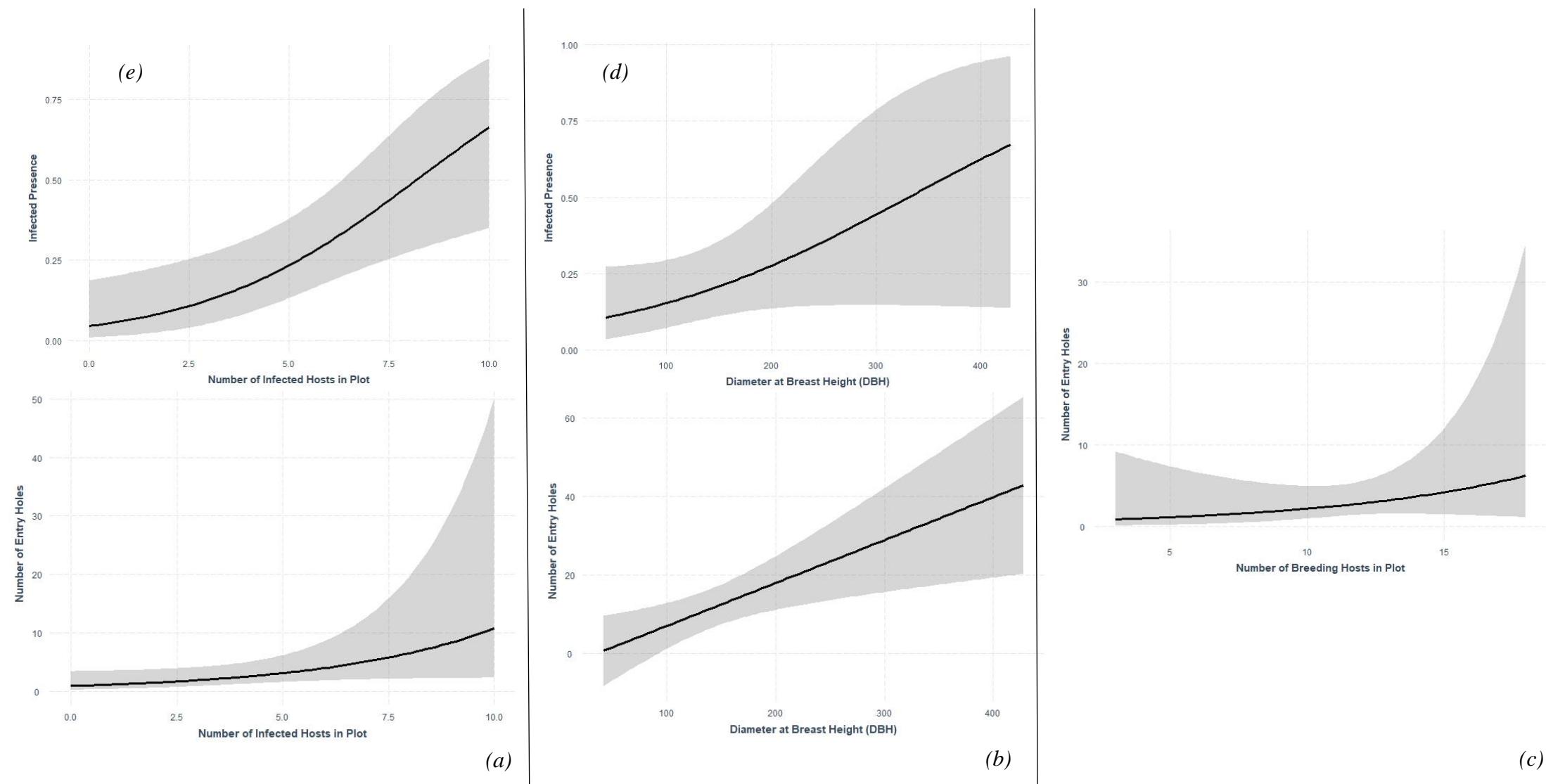


Figure 2.3: Variables that significantly influenced the severity of PSHB infestations (a – c) and the probability of PSHB infestations (d-e) at the George Botanical Gardens ($n = 10$ plots). (a) The effect of breeding host abundance on infestation severity. (b) The effect of tree size on the infestation severity. (c) The effect of the number of infected hosts in a plot on infestation severity. (d) The effect of tree size on infestation probability. (e) The effect of number of infested trees in a plot on infestation probability.

2.4.3) Distance to infested host

A total of 53 infested breeding hosts were used as basis to investigate progress of infestations (i.e. possible patterns of movement from an infested breeding host to other trees). Inter-tree distance data analysed with linear modelling procedures on log transformed data returned a significant result ($F = 11.75$, $df = 5$, $P = 2.03^{-10}$). The Tukey post-hoc test revealed that the distance between an infested reproductive host and its nearest infested host was significantly larger as compared to the nearest healthy host, irrespective of when considering any host (breeding or not), any breeding host (of any species) or conspecific species (Figure 2.4).

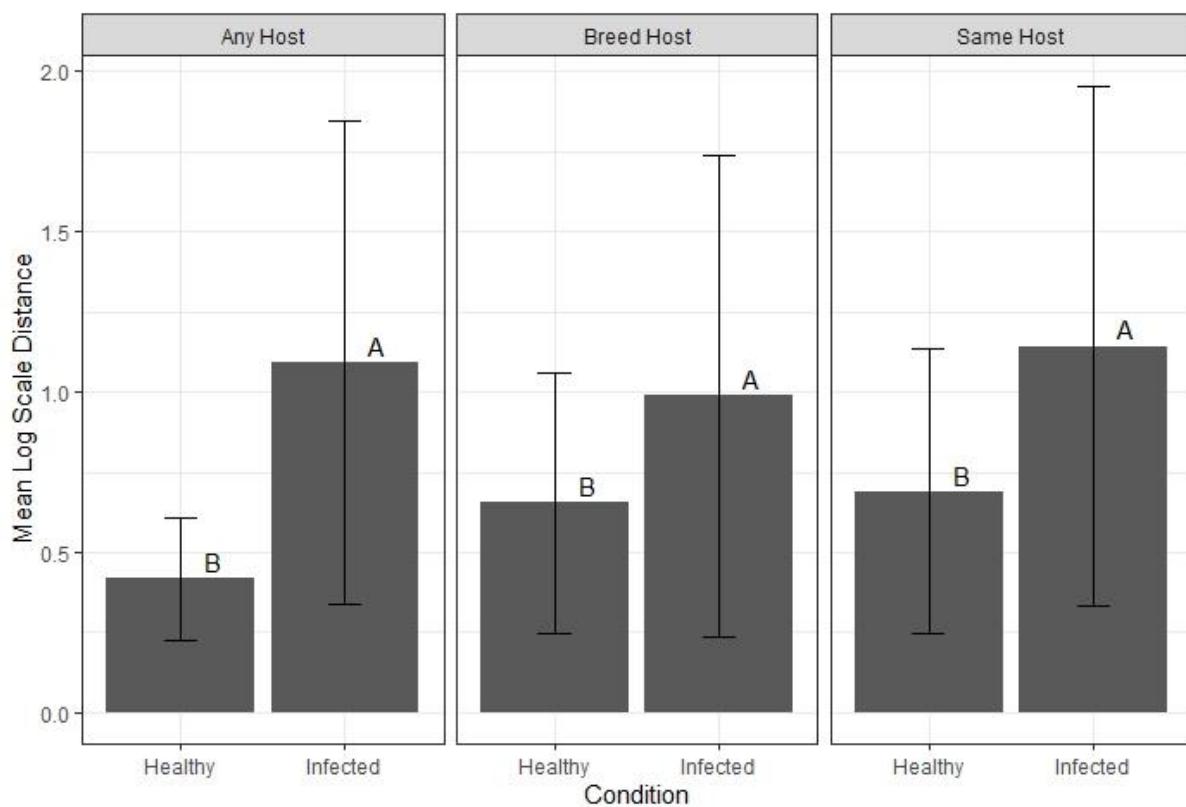


Figure 2.4: Mean log distance (m) from an infested breeding hosts to nearest other host (healthy and infested) categorised as either any host (i.e. breeding and non-breeding hosts), breeding hosts only (irrespective of breeding host species identity) and conspecific breeding host species. Different letters above bars indicate significantly different means (5% level).

2.4.4) Progress of infestation at the plot level (increase in probability of infestation over time)

At the start of the monitoring in June 2019, a total of 54 tree individuals were infested compared to 83 trees after the final monitoring survey in September 2020. Using data on the probability of increase in infestations (i.e. number of trees per plot) between the two monitoring times the best model had an AICc value of 79.45 and a weight of 0.09 (df = 3, LogLink = -36.20). Twenty-two competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, nine variables were retained, of which none were significant (Table 2.7). Only one environmental variable, human impact was positively correlated to progress of infestation and marginally significant.

Table 2.7: Model-averaged estimates of environmental variables predicting progress of infestation by PSHB at plot level.

Variable	Estimate	SE	Relative importance	Number of containing models	Z-value	P-value
Intercept	-0.425	0.981	-	-	0.424	0.671
Diameter at breast height	-0.303	0.194	0.66	12	1.478	0.140
Human impact	4.059	2.052	0.59	13	1.882	0.060
Breeding host abundance	0.069	0.046	0.33	8	1.416	0.157
Distance from water	0.020	0.012	0.40	10	1.562	0.118
Holes at time 1	0.003	0.002	0.18	4	1.549	0.121
Canopy cover	0.018	0.013	0.11	3	1.292	0.196
Tree abundance	-0.025	0.017	0.07	2	1.387	0.166
Infested breeding hosts time 1	0.115	0.112	0.04	1	0.975	0.329
Natural impact	3.622	2.305	0.03	1	1.485	0.138

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

2.4.5) Progress of infestation at the individual level (increase in severity of infestation over time)

The total number of PSHB holes counted at the end of the monitoring period was 1726 from 83 tree individuals (mean = 21.31 ± 41.45 SE). A maximum of 300 holes and a minimum of 1 hole was recorded per individual. For infested tree individuals, 62.19% showed an increase in their number of holes over time. Analyses to determine which factors may lead to a faster increase in infestation severity over time were first conducted on data from all infested individuals in which tree species could not be accounted for due to too few individuals of many species showing increase in number of holes. For the total data set, the best model had an AICc value of 463.61 and a weight of 0.15 ($df = 5$, LogLink = -226.41). Ten competing models were retained after model selection procedures. After model averaging procedures, eight variables were retained of which two were significant (Table 2.8). Distance to water was positively correlated to a faster increase in severity of infestation over time and marginally significant. A faster increase in severity of infestation over time was positively correlated to an increase in the number of breeding hosts in a plot but negatively correlated with an increase in canopy cover (Table 2.8, Figure 2.5).

Table 2.8: Model-averaged estimates of environmental variables predicting progress of severity by PSHB at the individual level without species as explanatory variable.

Variable	Estimate	SE	Relative importance	Number of containing models	Z-value	P-value
Intercept	3.242	0.945	-	-	3.381	0.001
Distance from water	0.022	0.012	0.59	6	1.822	0.068
Breeding host abundance	0.184	0.051	1.00	10	3.575	0.0004
Canopy cover	-0.056	0.017	1.00	10	3.304	0.001
Infested host abundance	0.147	0.098	0.43	4	1.479	0.139
Distance nearest infested breeding host	-0.009	0.008	0.15	2	1.102	0.270
Human impact	-0.047	0.035	0.16	2	1.333	0.183
Diameter at breast height	0.0004	0.000	0.07	1	0.946	0.344
Holes at time 1	0.001	0.002	0.06	q	0.772	0.440

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

The second analyses included host species as an explanatory variable (reduced data set as many host taxa had insufficient numbers of individuals with increased infestations over time). For this data set, the best model had an AICc value of 377.23 and a weight of 0.14 (df = 5, LogLink = -183.11). Thirteen competing models were retained after model selection procedures. After model averaging procedures, seven variables were retained of which two were significant (Table 2.9).

Table 2.9: Model-averaged estimates of environmental variables predicting progress of severity by PSHB at the individual level by including species as explanatory variable.

Variable	Estimate	SE	Relative importance	Number of models containing	Z-value	P-value
Intercept	2.187	0.781	-	-	2.762	0.006
Diameter at breast height	0.002	0.001	1.00	13	3.062	0.002
Infested host abundance	0.153	0.094	0.49	6	1.590	0.112
Species	-0.468	0.130	1.00	13	3.543	0.0004
Breeding host abundance	0.067	0.052	0.24	3	1.255	0.210
Distance from water	0.017	0.012	0.41	6	1.446	0.148
Human impact	-0.057	0.030	0.22	3	1.869	0.062
Holes at time 1	0.003	0.003	0.19	3	1.068	0.286

Relative importance: Sum of Akaike weights over all models in the top subset in which the variable was included.

Increased tree diameter was positively correlated to an increase in severity of infestations over time (Figure 2.5). As expected, host species identity also significantly affected the increase in severity of infestation between the first and the last sampling period and is likely driving much of the differences between the results of the two data sets (full data set and reduced data set). Post-hoc analyses revealed that the significance of this factor was driven by a faster than expected increase in the severity of infestations on *D. glabra*, *H. lucida* and *Q. robur* (data not shown).

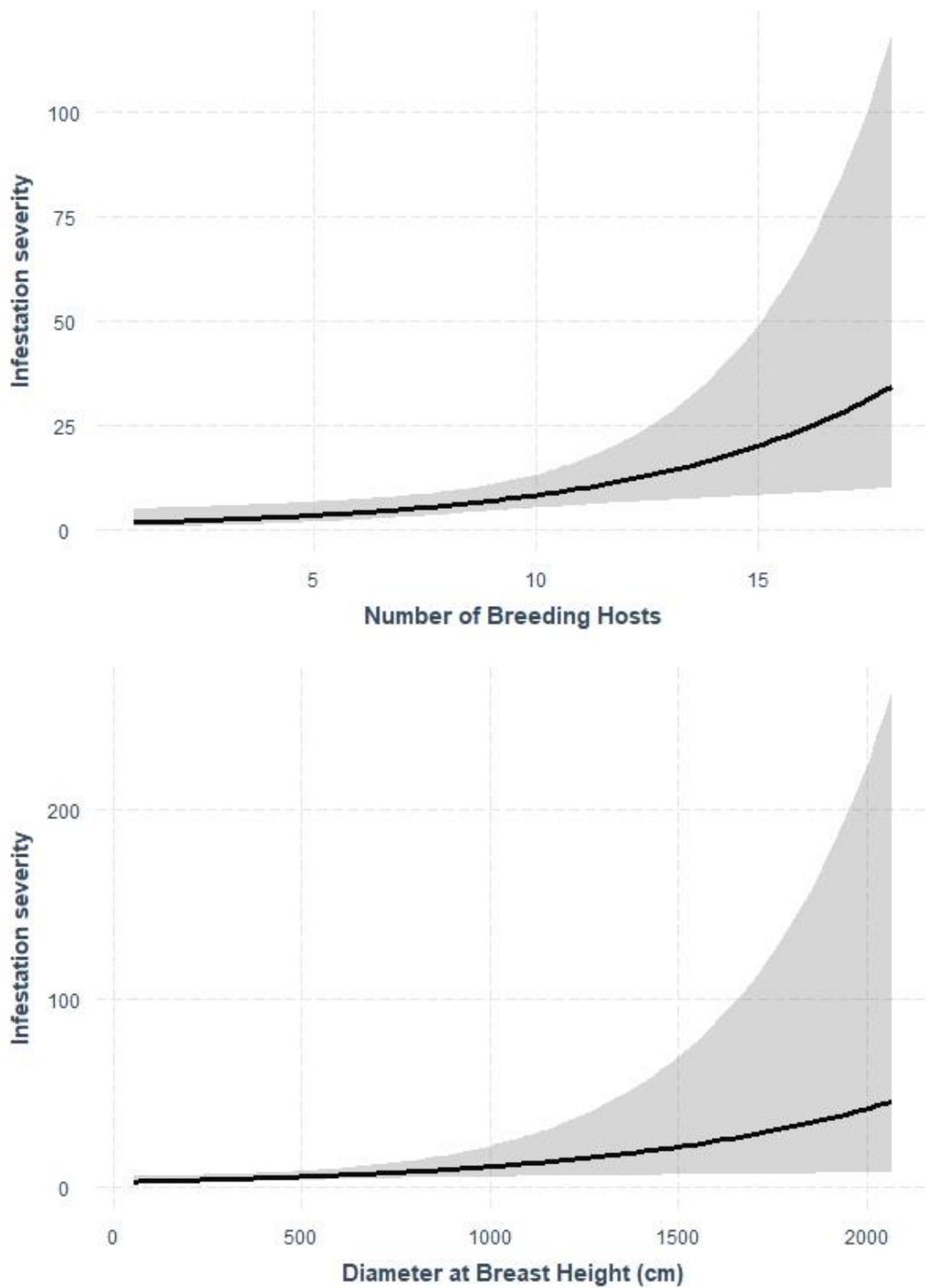


Figure 2.5: Variables that significantly affected the severity of individual trees. (a) – The effect of infestation severity (increase in holes) on the number of breeding hosts found in plot. (b) – The effect of infestation severity (increase in holes) on the diameter at breast height of trees.

2.4.6) Preferential colonization

A total of 598 tree individuals were of species that have had at least one individual with PSHB infestations in 27 plots where any infestations occurred across the sampling area. Eighty-three individuals, representing 18 species, of these were infested by PSHB. Of the 18 species that could be included in analyses, 10 species were found to significantly differ in their observed infestation probability versus what was expected by chance (Table 2.10). Eight host species were infested at probabilities that were expected by chance and does not seem to be preferentially selected for by PSHB. These included two reproductive host species and six non-reproductive host species. Eight species had a higher chance of being infested by PSHB than was expected by chance. These taxa therefore seem to be selected for by the beetle and include *Afrocarpus falcatus*, *Diospyros whyteana*, *Ekebergia capensis*, *Halleria lucida*, *Quercus robur*, *Sparrmannia africana*, *Vepris lanceolata*, and *Virgilia oroboides* subsp. *ferruginea*. Most of these are known to be breeding hosts for PSHB except *Diospyros whyteana*, *Ekebergia capensis*, and *Virgilia oroboides* subsp. *ferruginea* that has not yet been confirmed as such. Two species with signs of infestations in the monitored plots were infested at lower probabilities than expected by chance and therefore are likely selected against. This included *Burchellia bubalina* and *Rapanea melanophloeos* neither of which are known as reproductive hosts.

Table 2.10: Chi-square test table showing the sample size (n), the Chi-squared statistic, *P-value*, Yates correction *p-value* for the preferential colonization of PSHB between species.

Species	Observed infected (%)	Expected infected (%)	Expected by chance	n	Chi-square statistic	Yates corrected p-value
<i>Afrocarpus falcatus</i>	31	16	Higher	29	6.258	0.020
<i>Burchellia bubalina</i>	4	15	Lower	26	7.037	0.016
<i>Canthium inerme</i>	10	10	Same	10	0	0.45
<i>Diospyros glabra</i>	26	16	Same	65	3.014	0.118
<i>Diospyros whyteana</i>	33	21	Higher	3	7.812	0.009
<i>Ekebergia capensis</i>	30	10	Higher	10	12.5	0.001
<i>Gymnosporia buxifolia</i>	10	14	Same	71	0.758	0.514
<i>Halleria lucida</i>	46	14	Higher	28	24.381	<0.001
<i>Nuxia floribunda</i>	16	13	Same	32	0.038	1
<i>Olinia ventosa</i>	20	25	Same	5	0.717	0.498
<i>Quercus robur</i>	67	14	Higher	6	58.284	<0.001
<i>Rapanea melanophloeos</i>	3	14	Lower	243	7.779	0.011
<i>Scutia myrtina</i>	14	14	Same	7	0	0.45
<i>Searsia chirindensis</i>	25	14	Same	8	3.854	0.074
<i>Searsia lucida</i>	8	15	Same	26	2.407	0.184

<i>Sparrmannia africana</i>	43	14	Higher	7	20.636	<0.001
<i>Vepris lanceolata</i>	33	17	Higher	6	6.827	0.014
<i>Virgilia oroboides</i>	25	13	Higher	16	4.678	0.047

2.5) Discussion

The Polyphagous Shot Hole Borer (PSHB) is a recent invader in South Africa that has caused disease and death of thousands of trees globally (Chen *et al.*, 2014; Coleman *et al.*, 2019; Eskalen *et al.*, 2013; Mendel *et al.*, 2013, Chapter 1). Information on its impact on native ecosystems in its invaded range is lacking, but *E. kuroshio*, a close relative, has caused destruction of native willows (*Salix* spp.) in the Tijuana River Valley, USA (Boland, 2016) with severe impacts on ecosystem processes and resilience (Boland *et al.*, 2020). The present study represents the first to investigate the ecological factors that aid in PSHB invasion into a native forest. Numerous native tree species in Afrotropical forests were colonized by PSHB and its symbiotic fungus. Breeding PSHB colonies was evident in nearly 10% of all native species surveyed and may suffer high mortality. Organisms such as arthropods associated with these species will also be threatened as each tree species are known to support unique biodiversity (Swart *et al.*, 2020). Normal ecosystem functioning of Afrotropical forests and surrounding local economies may therefore be at risk (Umeda *et al.*, 2016). As expected, areas that were in close proximity to outbreak areas, especially those with a high abundance of reproductive hosts and in more open pioneer forest, were more likely to be invaded at this early stage in the invasion process (Choudhury *et al.*, 2020; Haas *et al.*, 2016; Holt *et al.*, 2003). However, distant sites that were frequented by tourists were also colonised by PSHB. Here, PSHB was found in climax forest that host large and mature tree individuals which, as was shown here, generally suffer from greater PSHB infestation probability and severity. The potential loss of large individuals of climax forest species such as *A. falcatus* is not only of ecological concern as the main emerging tree in these forest canopies (Geldenhuys & Von Dem Bussche, 1997; Lübbe & Geldenhuys, 1991), but social and economic impacts are expected as some individuals in the study region are South African champion trees (Golding & Geldenhuys, 2003) that generate income for surrounding poor communities as tourist attractions (Saayman *et al.*, 2012).

2.5.1) Factors that influence invasibility of Afrotropical forests

When self-dispersing, individuals move from infestation epicentres (urban environments in this study) into the surrounding forest edges (Muirhead *et al.*, 2006). As this study was conducted during the very early stages in the invasion process, it was therefore not unexpected that areas closer to infestation epicentres suffered from greater infestation levels (Grousset *et al.*, 2020; Haack *et al.*, 2010; Jacobi *et al.*, 2012; Kautz *et al.*, 2011, Chapter 1). The most likely explanation for PSHB presence in climax forest sites, sites distant from other invaded areas and the urbanised areas, is through long-distance human-mediated transport as contaminants of firewood. A high abundance and diversity of breeding hosts in both pioneer habitats and in climax forest ensures that PSHB has abundant resources across the entire southern Afrotropical forest complex, increasing its chances of establishment. PSHB also has haplodiploid mating system (Cooperband *et al.*, 2016; Stouthamer *et al.*, 2017), increasing chances of establishment even with low propagule pressure (Chapter 1).

As with other studies on the epidemiology of pests and diseases, it was found that an increase in breeding host abundance (or density) significantly correlated to an increase in the number of PSHB infestations in plots (Antonovics *et al.*, 1997; Burdon *et al.*, 1995; Carlsson *et al.*, 1990; Choudhury *et al.*, 2020; Gilbert *et al.*, 2002; Roy, 1993). A high density of hosts increases the available resources in the vicinity of a dispersing population and the chances of locating a host with minimal dispersal time. This pattern is especially concerning when considering the high densities of *D. glabra*, *H. lucida* and *V. oroboides* along southern Afrotropical forest edges (Coetsee & Wigley, 2013; Phillips, 1931). These were identified as a PSHB reproductive host (in the present study or before; Chapter 1) and may therefore suffer high mortality in the future. They are amongst the main contributors to forest succession (especially *V. oroboides*) as nursery plants for climax forest trees and protects forest interiors from harsh external factors such as fire and wind (Coetsee *et al.*, 2013; Phillips, 1926). Local decimation of these species could hold disastrous consequences for normal forest ecology and for the biota directly dependent on them (van der Colff *et al.*, 2015; Machingambi *et al.*, 2014).

Contrary to what was expected, disturbances in monitoring sites studied here, including those from natural sources or from humans, did not increase chances of colonization by PSHB (Boland, 2016; Coleman *et al.*, 2019; Keesing *et al.*, 2006). This is either an indication of the major role of human-mediated dispersal into nearly pristine areas, or to a preference for apparently healthy hosts (Kühnholz *et al.*, 2003). The former is more likely as even when PSHB

can easily infest healthy hosts (Kühnholz *et al.*, 2003), most ambrosia beetles prefer stressed trees in their native and invaded ranges (Boland, 2016; Ploetz *et al.*, 2013). This has not yet been shown for PSHB, however, if tree stress is found to be a contributing factor to PSHB epidemiology, a major natural (e.g. storm or fire) or anthropogenic disturbance (e.g. development of road or plantation forestry) in future could have catastrophic consequences. In addition, distance to surface water did not increase the invasibility of sites as was documented for *E. kuroshio* in the USA (Boland, 2016; Boland *et al.*, 2020). Climatically, Afromontane forests here differ substantially from the valley that formed part of the studies in the USA in that they receive precipitation year-round (Swart *et al.*, 2018). Moisture content of the trees in the present study, which is usually positively correlated with symbiotic fungal growth (Rudinsky, 1962), may therefore not vary considerably from the edges of surface water to deeper in forests, which may be the case for trees in the Boland & Woodward (2019) study. Host trees evaluated in the present study also likely does not have the high water requirements as expected for the riverine specialist *Salix* species that formed the focus of the studies on *E. kuroshio* (Boland, 2016; Boland *et al.*, 2020).

2.5.2) Factors that increase the severity and probability of infestation by PSHB of susceptible host individuals

Probability of infestation (whether a tree is infested or not) and severity in infestation (increase in number of entrance holes) for breeding hosts at the George Botanical Garden were positively correlated with tree size, even when PSHB can easily colonize small-diameter host individuals (Coleman *et al.*, 2019; Eskalen *et al.*, 2013). Coleman *et al.*, (2019) found no significant difference in DBH between PSHB infested and non-infested trees in the invaded range in California, but the opposite was found in the native range in Southwestern China. The DBH of trees infested by *E. kuroshio* were also larger than that of uninfested trees in California (Coleman *et al.*, 2019). Probability of infestations generally increases with tree size for these species, likely because larger hosts are easier to find in the landscape (Choudhury *et al.*, 2020; Mayfield *et al.*, 2013). As PSHB is a diurnal-active species, it probably uses visual cues (host silhouette diameter) along with kairomones to locate potential hosts as was found for the redbay ambrosia beetle (Fraedrich *et al.*, 2008; Frank & Ranger, 2016; Mayfield *et al.*, 2013). Severity of infestations (number of holes) on hosts may also increase on larger trees due to the ease of finding these, but also due to higher availability of abundant resources i.e. a greater volume of xylem which allows for construction of longer galleries and more brood production (Mayfield

et al., 2013; Ranger *et al.*, 2016). Larger trees are also some of the first attacked (and have larger densities of beetle populations) for other ambrosia beetles such as *Xyleborus glabratus* (Kendra *et al.*, 2013). Whether this would translate into these larger tree individuals suffering symptoms first as was documented for *X. glabratus* on Laurence remains to be seen (Kendra *et al.*, 2013; Umeda, Eatough Jones, *et al.*, 2019), but some evidence suggest that smaller trees are at higher risk of rapid death due to PSHB infestations in California (Coleman *et al.*, 2019).

The number of infested hosts in a plot also increased both the likelihood of infestations and the severity of infestations by PSHB. This is to be expected as, a high propagule pressure will exist with a large number of infested tree individuals. As expected, the severity of infestations also increased with an increase in breeding host abundance, as these are particularly good for rapid increase in beetle population numbers. Higher dominance of non-breeding hosts in the environment may therefore suppress the spread of future infestations (Keesing *et al.*, 2006; Prospero *et al.*, 2017). As breeding hosts succumb to PSHB, the ratio of non-breeding and non-hosts will increase, possibly leading to an “infestation-plateau” or “infestation carrying-capacity”. Long-term monitoring of the plots set out in the current study is therefore highly recommended to determine at what level infestation rates may significantly decrease. Based on evidence in the current study, this level might be very low and high infestation levels are therefore expected across the entire southern Afrotropical forest complex. Additionally, if the infestation continues to expand, some of the current non-reproductive host tree species might become more suitable to sustain beetle breeding due to continued colonization by its symbiotic *F. euwallaceae* fungus as beetles attack almost indiscriminately when propagule pressure is high (Eskalen *et al.*, 2013).

As was found for PSHB infestation at the plot level, an increase in tree injury level (human or otherwise) and distance to human activities (e.g. roads) did not increase the likelihood of infestations and the severity of infestations. Therefore, PSHB seemingly infests native hosts in these forests regardless of their health status (Umeda *et al.*, 2019), increasing chances of their unremitting spread into pristine habitats. More surprisingly, results indicated that distance to nearest infested breeding host did not increase the likelihood of infestations and the severity of infestations as expected (Choudhury *et al.*, 2020; Mendel *et al.*, 2017; Wichmann *et al.*, 2001). This result was echoed when considering inter-tree distances between infested breeding hosts to nearest healthy and infested conspecific host, any breeding host and any non-breeding host. In fact, distance to nearest healthy hosts were always significantly smaller than to infested hosts. This is partly explained by the preference of PSHB to colonize large tree individuals and

those of any health status. However, results indicated that PSHB also preferentially selected for certain plant species in plots. Eight out of the 19 tree species that was confirmed to be colonised by either PSHB or *F. euwallaceae* had a higher number of infested individuals than that expected by chance. Six of these were confirmed as breeding hosts here (*A. falcatus*, *H. lucida*, *Q. robur* (exotic), *S. africana*, and *V. lanceolata*) or before (*Virgilia oroboides*, Chapter 1). The other two (*D. whyteana* and *E. capensis*) are suspected PSHB breeding hosts but could not yet be confirmed. PSHB therefore finds these hosts suitable, leading to a higher invasion success (Lantschner *et al.*, 2020). *Diospyros glabra* and *G. buxifolia*, although breeding hosts, were infested seemingly at random and all individuals of these species (irrespective of size or health status) may be easily colonised by the beetle. Two host species (*B. bubalina* and *R. melanophloeos*) had lower numbers of colonised individuals than that what was expected by chance. PSHB may therefore usually avoid these, or usually have unsuccessful attempts at reaching their vascular tissues for fungal colonization. Even though the fungus can colonize the vascular tissues of these, the beetle is unable to capitalize on this indicating that they, along with all other plant species that did not sustain PSHB or its symbiont throughout this study, may produce allomones with effective activities against initial attack by the beetle (and not the fungus). All other taxa that were able to host *F. euwallaceae*, but not PSHB, were infested by PSHB at random. These therefore likely do not produce allomones that deter initial attack by PSHB but can deter its colonization even after establishment of *F. euwallaceae*. These hosts may therefore still be vulnerable to fusarium wilt (Bonilla-Landa *et al.*, 2018).

2.5.3) Progression of infestation

At the plot level there were only 29 additional trees infested after the year of monitoring. Not surprisingly there were no factors at the plot level that could indicate reasons for this increase as sample size was too low. This slow increase is likely due to the early stage of invasion in which this study took place and rapid increase in the rate of infestations are expected over time (Chen *et al.*, 2014; Stouthamer *et al.*, 2017). The severity of infestation did however increase on many host individuals, and especially for some of the breeding hosts (*D. glabra*, *H. lucida* and *Q. robur*). In addition to host identity, tree size was positively correlated to the increase in infestations adding credence to the hypotheses that larger hosts are more easily found or can sustain higher populations. When the overwhelming effect of host species on increase in infestations was not included in analyses, the number of breeding hosts were positively correlated to infestation rate, as expected under the same aforementioned hypotheses as both

host size and host density relates to the number of available resources and the ease of how to find it. However, an increase in canopy cover was negatively correlated with decreased infestation rate. Higher populations of ambrosia beetles are usually found at forest edges (Igeta *et al.*, 2004). The canopy layer of the forest protects the internal forest layer from direct solar radiation (Geldenhuys, 1992). Higher temperatures at forest edges and in pioneer forest where canopy cover is low might improve climatic conditions for PSHB into its optimal breeding range (24 to 30°C) (Umeda & Paine, 2019) or increase the ease in which it can spread (Mendel *et al.*, 2017; Rassati *et al.*, 2016). The first severe impacts of PSHB in these forests will therefore likely be realised in these pioneer communities that will have negative indirect impacts on climax forest. These edges will also sustain large breeding populations of PSHB that will easily move into climax communities. In the process, more open canopies will be created (from dead or diseased trees), forming a positive feedback loop.

2.5.4) Management considerations

Tree mortality during this one year survey was very low (only two trees have died due to beetle activity), however, a high number of mortalities is expected over time, especially for the reproductive hosts. Early detection, management and control of infestations are vital to mitigate future impacts (Rabaglia *et al.*, 2008). This study identified some of the hosts that are currently most at risk during this early stage of infestation and these can form the basis of programs designed to monitor the presence and movement of PSHB throughout this forest complex. Focussing on areas close to urban fringes and in forests where visitors frequent would increase the likelihood of early detection. Once detected, management will be very difficult as removal of highly colonized reproductive hosts would not be possible (due to access problems or environmental sensitivity) or very expensive (Umeda *et al.*, 2016). This is however still an option and should be encouraged, in the urban areas next to these forests. As was shown in the present study, an increase in infested host abundance increased both the likelihood of infestations and the severity of infestations. Therefore, as was previously recommended (Chen *et al.*, 2020, Chapter 1), removal of heavily infested (< 10 holes as nearly all of these showed increased attacks) reproductive hosts in urban areas will reduce propagule pressure, not only on the native forests, but also in the urban areas themselves. Infested wood needs to chipped (<5 cm) and solarized (Chen *et al.*, 2020; Jones & Paine, 2015). For native forests, the development of a biological control agent would be essential as chemical control is not an option. These studies have now been initiated in the USA (Stouthamer pers. com.) and in South

Africa, but it may take a long time to develop an effective agent. In the meantime, all movement of wood into these forests should be halted as this is the most likely pathway for infestations at new sites, especially in deep forest. Using locally-sourced wood is not an option due to the high value of native trees and the proximity of PSHB populations. It is therefore recommended that only charcoal-based products are allowed into these forests by visitors.

2.6) References

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Chapter 3

Future carbon storage- and economic losses due to invasion of an Afrotropical forest complex by the Polyphagous Shot Hole Borer beetle in South Africa

3.1) Abstract

Invasive pests have major impacts on native forests by altering tree species composition, forest succession and nutrient cycling. This has lasting economic and social impacts such as decreased carbon storage value and decreased value of provisioning, regulating and cultural services. Here the future carbon storage loss and associated economic impact due to the recent invasion of an Afrotropical forest complex by the Polyphagous Shot Hole Borer beetle (PSHB), *Euwallacea fornicatus*, in South Africa were estimated. Data on current carbon storage of trees and PSHB infestation levels were collected in 51 plots across the ca. 55 000 ha Knysna/Tsitsikamma forest complex and used to estimate future carbon storage- and economic loss due to tree mortality. 18 tree species in 27 plots were affected by PSHB. Total carbon storage in trees on all plots was ca. 136 Mg, amounting to ca. 14.48 Mt across the entire forest complex. Using low approximations of tree mortality, it was estimated that ca. 2 000 kg carbon will be lost/released in the 27 plots when only a small proportion of currently infested trees succumb. When the beetle has spread throughout the entire forest complex, ca. 1.2 Mt of the stored carbon will be lost/released. Current economic value of the forest complex is estimated at ca. \$ 241 780 818 (R 3.55Bn). Therefore, ca \$ 20 540 841 (R 301 266 352) (8.5%) of this may be lost due to future PSHB impact. If not mitigated, the PSHB beetle invasion into natural forests, and the Afrotropical forest in particular, may therefore have much more substantial ecological and economic impacts than previously estimated. Curbing the spread of this invasive pest into natural ecosystems should therefore be a priority.

3.2) Introduction

Increases in global atmospheric CO₂ concentrations disrupts ecosystem carbon balance and is a major threat for climatic stability (Beedlow *et al.*, 2004). Ensuring normal carbon sequestration in forest environments is one of the most important ways to mitigate climate change (Yohannes *et al.*, 2015), as living trees are an important atmospheric carbon sink (Backéus *et al.*, 2005; Dugan *et al.*, 2018). In addition, forests are also biodiversity hot spots (Chazdon & Uriarte, 2016; Funk *et al.*, 2019), and are have high economic and social value (Ashton, 1969). For example, forests provide goods and services including timber and non-wood products for fuel as well as food and medicine (Streck & Scholz, 2006). Forests also regulate local climatic conditions, providing regional cooling that maintain moisture supply to agricultural lands (Lawrence & Vandecar, 2015). Unfortunately forests are under threat from human development, agriculture, deforestation and wildfires (Curtis *et al.*, 2018). Many forests are now so severely degraded that their abilities to sequester carbon are at risk (Ioki *et al.*, 2014).

In addition to humans, insect pests can cause serious harm to tree health and lead to forest degradation. Changing local climates alter population dynamics of native insects and can facilitate the spread and establishment of non-native pest species (Hunt *et al.*, 2006). Insect outbreaks in forests, whether native or non-native, can result in large scale dieback and, given that trees are relatively large and long-lived, the loss of only a few can have major impacts on forest structure (Gower, 2003; Roy *et al.*, 2014). However, exotic pests are usually more threatening to forest ecosystems than outbreaks of native species (Di Castri, 1989; Pimentel, 1986), and in many cases even more threatening than major abiotic disturbances (e.g. fire and wind), because they often target certain species, lack natural control and can therefore continuously increase their effect on target species (Lovett *et al.*, 2006). For example, the non-native *Xyleborus glabratus* (Curculionidae, Scolytinae) has nearly eradicated plants in the Lauraceae in south-eastern North America (Hughes *et al.*, 2015). As tree pests become a more serious problem worldwide (Evans & Finkral, 2010; Wingfield *et al.*, 2010), there is increasing concern on how these may impact not only ecosystem biodiversity and ecology, but also carbon sequestration and, by implication, their economic and social benefits (Aukema *et al.*, 2011; Pimentel, 2001; Simberloff, 2000). When mortality of mature forest trees increases due to pest disturbances, carbon is released from the tissues into the atmosphere (Chambers *et al.*, 2001) and forest structure can shift to predominance of younger trees that store less biomass (Fleming,

2000; Kasischke *et al.*, 1995). The interacting effects of a changing climate on host trees and the increasing arrival of non-native pests worldwide is making it increasingly important to predict the impact of non-native insect pests on forest trees (Brockerhoff & Liebhold, 2017; Gower, 2003).

The Polyphagous Shot Hole Borer beetle (PSHB), *Euwallacea fornicatus* (Scolytinae), is native to South-East Asia (Stouthamer *et al.*, 2017), but has invaded agricultural, urban and native systems in California, Hawaii and Israel (Mendel *et al.*, 2012; Rabaglia *et al.*, 2006; Rugman-Jones *et al.*, 2020). The pest has recently also been detected in South Africa (Paap *et al.*, 2018; Stouthamer *et al.*, 2017) where currently it forms the largest global outbreak. It colonises trees and inoculates these with its fungal symbionts including the pathogen *Fusarium euwallaceae* (Hypocreales: Nectriaceae). This fungus serves as the main food source for the beetles (Eskalen *et al.*, 2013, Mendel, *et al.*, 2013; Freeman *et al.*, 2013; O'Donnell *et al.*, 2015) and can colonise woody tissues of many plant taxa. As a result, PSHB can infest a wide number of tree species (Umeda *et al.*, 2016), and it has one of the widest host ranges for any forest pest with host species recorded in more than 50 plant families globally, many of which can succumb due to the actions of the beetle/fungus system (Boland, 2016; Eskalen *et al.*, 2013, Stouthamer, *et al.*, 2013; Lynch *et al.*, 2020). Even though recently established, the fungus has been isolated from more than 100 different plant species in South Africa and the beetle can colonize (and likely kill) at least 30 of these (Chapter 1). The most significant current global impacts of the PSHB invasion are in urban and agricultural environments, but the PSHB is also starting to move into native environments. This will undoubtedly have massive economic and social impacts. For example, a recent estimation of impacts of this invasion indicates a baseline social cost of \$ 27.12bn to South Africa, ca. 1% of the country's GDP (de Wit *et al.*, 2021, unpublished data). Most of this cost will be needed to mitigate impacts in urban environments, but between US\$ 107 and US\$ 138 million loss is expected for native South African forests. This was however based on only very limited data and using a low estimated tree mortality of 5% (de Wit *et al.*, 2021, unpublished data).

While the infestation continues to rise in the urban environments of South Africa, we have little idea of the impacts that a largescale outbreak of PSHB will have on native forests (Paap *et al.* 2018; Chapter 2). Most reliant data have been collected in very recently invaded areas of Afrotropical forests along the south coast in the Knysna/Tsitsikamma forest complex (Chapter 2). About 21 of the globally targeted plant families also occur in Afromontane forests in South Africa (Geldenhuys, 1992), and at least 18 (one exotic) species in 13 families have

been already been recorded to host the fungus in this complex (Chapter 2). The beetle is able to breed in many of these species and mortality in these hosts are expected to be high (Eskalen *et al.*, 2013, Stouthamer, *et al.*, 2013; Owens *et al.*, 2018). Invasion of these forests by PSHB may therefore exacerbate the already high impacts of anthropogenic pressure on biodiversity and ecosystem function in these sensitive systems (Foley *et al.*, 2007; Geldenhuys, 1991). For example, the local extinction of important tree species due to targeted attacks by PSHB will change biotic turnover, which will lead to a change in functional diversity, complexity, vegetative biomass, and carbon storage (Guariguata & Ostertag, 2001). As mature (climax) forest stands often have larger and more established climax tree individuals, and pioneer (edge) forest stands often have pioneer species consisting of smaller trees, and less established climax tree species (Geldenhuys, 1991; Matlack, 1994), impact on carbon sequestration is expected to be more severe in climax forest communities.

The main aim of this study was to estimate the possible future impact on 1) carbon storage and 2) social costs, of PSHB invasion into an Afrotropical forest complex in South Africa. Allometric models were used to calculate the carbon biomass of trees in both edge and mature forest stands and use current PSHB infestation data to more accurately predict future losses in the abovementioned parameters. It was hypothesised that mature forest stands will have more carbon biomass and therefore a higher carbon stock than forest edges (pioneer communities), but that forest edges will experience a greater loss of carbon biomass due to higher infestation rates. This, as forest edge habitat is in closer proximity to outbreak areas in urban environments.

3.3) Materials and Methods

3.3.1) Study area

I used data collected from monitoring plots set up from the Outeniqua to Tsitsikamma forests and surrounds as described in the previous chapter (Chapter 2). These data were collected between June 2019 and September 2020, in an effort to assess the impact and potential effects of PSHB on Southern Afrotropical forests in the Southern Cape of South Africa. The area has only recently been invaded by this pest (likely around 2014) and the PSHB beetle has only started to infest some of the native vegetation. The study area ranged from George (33.9425 S, 22.4613 E) in the west to Tsitsikamma (33.9641 S, 23.8997 E) in the east. The area is characterised by a mosaic of Afromontane forest and moderately tall shrubby fynbos (Mucina *et al.*, 2006). A total of 906 forest patches with an estimated total of 60561 ha occur in the

Southern Cape (Geldenhuys 1991). The climate is a transitional to the subtropical/tropical and temperate regions (Gadow *et al.*, 2016). It receives all-year rainfall which peaks in March, October and November (Geldenhuys, 1991; Phillips, 1931), but patches can vary in climate. The overall area receives <1500 mm rain annually (varies between 700 mm to 1230 mm) with mean maximum temperatures 23.8°C for February to 18.2°C for August (Geldenhuys, 1991; Rutherford & Westfall, 1986).

3.3.2) Data Collection

In total there were 51 plots spread across 12 sites in Southern Cape forests with each plot 10 m x 15 m (150 m²) in size. Each tree (trees larger than 30 mm circumference/7 mm diameter) in the plot were identified to species. Each plot was categorized as “mature” (average diameter of trees in plot \geq 10 cm) or “edge/pioneer” (average diameter of trees in plot \leq 10 cm) based on how developed the trees were and was representative of either mature forest habitat or forest edge habitat. Pioneer species are species whose seeds can only germinate in forest gaps or at the edge and were common in edge plots. Climax/mature species are those whose seeds can germinate in full shade (Swaine & Whitmore, 1988) and were dominant in mature plots. Plots were therefore classified with trees of large average diameter (dominated by old-growth flora) as “mature” as there is little sunlight penetrating through the canopy (Santos *et al.*, 2008). In total, 24 plots were characterised as edge (pioneer) forest habitat and 27 plots represented mature forest (Addendum B). For each tree individual in the plots, the species was determined, diameter measured at breast height (~1.3 m) and recorded whether it was infested by PSHB. For individuals that were multi-stemmed, each stem of the individual was measured. Infested refers to the presence of at least one hole of PSHB as described in Chapter 2. Whether the host was a breeding host (in which the beetle can reproduce), non-breeding host (in which the beetle cannot reproduce but successfully transfer its fungal symbionts) or non-hosts (in which neither the beetle nor the fungus was ever confirmed) was documented as outlined in Chapter 2. For each tree species data was obtained on its wood specific gravity (g.cm⁻³) from the Global Wood Density and African Wood Density databases (<http://db.worldagroforestry.org/> and <http://apps.worldagroforestry.org/treesandmarkets/wood/>). The inclusion of wood density data in allometric equations generally improves estimates of carbon stocks (Mensah, Veldtman, & Seifert, 2016). For tree species that lacked wood specific gravity information in these databases, genus or family-level averages were calculated (Table 3.1). For *Elaeodendron*, *Olinia* and *Pterocelastrus* wood densities were compiled from closely related species. Tree

height was not determined due to difficulties in working in these dense and diverse forest systems. It is also not a prerequisite for fairly accurate estimations of tree carbon storage (Williams & Schreuder, 2000).

3.3.3) Calculation of carbon storage

Aboveground biomass of trees in forests is commonly inferred from ground census data (Chave *et al.*, 2015) as implemented in allometric models using input from tree biometric measurements (Brown 1997). However, there are very few aboveground and belowground carbon assessments for forests in South Africa, mainly due to the small size of woody biomes in the country (Rahlaa *et al.*, 2012). To calculate aboveground carbon storage for each tree individual, three equations commonly applied in tree allometry studies were used. For tree individuals that were multi-stemmed, carbon storage for each stem was individually calculated and added their totals.

Equation 1: The aboveground biomass was estimated for each individual following the general allometric model developed by Chave *et al.*, (2005):

$$AGB = p \times \exp (-0.667 + 1.784 \ln(D) + 0.207(\ln(D))^2 - 0.028(\ln(D))^3)$$

Where AGB , p , and D represent aboveground biomass (kg), wood density (g.cm^{-3}) and diameter (cm) at breast height, respectively.

Equation 2: Aboveground biomass was estimated for each individual using only diameter at breast height data following the allometric model developed by Mangwale *et al.*, (2017):

$$AGB = 0.127(D)^{2.335}$$

Equation 3: Aboveground biomass was also calculated following the equation developed by Mewded & Lemessa, (2019) by only using data on tree diameter at breast height:

$$AGB = 34.4703 - 8.0671(D) + 0.6589(D)^2$$

Belowground biomass for each tree individual was estimated following the regression equation developed by Cairns *et al.*, (2003) which estimates below ground biomass (BGB) from aboveground biomass:

$$BGB = \exp (-1.085 + 0.926 \ln(AGB))$$

Both aboveground biomass and below ground biomass values generated were multiplied by 0.5 and added together to convert it to woody carbon mass for the entire tree individual (Janetos *et al.*, 1997).

3.3.4) Comparison between aboveground biomass indices and choice of model

To assess the difference in aboveground biomass values estimated from the three above ground biomass estimators, an ANOVA was used to test the null hypothesis of no differences in mean above ground biomass estimated using total above-ground carbon storage estimated per plot (i.e. all individuals per plot) after testing for normality with a Shapiro-Wilks test in R (R Development Core Team, 2020).

3.3.5) Possible impact of PSHB on mature forest vs. edge habitat

I first calculated the total standing carbon (including above and below ground) per plot using data collected for each tree individual. Hereafter total standing carbon was compared between mature forest and forest edge habitats (pioneer) using a Mann-Whitney U test in order to determine which habitat type is more important in terms of carbon storage per unit area. How much of each habitat type is present in the area defined as the Garden route between George (33.9425 S, 22.4613 E) in the west to Tsitsikamma (33.9641 S, 23.8997 E) in the east was determined from satellite imagery (Google earth: <https://www.google.com/earth/>) and forest region data (Holness & Bradshaw, 2010). The total area covered by indigenous forest and the length (perimeter) of the edge habitat of these forests was determined using the polygon tool in the application. In the Garden Route forests, Swart *et al.* (2018) found that arthropod assemblage composition changed at 20 m from the forest edge. Watkins *et al.* (2003) found rapid changes in understorey plant assemblage occur at 30 m from the forest edge, and Wagner (1980) concluded stem density and regeneration changes were found at 50 -70 m from the edge. In Douglas-fir forests, Chen *et al.* (1992) found microclimate patterns to influence edge up to 240 m. Many authors found that there is variability in edge patterns across different forest types and regions, edge was therefore standardized at 50 m by considering results from studies in Afrotropical forests. The length of the edge habitat was therefore multiplied by 50 m in order to obtain the total estimated area of forest edge (pioneer) habitat in the region. This only accounts for the margins of forest edge and not the inner edges caused by canopy gaps, rivers and other disturbances. The total amount of carbon in both mature and edge habitat types across

the region was therefore estimated, assuming that tree composition and size was fairly consistent.

In order to calculate possible impact of PSHB on carbon storage in these Afrotropical forests survey data was used to determine the current infestation levels of PSHB on each tree individual and species in plots that have at least one tree with PSHB infestation (i.e. in plots in which the beetle has been confirmed) in order to minimize skewing of data due to the beetle not present in all plots yet (Chapter 2). Given that PSHB is a recent invader in the country, the actual impact of PSHB on each tree species is not known. Many breeding-host tree species in urban areas can succumb to the beetle quite rapidly (1 – 10 years) (e.g. *Q. robur*, *Platanus x acerifolia*, and *Acer* species) and may suffer nearly 100% mortality. Other species may take much longer to succumb and only a small proportion of individuals may die. This is especially true for non-breeding host species. The present study therefore used the precautionary principle to estimate current and future carbon storage loss that may be caused by PSHB invasion into these forests.

For short-term impact it was estimated (based on expert opinion) that ca. 25% of currently infested breeding host species will succumb to beetle infestations and that 3% of currently infested non-breeding hosts will succumb (Prof. F. Roets, Prof. Z.W. de Beer, Dr. T. Paap). Using data from field surveys calculations included (1) carbon loss estimated per species that is currently infested by adding the carbon mass of all individuals and multiplying it by either 25% or 3% depending on host status and (2) the total estimated carbon storage lost due to PSHB in each infested plot by also multiplying the carbon weight of all infested individuals per plot by either 25% or 3% (depending on host status) and adding these. It was hypothesised that the carbon loss impact of the beetle will in the short-term (time 1) be greater in forest edge habitats than in mature forest, given the proximity of edge habitats to urban and anthropogenically altered areas (Chapter 2). This hypothesis was tested by conducting a Mann-Whitney U test using these plot data between estimated carbon losses in mature forest plots vs. those of forest edge plots.

The total carbon storage lost in these forests over the long term was estimated given the minimal data currently available. Since its first arrival in the area ca. in 2014 (6 years ago) the PSHB beetle has only started to invade natural systems recently. It is therefore likely that there will be an increase in (1) percentage of individuals of a species currently infested with PSHB in plots, (2) increase in the number of individuals that will ultimately succumb due to

infestations (due to increased number of attacks on these individuals) and (3) an increase in area of extent of current infestation over time (due to spread). The precautionary principle was again used, and it was estimated that at the height of forest infestation that there will be (1) at least a doubling of the current percentage of individuals of each species infested with PSHB per plot across these forests (with a maximum of 100%), (2) that at least an average of 40 % of breeding hosts and 5% of non-breeding hosts will succumb, and that the beetle would have spread to all forest areas in the region. It was assumed that no additional breeding host or non-breeding host taxon will become evident during this time even though this is highly unlikely. Using these data, total estimated carbon storage loss across the region and in the two habitat types respectively could be calculated using area of extent data.

Using data from field surveys the following were estimated (1) carbon loss expected per species (and per individual) across all forested areas by using data on the carbon mass of all individuals in monitoring plots (area of 4050 m² and 3600 m² of edge and mature forest respectively) in relation to the total forested area (and between the two forest habitat types) and subtracting the appropriate percentage for projected PSHB loss and (2) the total estimated carbon storage lost due to PSHB across the study area (and between the two forest habitat types) by also subtracting the appropriate percentage carbon weight of all individuals of all impacted species estimated to occur within the region. It was assumed that tree species composition was constant in each habitat type across the sampling region. It was hypothesised that the carbon loss impact of the beetle will, in the long term (time 2), be greater in mature forest habitats than in forest edge habitats, given its affinity towards larger tree individuals (Chapter 2) that abound in mature forests. This hypothesis was tested by conducting a Mann-Whitney U test on tree assemblage data collected in each plot, across all plots surveyed in the current study (not just those that showed presence of PSHB infestations).

3.3.6) Estimated cost of PSHB invasion.

The monetary value of trees in natural forests have not been determined. This is a complex task as many factors need to be assessed including; carbon value, the value of provisioning services, regulating services, habitat services and cultural services (Holmes *et al.*, 2009; Turpie *et al.*, 2017; de Wit *et al.*, 2021, unpublished data). A study by de Wit *et al.* (2021, unpublished data) estimated the per hectare value of South African forests based on international studies (Brenner *et al.*, 2010; De Groot *et al.*, 2012, 2013) using the benefit-transfer method as between US\$ 6 642.87 per hectare and US\$ 2 111.23 per hectare with a mean of US\$ 4 380 per hectare. Using

this mean value and the estimated percentage loss of natural forests calculated in the present study (differentiating between edge and mature forest), the expected loss in monetary value due to PSHB invasion in the Garden Route research area of the current study could be estimated.

3.4) Results

3.4.1) Comparison between aboveground biomass indices and choice of model

There was no significant difference between the mean estimated biomass per equation ($F=0.002$; $df=2$; $p=0.998$). The estimations as calculated following Chave *et al.* (2005), Equation 1) was therefore used as this equation also incorporated wood density data. To this the estimated below ground biomass was added and the total mass of carbon stored per tree individual and per species in the monitoring plots was calculated (Table 3.1).

Table 3.1: All tree species encountered across all 51 monitoring plots in this study. Represented is the mean carbon mass (kg) (aboveground and belowground biomass) across all individuals measured per species in all the plots, whether it is a breeding host (**BH**); non-breeding host (**NB**); or a non-host (NH), its wood density (WD) and the source of where the wood density data was obtained/compiled from. Also provided is expected percentage carbon lost for each species (and in each habitat type) that is currently infested in these plots, assuming that 25% of breeding hosts and 3% of non-breeding hosts will succumb in the short term.

Species	Mean Carbon mass (kg)	Mean Carbon Loss (kg)	% C Loss Mature	% C Loss Edge	Host Type	WD (g.cm ³)	Source of WD
<i>Acacia mearnsii</i>	1.31	0	0	0	NH	0.6632	GWD
<i>Acacia melanoxylon</i>	109.49	0	0	0	NH	0.5381	GWD
<i>Acokanthera oppositifolia</i>	0.26	0	0	0	NH	0.784	GWD
<i>Afrocanthium mundianum</i>	25.25	0	0	0	NH	0.6431	Average from <i>Canthium</i> genus
<i>Afrocarpus falcatus</i>	389.98	291.76	27.71	6.78	BH	0.52	AWD
<i>Allophylus decipiens</i>	22.44	0	0	0	NH	0.4942	Average from <i>Allophylus</i> genus
<i>Apodytes dimidiata</i>	184.35	0	0	0	NH	0.61	GWD
<i>Buddleja saligna</i>	64.98	0	0	0	NH	0.753	Estimate from Family level
<i>Burchellia bubalina</i>	7.12	30.80	0.35	0	NB	0.71	Estimate from Family level
<i>Canthium inerme</i>	46.3	92.25	0	3.84	NB	0.6431	Average from <i>Canthium</i> genus
<i>Carissa bispinosa</i>	2.18	0	0	0	NH	0.95	AWD

<i>Cassine peragua</i>	59.31	0	0	0	NH	0.754	Estimate from Family level
<i>Cassine schinoides</i>	13.56	0	0	0	NH	0.72	Estimate from Family level
<i>Celtis africana</i>	24.2	0	0	0	NH	0.7448	GWD
<i>Chionanthus foveolatus</i>	2.14	0	0	0	NH	0.6785	Average from <i>Chionanthus</i> genus
<i>Clutia pulchella</i>	3.03	0	0	0	NH	0.58	Estimate from Family level
<i>Crassula ovata</i>	3.15	0	0	0	NH	0.24	Estimate from Family level
<i>Cunonia capensis</i>	91.31	0	0	0	NH	0.6598	GWD
<i>Curtisia dentata</i>	139.14	0	0	0	NH	0.7802	GWD
<i>Diospyros dichrophylla</i>	70.39	0	0	0	NH	0.7583	Average from <i>Diospyros</i> genus
<i>Diospyros glabra</i>	12.58	23.00	0	16.28	BH	0.7583	Average from <i>Diospyros</i> genus
<i>Diospyros whyteana</i>	38.43	0	1.27	0	NB	0.7583	Average from <i>Diospyros</i> genus
<i>Dovyalis rhamnoides</i>	1.02	0	0	0	NH	0.579	Average from <i>Dovyalis</i> genus
<i>Ekebergia capensis</i>	226.47	951.01	31.63	1.73	NB	0.5016	GWD
<i>Elaeodendron croceum</i>	49.83	0	0	0	NH	0.7147	<i>Ilex crocea</i> mean
<i>Euryops virgineus</i>	4.79	0	0	0	NH	0.5	Estimate from Family level
<i>Ficus burtt-davyi</i>	18.72	0	0	0	NH	0.441	Average from <i>Ficus</i> genus
<i>Ficus sur</i>	17.51	0	0	0	NH	0.48	AWD
<i>Gardenia thunbergia</i>	1.59	0	0	0	NH	0.6721	Average from <i>Gardenia</i> genus
<i>Gonioma kamassi</i>	35.66	0	0	0	NH	0.7773	GWD
<i>Gymnosporia buxifolia</i>	25.12	8.77	0.14	2.04	BH	0.55	Average from <i>Gymnosporia</i> genus
<i>Halleria lucida</i>	38.13	43.43	2.98	12.49	BH	0.715	GWD
<i>Harpephyllum caffrum</i>	220.81	0	0	0	NH	0.5123	Average from Family
<i>Ilex mitis</i>	123.75	0	0	0	NH	0.5719	GWD
<i>Kiggelaria africana</i>	60.63	0	0	0	NH	0.633	GWD
<i>Maytenus acuminata</i>	0.61	0	0	0	NH	0.6265	GWD
<i>Maytenus peduncularis</i>	1.17	0	0	0	NH	0.632	GWD
<i>Mystroxylon aethiopicum</i>	0.9	0	0	0	NH	0.678	GWD
<i>Nuxia floribunda</i>	174.04	38.86	1.12	3.95	NB	0.646	GWD
<i>Ochna arborea</i>	8.49	0	0	0	NH	0.913	GWD
<i>Ocotea bullata</i>	509.54	0	0	0	NH	0.6528	GWD
<i>Olea capensis</i> subs. <i>macrocarpa</i>	54.83	0	0	0	NH	0.8051	GWD

<i>Olinia ventosa</i>	242.7	687.49	0	28.63	NB	0.79	Average of <i>Plectonia</i> genus
<i>Osteospermum moniliferum</i>	0.59	0	0	0	NH	0.3	GWD
<i>Pinus pinaster</i>	0.57	0	0	0	NH	0.4505	Average from <i>Pinus</i> genus
<i>Pittosporum viridiflorum</i>	19.32	0	0	0	NH	0.633	GWD
<i>Platylophus trifoliatus</i>	210.53	0	0	0	NH	0.483	GWD
<i>Podocarpus latifolius</i>	17.58	0	0	0	NH	0.54	AWD
<i>Polygala myrtifolia</i>	4.04	0	0	0	NH	0.801	Average from Family
<i>Pterocelastrus tricuspidatus</i>	105.21	0	0	0	NH	0.788	Mean from <i>P.echinatus</i>
<i>Quercus robur</i>	1019.59	618.14	27.82	0	BH	0.6332	GWD
<i>Rapanea melanophloeos</i>	50.18	33.46	0.22	13.12	NH	0.7323	GWD
<i>Rhamnus prinoides</i>	7.59	0	0	0	NH	0.5793	Average from <i>Rhamnus</i> genus
<i>Rhoicissus tomentosa</i>	4.42	0	0	0	NH	0.73	Estimate from Family level
<i>Robsonodendron eucleiforme</i>	31.95	0	0	0	NH	0.721	Estimate from Family level
<i>Rothmannia capensis</i>	22.6	0	0	0	NH	0.6415	Average from <i>Rothmannia</i> genus
<i>Scolopia mundii</i>	1.03	0	0	0	NH	0.8103	Average from <i>Scolopia</i> genus
<i>Scutia myrtina</i>	27.84	37.53	0	1.56	NB	0.904	Average from <i>Scutia</i> genus
<i>Searsia chirindensis</i>	208.74	72.52	1.51	0.43	NB	0.6202	Average from <i>Searsia</i> genus
<i>Searsia lucida</i>	27.31	43.43	0.06	3.41	NB	0.6202	Average from <i>Searsia</i> genus
<i>Searsia pallens</i>	132.08	0	0	0	NH	0.6202	Average from <i>Searsia</i> genus
<i>Sideroxylon inerme</i>	134.75	0	0	0	NH	0.857	GWD
<i>Sparrmannia africana</i>	3.26	3.81	0	0.48	BH	0.56	Estimate from Family level
<i>Syzygium cordatum</i>	10.69	0	0	0	NH	0.645	GWD
<i>Trichocladus crinitus</i>	4.1	0	0	0	NH	0.632	Estimate from Family level
<i>Trimeria grandifolia</i>	23.56	0	0	0	NH	0.61	Estimate from Family level
<i>Vepris lanceolata</i>	80.3	137.96	1.68	5.26	BH	0.719	GWD
<i>Virgilia oroboides</i>	54.62	78.01	3.51	0	NB	0.7358	Average from Family

GWD – Global Wood density Database; **AWD** – African Wood density Database

Averages for genus and family wood densities were calculated from the **GWD**.

Of the 68 tree species encountered, 18 were affected by PSHB and 7 of the affected species are breeding hosts, while 11 are non-breeding hosts. For edge habitats *Olinia ventosa* (28.63 %) showed the highest percentage carbon mass loss per species followed by *Diospyros glabra* (16.28 %) and *Rapanea melanophloeoes* (13.12 %) (Table 3.1). For mature habitats *Ekebergia capensis* (31.63 %) showed the highest percentage carbon mass loss per species followed by *Quercus robur* (27.82 %) and *Afrocarpus falcatus* (27.71 %) (Table 3.1).

3.4.2) Possible impact of PSHB on mature forest vs. edge habitat

A total of 1682 individual trees of 68 species were assessed for possible infestation across the 51 monitoring plots. Out of these plots, 24 were characterised as edge (pioneer forest) plots and 27 as mature forest plots. Overall, mature habitats had a higher total carbon mass compared to edge habitats (Table 3.2). A significant difference between the mean estimated carbon mass per plot between the mature forest and edge forest sites was detected ($U = 626; p < 0.001$). Mature forest plots had a higher mean total standing carbon ($4171.97 \pm \text{SE } 914.23 \text{ kg C plot}^{-1}$) than edge forest plots ($987.50 \pm \text{SE } 97.77 \text{ kg C plot}^{-1}$).

Table 3.2: Short-term impact of PSHB on carbon mass loss of the 27 infested plots in mature and edge forest habitats. The following is given in the table: The total carbon mass (kg) of all 27 plots; the mean ($\pm \text{SE}$) carbon mass (kg) per plot; the total carbon mass (kg) loss; the mean ($\pm \text{SE}$) carbon mass (kg) lost per plot; percentage carbon lost per habitat type; total abundance of trees in habitat type; total infested abundance of trees in habitat type and percentage of infested trees per habitat type.

	Mature habitat	Edge habitat
Total C in 27 plots	36232.51	14610.9
Mean C per plot	3019.38 ± 397.40	974.06 ± 91.46
Total C Lost in 27 plots	1629.11	367.79
Mean C Lost per plot	135.76 ± 33.13	24.52 ± 6.40
%C Lost per plot	4.50%	2.52%
Total tree Abundance	312	565
Total infested tree Abundance	29	54
% Infested Trees	9.29%	9.56%

3.4.3) Short-term carbon mass loss

Only 27 of the 51 plots were infested by PSHB and out of these, 12 were recognized as mature and 15 as edge habitats. The total carbon mass loss between mature and edge forest habitats in the short term (25 % of breeding hosts and 3% of non-breeding hosts that will succumb) showed

a significant difference ($U = 161; p < 0.001$). Even though edge habitats have more infested trees and a higher abundance of trees, mature habitats will have a higher estimated mean carbon mass ($135.76 \pm \text{SE } 33.13 \text{ kg C plot}^{-1}$) loss compared to edge habitats ($24.52 \pm \text{SE } 6.40 \text{ kg C plot}^{-1}$). This equates to 4.5% C loss in mature plots and 2.52% C loss in edge plots (Table 3.2).

3.4.4) Long-term carbon mass loss

The total carbon mass loss between mature and edge habitats (all 51 plots) for prediction time 2 (40 % of breeding hosts and 5% of non-breeding hosts that will succumb) showed significant difference ($U = 520; p < 0.001$). Mature habitats had a higher expected mean carbon mass ($352.77 \pm \text{SE } 76.70 \text{ kg C plot}^{-1}$) loss per plot compared to edge habitats ($88.29 \pm \text{SE } 20.72 \text{ kg C plot}^{-1}$). Edge habitat had a slightly higher expected percentage carbon mass loss (8.94%) than mature habitat (8.46%). The total expected carbon mass loss in the long term was 11.64 Mg (9.52 Mg for mature and 2.12 Mg for edge habitats) (Table 3.3).

Table 3.3: Long-term impact of PSHB on carbon mass loss of all the 51 plots in mature and edge forest habitats. The following is given in the table: The total carbon mass (kg) of all 51 plots; the mean ($\pm \text{SE}$) carbon mass (kg) per plot; the total carbon mass (kg) loss; the mean ($\pm \text{SE}$) carbon mass (kg) lost per plot; percentage carbon lost per habitat type; total abundance of trees in habitat type; total infested abundance of trees in habitat type and percentage infested trees per habitat type.

	Mature Habitat	Edge Habitat
Total C in 51 plots	112643.17	23699.98
Mean C per plot	4171.97 ± 914.23	987.50 ± 97.77
Total C Lost in 51 plots	9524.7	2119
Mean C Lost per plot	352.77 ± 76.70	88.29 ± 20.72
% C Lost per plot	8.46%	8.94%
Total tree Abundance	825	858
Total infested tree abundance	58	108
% Infested trees	7.03%	12.59%

The total carbon mass for all 51 plots is ca. 136.34 Mg C (Table 3.4). Plot 54 had the highest total expected carbon mass loss for the short term (355.66 kg), while plot 2 had the highest expected total carbon loss for the long term (1663.72 kg) (Table 3.4). The total expected carbon mass loss for all plots and both habitats for the short term was 1996.90 kg and for the long term 11643.70 kg.

Table 3.4: All 51 infested plots categorized by Edge and Mature habitat. Table shows plot variables for carbon loss expected at time 1 (Short term) and 2 (Long term). **Abundance** - Total number (n) of trees per plot. **TCM** - Total carbon mass (kg) per plot. **CLT1** – Total carbon mass (kg) loss per plot short term. **CLT2** – Total carbon mass (kg) loss per plot long term. **%Lost1**: percentage carbon mass loss in the short term of the total carbon weight per plot. **%Lost2**: percentage carbon mass loss in the long term of the total carbon weight per plot. The sum and means of each variable are given below each habitat table.

Plot	Habitat	Abundance	TCM	CLT1	CLT2	%Lost1	%Lost2
1	Edge	35	679.80	18.02	57.69	2.65%	8.49%
2	Mature	55	3967.47	0.00	1663.72	0.00%	41.93%
3	Mature	34	2752.99	0.00	211.71	0.00%	7.69%
4	Mature	31	2039.06	157.85	505.32	7.74%	24.78%
5	Mature	13	2716.42	121.13	387.62	4.46%	14.27%
6	Mature	43	2007.31	0.00	502.52	0.00%	25.03%
8	Edge	61	539.22	11.99	38.58	2.22%	7.15%
9	Edge	50	1800.72	15.49	50.03	0.86%	2.78%
10	Edge	45	1314.42	4.22	13.64	0.32%	1.04%
11	Edge	36	887.62	0.00	23.60	0.00%	2.66%
12	Edge	21	1495.50	6.59	21.19	0.44%	1.42%
13	Edge	40	984.86	27.57	88.75	2.80%	9.01%
14	Edge	34	1319.56	6.81	21.93	0.52%	1.66%
15	Edge	45	888.44	10.04	32.18	1.13%	3.62%
16	Edge	56	662.40	7.70	24.95	1.16%	3.77%
17	Mature	23	1911.77	13.69	44.39	0.72%	2.32%
18	Mature	31	2656.77	0.00	109.75	0.00%	4.13%
19	Mature	33	1587.67	74.51	239.42	4.69%	15.08%
20	Edge	31	579.23	0.00	136.38	0.00%	23.54%
21	Edge	40	803.73	74.31	238.58	9.25%	29.68%
22	Edge	24	483.44	0.00	24.70	0.00%	5.11%
23	Edge	40	417.53	0.00	37.40	0.00%	8.96%
24	Edge	55	881.03	73.20	235.13	8.31%	26.69%
25	Edge	20	496.30	5.09	16.58	1.03%	3.34%
26	Edge	26	704.57	0.00	10.05	0.00%	1.43%
27	Edge	19	1016.96	4.72	15.30	0.46%	1.50%
28	Edge	22	962.38	64.38	206.44	6.69%	21.45%
29	Mature	42	2231.43	0.00	778.07	0.00%	34.87%
30	Mature	49	2302.26	0.00	308.70	0.00%	13.41%
31	Edge	43	1642.59	0.00	291.22	0.00%	17.73%
32	Edge	22	765.60	37.68	120.59	4.92%	15.75%
33	Edge	20	1152.34	0.00	0.00	0.00%	0.00%
34	Mature	25	1650.13	309.92	875.22	18.78%	53.04%
35	Edge	37	552.55	0.00	414.08	0.00%	74.94%
36	Mature	7	3532.32	61.45	196.75	1.74%	5.57%
37	Mature	17	4273.21	43.24	138.76	1.01%	3.25%

38	Mature	43	2581.79	0.00	19.88	0.00%	0.77%
39	Mature	26	7116.67	0.00	104.31	0.00%	1.47%
40	Mature	23	24779.54	0.00	132.76	0.00%	0.54%
42	Mature	27	3357.80	0.00	122.04	0.00%	3.63%
43	Mature	10	10252.60	0.00	204.14	0.00%	1.99%
44	Mature	45	1313.92	67.28	216.20	5.12%	16.45%
45	Mature	29	2754.09	0.00	102.17	0.00%	3.71%
46	Mature	36	5547.40	0.00	101.85	0.00%	1.84%
47	Mature	25	4227.58	30.29	96.93	0.72%	2.29%
48	Pioneer	36	2669.20	0.00	0.02	0.00%	0.00%
49	Mature	37	2380.14	0.00	290.69	0.00%	12.21%
51	Mature	14	2464.98	90.77	290.47	3.68%	11.78%
52	Mature	28	1722.42	0.00	5.34	0.00%	0.31%
53	Mature	39	5310.62	303.32	854.28	5.71%	16.09%
54	Mature	40	5204.84	355.66	1021.69	6.83%	19.63%
<i>Sum:</i>		1683	136343.15	1996.90	11643.70		
<i>Mean:</i>		33	2673.40	39.15	228.31		

3.4.5) Estimated carbon loss across Garden Route Forest Regions

The total tree inventory area of all 51 plots was 0.765 ha in size (0.405 ha in edge and 0.360 ha in mature forest plots). The total carbon mass for all plots and the corresponding habitat was used to calculate total carbon mass per hectare. The total carbon mass per hectare for edge habitats was estimated at $65.83 \text{ Mg C ha}^{-1}$ and for mature habitats at $278.13 \text{ Mg C ha}^{-1}$. The mean of both the habitats was $171.98 \text{ Mg C ha}^{-1}$. Using the mean carbon mass loss per plot per habitat type, the mean expected carbon mass loss per hectare for each was calculated, which was $5.89 \pm \text{SE } 1.37 \text{ Mg C ha}^{-1}$ for edge habitats and $23.52 \pm \text{SE } 6.16 \text{ Mg C ha}^{-1}$ for mature habitats.

The values calculated for each habitat type were used to estimate the total mass of carbon and total expected carbon mass loss per ha for each of the forest region zones, distinguishing between edge and mature habitat types (Table 3.5). First, a total of 55201.1 ha for the entire forest region in the Garden Route was estimated using Holness & Bradshaw (2010) (Table 3.5). The total size (ha) of each forest zone was used to estimate edge and mature habitats area per forest regions by multiplying each regional total area (ha) by a calculated estimation of the perimeter (50 m edge) of these regions. Mature forest was estimated to cover 51101 ha of the total land area while edge habitat was estimated to cover 4099 ha of land area. The total estimated carbon for edge habitats was 266.29 Gg and for mature habitats 10418 Gg (Table 3.5). Total estimated carbon loss across the entire forest complex for mature habitats was 1.38

Megatonnes (Mt) and for edge habitats 0.02 Mt considering if PSHB would expand into all the forest regions and only infest currently infested species across these regions.

Table 3.5: List of most of the forested zones across the Garden Route following Holness *et al.* (2010). **P** – A rough measurement of the perimeter of these forested zones. **E(ha)** – Hectare calculated for edge habitats using perimeter multiplied by 50 m edge penetration. **M (ha)** – Hectare calculated for mature habitats using perimeter. **ETC** – Total carbon mass Gigagrams (Gg) for edge habitats. **MTC** – Total carbon mass (Gg) for mature habitats **Edge TCL** – Total carbon mass lost in (Gg) for edge habitat regions using carbon mass lost data from infested plots. **Mature TCL** - Total carbon mass lost (Gg) for mature habitat regions using carbon mass lost data from infested plots.

Forested Zone	P (m)	E (ha)	M (ha)	ETC	MTC	Edge TCL	Mature TCL
Groenvlei coastal forest	21713	108.57	1713.04	7.15	476.45	0.64	40.29
Keurbooms Thicket-Forest	49932.4	249.66	2244.04	16.44	624.14	1.47	52.78
Kromrivier Thicket-Forest	16161	80.81	1197.00	5.32	332.92	0.48	28.15
Olienboomkop Grassy Fynbos-Forest	1365	6.60	0.00	0.43	0.00	0.04	0.00
Outeniqua Forest	908	4.54	1.16	0.30	0.32	0.03	0.03
Outeniqua Montane Forest	7987	39.94	273.87	2.63	76.17	0.24	6.44
Outeniqua Plateau Forest	201845	1009.23	22240.08	66.44	6185.66	5.94	523.04
Piesang River Fynbos-Forest	72546	362.73	1376.57	23.88	382.87	2.14	32.37
Storms River Thicket-Forest	20118	100.59	391.61	6.62	108.92	0.59	9.21
Tsitsikamma Coastal Plateau Forest	48429	242.15	1182.16	15.94	328.79	1.43	27.80
Tsitsikamma Dune Forest	6254	31.27	141.03	2.06	39.22	0.18	3.32
Tsitsikamma Forest Fynbos	22586	112.93	639.47	7.43	177.86	0.66	15.04
Tsitsikamma Fynbos Forest	5986	29.93	137.57	1.97	38.26	0.18	3.24
Tsitsikamma Mountain Forest	68457	342.29	1455.32	22.53	404.77	2.01	34.23
Tsitsikamma Plateau Forest	108988	544.94	11859.76	35.88	3298.57	3.21	278.92
Tsitsikamma Riverine Forest	56320	281.60	1339.70	18.54	372.61	1.66	31.51
Wilderness Forest-Thicket	32877	164.39	967.22	10.82	269.01	0.97	22.75
Wolwe River Fynbos-Forest	71220	356.10	3720.30	23.44	1034.73	2.10	87.49
Wolwedans Thicket-Forest	6211	31.06	221.95	2.04	61.73	0.18	5.22
Sum:	819903.4	4099	51101	269.87	14213.01	24.13	1201.81

3.4.6) Estimated cost of PSHB invasion.

To estimate the total cost for edge and mature habitats for forested zones the mean value for forests per hectare (US\$ 4 380) from (de Wit *et al.* 2021, unpublished data) was used and multiplied with the calculated area of edge and mature habitats as seen in Table 3.5. The total edge and mature habitat values amounted to \$ 17 954 899 and \$ 223 825 919, respectively. The total expected future costs for edge and mature habitats due to tree mortality caused by PSHB invasion amounted to \$ 1 605 168 and \$ 18 935 673, respectively (Table 3.6). Using carbon loss percentages data (8.94% for edge and 8.46% for mature habitats), the economic cost per

hectare was calculated which amounted to \$ 391.57 ha⁻¹ for edge and \$ 370.55 ha⁻¹ for mature habitats. In South African Rand estimated costs of invasion in this small forest complex is therefore estimated at R 23 601 106 for edge and R 278 414 987 for mature habitats (exchange rate of \$1 = R14.70).

Table 3.6: Economic value and costs of each forested zone described by Holness *et al.* (2010). Values and costs are given for both habitats edge and mature. Value is the total value of each zone and habitat (US \$) and costs is the total long-term carbon lost cost from PSHB for each habitat (US \$).

Forested Zone	Edge habitat Value	Mature habitat Value	Edge habitat cost	Mature habitat cost
Groenvlei coastal forest	475 515	7 503 093	42 511	634 762
Keurbooms Thicket-Forest	1 093 520	9 828 886	97 761	831 524
Kromrivier Thicket-Forest	353 926	5 242 838	31 641	443 544
Olienboomkop Grassy Fynbos-Forest	28 908	0 000	2 584	0 000
Outeniqua Forest	19 885	5 081	1 778	0 430
Outeniqua Montane Forest	174 915	1 199 529	15 637	101 480
Outeniqua Plateau Forest	4 420 406	97 411 529	395 184	8 241 015
Piesang River Fynbos-Forest	1 588 757	6 029 377	142 035	510 085
Storms River Thicket-Forest	440 584	1 715 252	39 388	145 110
Tsitsikamma Coastal Plateau Forest	1 060 595	5 177 839	94 817	438 045
Tsitsikamma Dune Forest	136 963	617 711	12 244	52 258
Tsitsikamma Forest Fynbos	494 633	2 800 879	44 220	236 954
Tsitsikamma Fynbos Forest	131 093	602 557	11 720	50 976
Tsitsikamma Mountain Forest	1 499 208	6 374 280	134 029	539 264
Tsitsikamma Plateau Forest	2 386 837	51 945 749	213 383	4 394 610
Tsitsikamma Riverine Forest	1 233 408	5 867 886	110 267	496 423
Wilderness Forest-Thicket	720 006	4 236 402	64 369	358 400
Wolwe River Fynbos-Forest	1 559 718	16 294 914	139 439	1 378 550
Wolwedans Thicket-Forest	136 021	972 119	12 160	82 241
<i>Sum:</i>	17 954 899	223 825 919	1 605 168	18 935 673

3.5) Discussion

Here it was shown that Afrotropical forests in the Southern Cape of South Africa store large masses of carbon, especially in mature forests dominated by larger tree individuals. Like other forests around the globe, Afromontane forests are therefore likely an important carbon sink (Gebeyehu *et al.*, 2019; Spracklen & Righelato, 2014) that is important for normal ecosystem functioning and for mitigating atmospheric carbon based climate change (Cavanaugh *et al.*, 2014; Mensah, Veldtman, Assogbadjo, *et al.*, 2016; Wu *et al.*, 2017). Unfortunately, it also became evident that this important resource is under severe threat from invasion by the

Polyphagous Shot Hole Borer Beetle. Current invasion into this system is in its infancy, and impacts are yet to be realised, but from the relatively low projections used in this study it may result in a net carbon loss in the range of $\sim 278.13 \text{ Mg C ha}^{-1}$ in mature forests. This will undoubtedly have massive implications for native biotic communities, especially as a selection of particular host tree species will be under severe pressure. The death of tree individuals, and local extinctions of some tree species, will also change normal ecological processes such as biotic interactions, gap formation, and nutrient and water cycling (Franklin *et al.*, 1987; Peet & Christensen, 1987) affecting organisms that are not directly associated with PSHB hosts. An increase in area of pioneer forest communities is expected at the expense of mature forests, with associated declines in taxa that depend on these climax forest communities (Carey & Johnson, 1995; Swart *et al.*, 2018). In addition to the impact on biota, the social cost of tree mortality at the height of infestation was estimated at $\sim \$ 370.55 \text{ ha}^{-1}$ for mature forests, which amounts to *ca.* $\$ 18\,935\,673$ loss for this small forest complex. If the PSHB beetle manages to invade the full extent of the $\sim 530\,000 \text{ ha}$ of native forests in SA, and estimates of tree mortality, stored carbon loss, and social costs are similar for all forest types, a total of 11.77 Mt carbon lost at a cost of $\$ 197\,217\,910$ may be realised. Potential loss to native forest ecosystems and society due to this new invasion is expected to be substantial and will be especially problematic in a developing country such as South Africa that have few resources to mitigate these impacts.

3.5.1) Possible impact of PSHB on mature forest vs. edge/pioneer habitat

From allometric equations it was calculated that mature forest habitats have a higher carbon biomass storage than edge/pioneer habitats, as was expected. This is because mature forests are dominated by larger trees that accumulate more biomass than young trees and pioneer species, at forest edges (Mildrexler *et al.*, 2020). Mature forest habitats were found to likely lose more carbon even in the short term (i.e., with the loss of a small proportion of only the currently infested individuals) due to PSHB invasion. This was contrary to my expectations as edge habitats are closer in proximity to the currently high infestations in the neighbouring urban areas which has been shown as an important predictor for infestation levels in these same forests (Chapter 2). In the 27 currently infested PSHB monitoring plots, an average of 2.4 trees were impacted by PSHB in mature forest habitats while an average of 4.5 trees were impacted in edge/pioneer forest habitats. Pioneer habitats also has a higher abundance of trees and house larger numbers of susceptible tree species than mature habitats, at least at the current stage of invasion (Chapter 2). Therefore, even though edge habitats have a higher abundance of trees

(and number of infested trees), the much smaller size of these compared to individuals in mature forests leads to substantial differences in expected loss of carbon (Dantas de Paula *et al.*, 2011).

The already high negative impacts on mature forest habitats are expected to be exacerbated in the longer term as more individuals, and likely additional species succumb to beetle infestations. Alarmingly, and not considered in the current study, there is also a tendency for PSHB beetles to preferentially infest larger host trees (Chapter 2). Also, like with many other tree pests, PSHB infestations can increase with abiotic disturbance events such as drought or storms (Boland, 2016; Boland & Woodward, 2019; Brockerhoff *et al.*, 2017), both events that often occur in the Afromontane forest systems in this region (Geldenhuys, 1982; Phillips, 1931). This would indicate that even the updated predictions of mortality of 40% breeding hosts and 5% non-breeding hosts used in the present study could still be an underestimate of actual long-term impact on carbon storage.

Even though mature habitats will lose more carbon biomass than edge habitats it is difficult to predict which habitat will suffer more in terms of alterations in ecosystem functions and biological diversity. Trees found in different contexts have been shown to house different biotic communities such as insects (Swart *et al.*, 2020). Individuals of species found in both mature and pioneer forest habitats are therefore important for maintaining biological diversity. Due to their large size, mature forest trees may contribute disproportionately to forest diversity, enhances carbon storage, and are often important for rare taxa (Ali *et al.*, 2019, 2020; Poorter *et al.*, 2015) as size may determine reproduction thresholds for species (Wright *et al.*, 2005). A dominant tree species in mature forests here was *Afrocarpus falcatus*, a tall emergent tree that may well prove to be a breeding host for PSHB (Geldenhuys & Von Dem Bussche 1997; Chapter 2). This tree also had the most carbon biomass weight compared to the other tree species assessed. Losing individuals of one of the most important carbon storage species in the forest could have drastic effects on future carbon stocks. Luckily, none of the *A. falcatus* individuals in our study plots have succumbed to PSHB and this species may have natural resistance. However, if found to suffer mortality in the future, negative effects will not only be experienced on carbon stocks, but also in the creation of steep microclimatic gradients from the removal of these particularly large individuals (Matlack, 1994). With the forests in the Southern Cape already severely fragmented (Geldenhuys, 1991), the remaining mature forest remnants my therefore suffer severely from PSHB invasion (Geldenhuys, 1997). Conversely, forest edges contain more tree taxa and support numerous biotas not found in mature forests.

Losing any particular tree species from these edge habitats could therefore also have severe impacts on biodiversity conservation.

Invasion by PSHB will very likely also alter disturbance regimes in Afrotropical forests. Edge and mature forest habitats experience differences in disturbance levels (Ziter *et al.*, 2014), with mature forest experiencing small disturbances in the form of canopy gaps created from irregular tree fall (Adie *et al.*, 2013) and forest edge habitats having increased disturbances driven by abiotic factors such as sunlight, wind and fire (Chen *et al.*, 1995, 1993, 1992; Laurance & Curran, 2008; Lawes *et al.*, 2000). Many of the forest edge specialist taxa are important for forest regeneration and protection (e.g. *Virgilia oroboides*) and losing these will have severe impacts on the ability of these forest remnants to buffer against biotic influences such as fire (Geldenhuys, 1982; Phillips, 1931). Tree mortalities caused by PSHB invasion will not only enhance disturbance regimes at the edge when trees start to rapidly die (Lovett *et al.*, 2006; Matlack, 1994), but will eventually spread this effect further into mature forest habitats where disturbance gaps will probably increase in both severity and frequency causing a negative feedback loop (Pickett *et al.*, 1987). Therefore, even though forest edges are predicted to have higher infestation levels than mature forest in the interiors of patches, edge habitats may act as a steppingstone for dispersal for PSHB, and its negative impacts, into mature forest habitats. Importantly, human mediated dispersal of PSHB directly into mature forests via contaminated wood and wood products (Chapter 2) will likely accelerate disturbance related negative impacts (Gippet *et al.*, 2019; Jacobi *et al.*, 2012; Umeda *et al.*, 2016).

3.5.2) Estimate carbon loss and social cost across Garden Route Forest Regions

I estimated aboveground carbon stocks for the study at $\sim 171.98 \text{ Mg C ha}^{-1}$ ($\sim 65 \text{ Mg C ha}^{-1}$ for edge and $\sim 278 \text{ Mg C ha}^{-1}$ for mature habitats). Estimates for mature forest habitats here were slightly higher than those for mature Afromontane forests in the midlands of KwaZulu-Natal, South Africa (250 Mg C ha^{-1}) and in the Eastern Arc Mountains of Tanzania (138-225.9 Mg C ha^{-1}) (Adie *et al.*, 2013; Marshall *et al.*, 2012). It was also higher than estimates for temperate podocarp forests in New Zealand ($169.1 \text{ Mg C ha}^{-1}$) (Coomes *et al.*, 2002) and an Afromontane forest on the Cape Peninsula of South Africa (113.4 Mg ha^{-1}) (Cardoso *et al.* 2017). However, similar to our results, a study by Midgley & Seydack (2006) conducted in a section covered by the present study, the Knysna forest, biomass was estimated at 500 Mg ha^{-1} (i.e. 250 Mg C ha^{-1} carbon). Carbon stocks in these particular forests therefore seem high for this latitude, making it very important in terms of climate change mitigation. This is also an order of

magnitude higher than normally found in urban forests such as in the USA (national average of 25.1 Mg C ha⁻¹) (Nowak & Crane, 2002), but this can vary according to city (e.g. Seattle, Washington ~140 Mg C ha⁻¹) (Hutyra *et al.*, 2011). Therefore, even though urban environments are under a larger threat from PSHB in terms of expected social costs (de Wit *et al.* 2021, unpublished data), native forests will likely experience more severe carbon loss, and the biological consequences of infestation will be much worse even if infestation levels in native forests remain low.

I estimated that ~1.20 Megatonnes of carbon can be lost from mature and ~0.02 Megatonnes from edge habitats in the future. Total expected carbon loss costs for edge and mature habitats in the long term were estimated at \$ 1 605 168 and \$ 18 935 673, respectively. This is quite low considering costs associated by invasion of the USA by e.g., the emerald ash borer (*Agrilus planipennis*) estimated to cost ca. \$ 10 billion the last decade (Kovacs *et al.*, 2010), but high considering the extent of the current study and the minimal resources available to manage this invasion. Costs in the current study do not include costs such as those associated with treatment, removal, and replacement of all affected individuals across the entire USA as in the previously mentioned study. In a study by Aukema *et al.* (2011) it was estimated that the economic impact of borers roughly annually accounted for \$ 1.7 billion in government expenditures in the USA. The USA nation-wide economic impact of invasive wood borers were estimated at \$ 2.1 billion per year (Pimentel *et al.*, 2005). Considering the economic cost per hectare, the values will be \$ 391.57 ha⁻¹ for edge and \$ 370.55 ha⁻¹ for mature habitats. Compared to the estimate damage from ambrosia beetle to loblolly pine forest in the United States (\$ 791.1 ha⁻¹) it is low (Susaeta *et al.*, 2016), however treatment, removal, and replacement costs are not included in our economic cost assessment. According to de Wit *et al.* (2021, unpublished data) the social costs of PSHB for primary forests is estimated at Int. \$ 121.6m, which is lower than the results of the current study (\$ 197.2m) in which more reliable data could be included. The invasion in the study plots is still very early and there has been thankfully no need for treatment, removal, or replacement in the forest habitats. However, it is important to consider these costs for future implications as the invasion will continue to rapidly spread.

3.5.3) Conclusions

In the present study prediction of future impacts on native forests were based on data collected and analysed independently for mature and pioneer forest habitats. This approach likely gives more accurate estimations than that used in a previous assessment of the impact of PSHB

invasion in South Africa (de Wit *et al.* 2021, unpublished data). Implementing the low projected impact levels used in the study indicated that previous estimations may be much lower than those realised in the future. However, continual monitoring of the same plots and sites used in this study into the future is suggested to adjust levels to more accurate predictions based on more data.

The selection of plots and certain areas could provide a better long-term guarantee of carbon stock loss. This study did not include the dead organic matter for soil carbon in calculations and if included it will increase the total carbon stocks per area, and that lost in the short and the long term. It also does not include or provide the carbon stocks fluctuations in a given time period, as trees will still grow in the short-term and may be replaced by non-susceptible species in the long term which will decrease the reduction in carbon stocks (Alexandrov, 2007). The infestation in this forest complex has only started to take hold and it is expected that in the future we will detect more susceptible tree species which would again increase our current estimations of carbon loss.

Increased release of CO₂ in forests are expected with increased tree mortality. However, some invasive bark and ambrosia beetles can suppress wood decay despite increasing the overall fungal diversity (introducing its own symbiotic fungi), but decreasing local diversity (by outcompeting native species) (Fukami *et al.*, 2010; Skelton *et al.*, 2019, 2020). If this is the case with PSHB, normal wood decay may be altered in unpredictable ways, with unknown consequences for normal ecosystem functioning and carbon release. It is therefore acknowledged that this antagonism has not been evaluated here but would be an important consideration for future study.

It must be emphasized that the figures in this study are precautionary. It was estimated that mature habitats will have a higher carbon mass loss in the long term than edge habitats. If infestations are going to spread more rapidly than thought, we might realise completely different estimates than here. Regular monitoring of infestation levels and spreads are therefore crucial for future estimations and predictions of carbon loss. Without careful management and public awareness, the beetle can easily be transported to uninfected areas (Haack *et al.*, 2010), increasing its rate of impact and, likely, also the number of tree species affected.

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Chapter 4

Concluding remarks

The Polyphagous Shot Hole Borer (PSHB) (Coleoptera: Curculionidae: Scolytinae), *Euwallacea fornicatus* is a significant threat to forests in its invaded range. Along with its symbiotic fungus *Fusarium euwallaceae*, it is responsible for fusarium dieback disease of trees (Freeman *et al.*, 2013) and have caused serious damage to trees in Israel, California and South Africa (Mendel *et al.*, 2012; Paap *et al.*, 2018). Before this study, nothing was known about its host range, probability and severity of infestation, and the potential impact on native forest ecology and economics. Therefore, the goal of the present study was to gather information on the factors that might affect PSHB current and future movement and its impact in Afrotropical forests.

Monitoring was conducted in 51 permanent plots that were scattered across the Garden Route of the Southern Cape, South Africa. These were monitored for a year for PSHB infestations on all tree individuals. It was therefore possible to detect and monitor changes in infestation levels of tree individuals and in plots, and to use this data to elucidate reasons for specific infestations and to predict future trends. In a space of one-year the number of infested trees increased by 29 and the majority of infested trees showed signs of increased infestation severity (62.19%). Although infestation rates at some sites were relatively high, tree mortality over this period was low, as only two trees have died due to beetle activity. This is likely a consequence of the short monitoring time and it is expected that numerous tree individuals will eventually succumb due to beetle activity in the future. It was shown that PSHB prefer to infest hosts that have a large diameter, similar to the results of workers on Scolytinae in other systems (e.g. Choudhury *et al.* 2020). In addition, the severity and probability of infestation increased when there were a high number of infested hosts in the vicinity and when breeding host density was high. Sites that were in closer proximity to outbreak areas, especially those with a high abundance of reproductive hosts and in more open pioneer/edge forest, were more likely to be infested. Distant sites more frequented by tourists which have larger host individuals may generally suffer greater from PSHB infestation probability and severity. It was also shown that the beetle preferentially select certain tree species in the landscape. All of these characteristics of the onset of the invasion into native Afrotropical forests by PSHB may help future monitoring and mitigation practices as these inform not only which sites to focus on, but also which specific tree individuals may be at increased risk of attack. For example, Monitoring and

mitigation can be focused on all sites in these forests where people regularly visit. These include sites that have fireplaces, but also those where tourist visit to view the large *Afrocarpus falcatus* trees. Large trees are preferentially invaded, but also, this particular species is infested at greater proportions than expected by chance. These monuments, and the sites in which they occur, should therefore be monitored regularly. At some sites, local vendors are encouraged sell products constructed from wood that is sourced from outside of these forests. This represents a substantial source of income for poorer communities and cannot be halted. Therefore, using properly treated wood for this purpose should be encouraged and goods should likely be inspected for possible infestations before being allowed to be displayed.

Invasive pests have major impacts on native forests by altering tree species composition, forest succession and nutrient cycling (Gower, 2003; Roy *et al.*, 2014). This has lasting economic and social impacts such as decreased carbon storage value and decreased value of provisioning, regulating and cultural services (Aukema *et al.*, 2011; Pimentel, 2001). In this study the potential carbon stock loss and associated economic impact was estimated using data collected from both mature and edge plot sites in Afrotropical forests, respectively. It was shown that Afrotropical forests have a high carbon stock biomass but is under severe threat from tree death caused by fusarium dieback spread by its PSHB vector. Mature habitats stored a higher carbon biomass than edge habitats and was also predicted to lose more carbon biomass from PSHB both in the short term and in the long-term. From the relatively low projections used in this study the impacts may result in a net carbon loss in the range of $\sim 278.13 \text{ Mg C ha}^{-1}$ in mature forests and $\sim 65 \text{ Mg C ha}^{-1}$ for edge forests. Therefore, even though edge habitats have a higher abundance of trees (and number of infested trees), the much smaller size of these trees compared to individuals in mature forests leads to substantial differences in expected loss of carbon biomass (Dantas de Paula *et al.*, 2011). The social cost of tree mortality at the height of infestation was estimated at $\sim \text{US\$ } 370.55 \text{ ha}^{-1}$ for mature forests, which amounts to *ca.* $\$ 18\,935\,673$ loss for this small forest complex. If the PSHB beetle manages to invade the full extent of the $\sim 530\,000 \text{ ha}$ of native forests in South Africa, and estimates of tree mortality, stored carbon biomass loss, and social costs are similar for all forest types, a total of 11.77 Mt carbon may be lost at a cost of $\$ 197\,217\,910$. According to de Wit *et al.*, (2021, unpublished data) the social costs of PSHB for primary/mature forests was estimated at Int. $\$ 121.6\text{m}$, which is therefore slightly lower than the results of the current study ($\$ 197.2\text{m}$). It is believed that the higher estimated socio-economic loss calculated here may represent more accurate results due to more refined data included.

The continuation of monitoring, management and prevention of spread of PSHB in these forests are of the utmost importance. Removing heavily infested hosts and especially reproductive hosts in urban areas will reduce propagule pressure, not only on the native forests, but also in the urban areas themselves. According to data from the native forests collected here, trees with more than 10 holes can be considered heavily infested as nearly all of these showed increased attacks over this one year period. When removed, infested wood needs to be chipped (<5 cm) and solarized (Chen *et al.*, 2020; Jones *et al.*, 2015). All movement of untreated wood products into forests should be halted as this is the most likely pathway to create new infestations at distant sites. For fires, the only reliable fuel source will be heavily treated products such as charcoal.

One benefit of PSHB invasion in South Africa is an increase in public awareness of invasive organisms and their potential impact, at least in heavily affected areas. This increase in public awareness has also provided in terms of public education and, combined with the creation of a multidisciplinary and multi-institutional Polyphagous Shot Hole Borer Research Network in South Africa, will help considerably in continued efforts in research, monitoring and control of invasive species.

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5) Addenda

Addendum A: Confirmed host plants of PSHB in South Africa.

Table 5.1: List of confirmed host plants of the Polyphagous Shot Hole Borer beetle in South Africa. The list includes only those taxa from which its primary fungal symbiont, *Fusarium euwallaceae*, or a beetle individual was successfully isolated, and identity confirmed using DNA sequence fingerprinting. Reproductive hosts include only those hosts that have been shown to be suitable for PSHB reproduction at least once. Non-reproductive hosts include those from which either PSHB or *F. euwallaceae* was isolated, but no evidence of PSHB reproduction has been verified.

Reproductive hosts			
Species	Family	Common name	Indigenous or exotic in South Africa
<i>Acacia mearnsii</i>	Fabaceae	Black wattle	Exotic
<i>Acacia melanoxylon</i>	Fabaceae	Blackwood	Exotic
<i>Acer buergerianum</i>	Aceraceae	Trident (Chinese) maple	Exotic
<i>Acer negundo</i>	Aceraceae	Boxelder	Exotic
<i>Acer palmatum</i>	Aceraceae	Japanese maple	Exotic
<i>Acer saccharinum</i>	Aceraceae	Silver maple	Exotic
<i>Anisodonta scabrosa</i>	Malvaceae	Rough-leaf African mallow	Indigenous
<i>Bauhinia galpinii</i>	Fabaceae	Pride of De Kaap	Indigenous
<i>Brachychiton discolor</i>	Malvaceae	Pink flame tree	Exotic
<i>Brachylaena discolor</i>	Asteraceae	Coast silver oak	Indigenous
<i>Calpurnia aurea</i>	Fabaceae	Wild laburnum	Indigenous
<i>Casuarina cunninghamiana</i>	Casuarinaceae	Beefwood	Exotic
<i>Combretum krausii</i>	Combretaceae	Forest bushwillow	Indigenous
<i>Combretum erythrophyllum</i>	Combretaceae	River bushwillow	Indigenous
<i>Diospyros glabra</i>	Ebenaceae	Cape star-apple	Indigenous
<i>Erythrina caffra</i>	Fabaceae	Coral tree	Indigenous
<i>Gleditsia triacanthos</i>	Fabaceae	Honey locust	Exotic
<i>Kiggelaria africana</i>	Achariaceae	Wild Peach	Indigenous
<i>Liquidambar styraciflua</i>	Altingiaceae	Sweetgum	Exotic
<i>Magnolia grandiflora</i>	Magnoliaceae	Southern magnolia	Exotic
<i>Persea americana</i>	Lauraceae	Avocado	Exotic
<i>Platanus x acerifolia</i>	Platanaceae	London plane	Exotic
<i>Podalyria calyptrata</i>	Fabaceae	Water blossom pea	Indigenous
<i>Populus nigra</i>	Salicaceae	Lombardy poplar	Exotic
<i>Populus simonii</i>	Salicaceae	Chinese cottonwood	Exotic
<i>Psoralea aphylla</i>	Fabaceae	Leafless fountain bush	Indigenous
<i>Psoralea pinata</i>	Fabaceae	Fountain bush	Indigenous
<i>Quercus palustris</i>	Fagaceae	Pin oak	Exotic
<i>Quercus robur</i>	Fagaceae	English oak	Exotic
<i>Ricinus communis</i>	Euphorbiaceae	Castor bean	Exotic
<i>Salix alba</i>	Salicaceae	White willow	Exotic

<i>Salix mucronata</i>	Salicaceae	Cape willow	Indigenous
<i>Sparrmannia africana</i>	Malvaceae	African hemp	Indigenous
<i>Trema orientalis</i>	Cannabaceae	Pigeon wood	Indigenous
<i>Viburnum odoratissimum</i>	Adoxaceae	Sweet viburnum	Exotic
<i>Virgilia oroboides</i>	Fabaceae	Keurboom	Indigenous
<i>Vepris lanceolata</i>	Rutaceae	White ironwood	Indigenous
<i>Wisteria sinensis</i>	Fabaceae	Chinese wisteria	Exotic

Non-reproductive hosts

Species	Family	Common name	Indigenous or exotic to South Africa
<i>Adansonia digitata</i>	Malvaceae	Baobab	Indigenous
<i>Afrocarpus falcatus</i>	Podocarpaceae	Outeniqua yellowwood	Indigenous
<i>Albizia adianthifolia</i>	Fabaceae	Flat crown	Indigenous
<i>Bauhinia purpurea</i>	Fabaceae	Butterfly orchid tree	Exotic
<i>Betula pendula</i>	Betulaceae	Silver birch	Exotic
<i>Bougainvillea</i>	Nyctaginaceae	Bougainvillea	Exotic
<i>Buddleja saligna</i>	Scrophulariaceae	False olive	Indigenous
<i>Calodendrum capense</i>	Rutaceae	Cape chestnut	Indigenous
<i>Camellia japonica</i>	Theaceae	Common camellia	Exotic
<i>Carya illinoiensis</i>	Juglandaceae	Pecan	Exotic
<i>Ceiba pentandra</i>	Malvaceae	Kapok	Exotic
<i>Cinnamomum camphora</i>	Lauraceae	Camphor tree	Exotic
<i>Citrus limon</i>	Rutaceae	Lemon	Exotic
<i>Citrus sinensis</i>	Rutaceae	Orange	Exotic
<i>Cordia caffra</i>	Boraginaceae	Septee tree	Indigenous
<i>Cussonia spicata</i>	Araliaceae	Cabbage tree	Indigenous
<i>Diospyros dichrophylla</i>	Ebenaceae	Star apple	Indigenous
<i>Diospyros kaki</i>	Ebenaceae	Persimmon	Exotic
<i>Diospyros lycioides</i>	Ebenaceae	Monkey plum	Indigenous
<i>Diospyros whyteana</i>	Ebenaceae	Bladdernut	Indigenous
<i>Dombeya rotundifolia</i>	Malvaceae	Wild pear	Indigenous
<i>Dovyalis caffra</i>	Salicaceae	Kei apple	Indigenous
<i>Ekebergia capensis</i>	Meliaceae	Cape ash	Indigenous
<i>Eriobotrya japonica</i>	Rosaceae	Loquat	Exotic
<i>Erythrina livingstoniana</i>	Fabaceae	Aloe coral tree	Exotic
<i>Erythrina lysistemon</i>	Fabaceae	Common coral	Indigenous
<i>Eucalyptus camaldulensis</i>	Myrtaceae	River red gum	Exotic
<i>Ficus carica</i>	Moraceae	Common fig	Exotic
<i>Ficus natalensis</i>	Moraceae	Natal fig	Indigenous
<i>Ficus sur</i>	Moraceae	Cape fig	Indigenous
<i>Fraxinus americana</i>	Oleaceae	American ash	Exotic
<i>Fraxinus excelsior</i>	Oleaceae	European ash	Exotic
<i>Grewia occidentalis</i>	Malvaceae	Cross berry	Indigenous
<i>Gymnosporia buxifolia</i>	Celestraceae	Common spikethorn	Indigenous
<i>Hakea salicifolia</i>	Proteacea	Willow-leaved hakea	Exotic
<i>Halleria lucida</i>	Stilbaceae	Tree fuchsia	Indigenous
<i>Harpephyllum caffrum</i>	Anacardiaceae	Wild plum	Indigenous

<i>Hibiscus rosa-sinensis</i>	Malvaceae	Chinese hibiscus	Exotic
<i>Ilex mitis</i>	Aquifoliaceae	Cape holly	Indigenous
<i>Jacaranda mimosifolia</i>	Bignoniaceae	Jacaranda	Exotic
<i>Leonotis leonurus</i>	Lamiceae	Wild tobacco	Indigenous
<i>Macadamia</i>	Proteacea	Macadamia	Exotic
<i>Malus domestica</i>	Rosaceae	Apple	Exotic
<i>Melia azedarach</i>	Meliaceae	Syringa	Exotic
<i>Morus nigra</i>	Moraceae	Black mulberry	Exotic
<i>Nuxia floribunda</i>	Stilbaceae	Forest elder	Indigenous
<i>Olea europaea</i> subsp. <i>africana</i>	Oleaceae	Wild olive	Indigenous
<i>Olea europaea</i> subsp. <i>europaea</i>	Oleaceae	Cultivated olive	Exotic
<i>Olinia ventosa</i>	Penaeaceae	Hard pear	Indigenous
<i>Osteospermum moniliferum</i>	Asteraceae	Bietou	Indigenous
<i>Platanus occidentalis</i>	Platanaceae	American plane	Exotic
<i>Platanus racemosa</i>	Platanaceae	Californian plane	Exotic
<i>Plumeria rubra</i>	Apocynaceae	Frangipani	Exotic
<i>Podocarpus henkelii</i>	Podocarpaceae	Henkel's yellowwood	Indigenous
<i>Protea mundii</i>	Proteacea	Forest sugar bush	Indigenous
<i>Prunus africana</i>	Rosaceae	African cherry	Indigenous
<i>Prunus avium</i>	Rosaceae	Sweet cherry	Exotic
<i>Prunus cerasifera</i>	Rosaceae	Cherry plum	Exotic
<i>Prunus nigra</i>	Rosaceae	Black plum	Exotic
<i>Prunus persica</i>	Rosaceae	Peach	Exotic
<i>Psidium guajava</i>	Myrtaceae	Guava	Exotic
<i>Quercus rugosa</i>	Fagaceae	Net leaf oak	Exotic
<i>Rapanea melanophloeos</i>	Primulaceae	Cape beech	Indigenous
<i>Robinia psuedoacacia</i>	Fabaceae	Black locust	Exotic
<i>Salix babylonica</i>	Salicaceae	Weeping willow	Exotic
<i>Schinus molle</i>	Anacardiaceae	Pepper tree	Exotic
<i>Schotia brachypetala</i>	Fabaceae	Weeping boer-been	Indigenous
<i>Searsia chirindensis</i>	Anacardiaceae	Red currant	Indigenous
<i>Searsia lansea</i>	Anacardiaceae	Karree	Indigenous
<i>Senegalia burkei</i>	Fabaceae	Black monkey-thorn	Indigenous
<i>Senegalia galpinii</i>	Fabaceae	Monkey-thorn	Indigenous
<i>Syzygium cordatum</i>	Myrtaceae	Waterberry	Indigenous
<i>Taxodium distichum</i>	Cupressaceae	Bald cypress	Exotic
<i>Ulmus procera</i>	Ulmaceae	English elm	Exotic
<i>Ulmus parvifolia</i>	Ulmaceae	Chinese elm	Exotic
<i>Vachellia karroo</i>	Fabaceae	Sweet thorn	Indigenous
<i>Vachellia sieberiana</i> var. <i>woodii</i>	Fabaceae	Paperbark thorn	Indigenous
<i>Virgilia divaricata</i>	Fabaceae	Keurboom	Indigenous
<i>Vitis vinifera</i>	Vitaceae	Grape	Exotic

Addendum B: Supporting photographs of study areas of edge and mature habitats.



Figure 5.1: Examples of the two habitat types included in the present study. Top: Typical edge/pioneer forest habitat. Bottom: Typical mature/climax forest habitat.