

ORIGINAL ARTICLE

Invasion of an Afrotropical forest complex by the polyphagous shot hole borer beetle

G. Townsend¹  | E. van Rooyen²  | M. Hill³  | W. De Beer⁴  | F. Roets² 

¹Forestry and Agricultural Biotechnology Institute, Department of Zoology and Entomology, University of Pretoria, PO Box 94, Grahamstown, 6140, South Africa

²Department of Conservation Ecology and Entomology, Stellenbosch University, JS Marais Building, Victoria Street, Stellenbosch, South Africa

³Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa

⁴Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

Correspondence

F. Roets, Department of Conservation Ecology and Entomology, JS Marais Building, Victoria Street, Stellenbosch University, Stellenbosch, South Africa.
Email: fr@sun.ac.za

Funding information

Tree Protection Cooperative Programme (TPCP); Forestry and Agricultural Biotechnology Institute (FABI); South African Department of Environment, Forestry, and Fisheries (DEFF)

Abstract

The polyphagous shot hole borer (PSHB), *Euwallacea fornicatus* Eichhoff (Coleoptera: Curculionidae, Scolytinae), is a significant tree-killing pest recently introduced into South Africa. Many native trees in urban settings are susceptible to infestation, but the presence of PSHB in natural ecosystems is unstudied. The presence and drivers of PSHB colonization in 1682 trees of 68 species were evaluated in 51 plots across a native Afrotropical forest complex in South Africa. Breeding colonies of PSHB were found in six native species (breeding hosts). An additional 11 species did not contain PSHB colonies but hosted its mutualistic fungus *Fusarium euwallaceae* Freeman et al. (Hypocreales: Nectriaceae). Invasibility increased when plots were closer to the urban infestation border, further away from surface water, and when containing a larger number of breeding hosts. Invasibility decreased with an increase in tree species richness. Polyphagous shot hole borers were found in climax forest distant to urban areas at sites frequented by tourists. The severity of infestation of trees increased with an increase in host diameter, breeding host abundance, and infested tree abundance. Probability of infestation increased with an increase in the number of infested trees. Infested trees were not spatially clumped. Instead, PSHB preferentially selected eight of the 17 native host species. And the data suggest that larger trees of these species may be more susceptible to PSHB. Eight species were infested at random and two were infested seemingly accidentally. Infestations increased more rapidly on larger trees and on those surrounded by a high abundance of breeding hosts. This study confirms that Afrotropical forests are highly susceptible to invasion by PSHB. Direct anthropogenic impact had no discernible effect on infestations, but humans aided spread of PSHB to distant sites. Halting movement of contaminated wood is important. Management of PSHB should focus on highly infested areas and trees as these increase the likelihood of further and more severe infestations.

KEYWORDS

ambrosia beetles, Coleoptera, Curculionidae, disease incidence, *Euwallacea fornicatus*, *Fusarium dieback* disease, *Fusarium euwallaceae*, host density, invasion biology, invasive pest, native forest, tree-killing pest

INTRODUCTION

The rate of biological invasions by highly destructive bark and ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) is on the increase globally (van Wilgen et al., 2020). Ambrosia beetles alter biological communities and degrade native forest ecosystems (Cobb et al., 2012;

Hughes et al., 2017). The polyphagous shot hole borer (PSHB), *Euwallacea fornicatus* Eichhoff, has recently invaded urban, agricultural, and natural environments in many parts of the world, killing thousands of trees (Mendel et al., 2012; Li et al., 2016; Gomez et al., 2019). Estimates of damage to economies are severe, and for South Africa alone damages amount to 18.45 billion international dollars, or about

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. *Entomologia Experimentalis et Applicata* published by John Wiley & Sons Ltd on behalf of Netherlands Entomological Society.

0.66% of the country's gross domestic product (de Wit et al., 2022).

In South Africa, PSHB was first discovered in 2012 (Stouthamer et al., 2017) but, aided by humans, it has now spread to nearly all provinces (van Rooyen et al., 2021). Polyphagous shot hole borer vectors a fungal mutualist within mandibular mycangia, *Fusarium euwallaceae* Freeman et al. (Hypocreales: Nectriaceae), that, together with the boring activities of the beetle in living woody hosts, can cause *Fusarium* dieback disease that often cause tree mortality (Freeman et al., 2013). This fungus serves as the food source for both larvae and adults (Freeman et al., 2013). Polyphagous shot hole borer has a particularly wide host range (Gomez et al., 2019; Mendel et al., 2012) and, in South Africa, has been identified from 162 hosts (van Rooyen et al., 2021; FABI, 2023). The beetle attempts to colonize hosts seemingly indiscriminately and may inoculate trees with *F. euwallaceae* even if they fail to establish breeding colonies. These 'non-reproductive' hosts often survive. However, when the beetle can readily establish reproductive colonies, these 'reproductive' trees may perish. In South Africa, PSHB reproduction has been recorded in at least 79 tree species of which 41 are indigenous (van Rooyen et al., 2021; FABI, 2023). Other reasons for the invasive success of PSHB include its small size (<2 mm), cryptic biology within woody tissues, high reproduction rate, and a haplodiploid reproductive strategy (van Rooyen et al., 2021). To date, no comprehensive or long-term surveys of PSHB infestations in native South African forests have been undertaken. The susceptibility of natural forest ecosystems to invasion depends on numerous biotic factors. For example, tree size may play a role as larger trees may be more susceptible to Scolytinae infestations (Smith & Hoffman, 2001; Kendra et al., 2013; Haas et al., 2016). Tree and canopy densities have been shown to influence many Scolytinae species' diversity and population densities, with some species being more abundant in open canopies at forest edges and others being more abundant in closed canopies (Maetô et al., 1999; Igeta et al., 2004; Holuša et al., 2021). Proximity to surface water has also been shown to increase *Euwallacea* infestations in riparian forests (Boland, 2016).

Increased propagule pressure is a major driver of establishment of invasive organisms in many ecosystems (Simberloff, 2009), therefore infestations could also increase as a result of increased abundance of infested breeding hosts (Christiansen et al., 1987; Ostfeld & Keesing, 2012), and a decreased distance to infested hosts (Wichmann & Ravn, 2001; Muirhead et al., 2006; Kautz et al., 2011). Some other important biotic factors may include tree species identity, tree species diversity, and individual tree health (Smith et al., 2008; Boland, 2016; Coleman et al., 2019). Abiotic factors such as temperature and moisture are also important for scolytine beetle movement and reproduction (Damon, 2000; Coyle et al., 2005). Anthropogenic factors such as tree mechanical injury and forest clearing also influence beetle abundance and tree attack (Holt et al., 2003; Ghazoul et al., 2015; Shimwela

et al., 2018; Choudhury et al., 2020). Understanding the relative importance of these factors in explaining the spread and impact of invasive species is critical for their management. South African forests 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 fires are also frequent and combined with anthropogenic disturbances – such as plant harvesting, agriculture, and tourism – increase the chances of PSHB establishment in native forests (Keesing et al., 2006; Boland, 2016; Coleman et al., 2019). Consequently, although not determined yet, PSHB may have established in the largest South African Afrotropical forest complex, as it is situated close to the PSHB-invaded towns of Knysna and George in the Western Cape province (Phillips, 1931; Mucina et al., 2006).

The main aims of this study were to determine (1) current PSHB infestation levels, (2) host preferences, and (3) factors that increase invasion success by PSHB in the largest Southern Afrotropical forest complex in South Africa. We evaluated both the factors that may affect the invasibility of these forests – including distance to infestation border, abundance of breeding hosts (host density), disturbance, native tree species richness, canopy cover, and distance to nearest surface water – and the factors that affect the susceptibility and severity of infestations of trees – including tree size, host density, infestation density, disturbance, canopy cover, distance to nearest surface water and distance to nearest infested breeding host – at this very early stage in the invasion process. In addition, the progression of infestations was followed over a 14-month period to determine which factors, including those mentioned above, lead to a faster increase in PSHB invasions in these natural forests.

MATERIALS AND METHODS

Site selection

Surveys to assess the level of PSHB infestation in Southern Afrotropical 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 1). Surveys were conducted at 11 sites that were chosen to represent a diverse array of impacts and ecologies (Appendix 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

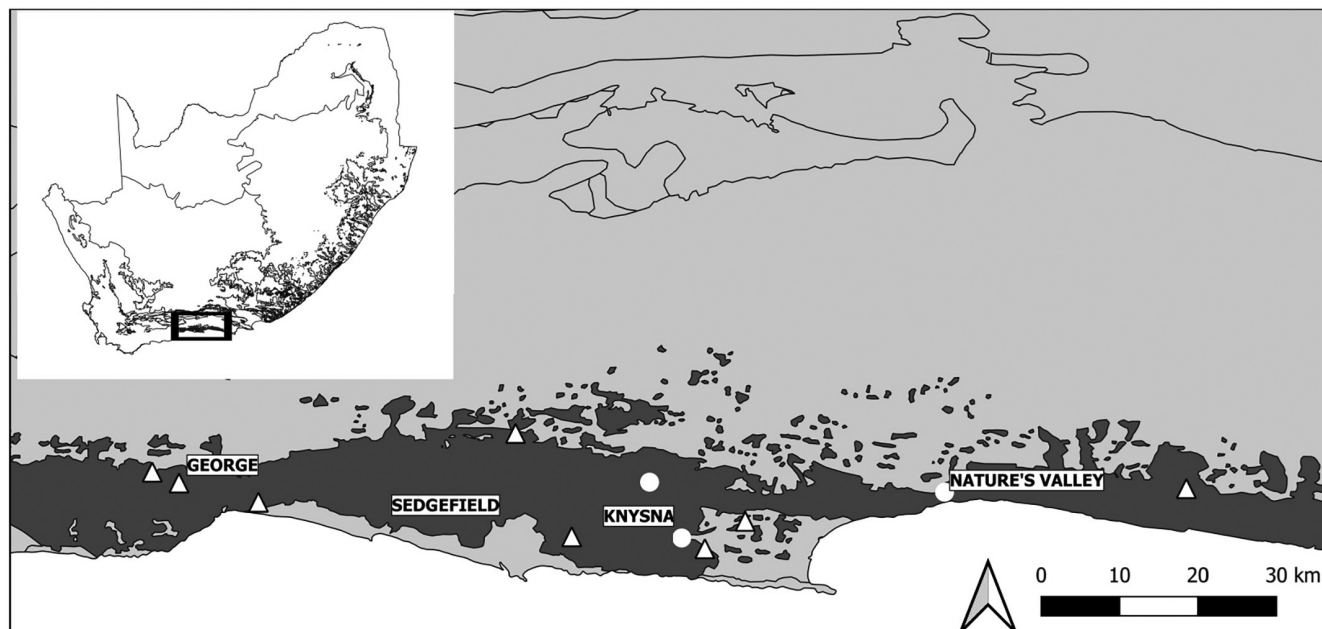


FIGURE 1 Map of the Southern Cape of South Africa depicting long-term monitoring sites for polyphagous shot hole borer (PSHB). Sites where evidence of PSHB infestations were found are indicated by triangles. The dark grey areas indicate forest cover.

at the George Botanical Garden's as this site showed substantial invasion by the beetle. Ten permanent monitoring plots were established at the George Botanical Garden's and at the Pledge Nature Reserve. Pledge Nature Reserve also showed signs of PSHB infestation but this area is smaller, contains many planted trees and was recovering from a fire which may have been confounding factors and therefore this area was not chosen as a focal site. At the other sites, the number of plots varied between 1 and 6 ($n = 51$). Plots were 15×10 m, at least 30 m apart, and selected to include many different tree species growing under a wide variety of conditions (e.g., different distances to surface water or nearest human impact).

Data collection

All permanent monitoring plots were surveyed ca. every 3 months during the study period (i.e., 4x). Surveys comprised all living trees/shrubs found in the plot that had a stem circumference of ≥ 3 cm at breast height regardless of health condition. Plot-level data variables that were recorded included: distance to surface water from the middle of each plot, distance to the border of closest known infestations (usually nearest urban border), abundance of trees of species that were confirmed or suspected as breeding hosts for PSHB at any time throughout the 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, fallen trees) 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 (e.g., cutting of branches, digging up of roots), total tree species richness and abundance, and the number of trees showing signs of PSHB colonisation (breeding hosts with extensive galleries) or attempted colonization (non-breeding hosts without beetle colonization but with colonization by *F. euwallaceae*). Host species included all species in which either the beetle or its fungal symbiont *F. euwallaceae* could be isolated in at least one tree across all sampling sites (confirmed host, see below), or in South Africa (van Rooyen et al., 2021). Breeding hosts included all species that contained at least one tree with more than 10 PSHB entry holes across all sites, or that showed signs of extensive gallery formation when opening the wood. Non-host species were those that showed no signs of PSHB infestations or attempted infestations across all sites throughout the study.

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 two observers), overall health condition of each tree was then scored as a rank between 1 and 5 (5 = completely healthy, 1 = dead/dying), tree injury scored as the percentage damage (average of two observers) 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 PSHB holes were counted (standardized from the base to the breast height of the tree, i.e., ca. 1.5 m). The mean flight height of PSHB is ca. 1.24 m (Byers et al., 2017). From each infested

breeding host (focal tree) in each plot, the distance to the nearest healthy and infested conspecific trees was measured. The distance from every focal tree to the nearest infested and healthy breeding host species (including any breeding host and not just conspecifics) was also measured. Lastly, the distance was measured from every focal tree to the nearest infested and healthy tree from any tree species that was confirmed to be infested by PSHB during the study period (i.e., the nearest confirmed non-reproductive host).

Infested tree confirmation

All trees were inspected for PSHB colonization or attempted colonization by evaluating trunks for entry holes of the correct size (ca. 0.85 mm) or other symptoms of boring beetles (such as sap flows and presence of frass, as highlighted in van Rooyen et al., 2021). When holes were present, bark was removed from the affected area using a sterile chisel to reveal any damage to the cambium and deeper wood tissue. Boring activity/colonization attempts were 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-specific symbiont of *E. fornicatus* in South Africa, and that it cannot spread without the help of its symbiotic beetle vector, confirmation of host status/successful colonization 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 fungal stained wood was removed and isolated following the methods of Paap et al. (2018). For genetic analysis, DNA was extracted from mycelia using the modified cetyl trimethylammonium bromide (CTAB) extraction (Lee et al., 1988; Wu et al., 2001). Thermocycling conditions followed Na et al. (2018) and O'Donnell et al. (1998). Amplification products were purified and sequenced by the Central Analytical Facilities (CAF) at Stellenbosch University.

Statistical analysis

Factors influencing the number of trees infested in plots (i.e., invasibility of a site)

The influence of selected variables on the number of infested trees per plot ($n=51$ plots) was tested using generalized linear mixed models (GLMMs) fitted to a zero-inflated Poisson distribution, with plot number nested in site as a random variable. This was done using the *glmmTMB* package (Brooks et al., 2017) in R v4.3.1 (R Development Core Team, 2020). The overall model incorporated the fixed plot effects of distance to infestation 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 model 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).

Factors influencing the severity and probability of infestation 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 Garden's (10 plots). As the total forested area is small, there is increased chance that all trees had a similar chance of infestation when only considering spatial extent. The effect of selected variables on the number of holes on trees of breeding host species was tested using generalized linear models (GLMs) fitted to a zero-inflated negative binomial distribution using the *glmmTMB* package in R. The overall model incorporated the fixed effects of dbh, abundance of breeding hosts within a plot (host density), abundance of infested breeding hosts within a 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 a tree was infested or not) instead of the numbers of holes on an individual. Therefore, we were able to test whether the abovementioned variables could lead not only to an increased severity of infestation on a particular tree (number of holes), but also whether these variables could influence the probability of infestation.

Movement and spread

To determine any patterns of colonization or attempted colonization by PSHB beetles in plots ($n=51$ plots), the distances between all infested breeding hosts and the nearest healthy and nearest infested conspecific trees, trees of any breeding host species, and trees of any species that can host the beetle or fungus (non-breeding hosts) were measured. The data was then log-transformed in R to enforce normality. Visual analyses of density plots and Q-Q plots (quantile-quantile plot) followed by Shapiro-Wilk

tests were used to assess the normality of the data. Data were non-normal and log-transformed to enforce normality. Inter-tree distances were compared using linear modelling (ANOVA) in R. Post-hoc analysis was performed on the inter-tree distances using a Tukey post-hoc test in R using the *multcomp* package (Hothorn et al., 2008). Using these data, it was possible to determine whether PSHB infestation of trees were clumped, i.e., whether attacks spread from reproductive hosts preferentially to any other possible host (breeding or non-breeding) simply due to its proximity to the source tree.

Progression of infestation at the plot level

To determine which factors may contribute to an increase in the number of trees that become infested over time, data collected during the first survey (June 2019) were compared with data 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 the number of infested trees per plot (from a total of 54 infested trees to 83 trees) was used as the response variable and the same plot-level variables were used as described previously as explanatory variables. In addition to these factors, the explanatory variables for the number of colonized breeding hosts per plot during the first sampling period and the total number of PSHB entry holes on breeding hosts per plot at the first sampling period were included. Greater increases in PSHB attack were expected in plots with a higher number of breeding hosts and more severe infestations on host trees. A GLMM fitted to a zero-inflated Poisson distribution using the *glmmTMB* package in R was used following procedures described before.

Progression of infestation at the tree level

To determine which factors may lead to a more rapid increase in infestation severity over time, data collected during the first sampling period were compared with data collected during the final sampling period. For these analyses, all trees infested with PSHB at any time during the sampling period from all plots were considered. Data were analysed using GLM procedures as outlined before. The overall model incorporated all fixed effects as outlined in the section 'Factors influencing the severity...', above. Variables added for this particular analysis included (1) the number of holes recorded during the first sampling period (i.e., we predicted an increase in infestations in trees with higher levels of initial infestations), (2) total number of holes on all breeding hosts in a plot during the first sampling period (i.e., we predicted a larger increase in infestations in trees that are surrounded by more severely infested breeding hosts), and (3) the distance to nearest infested breeding host as of the first sampling period (i.e., we predicted

that close proximity to an infested breeding host would increase the chances of increased infestation severity). Best candidate models and model averaging procedures followed those previously outlined. Analyses to determine which factors may lead to a more rapid increase in infestation severity over time were first conducted on data from all infested trees in which tree species could not be accounted for due to too few trees of many species showing an increase in the number of holes. In addition, abovementioned analyses were repeated, but included host identity as an explanatory variable to determine whether certain host species have the potential for faster colonization by PSHB than others. For this analysis only those host species that had at least three infested trees and more than five trees in total were included. Post-hoc analyses were performed on the different species using a Tukey test in R in the *multcomp* package (Hothorn et al., 2008).

Preferential colonization

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

RESULTS

The presence of PSHB was confirmed at eight of the 11 monitoring sites and in a total of 27 of the 51 plots at the end of the monitoring period (Appendix 1). These sites were spread across the survey area (Figure 1) but most, e.g., those in the George Botanical Garden's and in the Pledge Nature Reserve, were near urban transition zones. However, PSHB infestations were confirmed in climax forest areas distant from the urban fringes such as at Jubilee Creek and Tsitsikamma. Both sites have fire-making facilities where visitors can import their own wood. Other plots that were not infested tended to be in deep forest and away from high human traffic.

A total of 1682 trees and 68 species were assessed for possible infestation across the 51 monitoring plots. Six of the assessed species were exotic, whereas the others were indigenous to the area (Table S1). Six native and one exotic species were confirmed as breeding hosts of PSHB, whereas 11 species were found to host the fungus without PSHB establishment (Table S1). Fifty plant species did not have any visible signs of attempted PSHB infestation

despite some of these occurring in high numbers in plots that also contained PSHB infested hosts. Only one host tree [*Vepris lanceolata* (Lam.) G.Don] died during the monitoring period likely due to PSHB infestations. The crowns of all other infested host trees did not show any signs of reduction in health at the end of the monitoring period despite some being heavily infested. External symptoms of infestations varied considerably on different tree species with some [e.g., *Diospyros glabra* (L.) De Winter, *Diospyros whyteana* (Hiern) F.White, *Halleria lucida* L., and *Quercus robur* L.] 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*, *Nuxia floribunda* Benth., and *Gymnosporia buxifolia* (L.) Szyszyl.]. Dried sap was also often observed around entry holes on recently colonised species such as *Rapanea melanophloeos* (L.) R.Br. ex Sweet, *V. lanceolata*, and *Virgilia oroboides* subsp. *ferruginea* B.-E. van Wyk. 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.

Factors influencing the invasibility of a plot

The best model had an AICc value of 153.5 and a weight of 0.16 (d.f. = 8, LogLink = -68.77). Ten competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, five variables were retained, all of which were significant (Table 1). The number of infested trees across all 51 survey plots decreased with an increased distance from an infestation border and an increase in the tree species richness within plots. The number of infested trees increased with an increase in abundance of breeding hosts within a plot, the percentage of trees with natural impact within a plot, and an increased distance from a plot to a water source (Table 1, Figure 2).

Factors influencing the severity and probability of infestation in susceptible hosts

These analyses only included the 10 plots from the George Botanical Garden's which contained a total of 423 trees. Here, in total 36 trees were infested (9% of all trees surveyed) of which 26 trees were breeding hosts (5% of all trees surveyed) (Table S2). When considering the severity of infestations on hosts the best model had an AICc value of 333.1 and a weight of 0.41 (d.f. = 5, LogLink = -161.58). No competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, three variables were retained, all of which were significant (Table 1). Here, trees with a larger diameter tended to have more holes, an increase in the number of breeding hosts within a plot increased the severity of infestations on trees, and the severity of infestations also increased with a higher

number of infested trees (breeding and non-breeding) within a plot (Table 1, Figure 3).

When considering the probability of infestations on hosts the best model had an AICc value of 106.3 and a weight of 0.18 (d.f. = 3, LogLink = -50.16). Six competing models were retained after model selection procedures. After model averaging procedures, two variables were retained, one of which was significant (Table 1). As with data on severity, the probability of infestation also increased with the number of infested trees (breeding and non-breeding) within a plot (Figure 4).

Movement and spread of PSHB

Normality tests showed the movement data to have a non-normal distribution (all Shapiro-Wilk tests: $P < 0.001$). In total 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 ($F_{5,260} = 11.75$, $P = 2.03 \times 10^{-10}$). The distance between an infested reproductive host and its nearest infested host was significantly larger when compared to the nearest healthy host, irrespective of the host type (breeding or not), any breeding host (of any species), or conspecific species (Figure 5).

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

At the start of the monitoring in June 2019, in total 54 trees were infested compared to 83 trees after the final monitoring survey in September 2020. No additional plots were infested. Using data on the probability of increase in infested trees per plot between the two monitoring periods the best model had an AICc value of 85.9 and a weight of 0.09 (d.f. = 6, LogLink = -30.9). Eleven competing models were retained after model selection procedures ($\Delta\text{AICc} \leq 2$). After model averaging procedures, one variable was retained, which was not significant (Table 2).

Progress of infestation at the tree 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 trees (mean \pm SE = 21.31 ± 41.45). A maximum of 300 holes and a minimum of one hole was recorded per individual. Infested trees showed an increase of 62% in the number of holes recorded over time. For the total data set, the best model had an AICc value of 382.5 and a weight of 0.16 (d.f. = 6, LogLink = -185.2). Eleven competing models were retained

TABLE 1 Model-averaged estimates of environmental variables predicting severity of polyphagous shot hole borer (PSHB) invasion within a plot and in susceptible hosts. Also shown are estimates for environmental variables predicting the probability of infestation by PSHB in susceptible hosts.

	Variable	Estimate	SE	Relative importance	No. containing models	Z	P
Infestation severity within a plot	Intercept	-1.187	0.839	-	-	-1.145	0.16
	Distance to infestation border	-0.383	0.120	1.00	10	-3.182	0.001
	Breeding host abundance	0.132	0.032	0.99	10	4.034	<0.001
	Tree species richness	-0.074	0.032	0.64	7	-2.299	0.022
	Distance from water	0.025	0.009	0.58	3	2.576	0.009
	Natural impact	3.74	1.63	0.53	3	2.292	0.022
Infestation severity of susceptible hosts	Intercept	-5.423	1.631	-	-	-3.325	0.088
	Diameter at breast height	0.019	0.005	0.99	1	3.826	<0.001
	Breeding host abundance	0.183	0.073	0.63	1	2.500	0.012
	Infested host abundance	0.293	0.113	0.83	1	2.599	0.009
	Overall tree injury	0.058	0.043	0.64	1	1.339	0.18
Infestation probability of susceptible hosts	Intercept	-2.871	0.506	-	-	-5.680	<0.001
	Diameter at breast height	0.003	0.002	0.51	3	1.890	0.058
	Infested host abundance	0.186	0.065	0.97	6	2.881	0.004

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

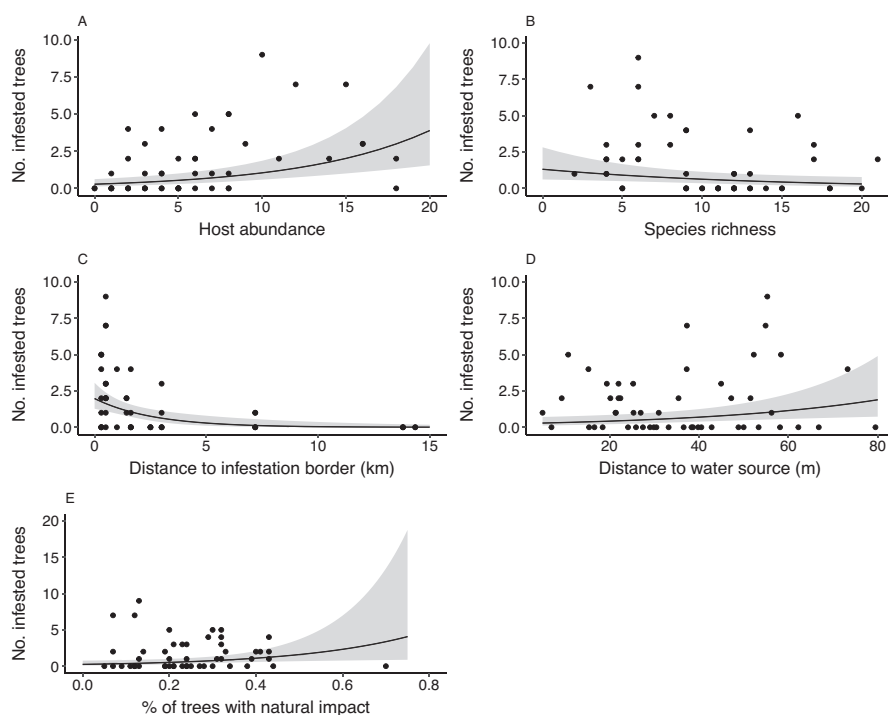


FIGURE 2 Regression estimates of the (A) effect of host species abundance per plot, (B) tree species richness per plot, (C) distance (km) of the plot to the infestation border, (D) distance (m) to water, and (E) natural impact on a plot on the number of polyphagous shot hole borer infested trees per plot. Shaded areas represent 95% confidence intervals.

after model selection procedures. After model averaging procedures, three variables were retained of which two were significant (Table 3). A faster increase in severity of infestation over time was positively correlated to an increase in diameter of trees and the number of breeding hosts in a plot (Table 3, Figure 6).

The second analysis included host species as an explanatory variable (reduced data set as many host taxa had insufficient numbers of trees with increased infestations over time). For this data set, the best model had an AICc value of 378.2 and a weight of 0.14 (d.f. = 6, LogLik = -183.11). Fifteen competing models were retained after model

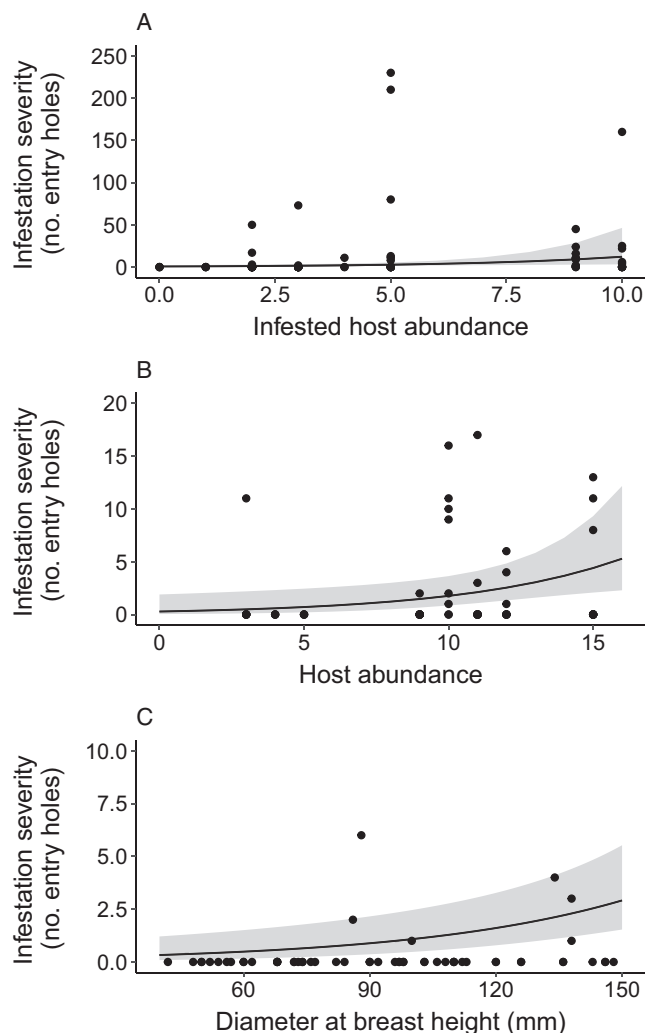


FIGURE 3 Regression estimates of the (A) effect of infested host tree abundance per plot, (B) number of breeding hosts per plot, and (C) tree size (diameter at breast height, mm) on the number of polyphagous shot hole borer entry holes per tree (infestation severity). Shaded areas represent 95% confidence intervals.

selection procedures. After model averaging procedures, three variables were retained of which two were significant (Table 3). An increase in severity of infestations over time was positively correlated to an increased tree diameter (Figure 6). 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 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* and *H. lucida* (data not shown).

Preferential colonization

A total of 598 trees belonged to species that have had at least one tree with PSHB infestations in the 27 plots where

infestations were recorded across the sampling area. Eighty-three trees of 18 species 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 vs. what was expected by chance (Table S3). Eight host species were infested at probabilities that were expected by chance and do not seem to be preferentially selected by PSHB (Table S3). These taxa 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 (Table S3). Most of these species are known to be breeding hosts (van Rooyen et al., 2021). Two species [*Burchellia bubalina* (L.f.) Sims and *R. melanophloeos*] with signs of infestations in the monitored plots were infested at lower probabilities than expected by chance and therefore are likely selected against (Table S3).

DISCUSSION

The polyphagous shot hole borer is a recent invader in South Africa that has caused disease and death of thousands of trees globally (Eskalen et al., 2013; Freeman et al., 2013; Chen et al., 2014; Coleman et al., 2019). Information regarding its impact on native ecosystems in its invaded range is lacking, but *Euwallacea kuroshio* Gomez & Hulcr, a close relative, has caused destruction of native willows (*Salix* spp.) in the Tijuana River Valley (CA, USA) (Boland, 2016) with severe impact on ecosystem processes and resilience (Boland & Uyeda, 2020). The present study is the first to investigate the ecological factors that aid PSHB invasion into a native forest. Numerous native tree species in Afrotemperate forests were colonized by PSHB and its symbiotic fungus. Breeding PSHB colonies were evident in nearly 10% of all native species surveyed which may suffer high mortality. Organisms associated with these species will also be threatened as each tree species is known to support unique biodiversity (Swart et al., 2020). Normal ecosystem functioning of Afrotemperate forests and surrounding local economies may therefore be at risk (Umeda et al., 2016).

Factors that influence invasibility of Afrotemperate forests

As expected, areas that were in close proximity to outbreak areas, especially those with a high abundance of reproductive hosts and in more open early-successional forest, were more likely to be invaded at this early stage in the invasion process (Holt et al., 2003; Haas et al., 2016; Choudhury et al., 2020). The most likely explanation for PSHB presence in climax forest sites, in sites distant from other invaded areas, and in urban areas, is through long-distance human-mediated transport as contaminants of firewood. Large and mature trees, expected in climax forest, were shown to generally suffer from greater PSHB infestation probability and

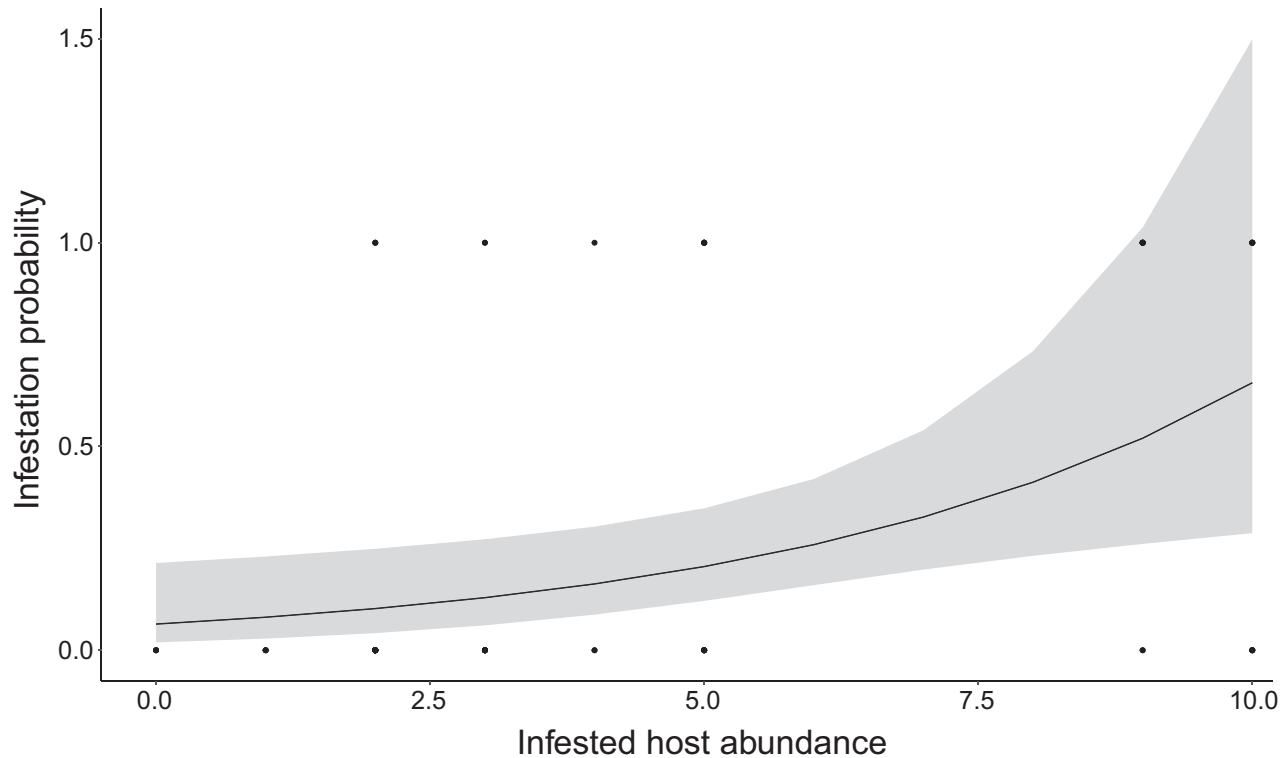


FIGURE 4 Regression estimates of the effect of infested tree abundance per plot on the probability of polyphagous shot hole borer infestation of trees. Shaded area represents 95% confidence interval.

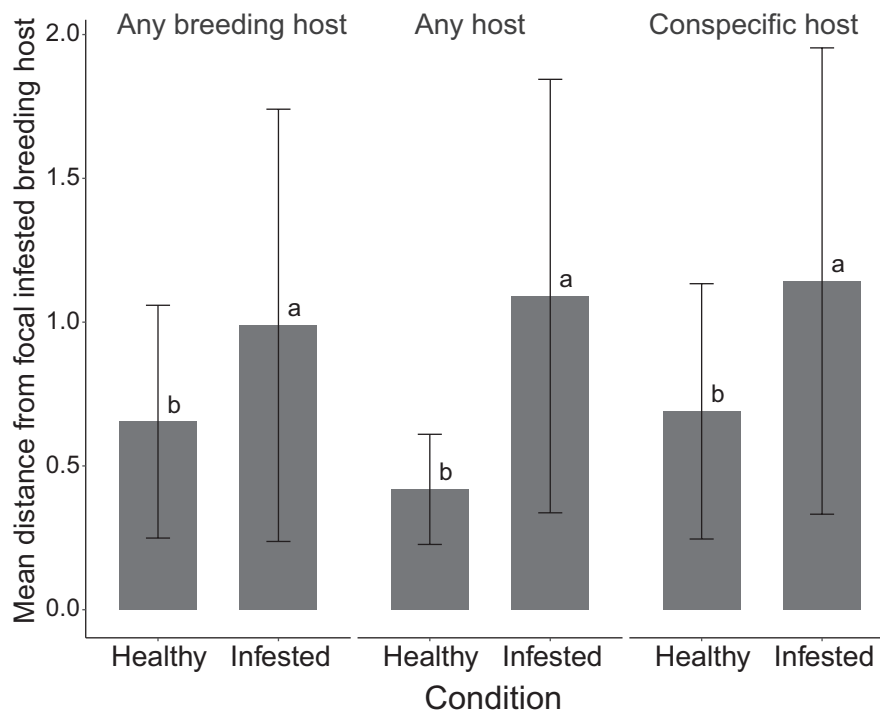


FIGURE 5 Mean (\pm SD) distance [log(m)] from a polyphagous shot hole borer infested breeding host tree to nearest other host (healthy and infested) categorised as either breeding hosts only (irrespective of breeding host species identity), any host (i.e., breeding and non-breeding hosts), and conspecific breeding host species. Means capped with different letters are significantly different (Tukey's honest significance test: $P < 0.05$).

severity. The potential loss of large trees of climax forest species such as *Afrocarpus falcatus* (Thunb.) C.N. Page is not only of ecological concern as the main emerging tree in these

forest canopies (Lübbe & Geldenhuys, 1991; Geldenhuys & Von Dem Bussche, 1997), but social and economic impacts are expected as some trees in the study region are South

TABLE 2 Model-averaged estimates of environmental variables predicting progress of infestation by polyphagous shot hole borer at plot level.

Variable	Estimate	SE	Relative importance	No. containing models	Z	P
Intercept	0.326	0.311	-	-	1.049	0.29
Distance to infestation border	-0.327	0.208	0.6	5	-1.572	0.12

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

TABLE 3 Model-averaged estimates of environmental variables predicting progress in severity of infestations by polyphagous shot hole borer at the tree level without species as explanatory variable and including species as explanatory variable.

	Variable	Estimate	SE	Relative importance	No. containing models	Z	P
Without species as explanatory variable	Intercept	-1.519	0.967	-	-	-1.571	0.12
	Breeding host abundance	0.164	0.061	0.92	11	2.706	0.006
	Infested host abundance	0.208	0.114	0.51	6	1.822	0.068
	Diameter at breast height	0.001	0.005	0.59	7	2.062	0.039
Including species as explanatory variable	Intercept	1.91	0.620	-	-	3.076	0.002
	Diameter at breast height	1.908	0.000	1.00	13	4.231	0.002
	Infested host abundance	0.173	0.091	0.49	6	1.904	0.056
	Species	-0.464	0.094	1.00	13	-4.919	<0.001

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

African champion trees (Golding & Geldenhuys, 2003) that generate income for surrounding poor communities as tourist attractions (Saayman et al., 2012).

As with other studies on the epidemiology of pests and diseases, an increase in breeding host abundance (or density) significantly correlated to an increase in the number of PSHB infestations in plots (Carlsson et al., 1990; Roy, 1993; Burdon et al., 1995; Antonovics et al., 1997; Gilbert et al., 2002; Choudhury et al., 2020). 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 the PSHB reproductive hosts *D. glabra*, *H. lucida*, and *V. oroboides* along southern Afrotropical forest edges (Phillips, 1931; Coetsee & Wigley, 2013). 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 (Phillips, 1926; Coetsee & Wigley, 2013). Local decimation of these species could hold disastrous consequences for normal forest ecology and for the biota directly dependent on them (Machingambi et al., 2014; van der Colff et al., 2015).

The biotic resistance hypothesis predicts that diverse native communities are more resistant to invasion (Fridley et al., 2007). In our study, the number of infested trees declined with an increase in tree species richness. Rassati et al. (2016) showed that in temperate deciduous forest stands in the northeast of Italy, higher tree species diversity resulted in lower densities of non-native ambrosia beetles, which significantly decreased their ability to establish in an area.

The exact cause for this is not known, but it is likely that as the species richness of a plot increases the number of suitable hosts decreases.

Natural disturbances in monitoring sites studied here increased the chances of colonization by PSHB (Keesing et al., 2006; Boland, 2016; Coleman et al., 2019). High numbers of injured and damaged trees may attract PSHB beetles to a certain area as stressed trees release kairomones which *Euwallacea* beetles are highly sensitive to (Hulcr & Stelinski, 2017). Even when PSHB can easily infest healthy hosts (Kühnholz et al., 2003), most ambrosia beetles prefer stressed trees in their native and invaded ranges (Ploetz et al., 2013; Boland, 2016). 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) could have severe negative consequences. In addition, increased distance from surface water resulted in increased invasibility of sites contrary to what was documented for *E. kuroshio* in the USA (Boland, 2016; Boland & Uyeda, 2020). Moisture content of the trees in the present study may be reduced further away from surface water, which may increase plant stress and increase their susceptibility to pests and pathogens (Swart et al., 2018, 2020).

Factors that increase the severity and probability of infestation of susceptible host trees

As hypothesized, probability of infestation (whether a tree is infested or not) and severity of infestation (increase in

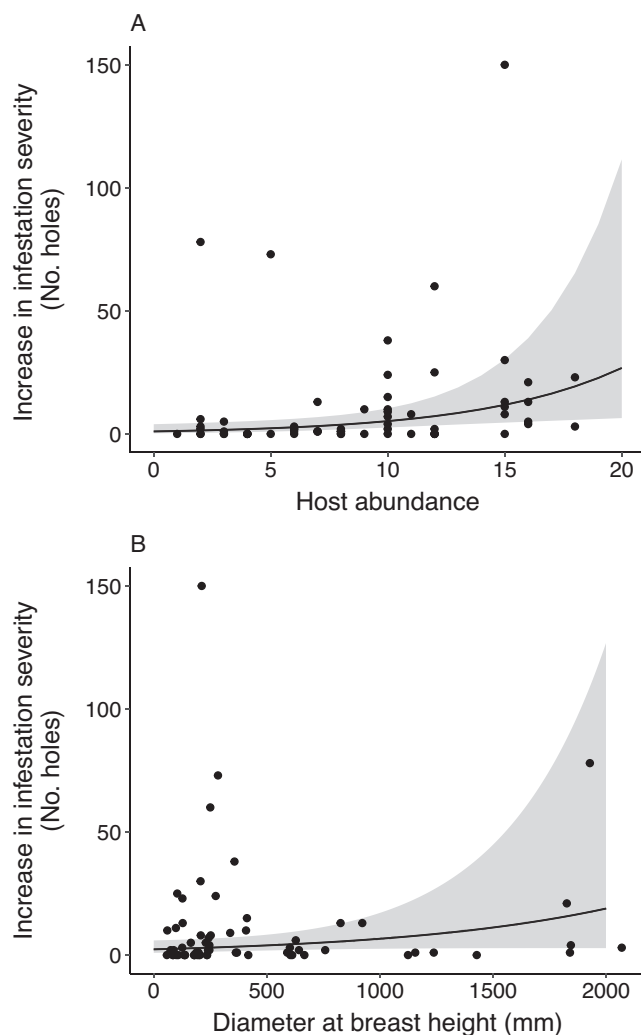


FIGURE 6 Variables that significantly affected the increase in severity of polyphagous shot hole borer infestations (no. holes) on trees between the first sampling period (June 2019) and the last sampling period (September 2020) including (A) the effect of the abundance of breeding hosts in a plot and (B) the effect of tree size (diameter at breast height, mm). Shaded areas represent 95% confidence intervals.

number of entrance holes) were positively correlated with tree size (dbh). Coleman et al. (2019) found no significant difference in dbh between PSHB-infested and non-infested trees in California (USA), but the opposite was found in the native range of PSHB in southwestern China. The average dbh of trees infested by *E. kuroshio* was, however, larger than that of uninfested trees in California (Coleman et al., 2019). Larger trees are some of the first attacked (and have larger densities of beetle populations) for other ambrosia beetles such as *Xyleborus glabratus* Eichhoff (Kendra et al., 2013). Larger trees present a larger surface area and emit more kairomones, making them easier to locate. Some Scolytinae may also visually select for larger trees by comparison of their silhouette and larger trees also have a higher probability of passively intercepting a flying beetle (Mayfield & Brownie, 2013). As PSHB is a diurnal-active

species, it likely uses visual cues (host silhouette diameter) along with increased expression of kairomones to locate potential hosts as was found for the redbay ambrosia beetle (Fraedrich et al., 2008; Mayfield & Brownie, 2013; Frank & Ranger, 2016). Severity of infestations on larger hosts may also increase due to greater availability of brood production resources (i.e., sapwood) (Mayfield & Brownie, 2013; Ranger et al., 2016; Choudhury et al., 2020). A high density of larger hosts therefore tends to have a higher pest and disease incidence in other examples of Scolytidae infestations (Anderson & May, 1986; Holt et al., 2003; Reynolds & Burke, 2011; Choudhury et al., 2020), but this has not been established for PSHB and more research would be needed to determine whether it has a true preference for larger (thicker) trees (Choudhury et al., 2020).

The number of infested hosts in a plot also increased both the likelihood and the severity of infestations by PSHB. This is to be expected as a high propagule pressure will exist with many infested trees. As expected, the severity of infestations also increased with an increase in breeding host abundance, as these species 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 & Cleary, 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.

Although at the plot level PSHB infestations increased with tree injury level, an increase in tree injury level and distance to human activities did not increase the likelihood of infestations and the severity of infestations on trees. 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 and severity of infestations, as expected (Wichmann & Ravn, 2001; Mendel et al., 2017; Choudhury et al., 2020). 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 could be due to trees intercepting beetles during flight and PSHB beetles opportunistically attacking trees 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 were confirmed to be colonised by either PSHB or *F. euwallaceae* had a higher number of infested trees than that expected by chance. Six of these species were confirmed as breeding hosts here or before: *A. falcatus*, *H. lucida*, *Q. robur* (exotic),

Sparrmannia africana L.f., *V. lanceolata*, and *V. oroboides* (van Rooyen et al., 2021). The other two (*D. whyteana* and *Ekebergia capensis* Sparrm.) are suspected PSHB breeding hosts but could not yet be confirmed. Polyphagous shot hole borer therefore finds these hosts suitable, leading to a higher invasion success (Lantschner et al., 2020). Two host species (*B. bubalina* and *R. melanophloeos*) had lower numbers of colonised trees than expected by chance. Even though the fungus can colonize the vascular tissues of these taxa, PSHB is unable to capitalize on this. This indicates that these hosts, 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. Other taxa that were able to host *F. euwallaceae*, but not PSHB, were infested by PSHB at random. These hosts probably 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).

Progression of infestation

At the plot level there were only 29 additional trees infested after the year of monitoring. Due to a low sample size, there were no factors at the plot level that could explain this increase. 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 is expected over time (Chen et al., 2014; Stouthamer et al., 2017). However, the severity of infestation did increase on many trees, especially for some of the breeding hosts (*D. glabra* and *H. lucida*). 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 located 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. The increasing severity of infestations in the breeding hosts *D. glabra* and *H. lucida* is concerning as both are early-successional forest species predominantly found on forest margins. *Halleria lucida* does also occur in deep forest (Geldenhuys, 1993). Both host species were found in high numbers in early-successional forest sites during this study. This could mean that the first severe impact of PSHB in these forests will be realised in early-successional forest communities. This will then have negative indirect impact on climax forest. These edges will also sustain large breeding populations of PSHB that can easily move into climax communities. In the process, more open canopies will be created (from dead or diseased trees), forming a positive feedback loop.

Management considerations

Early detection, management, and control of infestations are vital to mitigate future impact (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 species 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 and the severity of infestations. Therefore, as was previously recommended (Chen et al., 2020), removal of heavily infested (>10 holes as nearly all of these trees 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 get chipped (<5 cm) and solarized (Jones & Paine, 2015; Chen et al., 2020). For native forests, the development of a biocontrol agent would be essential as chemical control is not an option. These studies have now been initiated in the USA (R Stouthamer, pers. comm.) and in South Africa (Nel et al., 2023), 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. Future studies should pursue why certain hosts are preferred over others and may involve PSHB host choice experiments, the identification of allomones against PSHB, or identifying tree vascular anatomical traits that may hamper fungal growth.

AUTHOR CONTRIBUTIONS

Garyn Townsend: Conceptualization (equal); data curation (equal); formal analysis (lead); investigation (equal); methodology (equal); visualization (equal); writing – original draft (equal); writing – review and editing (lead). **Elmar van Rooyen:** Conceptualization (equal); data curation (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Martin Patrick Hill:** Conceptualization (equal); funding acquisition (equal); project administration (equal); supervision (equal); writing – review and editing (supporting). **Wilhelm de Beer:** Funding acquisition (equal); project administration (equal); supervision (lead). **Francois Roets:** Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (equal);

investigation (equal); methodology (equal); project administration (lead); resources (equal); supervision (lead); validation (equal); visualization (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

The South African Department of Environment, Forestry, and Fisheries (DEFF) and the Natural Resource Management Programme are thanked for funding, noting that this publication does not necessarily represent the views or opinions of DEFF or its employees. The Tree Protection Cooperative Programme (TCP) and the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, are acknowledged for funding and infrastructure support.

CONFLICT OF INTEREST

The authors have no conflict of Interest with this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

G. Townsend  <https://orcid.org/0000-0001-9128-961X>
 E. van Rooyen  <https://orcid.org/0000-0002-7700-6764>
 M. Hill  <https://orcid.org/0000-0003-0579-5298>
 W. De Beer  <https://orcid.org/0000-0001-9758-8987>
 F. Roets  <https://orcid.org/0000-0003-3849-9057>

REFERENCES

- Anderson RM & May RM (1986) The invasion, persistence and spread of infectious diseases within animal and plant communities. *Philosophical Transactions of the Royal Society of London B* 314: 533–570.
- Antonovics J, Thrall PH & Jarosz AM (1997) Genetics and the spatial ecology of species interactions: the *Silene-Ustilago* system. *Spatial Ecology - The Role of Space in Population Dynamics and Interspecific Interactions* (ed. by D Tilman & P Kareiva), pp. 158–180. Princeton University Press, Princeton, NJ, USA.
- Bartoń K (2009) MuMIn: Multi-model inference. R Package v.1.43.6, 75.
- Berliner D (2011) Systematic Conservation Planning for the Forest Biome of South Africa: An Assessment of the Conservation Status of South Africa's Forests and Recommendations for their Conservation. PhD Dissertation, University of Capetown, Capetown, South Africa.
- Boland JM (2016) The impact of an invasive ambrosia beetle on the riparian habitats of the Tijuana River Valley, California. *PeerJ* 4: e2141.
- Boland JM & Uyeda KA (2020) *The Ecology and Management of the Kuroshio Shot Hole Borer in the Tijuana River Valley (Issue Year 5)*. US Navy & Southwest Wetlands Interpretive Association, Imperial Beach, CA, USA.
- Bonilla-Landa I, de la Cruz OL, Sánchez-Rangel D, Ortíz-Castro R, Rodríguez-Haas B et al. (2018) Design, synthesis and biological evaluation of novel fungicides for the management of *Fusarium* dieback disease. *Journal of the Mexican Chemical Society* 62: 86–98.
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW et al. (2017) GlmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling. *The R Journal* 9: 378–400.
- Burdon JJ, Ericson L & Muller WJ (1995) Temporal and spatial changes in a metapopulation of the rust pathogen *Triphragmium ulmariae* and its host, *Filipendula ulmaria*. *Journal of Ecology* 83: 979–989.
- Burnham KP & Anderson DR (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York, NY, USA.
- Byers JA, Maoz Y & Levi-Zada A (2017) Attraction of the *Euwallacea* sp. near *forficatus* (Coleoptera: Curculionidae) to quercivorol and to infestations in Avocado. *Journal of Economic Entomology* 110: 1512–1517.
- Carlsson U, Elmquist T, Wennstrom A & Ericson L (1990) Infection by pathogens and population age of host plants. *Journal of Ecology* 78: 1094–1105.
- Chen Y, Coleman TW, Graves AD, Meeker JR & Seybold SJ (2014) Host range of the invasive polyphagous shot hole borer. *California Forest Pest Council Annual Meeting*. 12–13 Nov 2014.
- Chen Y, Coleman TW, Poloni AL, Nelson L & Seybold SJ (2020) Reproduction and control of the invasive polyphagous shot hole borer, *Euwallacea* nr. *forficatus* (Coleoptera: Curculionidae: Scolytinae), in three species of hardwoods: effective sanitation through felling and chipping. *Environmental Entomology* 49: 1155–1163.
- Choudhury RA, Er HL, Hughes MA, Smith JA, Pruett GE et al. (2020) Host density dependence and environmental factors affecting laurel wilt disease incidence. *Plant Pathology* 70: 676–688.
- Christiansen E, Waring RH & Berryman AA (1987) Resistance of conifers to bark beetle attack: searching for general relationships. *Forest Ecology and Management* 22: 89–106.
- Cobb RC, Chan MN, Meentemeyer RK & Rizzo DM (2012) Common factors drive disease and coarse woody debris dynamics in forests impacted by sudden oak death. *Ecosystems* 15: 242–255.
- Coetsee C & Wigley BJ (2013) *Virgilia divaricata* may facilitate forest expansion in the afrotemperate forests of the southern Cape, South Africa. *Koedoe* 55: 1–8.
- Coleman TW, Poloni AL, Chen Y, Thu PQ, Li Q et al. (2019) Hardwood injury and mortality associated with two shot hole borers, *Euwallacea* spp., in the invaded region of southern California, USA, and the native region of Southeast Asia. *Annals of Forest Science* 76: 1–18.
- van der Colff D, Dreyer LL, Valentine A & Roets F (2015) Invasive plant species may serve as a biological corridor for the invertebrate fauna of naturally isolated hosts. *Journal of Insect Conservation* 19: 863–875.
- Coyle DR, Booth DC & Wallace MS (2005) Ambrosia beetle (Coleoptera: Scolytidae) species, flight, and attack on living eastern cottonwood trees. *Journal of Economic Entomology* 98: 9.
- Damon A (2000) A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research* 90: 453–465.
- Eskalen A, Stouthamer R, Lynch SC, Rugman-Jones PF, Twizeyimana M et al. (2013) Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. *Plant Disease* 97: 938–951.
- FABI (Forestry and Agricultural Biotechnology Institute) (2023) *The Polyphagous Shot Hole Borer (PSHB) and its Fungus in South Africa*. <https://www.fabinet.up.ac.za/pshb> (accessed on 23-11-2023)
- Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield AE et al. (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the Southeastern United States. *Plant Disease* 92: 215–224.
- Frank SD & Ranger CM (2016) Developing a media moisture threshold for nurseries to reduce tree stress and ambrosia beetle attacks. *Environmental Entomology* 45: 1040–1048.
- Freeman S, Sharon M, Maymon M, Mendel Z, Protasov A, Aoki T, Eskalen A, O'Donnell K (2013) *Fusarium euwallaceae* sp. nov.—a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia* 105: 1595–1606.
- Fridley JD, Stachowicz JJ, Naeem S, Sax DF, Seabloom EW, Smith MD, Stohlgren TJ, Tilman D, Holle BV (2007) The invasion paradox: reconciling pattern and process in species invasions. *Ecology* 88: 3–17.

- Geldenhuys CJ & Von Dem Bussche GB (1997) Performance of *Podocarpus falcatus* provenances in South Africa. *Southern African Forestry Journal* 178: 15–24.
- Ghazoul J, Burivalova Z, Garcia-Ulloa J & King LA (2015) Conceptualizing forest degradation. *Trends in Ecology and Evolution* 30: 622–632.
- Gilbert GS, Ferrer A & Carranza J (2002) Polypore fungal diversity and host density in a moist tropical forest. *Biodiversity and Conservation* 11: 947–957.
- Golding JS & Geldenhuys CJ (2003) Methods and procedures for the selection of champion trees in South Africa for protection in terms of the National Forests Act of 1998. *Department of Water Affairs and Forestry 1998* (August): 1–21.
- Gomez DF, Lin W, Gao L & Li Y (2019) New host plant records for the *Euwallacea fornicatus* (Eichhoff) species complex (Coleoptera: Curculionidae: Scolytinae) across its natural and introduced distribution. *Journal of Asia-Pacific Entomology* 22: 338–340.
- Haas SE, Cushman JH, Dillon WW, Rank NE, Rizzo et al. (2016) Effects of individual, community, and landscape drivers on the dynamics of a wildland forest epidemic. *Ecology* 97: 649–660.
- Holt RD, Dobson AP, Begon M, Bowers RG & Schaubert EM (2003) Parasite establishment in host communities. *Ecology Letters* 6: 837–842.
- Hothorn T, Bretz F & Westfall P (2008) Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346–363.
- Holuša J, Fiala T & Foit J (2021) Ambrosia beetles prefer closed canopies: a case study in oak forests in central Europe. *Forests* 12: 1223.
- Hughes MA, Martini X, Kuhns E, Colee J, Mafra-Neto A et al. (2017) Evaluation of repellents for the redbay ambrosia beetle, *Xyleborus glabratus*, vector of the laurel wilt pathogen. *Journal of Applied Entomology* 141: 653–664.
- Hulcr J & Stelinski LL (2017) The ambrosia symbiosis: from evolutionary ecology to practical management. *Annual Review of Entomology* 62: 285–303.
- Jones ME & Paine TD (2015) Effect of chipping and solarization on emergence and boring activity of a recently introduced ambrosia beetle (*Euwallacea* sp., Coleoptera: Curculionidae: Scolytinae) in southern California. *Journal of Economic Entomology* 108: 1852–1859.
- Igeta Y, Esaki K, Kato K & Kamata N (2004) Spatial distribution of a flying ambrosia beetle *Platypus quercivorus* (Coleoptera: Platypodidae) at the stand level. *Applied Entomology and Zoology* 39: 583–589.
- Kautz M, Dworschak K, Gruppe A & Schopf R (2011) Quantifying spatio-temporal dispersion of bark beetle infestations in epidemic and non-epidemic conditions. *Forest Ecology and Management* 262: 598–608.
- Keesing F, Holt RD & Ostfeld RS (2006) Effects of species diversity on disease risk. *Ecology Letters* 9: 485–498.
- Kendra PE, Montgomery WS, Niogret J & Epsky ND (2013) An uncertain future for American Lauraceae: a lethal threat from redbay ambrosia beetle and laurel wilt disease (A review). *American Journal of Plant Sciences* 4: 727–738.
- King KC & Lively CM (2012) Does genetic diversity limit disease spread in natural host populations. *Heredity* 109: 199–203.
- Kühnholz S, Borden JH & Uzunovic A (2003) Secondary ambrosia beetles in apparently healthy trees: adaptations, potential causes and suggested research. *Integrated Pest Management Reviews* 6: 209–219.
- Lantschner MV, Corley JC & Liebhold AM (2020) Drivers of global Scolytinae invasion patterns. *Ecological Applications* 30: e02103.
- Lee SB, Milgroom MG & Taylor JW (1988) A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. *Fungal Genetics Reports* 35: 23.
- Li Y, Gu X, Kasson MT, Bateman CC, Guo J et al. (2016) Distribution, host records, and symbiotic fungi of *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) in China. *Florida Entomologist* 99: 801–804.
- Low AB & Rebelo AG (1996) *Vegetation of Southern Africa, Lesotho and Swaziland: A Companion to the Vegetation Map of South Africa, Lesotho and Swaziland*, Department of Environmental Affairs and Tourism, Pretoria, South Africa.
- Lübbe WA & Geldenhuys CJ (1991) Regeneration patterns in planted and natural forest stands near Knysna, Southern Cape. *South African Forestry Journal* 159: 43–50.
- Machingambi NM, Roux J, Dreyer LL & Roets F (2014) Bark and ambrosia beetles (Curculionidae: Scolytinae), their phoretic mites (Acari) and associated *Geosmithia* species (Ascomycota: Hypocreales) from *Virgilia* trees in South Africa. *Fungal Biology* 118: 472–483.
- Maetò K, Fukuyama K & Kirton LG (1999) Edge effects on ambrosia beetle assemblages in a lowland rain forest, bordering oil palm plantations, in peninsular Malaysia. *Journal of Tropical Forest Science* 11: 537–547.
- Mayfield AE & Brownie C (2013) The redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) uses stem silhouette diameter as a visual host-finding cue. *Environmental Entomology* 42: 743–750.
- Mendel Z, Protasov A, Maoz Y, Maymon M, Miller G et al. (2017) The role of *Euwallacea* nr. *fornicatus* (Coleoptera: Scolytinae) in the wilt syndrome of avocado trees in Israel. *Phytoparasitica* 45: 341–359.
- Mendel Z, Protasov A, Sharon M & Zveibil A (2012) An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. *Phytoparasitica* 40: 235–238.
- Mucina L, Geldenhuys CJ, Rutherford MC, Powrie LW, Lötter MC et al. (2006) Afrotemperate, subtropical and azonal forests. *The Vegetation of South Africa, Lesotho and Swaziland* (ed. by L Mucina & MC Rutherford), pp. 584–615. South African National Biodiversity Institute, Pretoria, South Africa.
- Muirhead JR, Leung B, Van Overdijk C, Kelly DW, Nandakumar K et al. (2006) Modelling local and long-distance dispersal of invasive emerald ash borer *Agrilus planipennis* (Coleoptera) in North America. *Diversity and Distributions* 12: 71–79.
- Na F, Carrillo JD, Mayorquin JS, Ndinga-Muniania C, Stajich JE et al. (2018) Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. of kuroshio shot hole borer (*Euwallacea* sp. nr. *fornicatus*) cause *Fusarium* dieback on woody host species in California. *Plant Disease* 102: 1154–1164.
- Nel WJ, Slippers B, Wingfield MJ, Yilmaz N & Hurley BP (2023) Efficacy of commercially available entomopathogenic agents against the polyphagous shot hole borer in South Africa. *Insects* 14: 361.
- O'Donnell K, Cigelnik E & Nirenberg HI (1998) Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90: 465–493.
- Ostfeld RS & Keesing F (2012) Effects of host diversity on infectious disease. *Annual Review of Ecology, Evolution, and Systematics* 43: 157–182.
- Paap T, de Beer ZW, Miglioni D, Nel WJ & Wingfield MJ (2018) The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. *Australasian Plant Pathology* 47: 231–237.
- Phillips JFV (1926) *Virgilia divaricata capensis* Lam. ('Keurboom'): a contribution to its ecology and silviculture. *South African Journal of Science* 23: 435–454.
- Phillips JFV (1931) Forest succession and ecology in the Knysna region. *Memoirs of the Botanical Survey of South Africa* 14: 1–327.
- Ploetz RC, Hulcr J, Wingfield MJ & de Beer WZ (2013) Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? *Plant Disease* 97: 856–872.
- Prospero S & Cleary M (2017) Effects of host variability on the spread of invasive forest diseases. *Forests* 8: 1–21.
- R Development Core Team (2020) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rabaglia R, Duerr D, Acciavatti R & Ragenovich I (2008) *Early Detection and Rapid Response for Non-Native Bark and Ambrosia Beetles*. USDA Forest Service, Forest Health Protection, Washington, DC, USA.
- Ranger CM, Reding ME, Schultz PB, Oliver JB, Frank SD et al. (2016) Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. *Journal of Integrated Pest Management* 7: 1–23.
- Rassati D, Faccoli M, Haack RA, Rabaglia RJ, Toffolo EP, Battisti A, Marini L (2016) Bark and ambrosia beetles show different invasion patterns in the USA. *PLoS One* 11: e0158519.

- Reynolds DL & Burke KL (2011) The effect of growth rate, age, and chestnut blight on American chestnut mortality. *Castanea* 76: 129–139.
- van Rooyen E, Paap T, de Beer ZW, Townsend G, Fell S, Nel WJ, Morgan S, Hill M, Gonzalez A, Roets F (2021) The polyphagous shot hole borer (PSHB) beetle: current status of a perfect invader in South Africa. *South African Journal of Science* 117: 9736.
- Roy BA (1993) Patterns of rust infection as a function of host genetic diversity and host density in natural populations of the apomictic crucifer, *Arabidopsis thaliana*. *Evolution* 47: 111–124.
- Saayman M, Rossouw R & Krugell W (2012) The impact of tourism on poverty in South Africa. *Development Southern Africa* 29: 462–487.
- Shimwela MM, Schubert TS, Albritton M, Halbert SE, Jones DJ et al. (2018) Regional spatial-temporal spread of citrus huanglongbing is affected by rain in Florida. *Phytopathology* 108: 1420–1428.
- Simberloff D (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics* 40: 81–102.
- Smith D, Smith I, Collett N & Elms S (2008) Forest health surveillance in Victoria. *Australian Forestry* 71: 188–195.
- Smith JP & Hoffman JT (2001) Site and stand characteristics related to white pine blister rust in high-elevation forests of southern Idaho and western Wyoming. *Western North American Naturalist* 61: 409–416.
- Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T et al. (2017) Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agricultural and Forest Entomology* 19: 366–375.
- Swart RC, Pryke JS & Roets F (2018) Arthropod assemblages deep in natural forests show different responses to surrounding land use. *Biodiversity and Conservation* 27: 583–606.
- Swart RC, Samways MJ, Pryke JS & Roets F (2020) Individual tree context and contrast dictate tree physiological features and arthropod biodiversity patterns across multiple trophic levels. *Ecological Entomology* 45: 333–344.
- Umeda C, Eatough Jones M & Paine T (2019) Irrigation and its effect on polyphagous shot hole borer attack. *Entomologia Experimentalis et Applicata* 167: 820–825.
- Umeda C, Eskalen A & Paine TD (2016) Polyphagous shot hole borer and *Fusarium* dieback in California. *Insects and Diseases of Mediterranean Forest Systems* (ed. by T Paine & F Lieutier), pp. 757–767. Springer, Cham, Switzerland.
- Wichmann L & Ravn HP (2001) The spread of *Ips typographus* (L.) (Coleoptera, Scolytidae) attacks following heavy windthrow in Denmark, analysed using GIS. *Forest Ecology and Management* 148: 31–39.
- van Wilgen BW, Measey J, Richardson DM, Wilson JR & Zengeya TA (2020) Biological invasions in South Africa: an overview. *Biological Invasions in South Africa* (ed. by BW van Wilgen, J Measey, DM Richardson, JR Wilson & TA Zengeya), pp. 3–31. Springer, Cham, Switzerland.
- de Wit M, Crooks D, Blignaut J, De Beer W, Paap T, Roets F, Van Der Merwe C, Van Wilgen BW, Richardson DM (2022) An assessment of the potential economic impacts of the invasive polyphagous shot hole borer (Coleoptera: Curculionidae) in South Africa. *Journal of Economic Entomology* 115: 1076–1086.
- Wu ZH, Wang TH, Huang W & Qu YB (2001) A simplified method for chromosome DNA preparation from filamentous Fungi. *Mycosystema* 20: 575–577.
- Yates F (1934) Contingency tables involving small numbers and the χ^2 test. *Supplement to the Journal of the Royal Statistical Society* 1: 217.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. List of tree species and their respective families found in all 51 monitoring plots. Summaries are presented for mean (\pm SE) diameter at breast height (cm), the percentage of trees of each species out of all trees of all species (dominance), polyphagous shot hole borer (PSHB) breeding host or not, the percentage of trees that were infested by PSHB, the percentage increase of infested trees from sampling period 1–4, and the percentage increase in the number of holes from sampling period 1–4.

Table S2. All tree species found in 10 polyphagous shot hole borer (PSHB) monitoring plots set out at the George Botanical Garden's. Presented is the dominance (% of all trees encountered), the number of trees infested with PSHB (% of infested trees of that species), and the average diameter at breast height (cm) of each species encountered.

Table S3. Test table showing sample size (n), χ^2 statistic, P, and Yates correction P for the preferential colonization of polyphagous shot hole borer between tree species.

How to cite this article: Townsend G, van Rooyen E, Hill M, De Beer W & Roets F (2024) Invasion of an Afrotropical forest complex by the polyphagous shot hole borer beetle. *Entomologia Experimentalis et Applicata* 172: 354–369. <https://doi.org/10.1111/eea.13415>

APPENDIX 1 Description of sites in the Southern Cape of South Africa that were monitored for polyphagous shot hole borer (PSHB) infestations.

Site	Plot	Coordinates	Tree richness	Tree abundance	PSHB	Site description
George Botanical Garden's	1	33°56'31.80"S, 22°27'41.90"E	4	35	Yes	Early-successional forest. Previously a pine plantation but has been rehabilitated for the past 15 years. Close to urban areas with 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. Close to urban areas.
	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 an urban area. Impacted by a severe fire in 2017 that damaged many trees. Contains numerous planted trees.
	22		20	31	No	
	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 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	