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From arrival to spread: improving the early-detection of alien wood-boring beetles

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Riassunto

L'introduzione di organismi esotici è un processo che avviene ormai da secoli ed è considerato a tutti gli effetti come una minaccia a livello globale. Dalla scoperta dell'America in Europa sono state introdotte circa 10.000 specie esotiche, principalmente piante e insetti. Tra questi ultimi hanno particolare rilevanza i coleotteri xilofagi, per lo più appartenenti alle famiglie scolitidi, cerambicidi e buprestidi. Questi insetti possono essere trasportati all'interno di pressoché qualsiasi tipo di materiale legnoso e, una volta arrivati e stabiliti nel nuovo ambiente, possono causare gravi danni al patrimonio forestale del paese di introduzione con gravi conseguenze sia sotto il profilo economico che ambientale. Da alcuni anni si è iniziato ad affiancare alle tradizionali metodologie di controllo diretto delle merci importate l'utilizzo di strumenti, quali trappole associate a sostanze attrattive, che permettono di aumentare le probabilità di intercettazione delle specie esotiche. Ad ogni modo, considerando le limitate risorse che vengono destinate per questo tipo di attività, diventa fondamentale avere a disposizione efficaci protocolli di monitoraggio ed individuare i punti a maggiore rischio di nuove introduzioni dove concentrare le risorse stesse. Questo non può però prescindere da una migliore conoscenza del processo di invasione biologica e delle tre fasi in cui esso viene comunemente diviso: l'arrivo, l'insediamento e la diffusione nel nuovo ambiente.

Questa tesi si pone due obiettivi principali: il primo è quello di migliorare l'efficacia degli esistenti protocolli di monitoraggio al fine di aumentare le possibilità di intercettazione di specie esotiche di insetti del legno sia in aree costiere che continentali, mentre il secondo è quello di approfondire i meccanismi che guidano il processo di invasione biologica.

Il primo studio riguarda lo sviluppo di un protocollo di monitoraggio utilizzabile per l'intercettazione di specie esotiche di insetti del legno nei porti europei e considera l'effetto di diverse tecniche di diffusione delle sostanze attrattive e di diversi modelli di trappole, nonché del loro posizionamento all'esterno o all'interno dell'area portuale, sulle possibilità di cattura delle specie esotiche stesse. I risultati indicano come l'utilizzo di trappole modello "*multi-funnel*", attivate contemporaneamente con più sostanze attrattive, rappresenti un'importante integrazione dei tradizionali metodi di ispezione. Essi inoltre suggeriscono l'impiego di trappole anche nelle aree circostanti il porto per rafforzare i programmi di monitoraggio.

Il secondo studio indaga come il volume annuale di importazioni e il paesaggio circostante i porti, espresso in termini di copertura e tipologia forestale (conifere vs latifoglie), influenzano la cattura e l'intercettazione di specie esotiche di insetti del legno. I risultati dimostrano come il monitoraggio dovrebbe essere effettuato utilizzando trappole sia all'interno che all'esterno dell'area portuale, concentrando gli sforzi di campionamento nei porti che ricevono annualmente i volumi più elevati di merci e nei boschi misti di latifoglie localizzati nei primi chilometri attorno al porto.

Il terzo studio confronta le catture di specie esotiche ottenute in aree portuali e in siti di riciclaggio o smaltimento di materiali legnosi, considerati come destinazione finale di parte degli imballaggi associati alle merci importate. Lo studio dimostra come tali siti possano essere considerati come utili punti da monitorare per aumentare le possibilità di intercettazione nelle aree continentali.

Il quarto e quinto studio indagano il ruolo delle principali variabili climatiche e ambientali sui meccanismi ecologici che regolano il processo di stabilizzazione e diffusione di scolitidi esotici rispettivamente a scala regionale e continentale. I risultati dimostrano che il processo di invasione non è casuale e che sia le variabili climatiche, in termini di temperatura e precipitazioni, che la diversità ambientale, in termini di composizione forestale, giocano un ruolo fondamentale nel modellare le comunità di specie esotiche e la loro distribuzione nel nuovo ambiente, ma che questo avviene in modo differente nei due gruppi principali di coleotteri scolitidi, gli scolitidi xilematici e quelli floematici.

Nel complesso, i cinque contributi di questa tesi forniscono indicazioni per aumentare le possibilità di intercettazione di specie esotiche di insetti del legno sia in aree costiere che continentali, descrivendo un approccio applicabile anche a scala internazionale. Allo stesso tempo contribuiscono ad approfondire i meccanismi che caratterizzano il processo di invasione degli insetti del legno, informazioni che risultano essere fondamentali in vista di futuri approcci applicativi volti alla riduzione dei danni ambientali ed economici attribuibili alle specie esotiche.

Summary

The introduction of alien species in new habitats is a global and growing problem worldwide. Since the discovery of the Americas about 10,000 species have been introduced in Europe, mainly plants and insects. Among the latter, wood-boring beetles (mainly Scolytinae, Cerambycidae and Buprestidae) are recognized as one of the most successful group of invaders. These insects can be transported in almost all types of woody materials and, once introduced and established, they can cause enormous damage to ecosystems and economies of the invaded countries. In addition to traditional control methods, in the last years several countries have implemented other kinds of tools, such as traps baited with attractive lures, in order to enhance the chances of alien wood-boring beetles interception. Resources available for these activities are however limited while the number of sites that should be monitored is extremely high. For this reason, it is of utmost importance to have reliable and efficient trapping protocols as well as to understand where surveillance efforts should be focused. However, the general solution of this problem is strictly related to a better comprehension of the three steps characterizing the invasion process: arrival, establishment and spread.

This thesis has two main aims: first, to enhance the early-detection of alien wood-boring beetles at high-risk sites located both in coastal and continental areas, and second, to gain insight into the mechanisms driving the alien wood-boring beetle invasion process.

The first study concerns the development of efficient early-detection protocols that can be adopted for the interception of alien wood-boring beetles arriving at European ports of entry. This is focused on how different use of attractive lures, different trap models and trap positions (inside ports vs surrounding areas) may influence the chances of interception of alien species. The results suggest the use of multi-funnel traps baited with different lures for monitoring alien wood-boring beetles. The deployment of traps outside the port is also recommended to validate the surveillance program.

The second study concerns how port size, in terms of amount of imported commodities, and characteristics of the landscape surrounding the ports, in terms of forest cover and forest composition, may affect the early-detection of alien wood-boring beetles. The results suggest that surveillance should be focused mainly in large ports and in the surrounding broadleaf forests, deploying traps simultaneously in both habitats.

The third study is focused on the comparison between ports of entry and wood waste landfills, considered as the final destination of a large part of wood packaging materials imported together with goods. The results show that wood waste landfills are a crucial site where detecting alien species associated with woody materials, even in continental areas far away from the coasts.

The forth and fifth studies investigate how the main climatic and environmental variables affect the establishment and spread of alien bark and ambrosia beetles at both regional and continental scale. The main results demonstrate that the invasion process of exotic scolytids is not random and that both climate and forest composition play a key role in shaping their communities and their spatial distribution in the invaded environment. However, these mechanisms occur differently according to the feeding guild of the two main groups of scolytids, the bark beetles and the ambrosia beetles.

In general, the five studies carried out in this thesis provide suggestions to increase the probability of an early-detection of alien species both in coastal and continental areas, describing a protocol applicable both at local and international scale. At the same time, they contribute to improve the knowledge about the mechanisms characterizing the invasion process of alien wood-boring beetles, information that can be of utmost importance for the development of future strategies focused to reduce economic and environmental damage due to alien species.

Chapter 1

Introduction

Alien species: a growing problem worldwide

Invasive alien species are defined as organisms introduced into environments located out of their natural range of distribution either intentionally or unintentionally (IUCN 2000). Enormous damage is done by alien species to ecosystems and economies (e.g. Vitousek et al. 1997; Pimentel et al. 2005; Colautti et al. 2006; Asner et al. 2008), and they are considered one of the biggest threat to biodiversity worldwide. Despite the ecological and economic effects of most alien species are of minor importance, some of them can significantly impact the functional properties of ecosystems, disrupt trophic webs, displace indigenous species, or threaten food and water supplies (Kenis et al. 2009). Considering the climate change and the increase of international trade, the number of species that can arrive and establish in a new environment will continue to increase in the next years (Roques 2010). For this reasons, in the last decades, the attention on "biological invasions" has exponentially increased (Lockwood et al. 2007).

Starting from the beginning of the Age of Discovery, man has voluntarily and involuntarily displaced organisms among continents (Mack et al. 2000), and this has resulted in the breakage of those physical barriers that in millions of years of evolution have diversified and made independent the biomes of the world (Holmes et al. 2009). It is difficult to determine when this process began, but surely during the last 25 years, we have been witnessing a sharp increase in the number of alien species introduced somewhere in the world (Hulme 2009). The phenomenon of the accidentally introduction of alien species is strongly influenced by globalization, and the pool of potential invaders – as well as both the geographic and taxonomic pattern of biological invasions – is strongly related to trends in human trade and transport (Perrings et al. 2005; Meyerson and Mooney 2007; Hulme 2009). Moreover, the income magnitude and the transport efficiency are known to drive the origin, frequency and magnitude of species introductions worldwide (Hulme 2009). In particular, the magnitude of imports can strongly influence the number of introduced species (Westphal et al 2008; Desprez-Loustau 2009; Roques et al. 2009; Marini et al. 2011) and the rate of new species introductions (Levine and D'Antonio 2003). However, as the income growth has been a global phenomenon, the characterization of the risk of new species introductions cannot avoid to take in consideration the volume, frequency, origin and destination of imports as well as the mechanism by which goods are transported and alien species become established and spread in a new area (Hulme 2009).

In this scenario, the availability of provisional and dispersal models, as well as efficient early-detection protocols, is becoming of utmost importance to try fighting the phenomenon, and it is well recognized that this possibility is strictly related to a better comprehension of the mechanisms driving the whole invasion process, meaning from the arrival of a species in a new environment, to its establishment and spread.

The three steps characterizing the invasion of alien species

Biological invasion can be divided into three distinct processes: the *arrival*, that is the process by which individuals of a given species are transported to new areas different from their native range; the *establishment*, that is the process by which popolulations grow to certain levels such that extinction is unlikely; the *spread*, that is the process by which a species expand its range into new areas (Liebhold and Tobin 2008).

It is well recognized the key role played by humans in favoring the arrival of alien species in a new environment (Hulme 2009). Every year a number of alien species are interecpeted at international ports of entry from commercial shipments of live plants, logs and row wood products, packaging materials and even in airline passenger baggage (Work et al. 2005; Brockerhoff et al. 2006; Haack 2006; Liebhold et al. 2006). Maritime ports and airports are the first points of entry for international trade and travelers, where commodities are imported from all over the world. In 2006, more than 90% of global trade was carried by sea with cargo-carrying fleet transporting more than 1 million deadweight tonnes (IMO 2008). It is obvious that such sites represent the points where the first detection of alien species is more probable. For example, in the USA, inspectors examine up to 2% of cargo arriving at maritime ports, airports and border crossings (Work et al. 2005). In any case, after the introduction, alien species are challenged by environmental and demographic stochastic forces that must be overcome to establish (Liebhold and Tobin 2008).

The establishment represent a critical phase as founder populations are usually small and consequently exposed at a greater risk of extinction (Liebhold and Tobin 2008). Despite the high number of species that have been introduced, most of them have failed to establish (South and Kenward 2001; Simberloff and Gibbons 2004). During this phase, a key role is played by the "*Allee effect*", which suggests that animal populations must be composed of a certain number of individuals to remain viable (Liebhold and Tobin 2008). Considering that there could be certain processes that may lead to a decrease of population growth with decreasing density, there may exist a threshold below which populations that are at a low density are driven toward extinction (Courchamp et al. 1999). This phenomenon can be influenced by several causes, among which failure to locate mates, inbreeding depression (Hopper and Roush 1993; Lande 1998) and low propagule pressure, which is defined as the rate at which invading populations arrive at a specific location (Liebhold and Tobin 2008). However, if a species is able to establish a self-sustaining population, than it can start the next step of the invasion, the spread.

The spread is often estimated as an increase in range radius over time (Liebhold and Tobin 2008). It can happen in two main forms: continuous spread or spread through long-distance dispersal. The continuous spread is the natural spread along a population front which is generally the result of coupling dispersal with population growth (Okubo 1980), whereas the long-distance dispersal is mainly driven by anthropogenic mechanisms. For example, species that evade the inspection process at ports of entry may be accidentally introduced in the internal part of a country following human activities: the most frequent scenario is when goods arriving at points of entry are moved – often across international boundaries by roads, rails and canals (Hulme 2009) – towards their final destination, such as industrial or commercial areas.

However, given the variation among insect species and their life history, there is a clear difference in the role of environmental, climatic and human-related variables in affecting their invasion process. Understanding which are these variables and how they affect each phase of the invasion is of critical importance to better understand why some species are more invasive than others as well as where surveillance efforts should be concentrated.

The case of alien wood-boring beetles

Around the world, a number of alien phytophagous insects become established every year and wood-boring beetles represent a significant proportion of these (Haack 2001, 2006; Work et al. 2005; Mattson et al. 2007). Wood-boring beetles have, in fact, several features that make them successful invaders. For instance, they are easily transported in wood products and wood packaging materials (Fig. 1) where they are sheltered from detection and adverse climatic conditions (Brockerhoff 2006). A number of measures have been taken to reduce the risk of such invasions. The pathway associated with wood packaging has been prioritized, and an International Standard for Phytosanitary Measures (ISPM-15) has been ratified, requiring treatments of these wood products (Brockerhoff et al. 2006). However, any preventive measure such as debarking, fumigation, irrigation, treatments with heat or chemicals, is able to completely prevent the risk of woody material infestation or re-infestation (Haack 2006; Skarpaas and Økland 2009). Moreover, considering that these insects include some of the most important forest pests causing significant economic damage on trees worldwide (Kirkendall and Faccoli 2010; Marini et al. 2011), it is clear the potential dangerousness of their introduction in non-native ranges.

Fig. 1 round wood and wood packaging materials stored in a port area (*Pictures by Gino Tallevi*).

Bark beetles, longhorn beetles and jewel beetles are the most commonly intercepted families of wood-boring beetles (Kirkendall and Faccoli 2010; Sauvard et al. 2010; Marini et al. 2011)**.**

Bark beetles are a subfamily of weevil insects (Coleoptera: Curculionidae) which include a group of about 6,000 species in at least 225 genera (Knížek and Beaver 2004). According to their feeding habits, these beetles are usually divided in phleophagous, xylomicetophagous and spermatophagous. The species belonging to the first group, also known as "true bark beetles", use phloem tissue as food and breeding site and they are characterized by a relatively high host specificity. The species belonging to the second group, also known as "ambrosia beetles", dig galleries within the xylem, use symbiotic fungi as nourishment and are recognized to be highly polyphagous species (Hulcr et al. 2007); the species belonging to the last group, also known as "seed borers", develop in seeds or woody fruits.. Around 20 alien species of scolytids are considered as established in Europe (Kirkendall and Faccoli 2010; Faccoli et al. 2012; Montecchio and Faccoli

2013) and two thirds of them are ambrosia beetles. This is mainly because the latter group of scolytids are characterized by several life-history traits, such as symbiotic trophic specialization with fungi, sib-mating behavior, cryptic life style and poliphagy that favor their arrival and establishment in a new environment (Atkinson et al. 1990; Kirkendall et al. 2008; Marini et al. 2011). Bark and ambrosia beetles mainly travel in wood and wood packaging materials (Haack 2001, 2006) whereas only a few species are likely to be transported in plants or plant parts (Kirkendall and Faccoli 2010).

Cerambycids is a large family including about 40,000 species worldwide, with larvae that may be found in trees, bushes and herbaceous plants (Cocquempot 2007; Cocquempot and Lindelöw 2010). However, they are mainly xylophagous borers of living, decaying or dead wood. At least 19 alien longhorn beetle species have established in Europe, among which mostly originated from Asia and Africa (Cocquempot and Lindelöw 2010). Frank (2002) suggested that alien longhorn beetles are usually imported as immature stages, whereas there are only few record of introductions of living adults (Bosmas 2006). The main pathways of arrival are related to the importation of house construction or building furniture (Cocquempot 2007), timber for pulp and wood-packaging materials (Hérard and Roques 2009).

Buprestids, also known as jewel beetles, is a large family including around 15,000 species worldwide (Paiero et al. 2012). Despite only two alien buprestids of minor importance have so far established in Europe (Denux and Zagatti 2010), there are other species that must be considered as potential threat to European forests, such as the emerald ash borer *Agrilus planipennis* (Fairmaire), which caused extensive damages to North American forests (Cappaert et al. 2005) and that it is currently expanding towards central Europe (Baranchikov et al. 2008), and the bronze birch borer, *A. anxius* Gory, which has recently been recommended for regulation (Augustin et al. 2012). The main pathways of transportation of these species are related to the importation of wood for industry, wood packaging materials, bonsai or the movement of fire wood (Augustin et al. 2012).

Prevention of alien species introduction along international trades includes inspection and treatment of imported commodities and associated woody materials (Liebhold et al. 2012), but these efforts cannot prevent all new introductions (Epanchin-Niell et al. 2014). When prevention does not succeed, early detection of new invasions can increase the possibilities of eradication or control, and reduce costs and damage (Epanchin-Niell and Hastings 2010). For this reason, the greatest opportunities for eradication or cost-effective control occur immediately after the insect introduction (Rabaglia et al. 2008). Several countries, such as USA, Canada, and New Zealand, have recently implemented trapping and sampling strategies to enhance the detection of alien wood-boring beetles (Haack 2001; Brockerhoff et al. 2006; Rabaglia et al. 2008), using, for example, traps baited with attractive lures (Fig. 2). However, early detection requires investments that can be costly (Epanchin-Niell et al. 2014) and become essential to have reliable and efficient protocols as well as to understand where to concentrate the surveillance efforts.

Fig. 2 baited multi-funnel traps set up in a port area close to imported round wood (*Picture by Gino Tallevi*).

Objectives and content of the thesis

The two main aims of this thesis are, first, to enhance the early-detection of alien wood-boring beetles at high-risk sites located both in coastal and continental areas, and second, to gain insight into the alien wood-boring beetle invasion process, disentangling the effect of environmental and climatic variables on their establishment and spread at different spatial scale. This thesis is composed, hence, by two main parts. The first one is focused on the arrival of alien wood-boring beetles and concerns the development of efficient early-detection protocols that can be adopted for the interceptions of alien wood-boring beetles arriving in Europe. In particular, I first investigated, from a technical point of view, which is the best trapping strategy to use at ports of entry (Chapter II) and, then, how this can be applied in both coastal (Chapter III) and continental areas (Chapter IV). The second part, instead, focuses on the establishment and spread of alien bark and ambrosia beetles. In particular, I investigated how forest heterogeneity, host tree species diversity and climatic variables may influence the establishment of alien ambrosia beetles at the regional scale (Chapter V) and the spread of both bark and ambrosia beetles at the continental spatial scale (Chapter VI). This last project has been developed between Italy and USA, in collaboration with USDA (Robert Haack) and the Michigan State University (Deborah McCullough).

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Chapter 2

Trapping wood-boring beetles in Italian ports: a pilot study

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Abstract

Invasive alien species cost forestry billions of euro every year and their early detection is becoming of utmost importance. The aim of this study is to improve some of the techniques available for trapping alien wood-boring beetles (Scolytinae, Cerambycidae, and Buprestidae) arriving at high-risk sites, such as ports. During 2009–2011, trapping carried out in four Italian seaports tested the comparative efficiency of different luring (single-lure vs. multi-lure traps) and trap designs (cross-vane vs. multi-funnel). In addition, trap captures within the ports were compared with those obtained in surrounding areas. Six out of 49 species trapped in 3 years of investigation were of alien origin: four Scolytinae and two Cerambycidae. The number of species trapped in multi-lure traps was as high as that resulting from the sum of the single-lure traps. The two trap designs performed equally well, but multi-funnel traps were more robust and easier to use in ports. In 2011, the number of species trapped in ports and surrounding areas was similar, although differently distributed. On a total of 26 species trapped in this experiment, nine were exclusive to ports, of which three aliens, eight were exclusive to surrounding areas, of which one alien, and nine were common to both habitats, of which one alien. In conclusion, we suggest the use of multi-funnel traps baited with different lures for monitoring alien wood-boring beetles in ports. Using traps outside the port is also recommended to validate the surveillance program.

Introduction

Invasive alien species involve a high cost for agriculture, horticulture, and forestry every year (Pimentel et al. 2005; Vilà et al. 2009). Considering climate change and the increase in international trade, which has broken down all barriers that have separated the world's biota into different and independent communities over millions years of evolution (Holmes et al. 2009), the number of species that can arrive and establish in a new environment will continue to rise in the coming years (Roques 2010a). Wood-boring beetles (mainly Scolytinae, Cerambycidae, and Buprestidae) include a number of invasive species (Kirkendall and Faccoli 2010; Sauvard et al. 2010; Marini et al. 2011) that represent an important threat to the biosecurity of all forested countries (Brockerhoff et al. 2006a). The wood-boring beetles may easily be transported in wood products, logs, timber, lumber, and wood packaging materials, where they can escape detection and, to some degree, shelter from adverse climatic conditions (Brockerhoff et al. 2006b). For these reasons, they are considered as greater successfully invaders (Haack 2006). An inventory of the phytosanitary interceptions in Europe on wood and wood products during the period 1995–2005 revealed that wood-boring beetles largely dominated the insect community associated to this pathway (Roques and Auger-Rozenberg 2006).

The early detection of alien species is of primary importance to improve the chance of eradication and provide better estimates of their arrival rates. Early detection is frequently achieved by the implementation of specific inspections and surveillance services activated by the national plant protection organizations under the coordination of international bodies (IPPC and EPPO), which develop standards aimed at intercepting and identifying incursions of quarantine pests (FAO 2011). However, large discrepancies have been observed between species interception, performed through the application of direct inspection methods, and species establishment during the same period (Haack 2006; Roques 2010b; Marini et al. 2011). Several countries, such as USA, Canada, and New Zealand, have recently implemented trapping and sampling strategies to enhance the detection of alien wood-boring beetles (Haack 2001; Tkacz 2002; Brockerhoff et al. 2006a; Rabaglia et al. 2008). Indeed, trapping can provide crucial information about the entry of a new organism and its potential establishment (Wylie et al. 2008). Moreover, considering the low number of individuals usually involved in the first phase of a biological invasion (Liebhold and Tobin 2008), the availability of trapping devices reliable at low population density is essential to the surveillance programs.

Trap efficiency in early detection of alien species strongly depends on both attractiveness of the lures used as bait and trap design. The common monitoring programs aim to obtain information on population density and phenology of a target species, usually using species-specific lures (e.g., sexual or aggregation pheromones). In surveillance programs of alien species, however, the aim is to trap a wide range of species (as many as possible), often from different families. These targets may require a number of specific lures and traps, increasing the general costs. Moreover, it is extremely difficult to predict which species may arrive, especially considering wood-boring beetles living in tropical or sub-tropical regions. The surveillance of wood-boring beetles is usually carried out by generic blends composed of kairomones, mainly (-)α-pinene and ethanol, simulating the bouquet of volatiles emitted by stressed or dying trees (Brockerhoff et al. 2006a). The kairomones are often complemented with bark beetle attractants (e.g., ipsenol, ipsdienol, or frontalin). The latter is known to be efficient for a restricted range of bark beetles (<30 species worldwide), such as species belonging to the genus *Ips* and *Dendroctonus* (Vité et al. 1972), while ethanol and (-)a-pinene are attractive for a wider range (more than 70 species) of bark beetles (e.g., *Tomicus*, *Hylastes*) and ambrosia beetles (e.g., *Xyleborus*, *Gnathotrichus*, *Xylosandrus, Monarthrum*) (Miller and Rabaglia 2009), as well as longhorn beetles and buprestids (Miller et al. 2011). For these reasons, simultaneous trapping performed with a combination of various generic and specific attractants (multi-lure technique) has been proposed to reduce the surveillance costs (Schwalbe and Mastro 1988; Brockerhoff et al. 2006a, 2012; Miller et al. 2011; Wong et al. 2012). Some studies, however, demonstrated a negative effect of (-)α-pinene toward ethanol in trapping some ambrosia beetles (Schroeder and Lindelöw 1989; Miller and Rabaglia 2009) or other wood-boring species (Wong et al. 2012). Some pheromones can also be incompatible because certain molecules inhibit the attraction to others, especially within closely related species (Brockerhoff et al. 2012). Thus, there is a need to further evaluate the potential of multi-lure technique for wood-boring beetles surveillance.

The trap designs most commonly used for wood-boring beetles are the cross-vane trap, the multi-funnel trap, and the German slot trap. A number of studies were carried out in the field to compare the efficiency of these traps in capturing wood-boring beetles, but the results varied according to the species, both within bark beetles (Czokajlo and Teale 1999; Flechtmann et al. 2000; Petrice et al. 2004; Stone et al. 2010) and other beetle families (Morewood et al. 2002; de Groot and Nott 2003). Despite the multi-funnel traps being the most commonly used for early detection (Brockerhoff et al. 2006a; Rabaglia et al. 2008), no comparative investigations have been reported in high-risk sites, such as ports.

A surveillance program should provide information not only on the arrival but also on the possible establishment of the alien species. Considering that alien insects trapped in the ports do not give such information, some authors highlighted the importance of a periodic tree health assessment carried out in the natural environments occurring close to both ports and other high-risk sites (Bashford 2008; Wylie et al. 2008; Britton et al. 2010). This purpose could be achieved by the integrated use of traps and susceptible plants of locally important species, called ‘‘sentinel-trees’’ (Wylie et al. 2008). In this way, captures by traps set up in the area surrounding a high-risk site could give useful information on the possible establishment of alien species in natural habitats and the reliability of the surveillance programs carried out in sites exposed to a high-risk of species introduction.

The main purpose of this research was to test various techniques to improve the trapping of wood-boring beetles in high-risk sites within the framework of the creation of a long-term monitoring program, involving all the main Italian international commercial ports. The study aimed at assessing the effect of different variables (lures, trap design, and trap position) on the insect catch. We compared the trapping performance of (i) single versus multi-lure traps, (ii) cross-vane versus multi-funnel traps, and (iii) traps deployed within the ports and in the surrounding areas.

Materials and methods

Study sites

Trapping tests were carried out for 3 years (2009–2011) in four international ports located in north-eastern Italy (Veneto and Friuli Venezia Giulia regions): Marghera (45° 27’ N, 12° 15’ E), Chioggia (45° 13’ N, 12° 16’ E), Trieste (45° 39’ N, 13° 46’ E), and Monfalcone (45° 47’ N, 13° 32’ E). These ports are the entry points for a large amount of solid commodities coming from every part of the world, roughly estimated by Assoporti (2012) at around 12 million tons per year for Trieste, 11 for Marghera, 3 for Monfalcone, and 2 for Chioggia. The trend is, however, different considering only wood and wood products, with a total amount of around 0.3 million tons for Trieste, 0.25 for Monfalcone, and 0.04 for Marghera and Chioggia (C.N.E.L. 2003).

In each site, the traps were suspended at about 2 m above the ground from suitable supports, such as buildings, wire fences, girders, or piles. The choice of traps position was made based on the permission given by the managers of the different ports, trying to maintain a distance of at least 50 m between each trap, to avoid interferences between the tested attractants. The lure dispensers were renewed according to their field life. The insecticide FERAG IDTM, provided by SEDQ (Spain), was placed inside each collector to ensure prompt insect death and thus to prevent escape and predation. Trapped adults of the target insects (Scolytinae, Cerambycidae, and Buprestidae) were stored in alcohol until morphological identification.

Single versus multi-lure traps

Ten hand-made cross-vane traps were set up within the ports of Marghera and Chioggia, five per site. The traps were composed of two white crossed panels (34 cm wide, 50 cm high) covered on the top by a 35 x 35 cm panel and fixed below to a wide white plastic funnel (35 cm diameter) screwed onto an insect collector (700 ml). In each port, four traps were each baited with one of four different lures (single-lure trap), while one was activated with all four lures (multi-lure trap). The tested lures included attractants generic for bark and wood-boring beetles (ethanol and (-) α-pinene), as well as three attractants specific for bark beetle (frontalin, ipsenol, and ipsdienol). Ipsenol and ipsdienol were tested together. Ethanol and (-)α-pinene were provided by Sigma-Aldrich (Germany) and poured into pierced plastic pouches of 200 ml to obtain a release rate of 0.2 g day^{-1} (60 days field life at 20°C) for ethanol and 0.75 g day^{-1} (90 days field life at 20 °C) for (-)α-pinene. Frontalin (release rate of 2.3 mg day^{-1}; 60 days field life at 20 °C), ipsenol (+50/-50; release rate of 0.15 mg day^{-1}; 90 days field life at 20 °C), and ipsdienol (release rate 0.15 mg day^{-1}; 90 days field life at 20 °C) were provided by Pherotech International Inc., Delta BC (Canada). The traps were set up on July 10, 2009 and checked every second week until October 16, 2009.

Cross-vane versus multi-funnel traps

One cross-vane (Polytrap PET, model 2010; Ecole d'ingenieurs de Toulouse-Purpan, France) and one multi-funnel (Pherotech) were set up within each of the four ports. The cross-vane traps were similar to those used in 2009, whereas the multi-funnel traps were composed of 12 black funnels with an insect collector screwed onto the last funnel. All traps were baited with a blend composed of ethanol, ipsenol, ipsdienol (as in 2009), (-)α-pinene (Ultra High Release, release rate of 2 g day^{-1}; 90 days field life, Pherotech), and 2-methyl-3-buten-2-ol (release rate of 0.01 g day^{-1}; 60 days field life at 20°C, Pherotech). In this trial, the frontalin tested in 2009 was replaced by 2-methyl-3-buten-2-ol as suggested in other protocols for monitoring bark and wood-boring beetles (Ibeas et al. 2007; Francardi et al. 2009; Dodds et al. 2010). Traps were set up on July 7, 2010 and checked every second week until October 14, 2010.

Ports versus surrounding areas

One multi-funnel trap was set up in each of the ports of Monfalcone, Trieste, and Marghera, and in natural or semi-natural areas close to each port. The first site, located 3 km from the port of Monfalcone, was an Austrian pine plantation (*Pinus nigra* Arnold); the second site, located 4 km from the port of Trieste, was an Austrian pine forest mixed with a few broadleaved species; the last site, located 3 km from the port of Marghera, was near a green-waste disposal. The traps were baited with the same multi-lure blend tested in 2010 (ethanol, (-)α-pinene, ipsenol, ipsdienol, and 2-methyl-3-buten-2-ol). Traps were set up on July 3, 2011 and checked every second week until October 12, 2011.

Data analysis

To compare the detection frequency of each species (presence/absence) in traps with different lures, designs, and habitats, the Fisher's exact test was used for the species with enough catch events in each season. As the arrival of commodities and wood packaging material potentially carrying wood-boring beetles is unpredictable and does not depend on season, we used each trap check as a replicate. Mean number of species trapped was analyzed using the generalized linear mixed models with Poisson distribution and log-link function for count data. Trap design, lure design, and habitat (inside/outside port) were considered as fixed factors, while site and collection date were considered as random factors. The number of trapped species was considered as dependent variable. Significance of effects was based on $\alpha = 0.05$. The statistical analyses were performed using the lmer function from the lme4 package in R (R Development Core Team 2011). All variables are reported as mean value ± standard error of the mean (SEM).

Results

During the 3 years of study (2009–2011), 49 wood-boring species were trapped. Scolytinae represented the most common and richest group with 33 species (1,043 specimens), followed by Cerambycidae (11 species and 104 specimens), and Buprestidae (5 species and 13 specimens). Most of the trapped species (43) were native to Europe but 6 were of alien origin: 4 Scolytinae (*Ambrosiodmus rubricollis*, *Cyrtogenius luteus*, *Xylosandrus crassiusculus*, *Hypothenemus eruditus*), and 2 Cerambycidae (*Neoclytus acuminatus*, *Xylotrechus stebbingi*). The bark beetles *Orthotomicus erosus* (234 specimens) and *Ips typographus* (185 specimens) were the most numerous European species; for some species, only one specimens were trapped (e.g., *Scolytus rugulosus* and *Pityokteines vorontzowi*). The ambrosia beetle *Xyleborinus saxesenii* was the only species found in all years and monitored sites, except in the natural area close to the port of Monfalcone. *C. luteus* was the most numerous alien species (19 specimens) and the most frequently trapped, while only one specimen of the alien longhorn beetle *X. stebbingi* was found.

Single versus multi-lure traps

The frequency of each single species trapped in the multi-lure trap was compared with that resulting from the sum of all single-lure traps. For those species with enough catch events, the results show no statistically significant differences for any of the tested species (Table 1).

Table 1 Number of specimens and detection frequency (%) of species of Scolytinae trapped in 2009 comparing single (SL) versus multi-lure (ML) in cross-vane traps (n = 14). Species are listed alphabetically. Legend: * indicates alien species; E: ethanol; F: frontalin; I: ipsenol/ipsdienol; P: (–)α-pinene. EFIP: trap baited simultaneously with the 4 lures (multi-lure).

	E	F	I	P	EFIP	SL %	ML %	*P*-value
Scolytinae								
Ambrosiodmus rubricollis (Eichhoff) *	-	-	1	-	-	7.1	0	-
Cyrtogenius luteus (Blandford) *	-	-	-	-	2	0	14.2	0.333
Hylurgus ligniperda (Fabricius)	-	2	14	1	43	57.1	35.7	0.467
Hylurgus micklitzi Wachtl	-	-	-	-	1	0	7.1	-
Ips sexdentatus (Borner)	-	-	1		2	7.1	21.4	0.4
Orthotomicus erosus (Wollaston)	-	2	3	2	14	42.8	35.7	1
Orthotomicus laricis (Fabricius)	-	1	5	-	83	28.5	35.7	0.444
Orthotomicus proximus (Eichhoff)	1	-	-	-	6	7.1	7.1	1
Pityogenes chalcographus (Linnaeus)	-	-	1	-	-	7.1	0	-
Taphrorychus bicolor (Herbst)	-	-	1		1	7.1	7.1	1
Xyleborinus saxesenii (Ratzeburg)	20	-	-	-	4	57.1	21.4	0.346
Xylosandrus crassiusculus (Motschulsky) *	1	-	-	-	-	7.1	0	-

On a total of 12 Scolytinae species trapped, 7 occurred in both traps, 3, of which two aliens, were trapped exclusively in single-lure traps, and 2, of which one alien, were trapped only in multi-lure traps. The alien *A. rubricollis* and the native *Pityogenes chalcographus* were trapped in traps baited only with ipsenol, while the alien *X. crassiusculus* was trapped in traps baited only with ethanol (Table 1). On the contrary, the alien *C. luteus* and the native *Hylurgus micklitzi* were trapped only in multi-lure traps. The mean number of trapped species per check (2.16 ± 0.56) was significantly higher in multi-lure traps than in each individual single-lure trap (Poisson GLMM, $P<0.01$), but only marginally higher (Poisson GLMM, $P = 0.06$) than the cumulated number (2.07 ± 0.43) (Fig. 1).

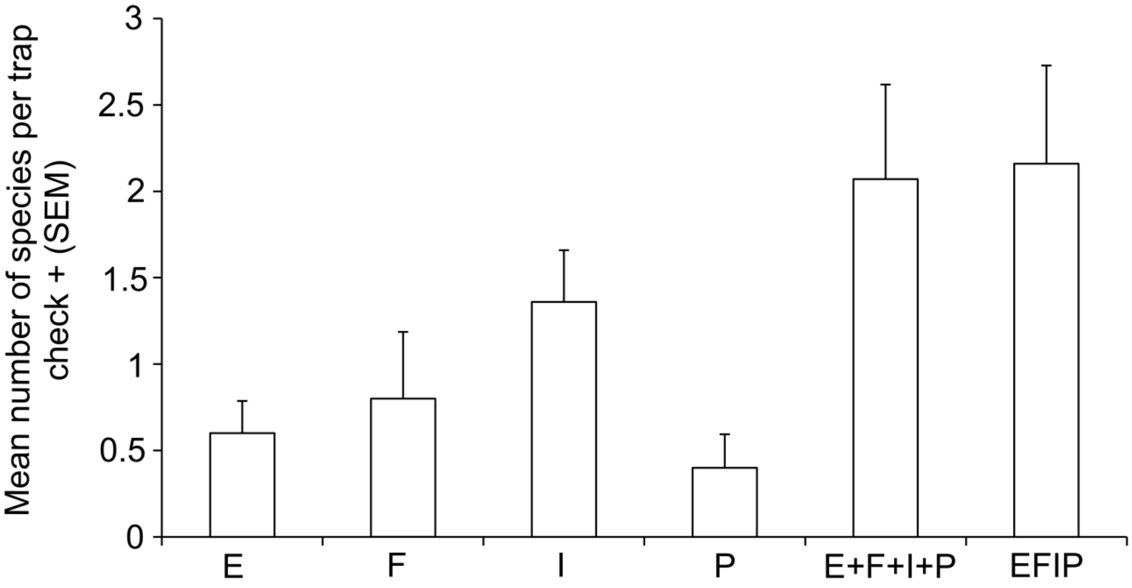

Fig. 1 Mean number of species trapped per check in 2009 according to the different tested lures. E ethanol; F frontalin; I ipsenol/ipsdienol; P (-)α-pinene; E+F+I+P cumulative mean number of species trapped by traps baited with individual lures; EFIP mean number of species trapped in the traps baited simultaneously with the 4 lures (multi-lure).

Cross-vane versus multi-funnel traps

Cross-vane and multi-funnel traps baited with the same multi-lure technique gave a similar trapping performance. The results show that for any of the species with enough catch events there were not statistically significant differences between trap designs (Table 2). Seven species were trapped exclusively in cross-vane traps, 11 species, of which three aliens, were trapped exclusively in multi-funnel traps and 15 species, of which one alien, were found in both trap designs. The longhorn beetles were more frequently trapped by multi-funnel traps, but the low number of cases did not allow testing (Table 2). The mean number of wood-boring species per check was 3.27 ± 0.40 in the cross-vane traps and 3.55 ± 0.46 in the multi-funnel traps (Poisson GLMM, $P = 0.52$).

Table 2 Number of specimens and detection frequency (%) of species of Scolytinae, Cerambycidae and Buprestidae caught in 2010 in the experiment comparing cross-vane (CV) vs. multi-funnel (MF) traps baited with the multi-lure technique (n=31). Species are listed alphabetically. Legend: * indicates alien species.

	CV	**MF**	**CV %**	**MF%**	***P*-value**
Scolytinae					
Carphoborus perrisi (Chapuis)	-	1	0	3.2	-
Crypturgus cinereus (Herbst)	2	-	6.4	0	0.333
Cyrtogenius luteus (Blandford) *	4	2	6.4	9.6	-
Hylastes ater (Paykull)	-	1	0	3.2	-
Hylurgus ligniperda (Fabricius)	25	43	45.1	54.8	1
Hylurgus micklitzi Wachtl	1	3	3.2	9.6	0.485
Ips acuminatus (Gyllenhal)	1	1	3.2	3.2	1
Ips amitinus (Eichhoff)	-	1	0	3.2	-
Ips cembrae (Heer)	1	1	3.2	3.2	1
Ips duplicatus (Sahlberg)	-	1	0	3.2	-
Ips sexdentatus (Borner)	39	18	41.9	38.7	1
Ips typographus (Linnaeus)	160	25	25.8	22.5	1
Orthotomicus erosus (Wollaston)	114	98	45.1	51.6	1
Orthotomicus laricis (Fabricius)	8	4	12.9	9.6	1
Pityokteines curvidens (Germar)	49	-	12.9	0	-
Scolytus multistriatus (Marsham)	1	-	3.2	0	-
Scolytus rugulosus (Müller)	-	1	0	3.2	
Xyleborinus saxesenii (Ratzeburg)	95	9	41.9	25.8	0.216
Xylosandrus crassiusculus (Motschulsky) *	-	3	0	3.2	-
Xyloterus lineatus (Olivier)	1	-	3.2	0	-
Cerambycidae					
Acanthocinus griseus (Fabricius)	2	-	6.4	0	-
Arhopalus rusticus (Linnaeus)	-	1	0	3.2	-
Chlorophorus pilosus (Forster)	-	2	0	3.2	-
Monochamus galloprovincialis (Olivier)	-	4	0	3.2	-
Neoclytus acuminatus (Fabricius) *	1	-	3.2	0	-
Spondylis buprestoides (Linnaeus)	14	17	19.3	22.5	0.659
Xylotrechus antilope (Schönherr)	-	1	0	3.2	-
Xylotrechus stebbingi Gahan *	-	1	0	3.2	-
Buprestidae					
Buprestis haemorrhoidalis (Herbst)	3	1	6.4	3.2	1
Buprestis novemmaculata Linnaeus	1	1	3.2	3.2	1
Buprestis octoguttata Linnaeus	1	-	3.2	0	-
Phaenops cyanea (Fabricius)	2	2	3.2	6.4	1
Phaenops knoteki (Reitter)	1	1	3.2	3.2	1

Ports versus surrounding areas

For those species with enough catch events, the trapping frequency was similar in ports and in surrounding areas (Table 3). On a total of 26 species, 9 species, of which two aliens, were trapped exclusively in the ports, 8 species, of which one alien, only in the surrounding areas, and 9, of which one alien, were common to both habitats. The mean number of trapped species per check did not differ significantly between habitats (1.39 ± 0.82 in the ports and 1.88 ± 0.26 in the surrounding areas) (Poisson GLMM, $P = 0.17$).

Table 3 Number of specimens and detection frequency (%) of species of Scolytinae and Cerambycidae caught in 2011 in the experiment comparing ports vs. surrounding areas (n=27) using multi-funnel traps baited with the multi-lure technique. Species are listed alphabetically. Legend: * indicates alien species.

	Port	Surrounding area	Port %	Surrounding area %	*P*-value
Scolytinae					
Ambrosiodmus rubricollis (Eichhoff) *	3	-	7.6	0	-
Carphoborus perrisi (Chapuis)	3	-	7.6	0	-
Crypturgus cinereus (Herbst)	1	2	3.7	7.6	1
Crypturgus pusillus (Gyllenhal)	1	-	3.7	0	-
Cyrtogenius luteus (Blandford) *	-	11	0	7.6	-
Hylastes ater (Paykull)	1	7	3.7	15.3	0.206
Hylesinus oleiperda (Fabricius)	-	4	0	3.7	-
Hylurgus ligniperda (Fabricius)	21	9	42.3	26.9	0.155
Hypoborus ficus Erichson	2	-	3.7	0	-
Hypothenemus eruditus Westwood *	2	1	7.6	3.7	1
Orthotomicus erosus (Wollaston)	-	1	0	3.7	-
*Pteleobius kraatzi (*Eichhoff)	2	-	3.7	0	-
Pityokteines curvidens (Germar)	1	-	3.7	0	-
*Pityokteines vorontzowi (*Jacobson*)*	-	1	0	3.7	-
Trypophloeus asperatus (Gyllenhal)	1	1	3.7	3.7	1
Xyleborus eurygraphus (Ratzeburg)	1	1	3.7	3.7	1
Xyleborus dispar (Fabricius)	1	1	3.7	3.7	1
Xyleborinus saxesenii (Ratzeburg)	9	30	22.2	25.9	1
Cerambycidae					
Acanthocinus griseus (Fabricius)	1	-	3.7	0	-
Arhopalus rusticus (Linnaeus)	-	1	0	3.7	-
Chlorophorus varius (Müller)	2	-	7.6	0	-
Monochamus galloprovincialis (Olivier)	-	6	0	14.8	-
Neoclytus acuminatus (Fabricius) *	2	-	3.7	0	-
Rhagium inquisitor Linnaeus	-	26	0	22.2	-
Spondylis buprestoides (Linnaeus)	4	18	21.7	33.3	0.232
Xylotrechus arvicola (Olivier)	-	1	0	3.7	-

Discussion

The main results emerging from this study indicate that the monitoring of wood-boring beetles at high-risk sites, such as the ports, can be usefully carried out with traps activated by multi-lure technique. In addition, the use of traps in areas surrounding the ports is a useful complement to port surveillance, giving information about the reliability of the latter and the possible establishment of alien species. The results showed that the multi-lure trap was as efficient as single-lure traps for Scolytinae species, both native and alien, suggesting no evident negative interactions among the tested lures. In 2009, three species were trapped only in single-lure traps. In the following years, however, among these, the alien species *X. crassiusculus* (in 2010) and *A. rubricollis* (in 2011), were trapped also in multi-lure traps, suggesting no negative reciprocal effect of the tested lures toward these species. Actually, reciprocal inhibition of the blend components may be of critical importance only if it completely prevents attraction (Wong et al. 2012). Although in some cases multi-lure technique has been reported as ineffective (Schroeder and Lindelöw 1989; Johansson et al. 2002; Miller and Rabaglia 2009; Brockerhoff et al. 2012), our results support previous studies in which kairomones and specific attractants were used simultaneously for the monitoring of wood-boring beetle species in high-risk sites (Brockerhoff et al. 2006a; Rabaglia et al. 2008; Wylie et al. 2008). The use of multi-lure technique allows the number of traps to be reduced, saving time on trap checking and decreasing the general costs for materials and manpower (Brockerhoff et al. 2012). Moreover, the use of a low number of traps can reduce the problems related to the possibilities of finding suitable and safe places for hanging the traps within the ports. Brockerhoff et al. (2006a) underlined that, when pheromones or other attractants are known for quarantine organisms or particularly important unwanted species, the best approach for their interception should be based on such specific attractants. Anyway, it is necessary to consider that it is extremely difficult to forecast which species may arrive, especially for species from tropical or sub-tropical regions. In addition, the chemical ecology of wood-boring beetles is well studied only for certain groups of species. For these reasons, the possibility to use traps attractive for a wide range of species is a good complement in an efficient monitoring program. Specific studies, nevertheless, are still needed to compare new sensitive detection methods in relation to the high cost required for their deployment. Comparison between traps baited with either single-lure or multi-lure is the first step toward the identification of effective monitoring programs (Wong et al. 2012).

Our results also show no significant difference in the trapping performance of the tested trap designs for both the number of species and the detection frequency of each species. The few trials previously carried out in high-risk sites did not focus on comparisons between trap designs, with papers reporting high efficiency for either the cross-vane trap (Wylie et al. 2008) or the multi-funnel trap (Brockerhoff et al. 2006a). A number of studies were carried out in forest, but also in this case the results varied according to the species, both within bark beetles (Czokajlo and Teale 1999; Flechtmann et al. 2000; Stone et al. 2010) and other families (Morewood et al. 2002; de Groot and Nott 2003), without suggesting a clear and unique interpretation. We found that multi-funnel traps are more suitable for ports as they are more resistant to adverse environmental conditions, such as high wind, and they are easier and quicker to set up. For the same reasons, multi-funnel traps are currently used in the goods warehouses of several western United States ports to detect alien pests (Rabaglia et al. 2008).

The results obtained in 2011 did not show significant differences between the number of species trapped in ports and surrounding areas, although the species complex in the two environments was largely different. In the surrounding areas the latter was affected mainly by the type of habitat, while in ports it depended mainly on type and amount of imported woody materials, which is largely unpredictable. Many other factors may also affect the reliability of the monitoring, such as port size and trap position. In large ports, more traps should be used in order to achieve the same probability of trapping insects, but this may conflict with accessibility and the unpredictable location of the insect sources. Our results confirm that the use of traps in areas surrounding the ports is useful for testing the accuracy of the monitoring program carried out within the port. This may offer an integrative approach to the study of biological invasions at risky sites, as suggested by Bashford (2008) and Rabaglia et al. (2008). Thus, the newly arrived alien species *C. luteus* was first trapped within the ports of Marghera (2009) and Chioggia (2010), and later (2011) in the area surrounding Marghera (Faccoli et al. 2012). In this case, trapping in ports was an effective alert tool.

Despite the pilot nature of this study, six species alien to Europe were captured, of which two Scolytinae found for the first time (*A. rubricollis* and *C. luteus*) (Faccoli et al. 2009, 2012, respectively). Among the four other alien species already known to have established in Europe, *X. crassiusculus* had not yet been recorded in north-eastern Italy.

Concerning *H. eruditus*, *X. stebbingi* and *N. acuminatus*, already established for a long time all over the country, it cannot be ascertained whether the captures in the ports corresponded to new arrivals from other continents or movements internal to Europe. For instance, the bark beetle *Ips duplicatus*, a species alien to Italy, has recently colonized north-eastern Europe from Siberia. In this case, we cannot suggest any hypothesis about the origin of the trapped individual. Our results confirm also that Italy, as well as France and Spain, provides climatic conditions suitable for the naturalization of tropical and sub-tropical species (Kirkendall and Faccoli 2010). Both the ambrosia beetle *A. rubricollis* and the bark beetle *C. luteus* are native to tropical Asia. However, whereas *A. rubricollis* was already established also in USA and Australia, for *C. luteus* this represents the first establishment outside its native range (Faccoli et al. 2012), confirming that the increased international trade of woody plants and wood packaging material, associated to climate change, will likely lead to new establishments of alien Scolytinae (Marini et al. 2011).

Early detection of alien species is of primary importance to increase the possibilities of their eradication. The settlement of an efficient monitoring protocol in high-risk sites such as the ports is, hence, a crucial point for future surveillance programs worldwide, as highlighted, for example, in the national surveillance program carried out in New Zealand (Brockerhoff et al. 2006a). Our study suggests the use of multi-funnel trap baited with multi-lure technique, associated to traps set up in habitats surrounding the ports, as a monitoring protocol suitable for the surveillance of alien wood-boring beetles. However, other steps should be taken to increase the efficiency of a surveillance program. Trap location within the ports appears to be an important factor because the area to be monitored can be extensive, especially in large ports. In this respect, it may be difficult to find a suitable balance between number of traps, port area, and strategic points where to locate the traps (for instance, where wood products are stocked). Lastly, it would be useful to extend the monitoring to a larger number of ports and to analyze how the presence or absence of forests in the areas surrounding the port may affect the catch and facilitate the establishment of alien wood-boring beetles. Because such a trapping system only captures adult insects whereas alien species can arrive in ports at different development stages, it has to be combined with more traditional inspection of the imported wood and wood products.

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Chapter 3

Improving the early detection of alien wood-boring beetles in ports and surrounding forests

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Abstract

International ports are generally considered the most likely points of entry for alien wood-boring beetles. A better understanding of the factors affecting their arrival and establishment at ports and their surrounding areas is of utmost importance to improve the efficacy and the cost-effectiveness of early detection programmes. Our work aimed at understanding how port size and the characteristics of the landscape surrounding the port, in terms of forest cover and forest composition, influence the occurrence of alien wood-boring beetles. From May to September 2012, 15 Italian international ports and the surrounding forests were monitored with multi-funnel traps baited with a multi-lure blend (a-pinene, ethanol, ipsdienol, ipsenol, methyl-butenol), three in each port and three in forests located 3–5 km away from the port. We identified both alien and native Scolytinae, Cerambycidae and Buprestidae beetles. Fourteen alien species, among which four are new to Italy, were trapped. Alien species richness was positively related to the amount of imported commodities at the port scale. Broadleaf forests surrounding ports received larger number of alien species than conifer forests. By contrast, total forest cover in the landscape surrounding ports was positively related to the occurrence of native but not alien species. The alien and native species richness was higher in the surrounding forests than in the ports. The simultaneous use of traps in ports with large volume of imported commodities and in their surrounding broadleaf forests can strongly increase the probability of alien wood-boring beetle interceptions. The identification of sites where the arrival and establishment of alien species is more probable, combined with an efficient trapping protocol, can substantially improve the efficacy of early detection. Similar approaches may be used in other countries as early warning systems to implement timely measures to eradicate or contain alien invasions at the European scale.

Introduction

Wood-boring beetles (Insecta, Coleoptera) are among the most important tree pests causing significant economic damage to forests world-wide (Brockerhoff et al. 2006a). These insects are easily transported in almost all types of woody material, where they can easily hide from detection (Brockerhoff et al. 2006b) and they are recognized as highly successful invasive group of species (Haack 2006; McCullough et al. 2006). Every year, new alien species are intercepted and recorded as established in both Europe and North America (Work et al. 2005; Kirkendall and Faccoli 2010). This trend is expected to continue given the increase of international trade which will intensify colonization pressure (Levine and D'Antonio 2003; Hulme 2009; Kenis et al. 2009; Marini et al. 2011). Moreover, climate warming may reduce the thermal limitations now hampering the establishment of species coming from tropical or subtropical regions (Roques 2010). In this context, the early detection of alien species is of primary importance to improve the chance of effective eradication and provide better estimates of their arrival rates (Brockerhoff et al. 2006a; Rassati et al. 2014a).

Although alien wood-boring beetles may arrive through fresh timber and plants for planting (Siitonen 2000; Piel et al. 2008; Liebhold et al. 2012), inspection data from USA and New Zealand indicate that crating, dunnage and pallets are the most common materials associated with these alien species (Haack 2001; Stanaway et al. 2001; Brockerhoff et al. 2006b). Pathway analyses indicated that international maritime ports are the most likely points of entry for alien wood-boring beetles because they receive large amounts of commodities that are commonly associated with wood packaging (Haack 2001; Brockerhoff et al. 2006a). In recent years, international cargo has been increasingly shipped in large containers that are difficult to inspect (Stanaway et al. 2001; McCullough et al. 2006). For this reason, several countries such as Australia, Canada, New Zealand and the USA combine traditional direct inspection of imported commodities with trapping programmes (Haack 2001; Brockerhoff et al. 2006a; Rabaglia et al. 2008; Wylie et al. 2008; Rassati et al. 2014a). Identifying the sites where the arrival and establishment of alien species is more probable is of utmost importance to improve the efficacy of early detection.

At the continental scale, the number of intercepted wood-boring beetles is often related to the volume of imported goods per state or country (Haack 2001; Huang et al. 2012). It is still unclear, however, if such a relation occurs also at the port scale. In addition, a few studies have investigated how the landscape surrounding high-risk sites can influence the establishment of alien species (Bashford 2008; Rabaglia et al. 2008). The surrounding landscape often consists of mosaics of urban areas, green spaces, crop fields or different types of forest where alien species are challenged by environmental and demographic stochastic forces that must be overcome to establish and spread (NRC 2002). For instance, there has been much debate on the effect of native tree density, diversity and distribution on the invasion success of wood-boring beetles (Brockerhoff, et al. 2006c; Colunga-Garcia et al. 2010). On the one hand, high tree species diversity in urban areas located around high-risk sites seems to be favourable for the establishment of alien species, as it can provide wide range of adequate hosts (Koch et al. 2011). On the other hand, urban areas with limited forest cover may hamper the invasion process as it cannot support many new insect establishments due to host limitation (Novak 1994; Koch et al. 2011). Moreover, an important role can be played by the composition and structure of forests located in these areas (Brockerhoff et al. 2006c). Considering that most alien wood-boring beetles are polyphagous feeders on broadleaf hosts (Kirkendall and Faccoli 2010; Marini et al. 2011), the presence of mixed broadleaf rather than conifer-dominated forests can facilitate the establishment of these species. Understanding the role of these factors is important in identifying which sites deserve special attention in surveillance programmes.

The main purpose of this study is to gain insights into the factors affecting the occurrence of alien wood-boring beetles in and around maritime ports. In particular, we investigated the relationships between the occurrence of alien wood-boring beetles and (i) the annual volume of imported commodities, (ii) the characteristics of the landscape surrounding each port in terms of forest cover and composition. First, as the amount of wood packaging materials is positively related to the amount of imported goods, we expected higher alien species richness in ports importing a high volume of commodities. Secondly, as most alien wood-boring beetles are polyphagous feeders on broadleaf hosts (Kirkendall and Faccoli 2010; Marini et al. 2011), we expected broadleaf forests to be more suitable to alien species invasions than conifer forests. Specifically, we will test whether it is sufficient to monitor only port areas or whether trapping should include both ports and surrounding forests. The analyses of the environmental factors affecting alien species occurrence will help identify the sites where the monitoring efforts should be concentrated to improve early detection effectiveness.

Materials and methods

Site selection and experimental design

Fifteen international ports located along the Italian peninsula and main islands (Sardinia and Sicily) were selected (Fig. 1, see Table S1, Supporting information). The ports covered a wide latitudinal gradient (min. Catania: 37°29’47″; max. Porto Nogaro: 45°47°49″) and were selected considering three statistically orthogonal factors: volume of solid commodities imported per year, proportion of forest cover in the 10 km surrounding each port and forest composition (conifer- vs. broadleaf-dominated forest) (see Table S1, Supporting information). Regarding the first two factors, we selected ports in order to provide the widest possible range of values. Due to the low number of available ports at the national scale, it was not possible to have a balanced experimental design between conifer (n = 9) and broadleaf (n = 6) forests. In each of the 15 selected sites, we sampled insects inside the port and in a surrounding forest. The distance between the sampling points inside and outside the port was between 3 and 5 km.

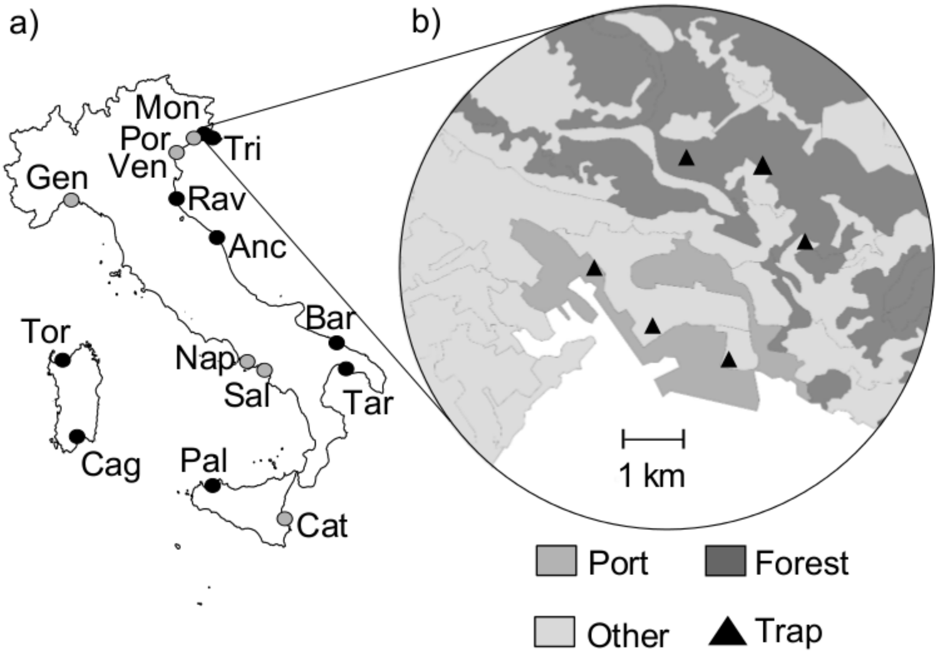

Fig. 1 (a) Geographical distribution of the 15 Italian surveyed ports and (b) an example of how the traps were set up in each site. Black circles on the map indicate ports surrounded by conifer forest and grey circles indicate ports surrounded by broadleaf forest. Port name abbreviations: Ancona (Anc), Bari (Bar), Cagliari (Cag), Catania (Cat), Genova (Gen), Monfalcone (Mon), Napoli (Nap), Palermo (Pal), Porto Nogaro (Por), Porto Torres (Tor), Ravenna (Rav), Salerno (Sal), Taranto (Tar), Trieste (Tri), Venezia (Ven).

The amount of imported commodities in 2011 was obtained from Assoporti (2012) or directly from the port authorities (Cagliari, Catania, Porto Torres and Trieste). We considered only the total volume of solid commodities because they are usually associated with wood packaging materials, while we excluded data about imported fluids such as oil or liquefied gas because they are not associated with wood packaging. Forest cover in the landscape surrounding each port was calculated in ARCGIS 10.0 (ESRI, Redlands, CA, USA) based on detailed digital aerial photographs (Google Earth). First, we digitized polygons representing both the port and the forest areas in Google Earth, excluding single trees or hedgerows with an area smaller than 100 m^2. Then using ARCGIS, we determined the centroid of each port and we established a 10-km radius buffer zone. Lastly, we calculated the percentage of landscape covered by forest after having excluded the sea area from the buffer zone. Each forest site selected as a survey point was classified visually by local forest health inspectors involved in the project into broadleaf or conifer forest according to the tree composition. The forest sites were representative of the dominant forest type occurring in the 10-km radius buffer around each port. Sites were assigned to one of the two forest categories when the dominant cover type was 80% or more. Conifer forests were mainly composed of pines, either Austrian *Pinus nigra* Arnold or Mediterranean *Pinus pinaster* Aiton and *Pinus pinea* Linnaeus pines, while broadleaf forests were always mixed stands composed mainly of oak *Quercus* spp., hophornbeam *Ostrya carpinifolia* Scop. and ash *Fraxinus* spp. species.

Trapping design and lures

In each of the 15 sites, six 12-unit black multiple-funnel traps (Econex, Murcia, Spain) were set-up, three inside the port and three in a forest site close to the port. The traps within the same environment (either port or forest) were at least 30 m apart. In Catania, only two traps were placed inside and outside the port. At each site, the traps were hung at about 2 m above the ground. All traps were baited with a generic multi-lure blend composed of (-) α-pinene (Ultra High Release, release rate of 2 g day^{-1}; 90 days field life at 20°C), ipsenol (+50/–50; release rate of 0.4 mg day^{-1}; 90 days field-life at 20°C), ipsdienol (release rate 0.4 mg day^{-1}; 90 days field-life at 20°C), 2-methyl-3-buten-2-ol (release rate of 11 mg day^{-1}; 90 days field-life at 20°C) and ethanol (release rate of 0.3 mg day^{-1}; 90 days field-life at 25°C) provided by Contech Enterprises Inc. (Victoria, BC, Canada). These lures had been tested earlier and attract a wide variety of wood-boring beetles (Rassati et al. 2014a). We did not add any liquid to the collection cups, and therefore used an insecticide (FERAG IDTM; SEDQ, Spain) to quickly kill the insects. We changed the lures after 3 months based on their expected field-life.

Trapping occurred from early May to late September 2012 (150 days). The number of trap checks varied from two to nine (average n = 6.7) in relation to restrictions to port access (see Table S1, Supporting information). The number of trap checks was not correlated with neither volume of import ($r_s = 0.22$, $P = 0.43$) nor forest cover in the landscape ($r_s = 0.43$, $P = 0.1$). Adults of the target groups (Scolytinae, Cerambycidae and Buprestidae) were stored in alcohol. Most individuals were identified on the basis of morphological features, but in a few cases, we used molecular techniques. In particular, DNA extraction was carried out following a salting out protocol based on the differential solubility of proteins and DNA at high salt concentrations (Patwary et al. 1994). The barcode region of the mitochondrial gene cytochrome oxidase I was amplified using universal primers (Folmer et al. 1994), and the obtained sequences were compared with those already deposited in the BoldSystem database (Ratnasingham and Hebert 2007). Species were classified either as native or alien according to the available literature (Wood and Bright 1992; Curletti 1994; Bense 1995; Pfeffer 1995). We considered as alien all those species that are not native to Italy. This category can include species that are already established, previously intercepted but not yet established, or never intercepted before. We decided to consider as alien also those species already established in Italy for three main reasons: as the general aim of our study is to identify priorities for identifying the sites with the highest risk of invasion, the abundance and diversity of the established alien taxa can provide a clear indication of the suitability of a site to be invaded; secondly, every year new individuals of established species can arrive at different points of entry where they are not present yet, establishing a new population and starting a new invasion; thirdly, new individuals can increase the genetic diversity of the established population, improve their fitness and modify the possible impacts on native ecosystems.

Data analysis

To account for the differences in trapping frequency and the variability due to the longer intervals between less-frequent trap checks, we used a generalized linear mixed-effects model to evaluate the effect of the time between trap checks on the mean number of species or abundance per trap. Then, we calculated the model residuals, and we used them as a response variable to test the effect of import volume (continuous variable), forest cover in the landscape (continuous variable), composition of forest site close to the port (categorical variable: conifer vs. broadleaf) and trap position (categorical variable: port vs. forest). The new response variable did not depend on the duration of the trapping. The model included the sampling site as a random factor to account for the spatial dependence of the sampling. The model was fitted using the ‘lme’ function in the package nlme for R version 2.15.1 (R Development Core Team 2012).

To compare the response of alien vs. native species, we used the same approach described above testing the effect of forest cover in the landscape, trap position and forest composition on native species richness and abundance. We did not include in the model the volume of imports as we did not expect any effect of this variable on native species.

Due to the relatively low number of replicates and the relatively high number of potential predictors, we used multi-model inference within an information-theoretic framework to evaluate the role of the selected variables in explaining species richness and abundance (Burnham and Anderson 2002). Our information-theoretic approach compared the fit of all the possible candidate models obtained by the combination of our predictors using second-order Akaike's information criterion (AICc) corrected for small samples. The AICc is a measure of relative model fit, proportional to the likelihood of the model and the number of parameters used to generate it. The best-fitting model is the one with the lowest AICc. In a set of n models, each model *i* can be ranked using its difference in AICc score with the bestfitting model ($\Delta AICc_i = AICc_i – AICc_{MIN}$). The difference in AICc values indicates the relative support for the different models. A model is usually considered plausible if its ΔAICc is below 2 (Burnham and Anderson 2002). From the set of plausible models, we omitted the models with uninformative parameters, that is models with ΔAICc below two but including only one additional parameter compared to the best model (Arnold 2010). For each model i, we also calculated an Akaike weight (w*i*), which is the probability that model i would be selected as the best-fitting model if the data were collected again under identical circumstances (Burnham and Anderson 2002). To gauge the relative importance of each predictor, we summed the w*i* across the models in the set (Σw*i*) in which the predictor occurred. The multi-model inference analyses were performed using the MuMin package for R (Barton 2010).

To test the similarity between the species recorded in each port and the surrounding forests, we used the Simpson's Similarity Index (Magurran and McGill 2010). A value close to 1 indicates that the two communities are very similar in the two environments. For each pair of port and surrounding forest, we computed the Simpson's Similarity Index for natives and aliens, separately. We used a one-way ANOVA to test difference in similarity between alien and native species.

To describe the influence of our selected factors on species composition, ordination methods were applied. The response variable was the species by site matrix based on species presence/absence. A preliminary detrended correspondence analysis (DCA) was performed. The largest DCA gradient length, expressed in standard deviation (SD) units of species turnover, of the first four DCA axes was below 3 SD units. Thus, the use of linear-based ordination models was appropriate for these data (ter Braak and

Smilauer 2002). A principal component analysis (PCA) was performed to extract the main part of the variability related to species composition. The factors trap position and forest composition were superimposed on the ordination plot to describe the similarity in species composition between ports and surrounding forests.

Results

General results

Overall, we collected 81 species of wood-boring beetles (see Table S2, Supporting information). Scolytinae represented the most abundant and diverse group with 49 species and 40,374 individuals, followed by Cerambycidae (26 species and 1,371 individuals) and Buprestidae (six species and eight individuals). Sixty-seven species were native (82.7%) and 14 (17.3%) were alien species, including 11 Scolytinae and three Cerambycidae. Among alien species, six Scolytinae [*Ambrosiodmus rubricollis* (Eichhoff), *Cyrtogenius luteus* (Blandford), *Gnathotrichus materiarius* (Fitch), *Hypothenemus eruditus* Westwood, *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford)] and two Cerambycidae (*Phoracantha recurva* Newman, *Xylotrechus stebbingi* Gahan) were already known to be established in Italy. The other six species were recorded for the first time in Italy [the Scolytinae *Liparthrum colchicum* Semenov, *Pseudothamnurgus scrutator* (Pandelle), *Xyleborus ferrugineus* (Fabricius) and *Xyleborus volvulus* (Fabricius)] or previously collected in earlier surveys [the scolytid *Ernoporicus caucasicus* (Lindemann) and the cerambycid *Cordylomera spinicornis* (Fabricius)] (Cola 1971) but were not considered to be established in Italy (see Table S2, Supporting information). No alien Buprestidae were trapped.

Of the eight alien species considered as established, one species was trapped exclusively in ports, two were trapped exclusively in the surrounding forests and five were found in both environments (see Table S2, Supporting information). Regarding the six species not considered as established, three were trapped only in ports and three only in the surrounding forests, while no species were trapped in both environments. Among the alien wood-boring beetles trapped exclusively in ports, two were species associated only with conifers and two only with broadleaf trees, while 75% of the species trapped exclusively in the surrounding forests were associated with broadleaf trees (see Table S2, Supporting information). Considering the alien species trapped in both environments, three were species associated only with broadleaf trees, one only with conifers and one with both conifer and broadleaf trees. The Scolytinae *C. luteus* and *X. germanus* were the most commonly collected species, with 220 and 104 individuals, respectively. By contrast, four species were represented by only one individual each, namely the Scolytinae *A. rubricollis* and *G. materiarius* and the Cerambycidae *C. spinicornis* and *P. recurva*.

Of the 67 native species, 10 were trapped exclusively in ports, 23 were trapped exclusively in the surrounding forests and 34 in both environments (see Table S2, Supporting information). The bulk of the species trapped exclusively in ports was associated only with conifers (60%), while the others were associated only with broadleaf trees (20%) or both broadleaf and conifer trees (20%). The opposite trend was observed considering the species trapped exclusively in the surrounding forests given that most were represented by species associated only with broadleaf trees (43%). Amongst the species trapped in both environments, the main part was associated with conifers (58%), while only 29% were associated exclusively with broadleaf trees. *Orthotomicus erosus* (Wollaston) and *Hylurgus micklitzi* Wachtl were the two most commonly collected Scolytinae, with 24,801 and 10,829 individuals, respectively, while *Spondylis buprestoides* (Linnaeus) was the most commonly collected cerambycid, with 840 individuals.

Species composition

The Simpson's Similarity Index for alien species between the port and the surrounding forests (0.1) was significantly lower than that for native species (0.69) ($P < 0.001$, $n = 15$).

For alien species, the first two principal components explained 33.4% and 14.8% of the total variation in species composition. The first axis indicated a clear separation between ports and surrounding forests, while the second axis was not associated with either trap position or forest composition (Fig. 2). Several ports located at very different latitudes presented similar alien species composition. For native species, the first two principal components explained 18.7% and 11.1% of the total variation in species composition. The first axis mostly separated sites according to latitude. Contrary to the results of alien species, several ports presented very similar native species composition with the corresponding surrounding forests.

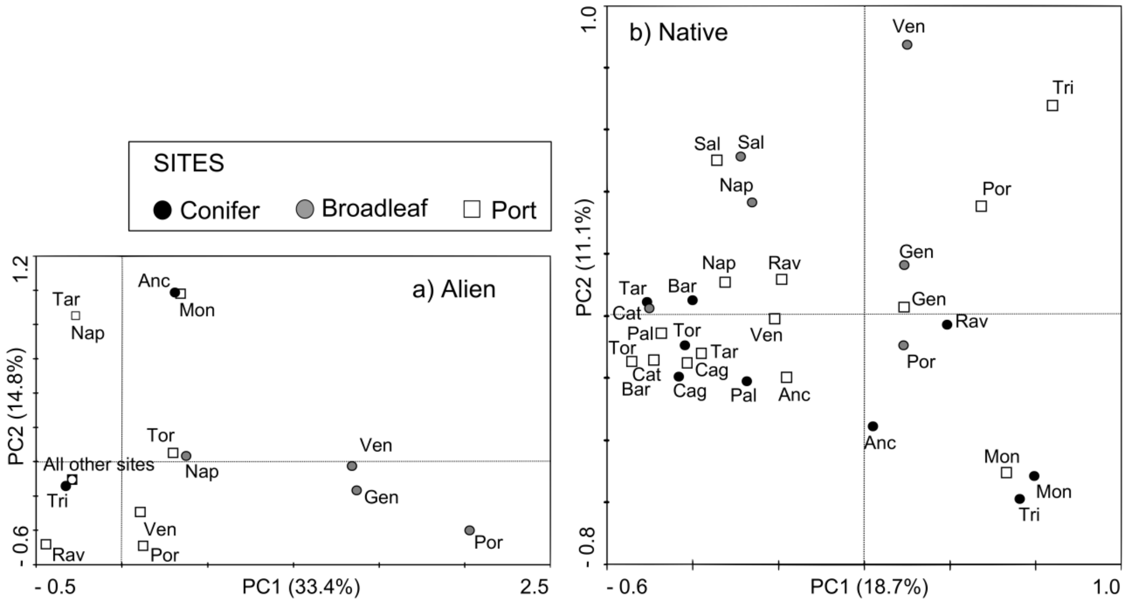

Fig. 2 Principal Component Analysis (PCA) plot for (a) alien and (b) native wood-boring beetles in ports and surrounding forests. Abbreviations of the sites are according to Fig. 1 and Table S1.

Drivers of species richness and abundance

We found three plausible models explaining the occurrence of total alien species in ports and surrounding forests (Table 1a). The model weights were generally high, indicating low model selection uncertainty. The sum of model weights of the variables indicated that the species richness was influenced mainly by trap position (Σw*i* = 0.82), composition of the forests surrounding ports (Σw*i* = 0.80), volume of imported commodities (positively, Σw*i* = 0.64) and interaction between trap position and composition of the forests surrounding ports (Σw*i* = 0.57). Forest cover in the landscape was the least important variable. The mean number of alien species standardized by the time between trap checks was higher in surrounding forests (0.041 ± 0.012) than in ports (0.006 ± 0.002) when the forests were mainly composed by broadleaf species (n = 6), while these values were similar for conifer forests (Fig. 3a).

We also found four plausible models explaining the alien wood-boring beetle abundance (Table 1b). The sum of the model weights of the variables indicated that the latter was influenced mainly by trap position (Σw*i* = 0.86), composition of the forests surrounding ports (Σw*i* = 0.86), interaction between trap position and forest composition (Σw*i* = 0.68) and volume of imported commodities (Σw*i* = 0.42). Forest cover in the landscape was the least important variable. As for species richness, the mean number of alien individuals standardized by the time between trap checks was higher in broadleaf forests (0.19 ± 0.22) than both in conifer forests (0.017 ± 0.011) and ports (0.015 ± 0.007). Considering separately the richness and abundance of species intercepted but not yet established, we did not find any significant effect of the tested variables.

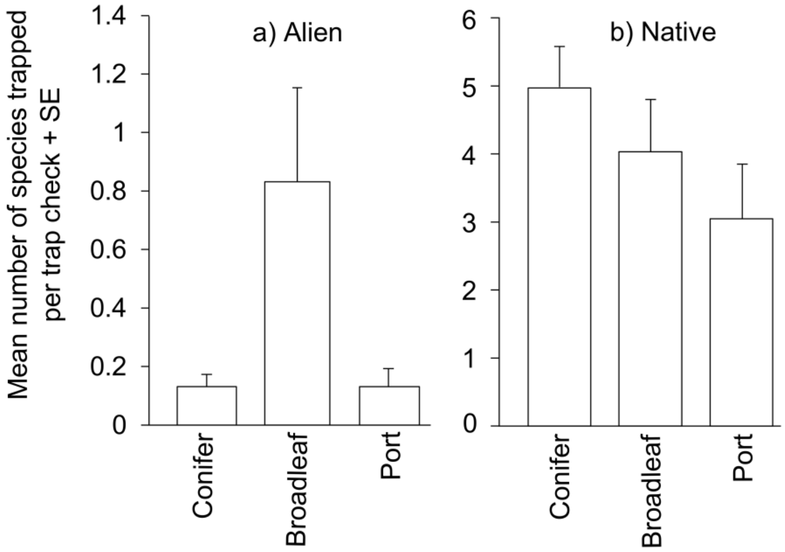

Fig. 3 Mean (+SE) number of alien and native species trapped per trap check in conifer forests, broadleaf forests and ports, standardized by the time between trap checks (number of species /duration of the sampling).

We found three plausible models explaining the native species richness (Table 1a). The model weights were generally high indicating low model selection uncertainty. The sum of model weights indicated that the native species richness was influenced by trap position (Σw*i* = 0.94) and positively by the forest cover in the landscape (Σw*i* = 0.69). The composition of the forests surrounding ports was the least important variable. The mean number of native species standardized by the time between trap checks was higher in conifer forests (0.25 ± 0.04) than both in broadleaf forests (0.18 ± 0.04) and ports (0.15 ± 0.03) (Fig. 3b). We also found three plausible models explaining the native wood-boring beetle abundance (Table 1b). The sum of model weights indicated that the latter was mainly influenced by trap position (Σw*i* = 0.93). The forest composition was the least important variable. The mean number of native individuals standardized by the time between trap checks was higher in conifer forests (24.7 ± 14.81) than both in broadleaf forests (6.33 ± 5.14) and ports (1.96 ± 0.47).

Table 1 Plausible candidate models (within 2 ΔAIC of the top model) explaining species richness and abundance of wood-boring beetles (Scolytinae, Cerambycidae, Buprestidae) trapped during the survey, separately for alien and native species. Models are ranked according to their second-order Akaike's information criterion (AICc). Parameter estimates and model weight (w_i) are reported. For each tested variable $\sum w_i$ indicates the sum of model weight

	Aliens					***Natives***			
	Best model	2nd	3rd	4th	∑ wi	Best model	2nd	3rd	∑ wi
a) Species richness									
ΔAICc	0	1.33	1.82	-	-	0	0.13	1.19	-
Model weight	0.27	0.14	0.11	-	-	0.34	0.32	0.18	-
Intercept	3.2^{e-01}	4.8^{e-01}	3.9^{e-01}	-	-	3.5^{e-01}	-2.2^{e-01}	7.9^{e-01}	-
Import	0.02	-	0.02	-	0.64	n.a	n.a	n.a.	-
Forest cover	-	-	-0.005	-	0.26	0.03	0.03	-	0.69
Forest composition	*	*	*	-	0.80	*	-	*	0.44
Trap position	*	*	*	-	0.82	*	*	*	0.94
Forest composition x Trap position	*	*	*	-	0.57	-	-	-	-
b) Abundance									
ΔAICc	0	0.91	1.09	1.10	-	0	1.34	1.71	-
Model weight	0.24	0.15	0.14	0.14	-	0.41	0.21	0.17	-
Intercept	8.9^{e-01}	6.8^{e-01}	8.5^{e-01}	1.1^{e-01}	-	7.0^{e-01}	4.4^{e-01}	3.6^{e-01}	-
Import	-	0.02	0.03	-	0.42	n.a	n.a	n.a.	-
Forest cover	-	-	-0.01	-0.01	0.39	-	0.02	-	0.36
Forest composition	*	*	*	*	0.86	-	-	*	0.32
Trap position	*	*	*	*	0.86	*	*	*	0.93
Forest composition x Trap position	*	*	*	*	0.68	-	-	-	-

* Indicates that the categorical variable was included in the model.

n.a. Not applicable: import volume was not tested for native species.

Discussion

Our nationwide study clearly identified where best to concentrate surveillance efforts to effectively intercept alien wood-boring beetles. In order to increase the probability of detecting alien species soon after their arrival, extensive monitoring programmes should be concentrated in ports with large volumes of imports and in the surrounding broadleaf forests, as the combination of these two conditions seems to provide the most favourable circumstances for trapping alien wood-boring beetles.

We found a significant effect of the volume of imported commodities on the alien species richness both in the ports and the surrounding forests indicating that import volume may have a key role in favouring alien invasions. Although a species can be already established, every year new individuals can arrive in the same points of entry or in areas where the species are not yet present, potentially establishing a new population and starting a new invasion. Previous studies have already shown the importance of several socio-economic indicators on alien species richness, but these analyses were mostly performed at the continental scale (Levine and D'Antonio 2003; Hlasny and Livingston 2008; Hulme 2009; Essl et al. 2011; Huang et al. 2012). For instance, Haack (2001) reported that the number of interceptions of alien wood-boring beetles in the US was positively correlated with the value of general imports per US state, but this relationship was not tested at smaller spatial scales. Wood packaging materials associated with imports are commonly found discarded at ports and such materials are often associated with alien wood-boring beetles (Haack 2001; Stanaway et al. 2001; Brockerhoff et al. 2006b). Wood packaging materials may transit through the ports to their final destination or, when broken and not usable, stored at the ports before being sent to companies authorized to recycle or destroy the wood. Therefore, there are many opportunities for adult insects to emerge from infested wood packaging and disperse in the surrounding habitats. In fact, despite ISPM-15 (IPPC 2013), some wood-boring beetles are apparently able to survive the approved treatments or colonize and develop in wood after them (Haack and Petrice 2009). Moreover, considering that some treatments may be improperly applied, either knowingly or because of faulty equipment or facilities (Haack and Petrice 2009), the risk related to the movement of wood packaging materials is still relatively high. Our results suggested that resources for surveillance programmes should be spatially concentrated in ports that import high volumes of solid commodities.

We also found that alien wood-boring beetle richness and abundance in the forest surrounding ports were strongly influenced by the tree composition, with broadleaf-dominated forests supporting more alien species and individuals than conifer-dominated forests. As the majority of alien species are generalist insects feeding on several broadleaf genera (e.g. ambrosia beetles) (Kirkendall and Faccoli 2010; Marini et al. 2011), broadleaf forests surrounding ports represent suitable habitat for their establishment. These areas have been recognized as high-risk sites in previous studies as the wood-boring beetles arriving at points of entry can disperse to neighbouring areas exploiting their ability to fly and find suitable hosts and habitats (Bashford 2008; Rabaglia et al.

2008). However, this result was independent of the amount of forest cover in the landscape. Although it could be expected that an area with limited forests would be less suitable for the establishment of introduced insects (Novak 1994; Koch et al. 2011), our results show that alien wood-boring beetles can be present even in landscapes with very low amounts of forest area. Previous studies have suggested that urban areas, which are usually characterized by a wide range of native and alien tree species in parks, gardens and along streets, may provide adequate tree hosts for invaders that would not otherwise become successfully established (Bashford 2008; Koch et al. 2011). The role of plantings in the landscape surrounding high-risk sites is, however, still largely under-investigated (Reed and Muzika 2010). The movement of alien insects from the port to the surrounding areas is almost inevitable; therefore, these areas are recognized as a crucial point in determining the success or the failure of the invasion process (Bashford 2008; Rabaglia et al. 2008). By contrast, for native species, we found that the proportion of forest cover in the surrounding landscape was positively related to the species richness, independently of the forest composition.

Lastly, we found that alien and native species richness and abundance were influenced by trap position, that is they were higher in the surrounding forests than inside the ports. The presence of woody materials in ports is, in fact, not constant over time and space, with commodities periodically unloaded, shipped or moved (Stanaway et al. 2001). Forest areas provide a more stable habitat with a larger variety of potentially suitable hosts and most of the wood-boring beetles emerging from wood packaging materials are not expected to reproduce inside ports but instead to fly away searching for suitable hosts. Our results confirmed this trend, as the communities of alien wood-boring beetles trapped in ports were clearly different than the beetle communities trapped in the surrounding forests. For these reasons, setting traps in both environments will increase the chances of detecting alien wood-boring beetles (Bashford 2008; Rabaglia et al. 2008; Wylie et al. 2008). This trapping protocol, integrated with the traditional inspections carried out by plant health inspectors, can strongly increase the possibility of early detection of alien species. Other countries, such as New Zealand and USA, have already implemented similar approaches, with a number of intercepted alien species attesting to the importance of such trapping programmes (Brockerhoff et al. 2006a; Rabaglia et al. 2008) and confirming their efficiency as an early warning system to trigger eradication or measures to contain the invasion. Regarding the native species, the results indicated that the wood-boring beetle communities trapped in ports were similar to those trapped in the surrounding forests, suggesting a potential exchange of species between the two environments. These individuals may fly from the forests towards the ports where they can colonize and complete development in wood packaging material (Haack et al. 2014), especially when bark is present (Haack and Petrice 2009). Traps can give information on the native species most commonly found in ports. These native species may constitute a pool of invaders that can be moved outside the country through international trade.

The development of early detection methods for alien species is a crucial step when implementing rapid response systems, effective eradication and suppression protocols for invasive pests (Pluess et al. 2012). If alien wood-boring and bark beetles are quickly detected, site-specific phytosanitary measures can be implemented and a timely action plan can be produced. However, due to the limited resources available for early detection, it is necessary to identify the most vulnerable locations where to concentrate surveillance efforts. Our countrywide survey of wood-boring beetles, with fourteen trapped alien species, among which four new to Italy, has provided clear indications of the most susceptible sites to invasions and has shown the efficacy of such a monitoring protocol. In particular, traps baited with attractive lures should be deployed both in ports and their surrounding forests, concentrating the efforts in ports with large volumes of imported commodities and in broadleaf forests surrounding them. Besides providing early detection of new invasions of alien pests, our trapping program can provide useful data on the geographical range of established alien species in support of regulatory controls or specific management programmes. Further benefits of our approach will be related to the potential reduction of the economic and environmental losses due to alien species. As traps and lures are fairly cheap and simple to use, pest management officials and the general public can be easily engaged in the fight against alien species (USDA-APHIS 2011). Significant challenges, however, still remain in the development of more effective surveillance tools for alien wood-boring beetles. In particular, more research efforts should be placed to develop more effective lure and trap systems for the large pool of potential alien wood-boring beetle species that may arrive in the future. At the same time, the analyses of native species trapped in ports can provide useful information on the species that constitute a pool of invaders that can be moved outside the country through international trades. Similarly, investigations on host preferences of commonly intercepted alien species could be conducted in both their native range and in the new environment (in a quarantine facility) to learn which tree species are most at risk as well as to help prioritize where surveys should be conducted.

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Data accessiblity

Alien and native species richness and abundance per site: data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.r7j3s (Rassati et al. 2014b). Parameters characterizing the 15 surveyed ports: uploaded as online supporting information.

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Table S1 Volume of imported solid commodities (Tons), percentage of forest cover in the 10 km radius buffer zone, composition of the forests surrounding ports and number of trap checks for each of the 15 surveyed sites. The sites are listed alphabetically.

Site	Abbreviation	Import (Tons)	Forest cover in the buffer (%)	Composition of the monitored forest	Number of trap checks
Ancona	Anc	1,962,907	18.84	Conifer	7
Bari	Bar	3,180,901	0.68	Conifer	5
Cagliari	Cag	4,857,495	1.27	Conifer	7
Catania	Cat	3,024,639	0.60	Broadleaf	3
Genova	Gen	17,689,731	21.08	Broadleaf	8
Monfalcone	Mon	2,748,047	25.59	Conifer	9
Napoli	Nap	9,408,437	0.63	Broadleaf	8
Palermo	Pal	3,747,938	6.19	Conifer	5
Porto	Por	517,684	4.79	Broadleaf	8
Porto Torres	Tor	1,961,443	2.14	Conifer	7
Ravenna	Rav	15,865,811	9.49	Conifer	7
Salerno	Sal	5,350,894	40.76	Broadleaf	8
Taranto	Tar	22,707,636	16.75	Conifer	2
Trieste	Tri	13,008,339	27.45	Conifer	9
Venezia	Ven	10,941,624	0.1	Broadleaf	8

Table S2 Abundance of wood-boring beetles (Scolytinae, Cerambycidae and Buprestidae) trapped during the survey inside ports and surrounding forests. “Status” indicate how the species is considered for Italy: native (Native), alien already established (Established) or alien intercepted but not yet established (Intercepted). “Host” indicate the categories of plant (broadleaf, conifer or herbaceous) or the plant species which are known as potential hosts of the trapped species. Species are listed alphabetically.

	Port	**Forest**	**Status**	**Host**
Scolytinae				
Ambrosiodmus rubricollis (Eichhoff)	1	-	Established	Broadleaf
Anisandrus dispar (Fabricius)	1	7	Native	Broadleaf
Carphoborus perrisi (Chapuis)	9	-	Native	Broadleaf
Crypturgus cinereus (Herbst)	5	19	Native	Conifer
Crypturgus mediterraneus Eichhoff	-	2	Native	Conifer
Crypturgus numidicus Ferrari	4	16	Native	Conifer
Cyrtogenius luteus (Blandford)	9	211	Established	Conifer
Ernoporicus caucasicus (Lindemann)	-	5	Intercepted	Broadleaf
Gnathotrichus materiarius (Fitch)	-	1	Established	Conifer
Hylastes attenuatus Erichson	1	21	Native	Conifer
Hylastes linearis Erichson	-	11	Native	Conifer
Hylastes opacus Erichson	1	34	Native	Conifer
Hylesinus oleiperda (Fabricius)	1	1	Native	Broadleaf
Hylurgus ligniperda (Fabricius)	148	1165	Native	Conifer
Hylurgus micklitzi Wachtl	527	10302	Native	Conifer
Hypoborus ficus Erichson	8	13	Native	Broadleaf
Hypothenemus eruditus Westwood	9	26	Established	Broadleaf
Ips amitinus (Eichhoff)	1	-	Native	Conifer
Ips sexdentatus (Borner)	347	1412	Native	Conifer
Ips typographus (Linnaeus)	136	41	Native	Conifer
Lymantor coryli (Perris)	-	8	Native	Broadleaf
Liparthrum colchicum Semenov	-	5	Intercepted	Broadleaf
Liparthrum mori (Aubé)	1	5	Native	Broadleaf
Orthotomicus erosus (Wollaston)	2526	22275	Native	Conifer
Orthotomicus laricis (Fabricius)	3	1	Native	Conifer
Orthotomicus proximus (Eichhoff)	22	29	Native	Conifer
Phloeotribus scarabeoides (Bernard)	1	-	Native	Broadleaf
Pityogenes calcaratus (Eichhoff)	1	5	Native	Conifer
Pityokteines curvidens (Germar)	1	-	Native	Conifer
Pityokteines spinidens (Reitter)	4	4	Native	Conifer
Pityokteines vorontzowi (Jacobson)	2	-	Native	Conifer
Pityophthorus buyssoni Reitter	-	1	Native	Conifer
Pityophthorus carniolicus Wichmann	1	9	Native	Conifer
Pseudothamnurgus scrutator (Pandellé)	-	2	Intercepted	Broadleaf
Pteleobius kraatzii (Eichhoff)	2	1	Native	Broadleaf
Scolytus multistriatus (Marsham)	6	11	Native	Broadleaf
Scolytus rugulosus (Muller)	-	1	Native	Broadleaf
Triotemnus ulianai (Gatti & Pennacchio)	1	3	Native	Herbaceous
Trypodendron lineatum (Olivier)	10	-	Native	Conifer
Xyleborinus saxesenii (Ratzeburg)	260	484	Native	Broadleaf/Conifer

Table S2. continued

Xyleborus dryographus (Ratzeburg)	-	12	Native	Broadleaf
Xyleborus eurygraphus (Fabricius)	13	7	Native	Conifer
Xyleborus ferrugineus (Fabricius)	6	-	Intercepted	Broadleaf/Conifer
Xyleborus monographus (Fabricius)	2	18	Native	Broadleaf
Xyleborus volvulus (Fabricius)	5	-	Intercepted	Broadleaf
Xylocleptes bispinus (Duftschmis)	2	2	Native	Clematis sp.
Xylocleptes biuncus Reitter	-	1	Native	Clematis sp.
Xylosandrus crassiusculus (Motschulsky)	1	21	Established	Broadleaf
Xylosandrus germanus (Blandford)	2	102	Established	Broadleaf/Conifer
Cerambycidae				
Acanthocinus aedilis (Linnaeus)	-	2	Native	Conifer
Acanthocinus griseus (Fabricius)	8	93	Native	Conifer
Arhopalus ferus (Mulsant)	-	9	Native	Conifer
Arhopalus rusticus (Linnaeus)	5	33	Native	Conifer
Arhopalus syriacus (Reitter)	-	8	Native	Conifer
Chlorophorus varius (Müller)	2	-	Native	Broadleaf/Conifer
Chlorophorus pilosus (Forster)	25	27	Native	Broadleaf
Cordylomera spinicornis (Fabricius)	1	-	Intercepted	Broadleaf
Gracilia minuta (Fabricius)	1	1	Native	Broadleaf
Hylotrupes bajulus (Linnaeus)	4	3	Native	Conifer
Monochamus galloprovincialis (Olivier)	8	129	Native	Conifer
Morimus asper (Sulzer)	-	1	Native	Broadleaf/Conifer
Niphona picticornis Mulsant	2	4	Native	Broadleaf/Conifer
Oxypleurus nodieri Mulsant	-	4	Native	Conifer
Parmena unifasciata (Rossi)	-	2	Native	Broadleaf
Penichroa fasciata (Stephens)	-	15	Native	Broadleaf/Conifer
Phoracantha recurva Newman	-	1	Established	Broadleaf
Pogonocherus hispidus (Linnaeus)	-	1	Native	Broadleaf
Pogonocherus perroudi Mulsant	-	7	Native	Conifer
Rhagium inquisitor Linnaeus	-	6	Native	Broadleaf/Conifer
Spondylis buprestoides (Linnaeus)	172	668	Native	Conifer
Stictoleptura cordigera (Fuessly)	1	1	Native	Broadleaf
Stromatium unicolor (Olivier)	-	1	Native	Broadleaf/Conifer
Trichoferus cinereus (Villers)	-	1	Native	Broadleaf
Trichoferus fasciculatus (Faldermann)	4	-	Native	Broadleaf/Conifer
Xylotrechus stebbingi Gahan	3	2	Established	Broadleaf
Buprestidae				
Buprestis haemorrhoidalis Herbst	2	1	Native	Conifer
Buprestis novemmaculata Linnaeus	1	-	Native	Conifer
Buprestis octoguttata Linnaeus	1	-	Native	Conifer
Coroebus undatus (Fabricius)	-	1	Native	Broadleaf
Dicerca berolinensis (Herbst)	-	1	Native	Broadleaf
Latipalpis plana (Olivier)	-	1	Native	Broadleaf
Total	4080	36294		

Chapter 4

Exploring the role of wood waste landfills in early detection of non-native wood-boring beetles

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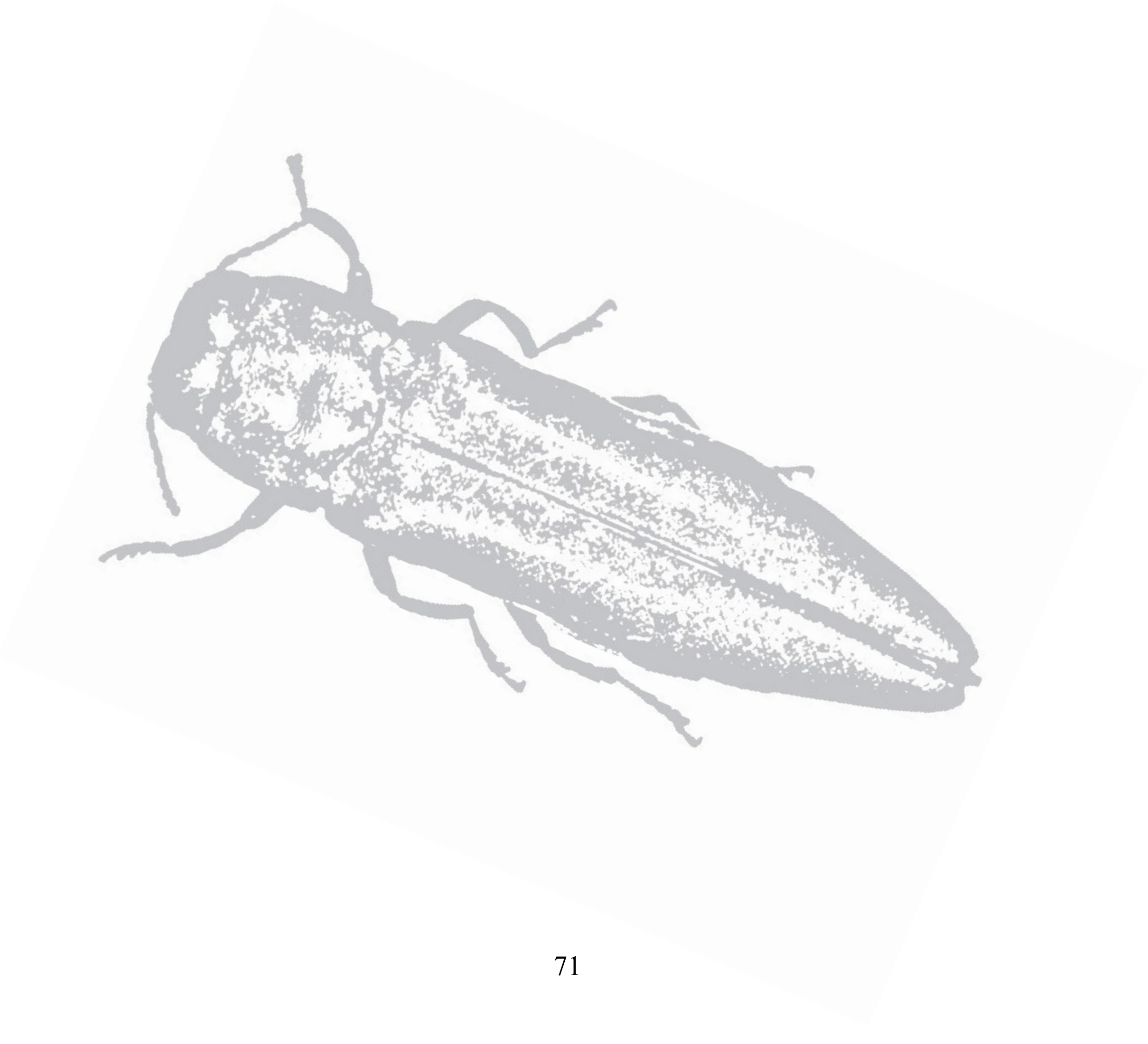

Abstract

Non-native wood-boring beetles (Coleoptera) represent one of the most commonly intercepted groups of insects at ports worldwide. The development of early detection methods is a crucial step when implementing rapid response programs so that non-native wood-boring beetles can be quickly detected and a timely action plan can be produced. However, due to the limited resources often available for early detection, it is important to identify the best locations where to concentrate surveillance efforts. The aim of this study was to investigate the role of wood waste landfills in the early detection of non-native wood-boring beetles. From June to September 2013, insects were collected in multi-funnel traps baited with a multi-lure blend (α-pinene, ethanol, ipsdienol, ipsenol, and methyl-butenol) at the main port and a nearby wood waste landfill in 12 Italian towns. Overall, 74 species of wood-boring beetles (Buprestidae, Cerambycidae, and Scolytinae) were trapped, among which eight were non-native to Italy. We found that species richness and species abundance of both non-native and native beetles were significantly higher in the wood waste landfill than in the ports. However, the non-native and native communities were similar in the two environments. The main conclusion emerging from this study is that wood waste landfills, given their similarity with ports of entry, should be considered when surveying for non-native wood-boring beetles. Therefore, within the framework of creating long-term monitoring programs that include both coastal and continental areas, both ports and wood waste landfills should be monitored to improve the probability for early detection of non-native species.

Introduction

Non-native wood-boring beetles are considered among the most dangerous forest pests worldwide with established populations of new species being reported nearly every year somewhere in the world (LaBonte et al. 2005; Work et al. 2005; Haack 2006; Kirkendall and Faccoli 2010). The rate of establishment of non-native wood-boring beetles is also increasing worldwide (Brockerhoff et al. 2014). For example, although wood-boring beetles represented only 11 % of the detected non-native species in the United States between 1800 and 1930, they represented 56 % of the new detections during 1980–2006 (Aukema et al. 2010). This pattern likely reflects the dramatic increase in volume of containerized shipping worldwide (Cullinane and Khanna 2000), which often entails the use of solid wood packaging materials such as crating, dunnage, and pallets. These materials represent the most common pathway of introduction for wood-boring beetles (Kenis et al. 2007; Zahid et al. 2008; Colunga-Garcia et al. 2009; DAISIE 2009) given that these insects develop under bark or inside wood where they can easily escape detection by inspectors and that the wood substrate itself protects these borers during transport (Haack 2001; Brockerhoff et al. 2006a; McCullough et al. 2006). Moreover, given that wood packaging materials are often manufactured from untreated, low-grade timber with residual bark (Haack and Petrice 2009), they can be infested by a wide variety of wood-boring beetles (Allen and Humble 2002; Evans 2007). In recognition of the threat posed by untreated wood packaging materials, an international standard (ISPM 15) was first approved in 2002 and was revised in 2013 (International Plant Protection Convention (IPPC) 2013). ISPM 15 details how wood packaging materials should be treated (e.g., minimum 56°C core temperature for 30 min for conventional heat treatment) prior to their use in international trade (Keiran and Allen 2004; Evans 2007). Although ISPM 15 has reduced the rate of infested wood packaging material, some treatments may be improperly applied, either knowingly or because of faulty equipment or facilities (Haack and Petrice 2009), and thus, live borers are still found occasionally in treated wood packaging, indicating that the risk of biological invasions through the wood pathway still exists (Haack et al. 2014).

Maritime ports and airports, where goods arrive from all over the world, are the primary points of entry for non-native species (Haack 2001, 2006; Brockerhoff et al. 2006b; McCullough et al. 2006; Wylie et al. 2008). When a non-native species arrives at a port of entry, it can potentially become established and spread either naturally into the surrounding areas (Bashford 2008; Rassati et al. 2014, 2015) or - if dispersal is human mediated - to disjunct sites located dozens or even hundreds kilometers from the original point of establishment (Piel et al. 2008; Hulme 2009; Colunga-Garcia et al. 2013). Because of the limited resources typically available for surveillance of non-native species and the high number of sites potentially exposed to non-native introductions (Colunga-Garcia et al. 2013), identification of the sites most vulnerable to establishment is important when designing surveillance efforts (Epanchin-Niell et al. 2014). Previous studies suggested, for instance, that timber importers, botanic gardens (Self and Kay 2005), or ornamental nurseries (Liebhold et al. 2012) are among the most at risk sites for pest introductions and therefore, should be considered when designing monitoring efforts for non-native pests. In the case of wood-boring beetles, much of the wood packaging materials associated with imports often goes to wood waste landfills (Buehlmann et al.

2009), and therefore, such sites may play a key role in the establishment of non-native species. It is important to recognize that only a small percentage of containers arriving through international trade is opened and inspected at the original port of entry (Haack 2001; Stanaway et al. 2001), with most commodities being instead transported directly to industrial or commercial areas (Colunga-Garcia et al. 2009). In such cases, the associated wood packaging materials are often discarded and sent to companies authorized to recycle or destroy the wood (Buehlmann et al. 2009). Although wood waste landfills have been already recognized as a potential site for establishment of wood-infesting insects (Auclair et al. 2005; Rabaglia et al. 2008), we are not aware of any studies that have empirically investigated the effectiveness of trapping non-native wood-boring beetles in such sites. The purpose of this study was to investigate the potential role of wood waste landfills, given that they serve as endpoints for wood packaging materials, in the early detection of non-native wood-boring beetles (Buprestidae, Cerambycidae, and Scolytinae). As wood waste landfills can receive wood packaging materials from many commercial routes, and often the wood packaging materials reside at such sites for longer periods of time than in the ports, we expected the wood waste landfills to have a relatively high species richness and species abundance of wood-boring beetles attesting to their value as monitoring sites, especially for early detection efforts in continental areas away from coastal ports.

Materials and methods

Selection of the experimental sites

The survey was carried out in 12 coastal towns located along the Italian peninsula and in the main Italian islands of Sardinia and Sicily in 2013 (Table 1). In each town, the port and the main wood waste landfill closest to the port were monitored. The selected ports were those that import large amount of solid commodities from every part of the world (Assoporti 2014) and therefore, should also receive large amounts of associated wood packaging materials. We selected those wood waste landfill sites that were authorized to destroy, recycle, and treat any kind of wood products. The distance between the selected ports and their paired wood waste landfill ranged from about 3–20 km (Table 1). Both ports and wood waste landfills were surrounded by a heterogeneous landscape, composed of mosaics of urban areas, green spaces, crop fields, and different types of forests.

Table 1 Port name, geographic coordinates and distance between the port and the nearby selected wood waste landfill (WWL) (km) for each of the 12 selected Italian towns where sampling occurred in 2013.

Town	**Port**	**Lat**	**Long**	**Distance between port and paired WWL**
Ancona	Ancona	43° 37'	13° 30'	19.86
Cagliari	Cagliari	39° 15'	09° 05'	6.10
Genova	Genova	44° 24'	08° 52'	2.87
Gorizia	Monfalcone	45° 47'	13° 32'	11.52
Napoli	Napoli	40° 50'	14° 16'	14.78
Palermo	Palermo	38° 08'	13° 21'	5.51
Ravenna	Ravenna	44° 28'	12° 15'	4.88
Salerno	Salerno	40° 40'	14° 44'	9.88
Sassari	Porto Torres	40° 53'	08° 39'	3.23
Trieste	Trieste	45° 39'	13° 15'	5.19
Udine	Porto Nogaro	45° 47'	13° 13'	4.58
Venezia	Marghera	45° 27'	12° 15'	2.71

Trapping design and lures

Six 12-unit, black funnel traps (Econex, Murcia, Spain) were set up in each of the 12 towns, with three traps placed inside the port and three traps in the nearby wood waste landfill. The commercial "dry-version" of the trap was used. A distance of at least 30 m was kept between traps. The tops of the traps were hung about 2 m off the ground, using suitable supports such as building structures, wire fences, and metal girders. All traps were in relatively open areas where insects could approach from several directions. Traps were baited with a multi-lure blend that was previously tested and found to be attractive to a wide variety of wood-boring beetles (Rassati et al. 2014) and composed of (-)α-pinene (ultra-high release, release rate of 2 g day^{-1}; 90-day field-life at 20°C), ethanol (release rate of 0.3 mg day^{-1}; 90-day field-life at 25°C), ipsenol (+50/-50; release rate of 0.4 mg day^{-1}; 90-day field-life at 20°C), ipsdienol (release rate 0.4 mg day^{-1}; 90-day field-life at 20°C), and 2-methyl-3-buten-2-ol (release rate of 11 mg day^{-1}; 90-day field-life at 20°C), all provided by Contech Enterprises Inc. (Victoria, BC, Canada). The collector cup of each trap was sprayed with an insecticide (Decis, Bayer Crop Science, Triangle Park, NC, USA) to quickly kill the trapped insects. The lure dispensers were changed after two months from the beginning of the monitoring, and the insecticide was renewed at each trap check. Trapping lasted for about 14 weeks during June through September 2013, with a number of trap checks varying from 2 to 8 (mean n = 5.8), depending on local restrictions regarding access to the ports and wood waste landfills. Despite some beetles, especially some early-season ambrosia beetles, may have been missed, we assumed that the trapped species provide a clear indication of the role that the tested sites may play in the early detection. Moreover, although the number of trap checks was different, the traps were exposed for the same period of time (mean ± SE = 96.3 days ± 4.53). Trapped wood-boring beetles (Buprestidae, Cerambycidae, and Scolytinae) were stored in alcohol until morphological identification. When needed, DNA extraction was conducted following a standard salting out protocol (Patwary et al. 1994). The barcode region of the mitochondrial gene cytochrome oxidase I was then amplified using universal primers (Folmer et al. 1994), and the resulting sequences were entered in the Bold System database (Ratnasingham and Hebert 2007). Once identified, beetles were then classified either as native or non-native species, including in the latter group both species newly intercepted or already established in Italy (Balachowsky 1949; Wood and Bright 1992; Curletti 1994; Bense 1995; Pfeffer 1995; Jendek 2006; Kubán 2006; Kirkendall and Faccoli 2010; Löbl and Smetana 2010; Knìzěk 2011).

Data analysis

To account for the differences in trapping frequency and the variability due to the longer intervals between less frequent trap checks, we used a general linear mixed effects model to evaluate the effect of the time between trap checks on the mean number of species or abundance per trap. The total number of non-native and native species (i.e., richness) and individuals (i.e., abundance), obtained after pooling together the collection data for all traps per site for the entire season, was the response variables. Then, we calculated the model residuals, and we used them as a response variable to test the effect of trap position (categorical variable: port vs. wood waste landfill). The new response variable did not depend on the duration of the trapping period. The model included the site as a random factor to account for the spatial dependence of the sampling. The model was fitted using the ‘lme’ function in the package nlme (Pinheiro et al. 2013) for R version 2.15.1 (R Development Core Team 2013). The non-native and native species abundance was log-transformed to improve linearity. The model included site as a random factor to account for spatial dependence of the trapping. All variables were reported as mean ± standard error of the mean (SEM). The detection frequency of non-native species with enough catch events (presence/absence) recorded in the two different environments (port and wood waste landfill) was compared with a Fisher's exact test. Finally, the Simpson's Similarity Index (Magurran and McGill 2010) was used to test for the similarity between species recorded in ports and wood waste landfills, where a value close to one indicates very similar insect communities in the two sampled environments. Differences in similarity between non-native and native species were tested by one-way ANOVA.

Results

Trapped beetles

During the survey, in total, 74 species of wood-boring beetles were trapped (Tables 2, 3). The total number of beetles caught was 11,255, with Scolytinae representing the most diverse and abundant group (42 species and 10,987 individuals), followed by Cerambycidae (23 species, 244 individuals), and Buprestidae (nine species and 24 individuals). Although most species were native (66), five Scolytinae (*Ambrosiodmus rubricollis* (Eichhoff), *Cyrtogenius luteus* (Blandford), *Gnathotrichus materiarius* (Fitch), *Hypothenemus eruditus* Westwood, *Xylosandrus germanus* (Blandford)), and three Cerambycidae (*Cordylomera spinicornis* (Fabricius), *Neoclytus acuminatus* (Fabricius), *Xylotrechus stebbingi* Gahan) were non-natives. Most of them were already known to be established both in Italy and other European countries. The cerambycid *C. spinicornis* had been collected in earlier surveys (Cola 1971; Rassati et al. 2015) but it is still not considered to be established in Italy.

Among the non-native species, *C. luteus* was the most abundant scolytid with 66 individuals collected, while *X. stebbingi* (56 individuals) was the most commonly collected cerambycid. Three species were represented by only one individual each (*A. rubricollis*, *X. germanus*, and *N. acuminatus*). Among native species, *Orthotomicus erosus* (Wollaston) (6,478 individuals) and *Ips sexdentatus* (Borner) (1,741 individuals) were the two most commonly collected native Scolytinae, while *Acanthocinus griseus* (Fabricius) and *Buprestis novemmaculata* L. were the most abundant native Cerambycidae and Buprestidae, with 47 and nine individuals, respectively.

Ports versus wood waste landfills

For non-native wood-boring beetles, we found significant differences both in species richness and abundance between the beetles collected at the ports and wood waste landfills. In particular, the mean number of non-native species trapped per site in wood waste landfills (1.50 ± 0.33) was significantly higher than in ports (0.75 ± 0.27) (GLMM, *P*<0.05, Fig. 1a). The same trend was found considering the mean number of individuals trapped per site (9.3 ± 5.66 at wood waste landfills vs 2.08 ± 1.03 at ports, GLMM, *P*<0.05, Fig. 1b). Among non-native species, one species was trapped exclusively in ports, two were trapped exclusively in wood waste landfills, and five were found in both environments (Tables 2, 3). Comparing the detection frequency, we found a significant difference only for the non-native scolytid *G. materiarius*, which was more frequently trapped in wood waste landfills than in ports (Table 2).

Table 2 Abundance (total number of individuals trapped during the season), detection frequency (%), results of the Fisher exact test, and native distribution for each Scolytinae trapped in the 2013 survey comparing ports and wood waste landfills (WWL) at 12 paired sites in Italy (n = 70[a]). * = P-value < 0.05. [a]: total number of trap checks performed in 2013 at all 24 trapping locations (12 ports and 12 landfills) + indicates non-native species A: Asia; E: Europe; N: North Africa; NA: North America; SA: South America

	Port	WWL	Port (%)	WWL (%)	P-value	Native to
Scolytinae						
+*Ambrosiodmus rubricollis* (Eichhoff)	0	1	0	1.4	-	A
Anisandrus dispar (Fabricius)	1	0	1.4	0	-	A, E
Carphoborus perrisi (Chapuis)	5	9	7.1	7.1	1	A, E, N
+*Cyrtogenius luteus* (Blandford)	15	51	8.5	10	1	A
Cryphalus piceae (Ratzeburg)	4	4	5.7	4.2	1	A, E, N
Crypturgus cinereus (Herbst)	3	25	4.2	22.8	*	E
Crypturgus cribrellus Reitter	2	-	2.8	0	0.33	E
Crypturgus mediterraneus Eichhoff	8	43	10	24.2	*	A, E, N
Crypturgus numidicus Ferrari	3	17	4.2	15.7	*	A, E, N
Crypturgus pusillus (Gyllenhal)	8	3	4.2	2.8	1	A, E
+*Gnathotrichus materiarius* (Fitch)	-	8	0	8.5	*	NA
Hylastes attenuatus Erichson	1	2	1.4	1.4	-	A, E
Hylurgus ligniperda (Fabricius)	81	569	51.4	65.7	0.15	A, E, N
Hylurgus micklitzi Wachtl	249	830	50	60	0.33	A, E, N
Hypoborus ficus Erichson	8	10	4.2	8.5	0.34	A, E, N
+*Hypothenemus eruditus* Westwood	-	2	0	2.8	-	SA?
Ips acuminatus (Gyllenhal)	8	-	5.7	0	*	A, E
Ips amitinus (Eichhoff)	1	-	1.4	0	-	A, E
Ips sexdentatus (Borner)	888	853	48.5	70	*	A, E
Ips typographus (Linnaeus)	85	196	10	27.1	*	A, E, N
Liparthrum mori (Aubé)	1	-	1.4	0	-	A, E
Orthotomicus erosus (Wollaston)	1405	5073	80	88.5	0.51	A, E, N
Orthotomicus laricis (Fabricius)	3	2	2.8	1.4	-	A, E, N
Phloeotribus cristatus (Fauvel)	1	-	1.4	0	-	A, E, N

Table 2 continued

Pityogenes calcaratus (Eichhoff)	1	-	1.4	0	-	A, E
Pityogenes chalcographus (Linnaeus)	3	1	4.2	1.4	0.48	A, E
Pityokteines spinidens (Reitter)	2	3	1.4	2.8	-	A, E
Pityokteines vorontzowi (Jacobson)	4	7	4.2	2.8	1	A, E
Pteleobius kraatzii (Eichhoff)	-	2	0	2.8	-	A, E, N
Scolytus amygdali Guerin	-	1	0	1.4	-	A, E, N
Scolytus multistriatus (Marsham)	16	9	5.7	5.7	1	A, E
Scolytus rugulosus (Muller)	1	3	1.4	2.8	-	A, E, N
Taphrorychus alni Pfeffer	-	1	0	1.4	-	E
Trypodendron lineatum (Olivier)	4	3	2.8	2.8	-	A, E, N
Trypophloeus binodulus Ratzeburg	1	-	1.4	0	-	A, E, N
Triotemnus ulianai (Gatti & Pennacchio)	-	2	0	2.8	-	E
Xyleborinus saxesenii (Ratzeburg)	129	294	48.5	62.8	0.14	A, E, N
Xyleborus eurygraphus (Fabricius)	1	11	1.4	7.1	0.08	A, E, N
Xyleborus monographus (Fabricius)	-	5	0	5.7	*	A, E, N
Xylocleptes bispinus (Duftschmis)	-	3	0	2.8	-	A, E, N
Xylocleptes biuncus Reitter	1	-	1.4	0	-	A, E, N
$^{+}$*Xylosandrus germanus* (Blandford)	-	1	0	1.4	-	A

Table 3 Abundance (total number of individuals trapped during the season), detection frequency (%), results of the Fisher exact test and native distribution for each Cerambycidae and Buprestidae trapped in the 2013 survey comparing ports and wood waste landfills (WWL) at 12 paired sites in Italy (n = 70). * P-value < 0.05. [a]: total number of trap checks performed in 2013 at all 24 trapping locations (12 ports and 12 landfills) $^{+}$ indicate non-native species A: Asia; E: Europe; N: North Africa; SCA: South-Central America; WA: West Africa

	Port	WWL	Port (%)	WWL (%)	P-value	Native to
Cerambycidae						
Acanthocinus griseus (Fabricius)	13	34	11.4	22.8	*	A, E
Aegomorphus clavipes (Schrank)	-	1	0	1.4	-	A, E, N
Arhopalus ferus (Mulsant)	-	1	0	1.4	-	A, E, N
Arhopalus rusticus (Linnaeus)	-	4	0	4.2	0.1	A, E, N
Arhopalus syriacus (Reitter)	-	3	0	4.2	0.1	A, E, N
Aromia moschata (Linnaeus)	2	-	2.8	0	-	A, E
Asemum striatum (Linnaeus)	-	1	0	1.4	-	A, E
Callimus angulatus (Schrank)	-	2	0	1.4	-	A, E, N
Chlorophorus glabromaculatus Goeze	3	5	4.2	7.1	0.61	E
Chlorophorus varius (Müller)	8	-	4.2	0	0.1	A, E
$^{+}$*Cordylomera spinicornis* (Fabricius)	2	-	2.8	0	0.33	WA
Hylotrupes bajulus (Linnaeus)	9	10	7.1	11.4	0.43	A, E, N
Leiopus nebulosus (Linnaeus)	-	1	0	1.4	-	A, E
Monochamus galloprovincialis (Olivier)	13	7	5.7	7.1	1	A, E, N
$^{+}$*Neoclytus acuminatus* (Fabricius)	-	1	0	1.4	-	SCA

Table 3 continued

Niphona picticornis Mulsant	-	1	0	1.4	-	A, E, N
Parmena solieri Mulsant	3	-	2.8	0	-	E
Penichroa fasciata (Stephens)	3	1	1.4	1.4	-	A, E, N
Rusticoclytus rusticus Linneaus	1	-	1.4	0	-	A, E, N
Spondylis buprestoides (Linnaeus)	28	7	7.1	4.2	0.61	A, E, N
Stictoleptura cordigera (Fuessly)	1	2	1.4	2.8	-	A, E, N
Trichoferus fasciculatus (Faldermann)	6	15	2.8	7.1	0.28	A, E, N
$^{+}$*Xylotrechus stebbingi* Gahan	8	48	11.4	20	0.13	A
Buprestidae						
Agrilus viridicaerulans Marseul	-	1	0	1.4	-	E, N
Buprestis haemorrhoidalis Herbst	-	4	0	5.7	0.1	A, E, N
Buprestis novemmaculata Linnaeus	2	7	2.8	7.1	0.28	A, E, N
Buprestis octoguttata Linnaeus	1	-	1.4	0	-	A, E
Eurythyrea micans (Fabricius)	1	-	1.4	0	-	E, N
Melanophila cuspidata (Klug)	1	-	1.4	0	-	A, E, N
Palmar festiva (Linnaeus)	-	3	0	2.8	-	E, N
Phaenops cyaneus (Fabricius)	1	-	1.4	0	-	A, E, N
Phaenops formaneki Jacobson	3	-	1.4	0	-	A, E

For native wood-boring beetles, we found significant differences in both species richness and abundance between ports and wood waste landfills. In particular, the mean number of native species trapped per site in wood waste landfills (12.6 ± 1.55) was significantly higher than in ports (9.6 ± 1.65) (GLMM, $P<0.05$, Fig. 1c). The same trend was found considering the mean number of native individuals trapped per site (626.81 ± 174.31 vs. 233 ± 80.94, respectively, GLMM, $P<0.05$, Fig. 1d). Among native species, 18 species were trapped exclusively in ports, 17 were trapped exclusively in wood waste landfills, and 31 were found in both environments. Comparing the detection frequency of native species, we found a significant difference for eight species, among which seven were more frequently trapped in wood waste landfills (the Scolytinae *Crypturgus cinereus* (Herbst), *Crypturgus mediterraneus* Eichhoff, *Crypturgus numidicus* Ferrari, *Ips sexdentatus* (Borner), *Ips typographus* (L.), *Xyleborus monographus* (Fabricius), and the cerambycid *A. griseus* (Fabricius)), and one (the scolytid *Ips acuminatus* (Gyllenhall)) was more frequently trapped in ports (Tables 2, 3). The Simpson's Similarity Index for non-native species between the ports and the wood waste landfills (0.83) was similar to that of native species (0.68) ($P = 0.34$, $n = 12$).

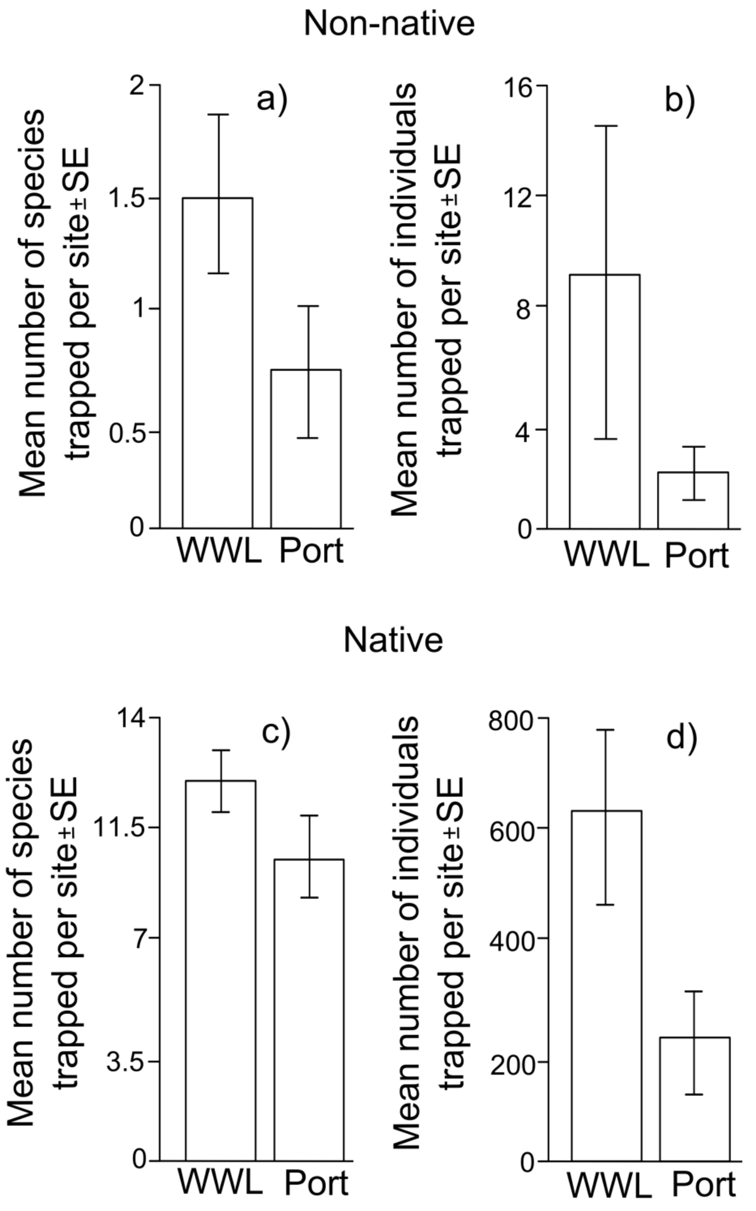

Fig. 1 Mean number of species and individuals (+SE) trapped per site in wood waste landfills (WWL) and maritime ports in 12 Italian towns over an approximate 14-week trapping period in 2013 (see text for details).

Discussion

The early detection of non-native species is basic to implementation of rapid response systems, and development of effective eradication and suppression protocols for invasive pests (Pluess et al. 2012). However, one of the first steps when developing early detection programs is to identify sites or habitats that are at high-risk of invasion and then to concentrate the surveillance efforts at these areas, including both coastal and continental sites (Epanchin-Niell et al. 2014). The present study suggested that wood waste landfills, as with ports, could be considered as useful sites for trapping non-native wood-boring beetles. Using baited traps to survey such sites could either potentially increase the probability of locating new non-native species or add information on the distribution of already established ones, therefore allowing for a timely response to implement eradication efforts or destroy infested materials.

We found that non-native species richness was higher in wood waste landfills than in ports, even though the composition of the wood-boring beetle communities was similar in the two environments. Ports, which receive large amounts of commodities associated with wood packaging materials, were previously identified as the most high-risk sites for non-native species introductions (Brockerhoff et al. 2006b; Bashford 2008; Rabaglia et al. 2008; Wylie et al. 2008; Rassati et al. 2014). However, despite the integrated use of trapping protocols and traditional inspection methods that strongly increase the probability of detecting non-native species soon after arrival at the ports (Brockerhoff et al. 2006b; Rabaglia et al. 2008; Rassati et al. 2014), some individuals may escape detection, become established, and spread naturally in the surrounding areas or at further distances when their dispersal is human mediated (Piel et al. 2008; Colunga-Garcia et al. 2013). For this reason, the identification of hotspots for invasion, such as sites handling significant volumes of timber and wood packaging materials, has been highlighted as a priority to enhance the efficacy of early detection programs (Self and Kay 2005; Ostrauskas and Tamutis 2012; Colunga-Garcia et al. 2013). Our results suggested that wood waste landfills, which can be considered as the last step in the life cycle of wood packaging materials (Buehlmann et al. 2009), can serve as high-risk sites for non-native wood-boring beetle invasion. In fact, despite that all the non-native species trapped in this trial were already established in Italy (Kirkendall and Faccoli 2010), they can provide useful indication of the suitability of a given site to be invaded, especially considering that every year new individuals can arrive in areas where the species are not yet present. For example, the non-native scolytid *C. luteus*, which has been reported in the Veneto (Faccoli et al. 2012) and Friuli Venezia Giulia (Rassati et al. 2015) regions of Italy until 2012, was trapped simultaneously at port and wood waste landfill in the Emilia Romagna region during this trial, underlining the potential role of both habitats in non-native species invasion. The deployment of traps baited with generic lures inside wood waste landfills should enhance detection of non-native wood-boring beetles and thereby act as an early warning system to trigger eradication programs or other measures to contain the spread of non-native species. Moreover, as broadleaf forest's surrounding points of entry have been already highlighted to be crucial sites for the interception of non-native species (Rassati et al. 2015), the simultaneous use of baited traps both in wood waste landfills and surrounding broadleaf stands could increase the possibility of trapping non-native wood-boring beetles.

We found that non-native species abundance was significantly higher in wood waste landfills than in ports. The amount of wood packaging materials present in wood waste landfills can be stored for longer periods of time than in ports, increasing the probability of non-native species emerging and dispersing from wood waste landfills. In ports, the type of woody materials, their amount, and their storage location are often unpredictable, which affects the possibility of establishing effective survey protocols and thus the probability to detect non-native species (Rassati et al. 2014). Typically, the number of sites involved in processing, destroying, recycling, or treating wood packaging materials is high within most countries and therefore, is important information to use when selecting survey sites. In this regard, previous studies indicated that, as a general rule, higher amounts of imported commodities in a given area increase the probability of non-native species introduction, and this has been demonstrated at both continental (Mack et al. 2000; Haack 2001; Marini et al. 2011; Huang et al. 2012; Liebhold et al. 2013) and port scale (Rassati et al. 2015). We suggest that this rule should be applied to wood waste landfills, adjusting for variation in the volume of handled wood packaging materials. A better understanding of how such wood is managed and treated at different wood waste landfills will enhance the decision process about where surveillance efforts should be focused. Another important issue to consider is the timeliness of trap collections at ports and wood waste landfills. On the one hand, given that landfill sites are usually located in continental areas, the capture of non-native species often provides little information as to where the non-native organism first entered the country, which is useful when developing eradication strategies. On the other hand, trapping at landfill sites can provide useful data on the geographical range of newly or recently arrived non-native species, which is useful when forming conservation and management strategies (Rassati et al. 2015).

Lastly, we found that both native species richness and abundance were significantly higher in wood waste landfills than in ports, although the species compositions were similar in the two environments. Wood waste landfills are usually less isolated compared to ports, and thus, the surrounding landscape often has greater amounts of green space, such as forests and parks, which would favor the in and- out exchange of native wood-boring beetles (Rassati et al. 2015). Trapping of native species in such high-risk sites is, however, still poorly investigated. In fact, a subset of these native species may actually fly from the local vegetation surrounding the landfills or emerge from wood packaging materials that were associated with either national or international trade. In support of this idea is a study by Hu et al. (2013) that used molecular data to suggest that the native cerambycid *Monochamus alternatus* Hope, which vectors the pinewood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer), has expanded its range westward within mainland China, most likely through the inadvertent transport of infested wood packaging materials associated with trade within China. Similarly, considering that the Scolytinae *I. typographus* and *I. acuminatus* are typically distributed within continental areas of Italy, their presence also in coastal regions suggests that they were moved to the coast in infested logs or wood packaging materials. As further support, consider that *I. typographus* and *I. acuminatus* have been commonly intercepted on wood packaging materials associated with Italian imports to the U.S.A., which would have departed Italy from its coastal ports (Haack 2001; Haack and Rabaglia 2013). Future studies should focus on understanding if the native species that are most commonly trapped in high-risk sites are also the most prone to be transported in wood packaging materials and thereby constitute a pool of invaders that can be moved outside the country through international trade.

Despite the measures undertaken to prevent the arrival of non-native species, the potential of new introductions is still high and appears to be increasing along with the increasing volume of international cargo and the numbers of potential countries of origin (Aukema et al. 2010; Kirkendall and Faccoli 2010; Colunga-Garcia et al. 2013; Haack et al. 2014). Our results suggest that the use of baited traps in wood waste landfills, which provided detection results comparable to ports, would be an effective strategy to increase the efficiency of early detection of nonnative species in continental areas far from the coast. The establishment of a monitoring network in both coastal and continental areas, with special attention to wood waste landfills, would likely increase the probability of detecting non-native wood-boring beetles compared with trapping primarily along coastal areas near ports. Moreover, given that countries often experience the highest invasion pressure in metropolitan and industrial areas, which represent the final destination of the imported goods (Colunga-Garcia et al. 2013), expanding our understanding on the dispersal and distribution of invasive species in urban and suburban areas represents the next major challenge to improving early detection strategies.

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Chapter 5

Habitat and host preferences of alien ambrosia beetles in European temperate forests

Rassati D, Faccoli M, Battisti A, Marini L

Submitted

Abstract

Ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) are recognized as a successful group of invasive species and their introduction can cause severe economic and biological consequences. In order to predict their impact on an invaded ecosystem, it is of utmost importance to understand the mechanisms driving their invasion process. The aims of this study were, first, to understand how climate, forest composition and forest structure can affect the establishment of alien ambrosia beetles at the regional spatial scale, and, second, to evaluate if these species, known as strongly polyphagous in their native range, show any preferences in terms of host tree species when introduced to Europe. In 2013 we sampled 33 forest stands located in north-east Italy that belonged to four different forest types, using both baited traps and log traps of five host tree species. We found a clear effect of forest type, forest structure and climate in shaping alien ambrosia beetle communities. In particular, chestnut dominated forests was the forest type with the highest alien species richness and abundance, values that were also affected positively by mean tree diameter and mean annual temperature. Moreover, log colonization was significantly affected by tree species, forest type and their interaction, with chestnut logs and oak dominated forests representing the favorite host and forest type, respectively. Such information can help to enhance management strategies and early-detection programs, indicating which habitat and host tree species are most susceptible to alien ambrosia beetles attack.

Introduction

Ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) are considered as one of the most successful group of invasive species worldwide (Haack 2006; Kirkendall and Faccoli 2010; Haack and Rabaglia 2013). They have a life-history characterized by symbiotic trophic specializations with fungi that limits the competition with native bark beetles (Kirkendall 1983), a cryptic life style that favors their movement across continents (Marini et al. 2011), and a sib-mating behavior that allows rapid establishment and spread in new environments (Jordal et al. 2011). First detections of these species often occur in coastal regions surrounding ports and in inland shipping sites (i.e. main towns, industrial and commercial areas) that receive international goods (Haack 2001, 2006; Brockerhoff et al. 2006; Liebhold et al. 2013). Forests represent, however, the environment where the establishment of alien ambrosia beetles is expected to be more probable (Bashford 2008;

Colunga-Garcia et al. 2009; Rassati et al. 2015). Although broadleaf forests have been reported to host more alien ambrosia beetles than coniferous forests (Rassati et al. 2015), just how forest composition and host availability can affect the invasion process is still largely under investigated.

While at large spatial scales, such as continents, the establishment of alien ambrosia beetles seems to be driven mainly by climatic factors (Marini et al. 2011), the effect of forest composition and structure may be much more important at smaller spatial scales. Given that ambrosia beetles can fly long distances [i.e. up to 40 km for *Trypodendron lineatum* (Olivier) (Salom and McLean 1991)], they are expected to be able to fly and colonize suitable resources across large areas (Park and Reid 2007). It is known that a key role is played by the health conditions of forest stands, with physiologically stressed forests being more attractive to ambrosia beetles than healthy ones (Ranger et al. 2010, 2012, 2013). Nevertheless, it is still unclear how alien ambrosia beetles behave when the invaded environment is characterized by the presence of mosaics of different forest types. Few attempts to compare scolytids communities occurring in forest plantations of different tree species have been performed in the tropics (Flechtmann et al. 2001; Dorval et al. 2004; Hulcr et al. 2007), which underlines the poor habitat specificity of this group of insects. These patterns are, however, poorly investigated in temperate regions (Hulcr et al. 2007), especially when considering alien ambrosia species. Given that forests can vary by tree species composition, tree age, elevation and microclimate, a different pattern in alien ambrosia beetle species richness and abundance would be expected when comparing different forest types.

Within the same forest type, stand characteristics can also play an important role in determining the structure of alien ambrosia beetle communities. Reed and Muzika (2010) have demonstrated that dendrometric parameters, such as stand age and forest structure, can influence presence and relative abundance of alien and native ambrosia beetles. Host density, for instance, may influence ambrosia beetles richness and abundance, which can be higher in areas with greater availability of host material (Park and Reid 2007; Reed and Muzika 2010). The microclimate also varies with stand structure affecting habitat and host selection of many saproxylic beetles (Fettig et al. 2007; Vodka et al. 2009). Hulcr et al. (2008a) found that sites with greater humidity favor the growth of symbiotic fungi and therefore supports more species of ambrosia beetles than drier ones. The effects of these variables on alien ambrosia beetle communities are still understudied despite that such information can be crucial when developing local management and conservation strategies.

Also the mechanisms involved in tree host selection are still unclear. Most ambrosia beetles do not produce long-distance pheromones and they are considered host generalists relying on generic cues of host decay such as ethanol (Beaver 1979; Ranger et al. 2010). Although this behavior has been shown in the beetles' native range (Hulcr et al. 2007), exceptions to this general rule do occur, especially in new invaded environments (Stone et al. 2007; Kendra et al. 2014). In the USA, for example, the invasive alien species *Xyleborus glabratus* is more attracted by the redbay (*Persea borbonia* L) than by other hosts such as sassafras (*Sassafras albidium* (Nutt.) Nees) and oak (*Quercus virginiana* Mill.) (Hanula et al. 2008). The identification of such host preferences may become an extremely important step in predicting the impact of alien ambrosia beetles on native tree species and allowing the potential identification of new volatiles that can improve the efficacy of trapping and early detection (Hanula et al. 2008; Hulcr et al. 2011; Kuhns et al. 2013).

To understand whether the invasion process of alien ambrosia species is driven by either stochastic or niche-based processes, we first investigated the effect of different forest compositions, their intrinsic features and climate on the structure of alien ambrosia beetles communities; second, we investigated if alien ambrosia beetles show any preferences in terms of host tree species, to better understand the mechanisms of host selection as well as to identify tree species that are potentially more susceptible to future invasions. Lastly, because alien species may directly and indirectly interact with native species sharing the same habitats and hosts, we compared habitat and host preferences of alien and native ambrosia beetles to identify specific selection patterns.

Materials and methods

Study areas

The study was conducted in 33 forest sites located in north-east Italy (Veneto and Friuli Venezia Giulia). Based on their composition, we first defined four forest types: oak-dominated forest, hop hornbeam-dominated forest, chestnut-dominated forest and beech-dominated forest. These forest types were chosen because they were among the most common types present in the study area and they also form a climatic gradient related to elevation (Table 1). We attempted to locate sites where the number of trees of the dominant species was at least 60% of the total. Due to difficulties in finding suitable sites for all tree species, it was not possible to have a perfectly balanced experimental design. We sampled eight oak-dominated forests, 11 hop hornbeam-dominated forests, six chestnut dominated forests and eight beech dominated forests. All selected forest sites appeared to be in good health with no current or recent insect or disease outbreaks obvious. At each site, species composition, tree density, basal area and mean diameter of all trees with a diameter larger than 10 cm were measured in one circular buffer area (10-m radius) around the point where the trap was placed (Table S1). In each site, to estimate the habitat diversity the Shannon index based on tree species abundance was calculated. Lastly, we estimated the mean annual amount of rainfall and the mean annual temperature for each of the selected forest site. The normal annual rainfall (1960-90) was interpolated using ordinary kriging from 347 metereological stations scattered throughout the study area, whereas mean annual temperature (1960-90) was interpolated from 73 meteorological stations (Marini et al. 2011) using ordinary kriging with external drift (Benavides et al. 2007). The geostatistical interpolations were computed using the Kriging Interpolator 3.2 extension for ArcView 3.2 (ESRI).

Table 1 Summary data for each selected forest type including the abbreviation code, number of sites sampled, dominant tree species, mean elevation (m), percent forest cover based on the whole forest area of Veneto region. These data are comparable to those characterizing the neighbouring region Friuli Venezia Giulia.

Forest type	**Code**	**N° of sites**	**Dominant tree species**	**Elevation**	**Cover % ***
Oak-dominated forest	OAK	8	*Quercus robur* L.	8.87 ± 1.77	0.34
Hop hornbeam-dominated forest	OST	11	*Ostrya carpinifolia* Scop.	380.1 ± 49.55	14.68
Chestnut-dominated forest	CHE	6	*Castanea sativa* Miller	410.5 ± 76.08	5.02
Beech-dominated forest	BEE	8	*Fagus sylvatica* L.	894.2 ± 93.28	19.72

* Data obtained from Del Favero (2006)

Trapping design and lures

In each forest site, one 12-unit black-funnel trap (Econex, Murcia, Spain) was hung to a lower tree branch about two meters from the ground, in the inner forest at least 50 meters from the forest edge. We used the “dry” version of the insect collector, which has a 4-cm diameter hole in the cup bottom that is covered with a wire mesh screen (0.5 mm mesh size) which allows water to drain. All traps were baited with ethanol (release rate of 0.3 mg day^{-1}; 90-days field-life at 25°C, provided by Contech Enterprises Inc., Victoria BC, Canada), which is known to attract a wide variety of ambrosia beetles (Miller and Rabaglia 2009). Moreover, each trap was sprayed with a fluoropolymer resin (FLUON), provided by BioQuip Products (California, USA), which prevented beetles from climbing out of traps (Graham and Poland 2012). The insect collector was sprayed with a contact insecticide (Decis, Bayer Crop Science, Triangle Park, NC, USA), to ensure prompt death of trapped insects. According to the expected field-life of the lures, they were changed after three months and the insecticide treatment was renewed monthly during each trap check. Traps were left exposed for five months (February - September 2013) and checked monthly (five sampling events). On each visit, all insects were removed, put in separate vials filled with ethanol and then brought to the laboratory for identification.

Host and habitat selection

In order to test for host selection by alien and native ambrosia beetles, log traps (10-15 cm diameter and 30-35 cm long) of five tree species (*Ostrya carpinifolia* Scop., *Castanea sativa* Miller, *Fagus sylvatica* L., *Fraxinus ornus* L., *Robinia pseudoacacia* L.) were cut at the end of January 2013. The first three species were chosen to correspond with the dominant tree species in three of the four sampled forest types; it was not possible to obtain oak logs for this study. *F. ornus* and *R. pseudoacacia* were instead chosen because they occurred in all forest types as minor species. At each site, three logs per each tree species were placed on the ground in five separate groups, each formed by logs of the same species. Log groups were located about 2.5 m from each other, and at least 60 m away from the funnel trap at each site. Logs were placed in the forest in late February 2013 and left until late June 2013, in order to allow colonization by both alien and native ambrosia beetles. Logs were then collected, brought to the laboratory, and individually closed within plastic rearing boxes. In order to allow for aeration, a 10-cm-diameter hole was opened at the bottom of each pot. The holes were covered with wire-mesh (0.5 mm) screening. The rearing boxes were stored at outdoor temperature in a warehouse and checked twice, once at the beginning of August and once in mid-September, collecting all the emerged beetles. All adults collected were stored in alcohol, identified to species level and then classified as either native or alien to Europe according to the available literature (Balachowsky 1949; Wood and Bright 1992; Pfeffer 1995; Kirkendall and Faccoli 2010; Knížek 2011).

Data analysis

Ambrosia beetle species richness and abundance

The effects of forest type (categorical variable), mean tree diameter (continuous variable) and tree density (continuous variable) on alien and native ambrosia species richness and abundance recorded with traps were tested by a general linear mixed-effects model (GLMM). Given that mean tree diameter and basal area were highly correlated ($r_{S\,=}\,0.85$, $P < 0.01$), only the first variable was included in the model. The mean number of either alien or native ambrosia beetles species (i.e. richness) or individuals (i.e. abundance) collected per trap-check was used as the response variable. The alien and native species abundance was log-transformed to improve linearity. The model included the study site ID nested within the check data as random factors to account for the spatial and temporal dependence of the sampling. We did not test interactions between the selected variables as we did not have any specific ecological hypotheses supporting these tests.

The effect of climate (mean annual temperature and mean annual amount of rainfall, continuous variables) and habitat diversity (expressed as the Shannon index, continuous variable) on alien and native species richness and abundance recorded with traps were tested by running a separate generalized linear mixed-effects model (GLMM) including the forest type among the random factors described above. We did not include climatic factors and the Shannon index in the models testing forest type and stand characteristics due to the very high collinearity between these groups of variables. For instance, beech-dominated forests were always located at colder temperatures than the other forest types. The mean number of alien and native ambrosia beetle species and individuals was the response variable. The alien and native species abundance was log-transformed to improve linearity. The GLMM analyses were performed using the ‘‘nlme’’ (Pinheiro et al. 2013) packages implemented in R (R Development Core Team 2014).

Due to the relatively low number of replicates (i.e., sites) per forest type and the relatively high number of potential predictors, we used multi-model inference within an information-theoretic framework to evaluate the role of the selected variables in explaining species richness and abundance (Burnham and Anderson 2002). Our information-theoretic approach compared the fit of all the possible candidate models obtained by the combination of our predictors using second-order Akaike’s information criterion (AICc) corrected for small samples. The AICc is a measure of relative model fit, proportional to the likelihood of the model and the number of parameters used to generate it. The best fitting model is the one with the lowest AICc. In a set of n models each model i can be ranked using its difference in AICc score with the best-fitting model ($\Delta AICc_i = AICc_i – AICc_{MIN}$). The difference in AICc values indicates the relative support for the different models. A model is usually considered plausible if its ΔAICc is below 2 (Burnham and Anderson 2002). From the set of plausible models we omitted the models with uninformative parameters, *i.e.* models with ΔAICc below two but including only one additional parameter compared to the best model (Arnold 2010). For each model i we also calculated an Akaike weight (w_i), which is the probability that model i would be selected as the best fitting model if the data were collected again under identical circumstances (Burnham and Anderson 2002). To gauge the relative importance of each predictor, we summed the w_i across the models in the set ($\sum w_i$) in which the predictor occurred. The multi-model inference analyses were performed using the MuMIn package for R (Barton 2010).

A potential problem with data obtained for many ecological observational studies is that the sampled variables may have a spatial component. This can result in spatial autocorrelation which causes problem for statistical methods that make assumptions about the independence of residuals (Legendre and Legendre 1998). We therefore tested for spatial autocorrelation using Mantel correlograms (Borcard and Legendre 2012). Specifically, we tested whether model residuals obtained for both alien and native species richness and abundance were spatially auto-correlated. To derive the residuals, we did not use the single best model as this would inevitably miss out some parameters of importance, but we built for both alien and native species richness and abundance a model (fitted with ML) that included all the parameters that were in models with $\Delta AIC < 2$. Mantel correlation coefficients were calculated for each lag interval and tested for significance with a permutation test, using 1,999 permutations. Each correlogram was tested for significance using a Bonferroni-corrected α of 0.01 (Legendre and Legendre 1998). The spatial autocorrelation analysis was performed using the "mpmcorrelogram" (Pinheiro et al. 2013) implemented in R (R Development Core Team 2014).

Ambrosia beetle species composition

Ordination methods were applied to unravel the influence of the different forest type on species composition. The response variable was the abundance of either alien or native ambrosia beetle species trapped per site with traps. A preliminary Detrended

Correspondence Analysis (DCA) was performed. The largest DCA gradient length, expressed in standard deviation (S.D.) units of species turnover, of the first four DCA axes was below 3 S.D. units. Thus, the use of linear-based ordination models was appropriate for these data (ter Braak and Šmilauer 2002). First, a Principal Component Analysis (PCA) was performed to extract the main part of the variability related to species composition. Second, a Redundancy Analyses (RDA) was applied using the forest type as a factor quantified by four dummy variables, and used a separate Monte Carlo permutation test with 999 permutations ($P > 0.05$).

Logs colonization

The effect of host tree species (categorical variables) and forest type (categorical variable) on the proportion of logs colonized by ambrosia beetle species was tested by a log-linear analysis for a three-way contingency table using VassarStats website (Lowry 2001). We constructed separate contingency tables for each ambrosia beetle species, where columns were designated as log species and rows were designated as colonization degree (colonized or non-colonized), combination that was tested in each forest type. For each ambrosia species, G-test statistics ($G2 \approx \chi2$) were calculated for the 3-way (colonization x forest type x log species) and 2-way (colonization x forest type and colonization x log species) interactive effects. We could not use generalized linear mixed-effects model (GLMM) with a binomial distribution or zero-inflated models due to the poor model performance, i.e. model diagnostics always indicated very poor model residual distribution.

Results

General results

Overall, we trapped ten species of ambrosia beetles, four alien and six native to Europe (Table 2). The most numerous alien species was *Xylosandrus germanus* (Blandford) (1,317 individuals) whereas *Ambrosiodmus rubricollis* (Eichhoff) was represented by only 4 individuals. *Xyleborinus saxesenii* (Ratzeburg) was instead the most abundant native species (1,163 individuals), whereas only one individual of *Trypodendrum signatum* (Fabricius) was trapped. From the logs we collected only 4 ambrosia beetle species, two alien and two native, for a total of 567 and five individuals respectively (Table 2). The most numerous alien species was *X. germanus* (399 individuals), while

Xyleborus dryographus (Ratzeburg) (four individuals) was the most numerous native species. The trapped alien species represented the 44.4% of the alien ambrosia beetles that are known to be established in Italy (Kirkendall and Faccoli 2010), whereas the trapped native species represented the 66.6% of ambrosia beetles native to Europe (Knížek 2011).

Table 2 Total number and relative abundance (%) of alien and native ambrosia beetles collected with traps or emerged from the logs. Species are listed alphabetically.

Species	**Abbreviation**	**Traps**		**Logs**	
		Total	**%**	**Total**	**%**
Alien species					
Ambrosiodmus rubricollis (Eichhoff)	*Amb rub*	4	0.14	168	29.37
Ambrosiophilus atratus Eichhoff	*Amb atr*	59	2.11	-	-
Xylosandrus crassiusculus (Ratzeburg)	*Xyl cra*	50	1.79	-	-
Xylosandrus germanus (Blandford)	*Xyl ger*	1345	48.21	399	69.75
Subtotal		1458	52.22	567	99.12
Native species					
Anisandrus dispar (Fabricius)	*Ani dis*	57	2.04	-	-
Xyleborinus saxesenii (Ratzeburg)	*Xyl sax*	1163	41.6	1	0.17
Xyleborus dryographus (Ratzeburg)	*Xyl dry*	66	2.36	4	0.69
Xyleborus monographus (Fabricius)	*Xyl mon*	38	1.36	-	-
Trypodendron domesticum (Linnaeus)	*Try dom*	7	0.25	-	-
Trypodendron signatum (Fabricius)	*Try sig*	1	0.03	-	-
Subtotal		1332	47.81	5	0.88
Total		2790		572	

Drivers of species richness and abundance

For alien ambrosia beetle species richness, two plausible models were selected indicating support for the effect of forest type and dendrometric parameters (Table 3a). The model weights of the variables indicated that species richness was mainly influenced by forest type ($\sum w_i = 0.99$) and positively by mean tree diameter ($\sum w_i = 0.93$). In particular, the highest number of alien species was trapped in chestnut-dominated forests, followed by oak-dominated forest, hop hornbeam-dominated forests and beech-dominated forest (Fig 1a). We also found five plausible models explaining the effect of climate and habitat diversity on the species richness of alien ambrosia beetles (Table 4a). The model weights of the variables indicated that the species richness was positively influenced by mean annual temperature ($\sum w_i = 0.73$).

For alien ambrosia beetle abundance, we found two plausible models indicating the effect of forest type and dendrometric parameters (Table 3b). The sum of the models weights of the variables indicated that their abundance was mainly influenced by forest type ($\sum w_i = 0.99$). As for species richness, the highest number of alien individuals was trapped in chestnut-dominated forest, followed by hop hornbeam-dominated forests, oak-dominated forests and beech-dominated forests (Fig 1b). We also found three plausible models explaining the effect of climate and habitat diversity on the abundance of alien ambrosia beetle species (Table 4b). The model weights of the variables indicated that the abundance was influenced mainly by mean annual temperature (positively, $\sum w_i = 0.54$).

For native ambrosia beetle species richness, we found three plausible models explaining the effect of forest type and dendrometric parameters (Table 3a). The highest number of native species was trapped in oak-dominated forests, followed by chestnut-dominated forests, hop hornbeam-dominated forests and beech-dominated forests (Fig 1c). We also found three plausible models explaining the effect of climate and habitat diversity on the native ambrosia beetle species richness (Table 4a). The model weights of the variables indicated that the species richness was positively influenced by mean annual temperature ($\sum w_i = 0.98$).

For native ambrosia beetle abundance, we selected two plausible models (Table 3b). The sum of models weights of the variables indicated that their abundance was mainly influenced by forest type ($\sum w_i = 0.99$). The highest number of alien individuals was trapped in oak-dominated forests, followed by hop hornbeam-dominated forests, chestnut-dominated forests and beech-dominated forests (Fig 1d). We also found two plausible models explaining the effect of climate and habitat diversity on the native ambrosia beetle abundance (Table 4a). The model weights of the variables indicated that abundance was positively influenced by mean annual temperature ($\sum w_i = 0.99$).

Table 3 Plausible candidate models (<2 ΔAIC of the top model) explaining the effect of forest type and dendrometric parameters on species richness and abundance of alien and native ambrosia beetles (Scolytinae) trapped during the survey. Models are ranked according to their second-order Akaike's information criterion (AICc). Parameter estimates and model weight (w_i) are reported. For each tested variable $\sum w_i$ indicates the sum of model weights.

	Alien			Native			
	Best	2nd	$\sum w_i$	Best	2nd	3rd	$\sum w_i$
a) Species richness							
ΔAICc	0.00	1.59	-	0.00	1.40	1.97	-
Model weight	0.64	0.29	-	0.48	0.24	0.18	-
Intercept	0.39	0.58	-	1.33	1.11	1.12	-
Forest type	*	*	0.99	*	*	*	0.99
Density	-	-1.6^{-04}	0.31	-	2.4^{e-04}	-	0.33
Mean diameter	3.93	3.73	0.93	-	-	0.84	0.27
b) Abundance							
ΔAICc	0.00	0.92	-	0.00	1.91	-	-
Model weight	0.48	0.30	-	0.54	0.20	-	-
Intercept	1.35	3.17	-	0.84	1.17	-	-
Forest type	*	*	0.99	*	*	-	0.99
Density	-1.2^{e-03}	-1.4^{e-03}	0.79	-	-3.7^{e-04}	-	0.27
Mean diameter	6.65	-	0.63	-	-	-	0.24

Table 4 Plausible candidate models (within 2 ΔAIC of the top model) explaining the effect of climate and habitat diversity on species richness and abundance of alien and native ambrosia beetles (Scolytinae) trapped with traps. Models are ranked according to their second-order Akaike's information criterion (AICc). Parameter estimates and model weight (w_i) are reported. For each tested variable $\sum w_i$ indicates the sum of model weight

	Alien						Native			
	Best	2nd	3rd	4th	5th	∑ wi	Best	2nd	3rd	∑ wi
a) Species richness										
ΔAICc	0.00	0.67	0.68	1.14	1.71	-	0.00	1.81	1.91	-
Model weight	0.25	0.18	0.18	0.14	0.11	-	0.51	0.20	0.19	-
Intercept	-0.40	-0.21	0.41	0.56	0.48	-	-1.10	-0.50	-1.00	-
T°	0.12	0.08	0.13	-	0.09	0.73	0.19	0.18	0.17	0.98
Rainfall	-6.6^{-04}	-	-5.8^{-04}	-	-	0.39	-	-3.2^{-04}	-	0.28
H Index	-	0.20	-	0.38	0.18	0.52	-	-	0.10	0.28
b) Abundance										
ΔAICc	0.00	0.85	1.50	-	-	-	0.00	1.86	-	-
Model weight	0.29	0.19	0.14	-	-	-	0.53	0.21	-	-
Intercept	-0.25	1.40	1.05	-	-	-	-2.2	-1.5	-	-
T°	0.15	-	-	-	-	0.54	0.33	0.32	-	0.99
Rainfall	-	-	-	-	-	0.26	-	-4.6^{-04}	-	0.28
H Index	-	-	0.33	-	-	0.33	-	-	-	0.25

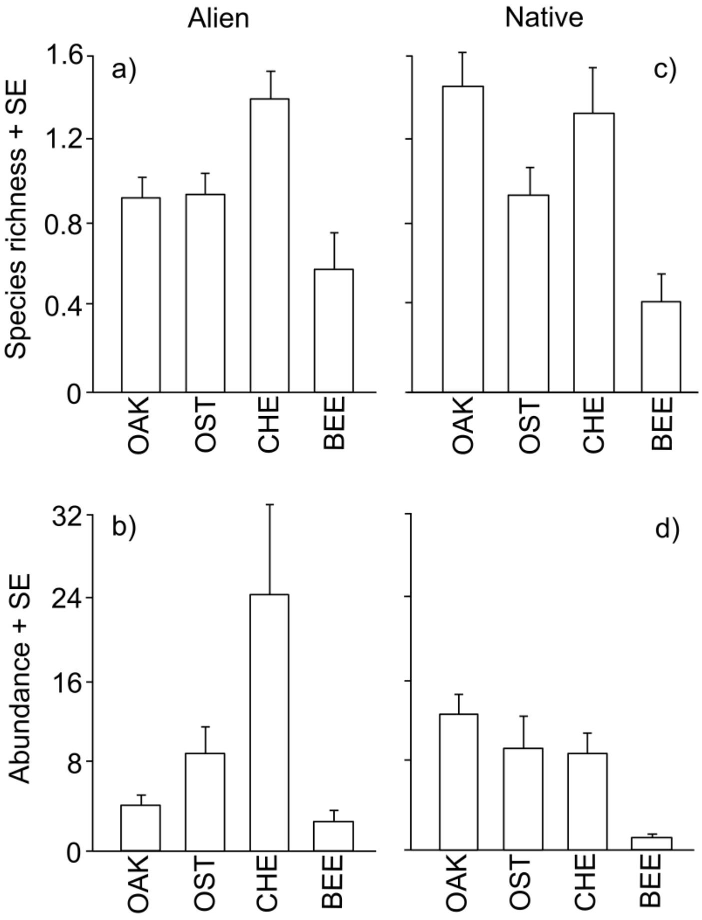

Fig. 1 Mean + standard error (SE) alien and native ambrosia beetle species and individuals recorded per trap check in the four forest types. Abbreviations are according to Table 1.

We did not find any spatial autocorrelation in the residuals of richness and abundance of both alien and native ambrosia beetles. At all the distances, the Mantel correlation values were close to zero.

Species composition

Considering the PCA analysis for alien ambrosia beetles, the first two principal components explained 61.8% and 23.3% of the total variation in species composition. Chestnut-dominated forests, oak-dominated forests and hop hornbeam-dominated forests showed quite comparable species assemblages (Fig 2a). Beech-dominated forests showed a similarity in species composition and were instead separated from those belonging to other forest types. Considering the PCA analyses for native species, the first two principal components explained 65% and 18.9% of the total variation in species composition. Oak and hop hornbeam-dominated forests showed similar species compositions, separated from those homogeneous of chestnut and beech-dominated forests (Fig 2b)

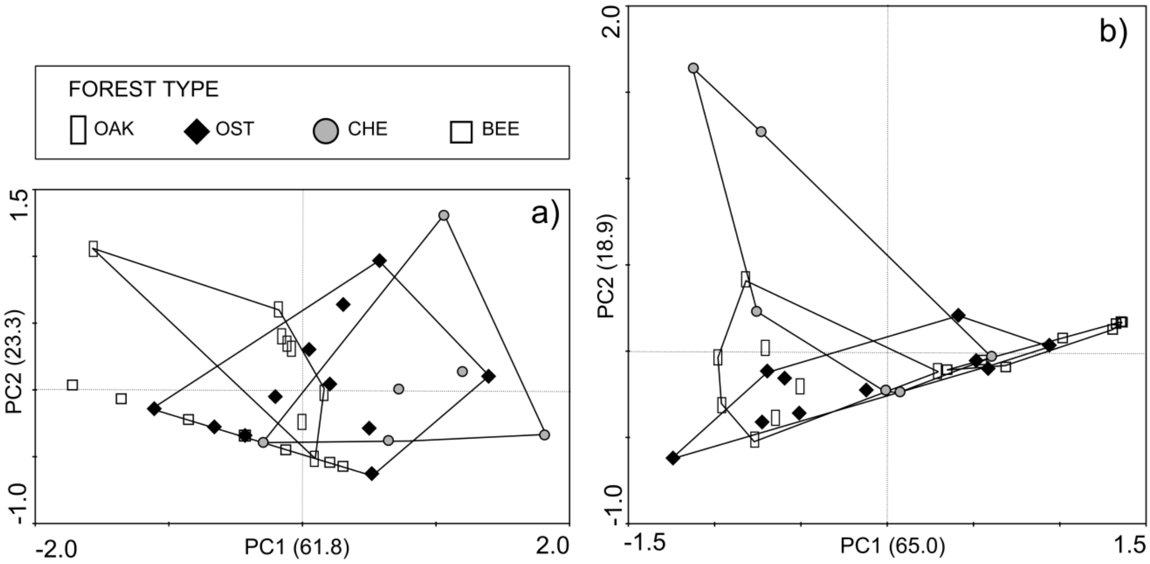

Fig. 2 PCA ordination diagrams of the 33 forest stands against the first two Principal Components separately for a) alien and b) native ambrosia beetle species. Abbreviations are according to Table 1.

Considering the RDA analyses for alien ambrosia beetles performed using forest types as factor, the first axis explained 23.3% of the total variation in ambrosia beetle species composition. *A. rubricollis* was found to be strongly associated with oak-dominated forests while species belonging to the genus *Xylosandrus* (*X. germanus and X. crassiusculus*) were found to be associated mainly with chestnut-dominated forests (Fig 3a). Also for native species, the first axis explained 36.2% of the total variation in ambrosia beetle species composition. The bulk of native species were not strictly associated with a certain forest type, while species belonging to the genus *Trypodendron* were found to be strongly associated with beech-dominated forests (Fig 3b).

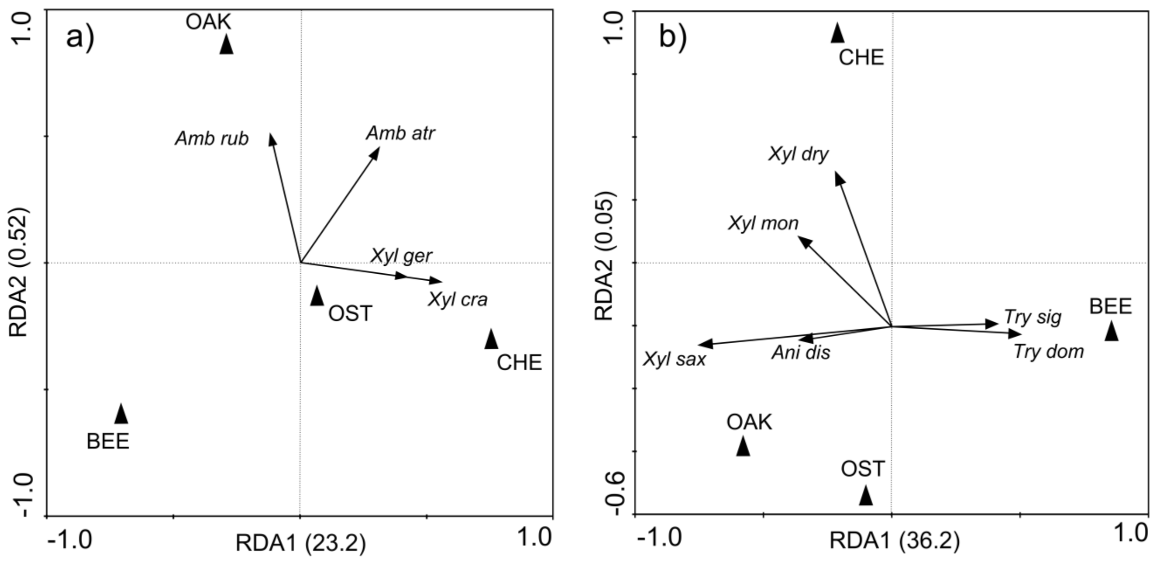

Fig. 3 RDA ordination diagrams of a) alien and b) native ambrosia beetle species occurring in the 33 forest stands against the first two canonical axes constrained by forest type. Abbreviations are according to Table 1 for forest types and Table 2 for alien and native ambrosia beetle species.

Drivers of log colonization

We found an effect of forest type and log species on the proportion of logs colonized by the ambrosia beetles species *X. germanus* and *A. rubricollis* (Table 5). In particular, we found that for both species the proportion of colonized logs was higher for chestnut than for the other tested species and for oak-dominated forest than in the other selected forest types (Fig 4a, b). We also found for both species an interaction between forest type and log species, i.e. the highest proportion of colonized logs was registered when chestnut logs were located in oak-dominated forests (Fig 4a, b).

Table 5 Results of log-linear analyses testing the effects of forest type, log species and their interaction on the proportion of logs colonized by the two ambrosia beetle species emerged from the logs.

		Xylosandrus germanus		*Ambrosiodmus rubricollis*	
Effects	*df*	G^2	*P*	G^2	*P*
Log species	4	60.96	<0.01	35.16	<0.01
Forest type	3	32.56	<0.01	35.86	<0.01
Log species x Forest type	31	106.72	<0.01	75.86	<0.01

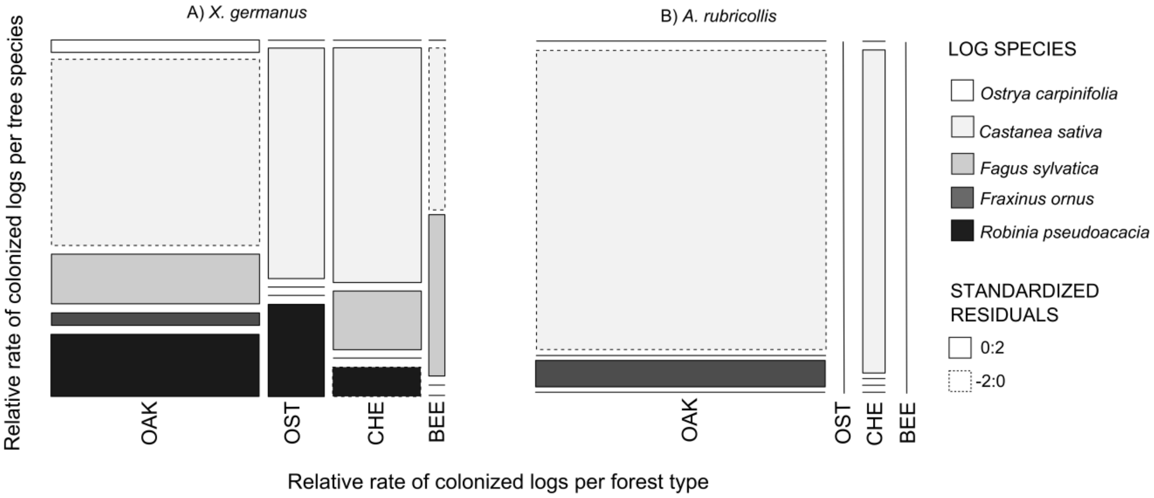

Fig. 4 Mosaic plots representing the proportion of logs colonized by the alien ambrosia beetle species *X. germanus* and *A. rubricollis* for host tree species and forest types. Vertical and horizontal sides of the boxes indicated respectively the proportion of logs per host tree species and forest type. Abbreviations are according to Table 1.

Discussion

Our study elucidated some patterns of establishment and spread of alien ambrosia beetle species within the invaded environment, highlighting that the invasion process was clearly linked to niche-based processes both at the the regional scale, where was driven by forest composition and climatic variables, and at the stand scale, where a key role is played by the forest structure. Moreover, also within forest stands, alien ambrosia beetles showed preferences in terms of host tree species, despite they are known to be highly polyphagous in their native range (Hulcr 2007). Such information is recognized as crucial in predicting the impact of alien species in the invaded ecosystems (Marini et al. 2011; Liebhold et al. 2013) and this is especially true for alien ambrosia beetles as the accidental introduction of these insects will likely become more and more common in the future (Haack 2006; Kirkendall and Faccoli 2010; Haack and Rabaglia 2013).

We found a clear effect of forest composition on both species richness and abundance of alien ambrosia beetles. Given that each forest type results from the interaction of a number of different factors such as macroclimate, topographic parameters and soil conditions, which shape the tree community and influence the microclimate within stands (Aussenac 2000), it is reasonable that alien ambrosia beetle species find certain forest types more suitable for their establishment than others. In particular, the presence of certain dominant tree species can influence both the beetles (Stone et al.

2007; Hulcr et al. 2008) and their symbiotic fungi (Rice et al. 2008), which can develop differently in different host tree species (Castrillo et al. 2012). Hence, alien ambrosia beetles coming from tropical areas change their behavior (Hulcr and Dunn 2011), shifting from being highly generalist (Fletchmann et al. 2001; Dorval et al. 2004; Hulcr et al. 2008a, b) to relatively specialist in habitat selection.

We also found a clear effect of climate on alien ambrosia beetle species richness. Previous studies have already emphasized the key role played by climatic variables, demonstrating that temperature and rainfall are strictly related with the number of alien ambrosia beetles established in the USA, but these studies were performed at a larger spatial scale (Marini et al. 2011; Reich et al. 2014). Our results confirmed the key effect of temperature at the regional scale, but not that of rainfall. In this regard it must be noted that our study sites were located in what is defined as the sub-humid area (De Martonne 1926) and the differences in annual amount of rainfall among them were probably not enough to affect the species richness of alien ambrosia beetle. On the contrary, the temperature regime characterizing forests located at the higher elevation range (i.e. beech-dominated forests), can limit the number of species present in the system affecting both the amount of available energy (Currie 1991) and the performance of the beetles (Reich et al. 2014) or that of their symbiotic fungi (Rice et al. 2008). This result reflects the distribution and number of alien ambrosia beetles across Europe, which decreases with increasing latitude (Kirkendall and Faccoli 2010).

Within forest stands, we found an effect of dendrometric parameters on alien ambrosia beetle communities. Ambrosia beetle diversity was positively affected by mean tree diameter whereas their abundance was negatively related to tree density. Previous studies reported that stand age and forest structure influenced ambrosia beetle diversity and the abundance of some species (Reed and Muzika 2010), but the effect of tree diameter was not clear. Locating host plants by phytophagous insects is often a complex process involving behavioral responses to both chemical and visual stimuli (Harris and Forster 1995) and in scolytids the importance of visual cues in host location is well recognized (Strom et al. 2001). A positive relation between tree diameter and host selection has been already observed in ambrosia beetles and it can be related to the fact that a greater volume of xylem allows for construction of longer galleries and more brood production relative to smaller diameter hosts (Mayfield and Brownie 2013). On the contrary, the negative effect of tree density was unexpected, and could be related to the influence of stand structure on the diffusion of volatiles from traps (Park and Reid 2007).

Turchin and Odendaal (1996) demonstrated that a pheromone trap surrounded by many competing vertical silhouettes can affect the estimation of beetle abundance and it is possible that this occurred also during this trial.

The colonization of logs by both *X. germanus* and *A. rubricollis* was significantly affected by tree species, forest type and their interaction. Ambrosia beetles are known to be highly polyphagous species, especially in their native ranges (Hulcr et al. 2007; Hulcr et al. 2008a, b) but exceptions may occur in invaded environments, with alien species showing a higher preference for certain host tree families or species (Stone et al. 2007; Hanula et al. 2008; Kendra et al. 2014). The clear preference for chestnut logs highlighted in the present study confirmed this idea. However, despite chestnut trees being shown highly attractive for these species in a previous experiment (Olivier and Mannon 2001), it is still unclear how host selection occurs. Recent studies indicated that a key role may be played by volatiles from both host and symbiotic fungi (Hulcr et al. 2011) whereas other factors, such as the wood density, water content and depth of sapwood seem to affect the density of attack, the depth of penetrations or the distribution of entry holes (Prebble and Graham 1957) but not the host selection (Hulcr et al. 2007). Despite the high attractiveness of chestnut logs, the highest rate of colonization was found in oak dominated forests rather than in chestnut forests. Oak-dominated forests are located at lower elevation range than all the other tested forest types, and are known to be characterized by warm and humid conditions that produce a microclimate (Mason 2004) probably favorable for the reproduction of beetles and associated fungi (Fettig et al. 2007; Hulcr et al. 2008b; Vodka et al. 2009). In general, chestnut and oak-dominated forests seem to be at higher risk of attack by alien ambrosia beetles. The lack of oak logs in our study, species already known to be a potential host of several ambrosia beetle species (Wood and Bright 1992; Pfeffer 1995), did not allow to conclude whether this species may be highly threatened by these insects.

Regarding native ambrosia beetle species, we found a significant effect of forest composition and climate on both species richness and abundance. Ambrosia beetle species native within a given area have co-evolved with their hosts and they are more adapted to certain habitats than others. In this respect, as composition and characteristics of forest stands change, the associated beetle communities also change. However, this trend is not observed in tropical regions where tree species composition does not seem to have any significant effect in shaping native ambrosia beetle communities, suggesting that ambrosia beetles might be more specialized in temperate than in tropical regions

(Beaver 1979; Hulcr et al. 2008a). On the contrary, the effect of climatic variables on native ambrosia beetles has been already highlighted worldwide (Hulcr et al. 2008a; Marini et al. 2011; Reich et al. 2014), with warmer and wetter areas supporting more species than colder and drier areas. However those trends are still largely under investigated and quantitative data on habitat and host selection of native ambrosia beetles are almost missing for all biomes (Hulcr et al. 2007).

The increase of international trade from tropical and sub-tropical regions and the expected changes in climate will likely favor the establishment of new alien ambrosia beetle species in the near future (Kirkendall and Faccoli 2010; Marini et al. 2011; Rassati et al. 2015). Our study highlighted that these beetles choose their environment as well as show preferences in terms of host species. This information may help developing management strategies and allow local detection programs to be more efficient. In particular, given that chestnut logs represented the most favored host, chestnut-dominated forests should be considered at high risk to be damaged either by ambrosia beetle species that are already established or by those that may be introduced in the future: for this reason monitoring programs should be focused within these forests, especially considering their economic importance (Pezzi et al. 2011). At the same time, oak-dominated forests were demonstrated to have intrinsic features that favor reproduction and host colonization by ambrosia beetles and future studies should evaluate the potential role that alien ambrosia beetles might have in oak decline (Sallè et al. 2014). Lastly, in order to assess the ecological impact of alien ambrosia beetle species on native beetle communities, more research should be focused on understanding how alien and native ambrosia beetles interact when they compete for the same resources.

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Table S1 Summary of stand and site characteristics (means ± SEM) for the tested forest types.

Forest type	**Diameter (m)**	**Basal area (m^2)**	**Density**
Oak-dominated forest	0.24 ± 0.01	36.57 ± 6.47	736.64 ± 43.04
Hop hornbeam-dominated forest	0.15 ± 0.01	16.32 ± 2.17	914.88 ± 113.72
Chestnut-dominated forest	0.24 ± 0.01	44.05 ± 8.32	875.79 ± 118.81
Beech-dominated forest	0.2 ± 0.02	29.45 ± 2.26	943.47 ± 58.33

Chapter 6

Exploring invasion patterns of exotic Scolytinae across the USA through ß-diversity analysis

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Submitted

Abstract

Scolytinae (bark and ambrosia beetles) represent one of the most successful groups of invaders but their invasions patterns are still unclear. In this study we aimed to understand how the different feeding habits of scolytines influence the spread and the assembly of their communities and which drivers influence these processes. We used data on the distribution of exotic and native scolytines in the continental 48 contiguous USA states. We used the beta-diversity index, partitioned into its species richness and species replacement components, to first analyze spatial autocorrelation using Mantel correlograms, and second to apply regression on distance matrices to test the direction and the shape of the association of the tested drivers on β-diversity. For exotic bark beetles, we found that β-diversity was composed by both species richness and species replacement. Their species richness differences were primarily affected by differences in imports values while temperature played a significant role in determining species replacement. For exotic ambrosia beetles, the β-diversity was determined more by species richness than species replacement. Their species richness differences were affected by differences in amount of rainfall between states while species replacement was affected by both rainfall and temperature differences. The different feeding habits of exotic bark and ambrosia beetles influenced their spread in the new environment. The lower dependency that bark beetles had on climate allows them to potentially colonize more areas within the USA, while exotic ambrosia beetles, being more dependent on climate, will be typically filtered by the environment.

Introduction

International movement of exotic forest insects represents a severe threat to forests worldwide (Holmes et al. 2009; Vilà et al. 2009; Gandhi and Herms 2010). Bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) represent one of the most successful groups of invaders, in part because they are easily transported in wood products and packaging materials (Brockerhoff et al. 2006a; Haack 2006; Haack and Rabaglia 2013). Continuing increases in international trade are expected to pose even greater risks of introduction by these organisms (Levine and D'Antonio 2003; Costello et al. 2007; Hulme 2009; Kenis et al. 2009; Marini et al. 2011; Bacon et al. 2012). For these reasons, improved understanding of the possible drivers of the invasion process of bark and ambrosia beetles is recognized as a research priority.

Although factors influencing geographic distribution and species richness of non-native species have been examined (e.g. Pyšek et al. 2010; Marini et al. 2011), drivers and mechanisms of assembly of exotic communities are less well-studied. In this context, analyses of β-diversity may provide insights into these processes (Leprieur et al. 2009; Winter et al. 2009; Marini et al. 2012). Spatial β-diversity can be generally defined as the extent of change in community composition, or degree of community differentiation, between different locations (Whittaker 1960). This differentiation can be partitioned in components, each representing distinct ecological processes: species richness difference, species replacement, and nestedness. Several indices have been proposed to quantify the individual components (Baselga 2010, 2012; Podani and Schmera 2011), which has generated some confusion given the ambiguous ecological interpretation of some indexes (e.g. Tuomisto 2010). A recently developed analytical framework partitions β-diversity into its species richness and replacement components (Podani and Schmera 2011). This framework can be effectively used to unveil potential drivers of community assembly and understand the invasion process in the landscape, but to date, there have been no studies investigating β-diversity patterns of Scolytinae.

Scolytinae are commonly divided into two main guilds according to their feeding habit (Wood 1982; Haack and Rabaglia 2013): phloem-feeding bark beetles, and xylem-inhabiting fungus-feeding ambrosia beetles. Bark beetles are characterized by relatively high host specificity (Knížek and Beaver 2004) given their reliance on specific phloem characteristics, tree defense chemistry, and nutritional quality (Byers 1989). Many ambrosia beetles are relative generalists; they bore into the sapwood but feed primarily on symbiotic fungi that can develop in either living or dead wood (Furniss and Caroline 1977). Feeding habit can strongly influence patterns of species richness of exotic Scolytinae (Marini et al. 2011) but it is still unknown whether these characteristics also affect the assembly mechanisms of their communities.

The first stage of the biological invasion process begins with the arrival of a pool of exotic species that constitutes the potential colonizers of a defined recipient region. The primary points of entry of exotic species are likely to be airports and seaports, which import goods from all over the world (Haack 2001, 2006; Knížek and Beaver 2004; Brockerhoff et al. 2006b; Liebhold et al. 2006; McCullough et al. 2006; Haack et al. 2014). A positive relationship has been shown between the value or volume of imported goods and the number of introduced exotic species (Marini et al. 2011; Huang et al. 2012; Brockerhoff et al. 2014; Rassati et al. 2015). After the initial introduction, exotic invaders must overcome a continuum of abiotic and biotic filters in order to successfully establish and spread in a new region (NRC 2002). Spread, i.e., the process by which an exotic species expands its range from a habitat in which it currently occurs to one in which it does not, is affected by multiple variables (Liebhold and Tobin 2008) and not all exotic species that arrive at a given point of entry are able to immediately spread within the new environment, as confirmed by the higher number of exotic species established in the coastal areas than within continental areas (Haack 2001; Marini et al. 2011; Haack and Rabaglia 2013; Liebhold et al. 2013).

The factors influencing the spread of exotic scolytines are still not clear. Scolytine distribution in the new environment should follow the rule of the distance decay of similarity, where the similarity between two locations often decreases as the distance between them increases (Nekola and White 1999). This is usually due to a combination of at least two, not mutually exclusive, mechanisms: the environmental filtering and the dispersal limitation of the species (Leprieur et al. 2009). It is still unclear, however, how this mechanism influences scolytine distribution, either exotic or native: in fact, as exotic species share the habitat with the more abundant native species, it becomes interesting to compare the processes shaping their communities. First, we predicted that the pool of exotic scolytine species arriving in the coastal areas are filtered by the environment as they spread into continental areas according to their feeding habit ("environmental filtering hypothesis" Keddy 1992). Changes in community composition would therefore reflect species-specific niche differences in adaptive responses that have evolved along environmental gradients. Hence, we expected that the patterns of spread would differ between bark and ambrosia beetles. Considering the relatively high host-specificity of bark-beetles, we would expect that their spread would be limited primarily by the presence of their host species or genus. In contrast, we would expect that ambrosia beetles would be less constrained by host availability but could spread within the new environment wherever the climate allows development of the beetle and its fungi. Second, along with the environmental filtering, dispersal limitations can shape differences in community composition. Despite this, it could be expected that the dissimilarity between both exotic and native scolytine communities should increase along with distance, so we predict that the common multiple-site introductions and human-assisted dispersal (Colunga-Garcia et al. 2013) will increase the homogenization of exotic species communities compared to native communities.

To gain insights into the scolytine invasion process, in the present study we used data on exotic Scolytinae occurring in the USA to quantify patterns of species richness difference and replacement of exotic β-diversity. Specifically, we first analyzed spatial autocorrelation of species richness and replacement separately for bark and ambrosia beetles across the continental USA. The shape of the spatial autocorrelation should indicate whether the spread of exotic bark and ambrosia beetles is a gradual or a discontinuous process characterized by jump dispersal ahead of the established core population (e.g. Robinet et al. 2011). Second, we investigated potential environmental drivers of β-diversity including climate, volume of imports, and forest composition variables. We predict that forest composition and climate would be the main drivers for bark and ambrosia beetles, respectively. Third, we compared exotic with native Scolytinae responses to identify important drivers of the assemblage of exotic and native species communities and whether these are similar or different.

Materials and methods

Study region

We used data from the continental 48 contiguous USA states. For each state, we had detailed and comparable information about the distribution of both exotic and native bark and ambrosia beetles in each state as follows.

Data on Scolytinae distribution

Information on the distribution of exotic and native Scolytinae was gathered from published and unpublished sources. We included in our analyses only those exotic species known to have established self-sustaining populations in the USA. The list of species naturalized in the USA was initially based on Wood (1977, 1982), Wood and Bright (1992), and Bright and Skidmore (1997, 2002). Updated data for exotic species was acquired from scientific literature published by Haack (2001, 2006), Rabaglia et al. (2006), Cognato et al. (2009). The lists used in this paper were current through 2010 and integrated with unpublished records of bark beetles in the USDA Forest Service, Early Detection and Rapid Response project (Atkinson unpublished data; Rabaglia et al. 2008; Haack and Rabaglia 2013). Despite the coarse spatial resolution of the data and considering that no information at a finer spatial resolution is currently available for this group, we believe that the data used in this paper can provide a sound basis for gaining insights into the potential drivers of Scolytinae β-diversity patterns across the USA.

Assigning Scolytinae to feeding habit

We split the Scolytinae into three groups: phloem-feeding species ("bark beetles"), fungus-feeding species ("ambrosia beetles") and seed-feeding species. In the first group we included both species which feed in the phloem layer and species which develop in twigs; in the second group we included species which bore into the wood and feed primarily on symbiotic fungi that grow along the tunnel walls; in the third group we included species which develop in seed or hard fruit. We excluded the latter from the analyses because it is represented by too few species for running a separate analysis.

Analyses of β-diversity

Species richness difference and species replacement

We used the recently proposed method by Carvalho et al. (2012) [see also Podani and Schmera (2011) and Legendre (2014)] to partition the compositional β-diversity into two components: species richness difference and species replacement. The general term β-diversity refers to the total compositional change between two sampling units irrespective of the process that originated each. The term "species richness difference" refers to the relative difference between the number of species that each site supports irrespective of any potential nestedness. In other words, it refers to the fact that one community may include a larger number of species than another, which may be due to various ecological processes (Legendre 2014). The term "species replacement" indicates that one or more species present in one site are substituted by different species at another site. It refers to the well-known fact that species tend to replace each other along ecological gradients that are sufficiently long (Legendre 2014). The two terms are therefore additive and can be generally defined as:

β-diversity = species replacement + species richness difference

The total compositional beta diversity between row cells is given by the Jaccard similarity index:

$\beta_{cc} = (b+c)/(a+b+c)$

where “a” is the number of species common to both sampling units, b and c are the number of species exclusive to the first sampling unit and to the second sampling unit, respectively. β_{cc} is bounded between zero (perfect similarity) and one (maximum possible dissimilarity). This proportional measure can be partitioned into its replacement and richness difference component as given below (Podani and Schmera 2011; Carvalho et al. 2012).

The species richness difference between two sampling units is given by:

$\beta_{rich} = |b-c| / (a+b+c)$

The replacement component, i.e. the substitution of n species in a given sampling unit from n species in another site, is defined using the β_{-3} (Cardoso et al. 2009):

$\beta_{-3}= 2*\min(b-c) / (a+b+c)$

where min(b,c) is the minimum number of exclusive species. This quantity is multiplied by 2 because each substitution involves two different species.

The indices were computed using the “vegdist” function in the package vegan (Oksanen et al. 2012) for R (R Development Core Team 2013).

Spatial autocorrelation

The pattern and significance of spatial autocorrelation across different geographical distance lags were examined using Mantel correlograms. First, we created a geographical distance matrix between the 48 USA states (see below). Second, we divided the distance matrix into n distance classes using Sturge’s rule to set the range of pairwise distances in each class (Legendre and Legendre 1998). Mantel correlation coefficients were calculated for each distance class and tested for significance with a permutation test (using 999 permutations) based on a progressive Bonferroni correction (alpha= 0.05; Legendre and Legendre 1998). As the Mantel correlogram was computed on a dissimilarity matrix (β-diversity), we coded the second distance matrix such that negative significant values of Mantel statistics corresponded to an increase in dissimilarity. On the other hand, positive significant values indicate that the communities are more similar than expected by chance (i.e. low beta-diversity). The response matrices were the replacement and species richness difference variables for bark and ambrosia beetles separately. All Mantel analyses were performed using the “mantel.correlog” function with default settings in the vegan package (Oksanen et al. 2012) implemented in R (R Development Core Team 2013).

Drivers

We included in the analyses several variables that can help to explain species richness difference and species replacement among different USA states. These variables are indicative of the volume of international trade (imports) and the environment for each USA state.

Geographical distance and area

We measured the geographical distance (XY) between each pair of USA states using the centroid projected in UTM WGS84 and then calculating a distance matrix between the sampling units using the “earth.dist” function in the fossil package (Vavrek 2012) for R. In addition, we considered the difference in extent (size) of the study regions (USA states), after the variable AREA was log-transformed to improve linearity and uniformity.

International trade

Wood packaging materials, such as pallets, inadvertently represent one of the most common means through which bark and ambrosia beetles are transported in international trade. Total imports (IMP) are therefore expected to be more related to the introduction potential of exotic Scolytinae than simply imports of wood products (e.g., lumber). For this reason, we used the difference in log-transformed total value of imports between study regions (USA states) as proxy for the number of exotic Scolytinae. We acquired data on the average value of goods imported during the period 2008–2010 from official economic statistics of the USA (Economic Census Bureau, US International Trade in Goods and Service FT900). We used data for the final destination of the imports rather than the first port of arrival because the vast majority of imports arrive in shipping containers that are not opened until they reach their final destination. See Marini et al. (2011) for more detail about the selection of this metric. We used Euclidean distance to compute the distance matrix for import.

Environmental distances

Two distance matrices based on two climatic variables were used based on monthly data: mean annual temperature (TEMP) and mean annual precipitation (RAIN). Both were derived from the WorldClim database and corresponded to the bioclimatic variables BIO6 and BIO12 respectively (Hijmans et al. 2005; 1 km resolution: 1960–1990 period). Both variables were averaged within each USA state. Moreover, we included in the analyses a distance matrix based on forest vegetation differences (FOR) between states using the Bray-Curtis dissimilarity index. We retrieved current information on the area covered by 31 forest type categories by state from the USDA Forest Service, Forest Inventory Data Online (FIDO) (USDA-FS 2010). Each category is defined as a physiognomically uniform group of plant associations sharing one or more dominant tree species. The FOR variable indicated whether two states are similar or different based on the number of forest types which are shared between them or are exclusive to the first or the second state. All environmental matrices were calculated using the “vegdist” function in the vegan package (Oksanen et al. 2012) for R.

Analyses of drivers of β*-diversity (richness difference and replacement)*

We used multiple regression on distance matrices (MRM) (Lichstein 2007) to test the overall direction and the shape of the association of the single environmental and geographical correlates on species replacement (β_{-3}) and richness difference (β_{rich}). MRM performs regression between a response matrix and any number of explanatory matrices. Each explanatory matrix contains distances or similarities between all pair-wise combinations of n objects (USA states) of ecological and environmental factors, or other attributes such as imports. Tests of statistical significance were done by permutation (Legendre et al. 1994). MRM allows for the inclusion of quadratic terms in the model to account for non-linearity in the relationships. For each predictor we tested both linear and quadratic terms. The MRM analysis was carried out with the ‘MRM’ function in the ecodist package (Goslee and Urban 2007) for R.

Second, we used hierarchical partitioning (HP) (Chevan and Sutherland 1991) to evaluate the relative importance of explanatory distance matrices in explaining variation in richness difference (β_{rich}) and species replacement (β_{-3}). HP jointly considers all possible models in a multiple regression and identifies the most likely causal factors. The analyses splits the variation explained by each variable into a joint effect together with the other explanatory variables and into an independent effect not shared with any other variable. HP was computed using the hier.part package (version1.0-3; Walsh and Mac Nally 2008) implemented in R. The estimated relative importance of each variable was represented by the size of its individual effect.

Results

General results

A total of 510 Scolytinae species were analyzed for the continental USA, including 51 exotic and 459 native species. The number of exotic bark and ambrosia beetles was similar (26 and 25, respectively), whereas there were many more native bark beetles (386) than native ambrosia beetles (73).

For exotic bark beetle species, we found a mean value of β_{rich} only slightly greater than β_{-3} (0.31 vs. 0.24) whereas, for exotic ambrosia beetles, the β_{rich} value was clearly higher than β_{-3} (0.50 vs. 0.17). In other words, if both species richness differences and species replacement contribute to differentiate exotic bark beetles communities at USA state scale, exotic ambrosia beetle communities differs mainly in terms of species richness, whereas species composition remains similar. For both native bark and ambrosia beetles we found similar values between species richness differences and species replacement, with β_{rich} that was slightly lower than β_{-3} for bark beetles (0.37 vs. 0.40) and slightly higher for ambrosia beetles (0.37 vs. 0.35).

Correlograms

When exotic bark beetle species were considered, the Mantel correlogram for β_{rich} showed no clear trend, (Fig. 1a) whereas for β_{-3} a significant positive autocorrelation at the smallest lag distance classes were apparent (Fig. 1b), indicating that neighboring states had similar exotic bark beetle species composition, while no clear trend was identified when the distance between them increased. The Mantel correlogram computed for exotic ambrosia beetle β_{rich} showed significant and positive autocorrelation at the smallest lag distance classes and a significant but negative autocorrelation at highest lag distance classes (Fig.1c), indicating that neighboring states had similar exotic ambrosia beetle species richness, whereas communities located in distant states were characterized by a different number of species. For the exotic ambrosia beetle β_{-3}, only a significant and positive autocorrelation at the smallest distance classes were apparent (Fig. 1d), indicating that neighboring states had a similar exotic bark beetle species composition whereas no clear trend was identified when the distance between states increased.

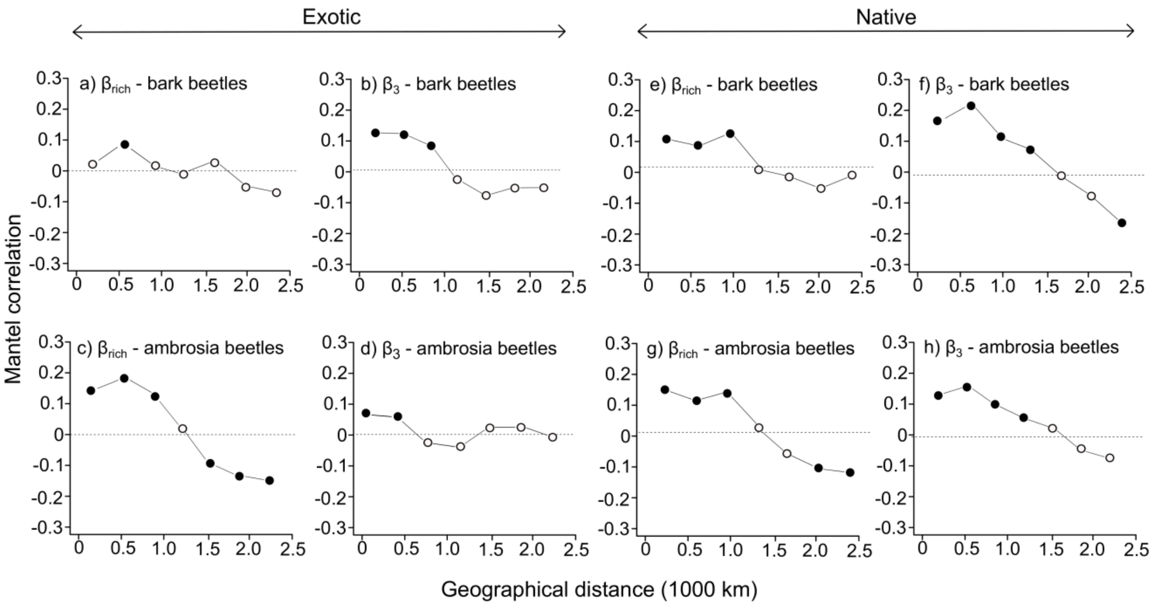

Fig. 1 Mantel correlogram for native and exotic bark and ambrosia beetle dissimilarity using β_{rich} and β_{-3}. Solid circles indicate significant positive or negative correlations (based on sequential Bonferroni corrections with $\alpha = 0.05$) between compositional dissimilarity and geographical distance between USA states (based on longitude and latitude of centroid of the different USA states). Open circles indicate non-significant correlation.

When native bark beetle species were evaluated, the Mantel correlogram for β_{rich} showed a significant and positive autocorrelation at small distance classes (Fig. 1e), indicating that neighboring states had similar native bark beetle species richness, while no clear trend was identified when the distance between them increased. For native bark beetles β_{-3} a significant and positive spatial autocorrelation at the smallest distance classes and a negative autocorrelation at highest distance class were present (Fig. 1f), indicating that neighboring states had similar native bark beetle species composition, whereas communities located in distant states were composed by different species. The Mantel correlogram computed for native ambrosia beetle species β_{rich} showed significant and positive autocorrelation at the smallest lag distance classes and a significant but negative autocorrelation at highest lag distance classes (Fig. 1g), indicating that neighboring states had similar native ambrosia beetle species richness, whereas communities located in distant states were characterized by different number of species. Instead, for the native ambrosia beetle β_{-3}, a significant and positive autocorrelation at the smallest and intermediate distance classes were apparent (Fig. 1h), indicating that neighboring states had similar native bark beetle species composition, whereas no clear trend was identified when the distance between states increased.

Drivers

For the exotic bark beetle species, we found a significant and positive association between β_{rich} and import distances, indicating that the higher the difference between two states in terms of volume of imported commodities, the higher is the difference in exotic bark beetle species richness (Table 1a). Moreover, we found a significant and positive association between exotic bark beetle β_{-3} and both geographical and environmental (temperature, rainfall and forest vegetation) distances (Table 1a), indicating that the higher the geographical distance or the differences in terms of mean annual temperature, mean annual rainfall and forest vegetation between two states, the more the two communities differ in terms of species composition.

For the exotic ambrosia beetle species, we found that β_{rich} was significantly and positively correlated with rainfall, forest vegetation and geographical distances (Table 1a), indicating that the higher is the geographical distance and the difference in terms of forest vegetation and mean annual amount of rainfall between two states, the higher is the differences in exotic ambrosia beetle species richness. Moreover, we found that their β_{-3} was positively related to temperature distances and negatively to rainfall distances (Table 1a). This indicated that the more diverse two states are in terms of mean annual temperature, the higher is the difference in ambrosia beetle species composition between them, whereas the opposite trend exists when considering the differences in the mean annual amount of rainfall.

For the native bark beetle species, we found that β_{rich} was significantly and positively correlated with both geographical and forest vegetation distances (Table 1b), indicating that the higher the geographical distance and the differences in terms of forest composition between two states, the higher is the difference in native bark beetle species richness. Moreover, we found that their β_{-3} was correlated positively with all the environmental and geographical distances (Table 1b). This indicated that the higher the geographical distance and the differences in terms of forest composition, mean annual temperature and mean annual amount of rainfall between two states, the more the two native bark beetle communities differ in terms of species composition. A similar trend was found for native ambrosia beetles β_{rich}, whereas their β_{-3} was significantly and positively associated only with rainfall, forest vegetation and geographical distances (Table 1b).

Table 1 Test results of the linear and quadratic effect of the geographical and environmental distance matrices on β_{rich} and β_{-3} for both exotic and natives Scolytinae in the United States, presented separately for bark and ambrosia beetles. The significance of the slopes is evaluated by regression on distance matrices with a permutation test (n=999). For each predictor both linear and quadratic terms were tested. R^2 indicates the cumulative variation explained by the linear term alone or by the linear and quadratic term together. Only significant terms are reported ($P<0.01$). XY: geographical distance; TEMP: difference in temperature between states; RAIN: difference in rainfall between states; FOR: difference in forest vegetation between states; AREA: difference in area between states; IMP: difference in imports between states. AREA and IMP were log-transformed.

a) Exotic species								
	Bark beetles				*Ambrosia beetles*			
	β_{rich}	R^2	β_{-3}	R^2	β_{rich}	R^2	β_{-3}	R^2
TEMP	-	-	0.023	0.138	-	-	0.010	0.027
TEMP^2	-	-	-	-	-	-	-0.001	0.038
RAIN	-	-	0.0001	0.042	5.4 $^{e-04}$	0.273	-0.0001	0.046
RAIN^2	-	-	-	-	-	-	-	-
XY	-	-	5.4 $^{e-04}$	0.049	9.7 $^{e-05}$	0.086	-	-
XY^2	-	-	-	-	-8.3 $^{e-08}$	0.154	-	-
IMP	0.126	0.082	-	-	-	-	-	-
IMP^2	-	-	-	-	-	-	-	-
AREA	-	-	-	-	-	-	-	-
AREA^2	-	-	-	-	-	-	-	-
FOR	-	-	0.173	0.028	0.41	0.088	-	-
FOR^2	-	-	-	-	-	-	-	-
b) Native species								
TEMP	-	-	0.019	0.108	0.015	0.048	-	-
TEMP^2	-	-	-	-	-	-	-	-
RAIN	-	-	0.0002	0.134	0.0002	0.064	0.0001	0.065
RAIN^2	-	-	-	-	-	-	-	-
XY	0.003	0.027	9.2 $^{e-05}$	0.168	4.5 $^{e-05}$	0.030	0.008	0.190
XY^2	-	-	-2.8^{e-08}	0.186	-5.5^{e-08}	0.078	-	-
IMP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
IMP^2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AREA	-	-	-	-	-	-	-	-
AREA^2	-	-	-	-	-	-	-	-
FOR	0.206	0.046	0.334	0.128	0.192	0.033	0.37	0.143
FOR^2	0.572	0.067	-	-	-	-	1.07	0.072

n.a. Not applicable: import volume was not tested for native species.

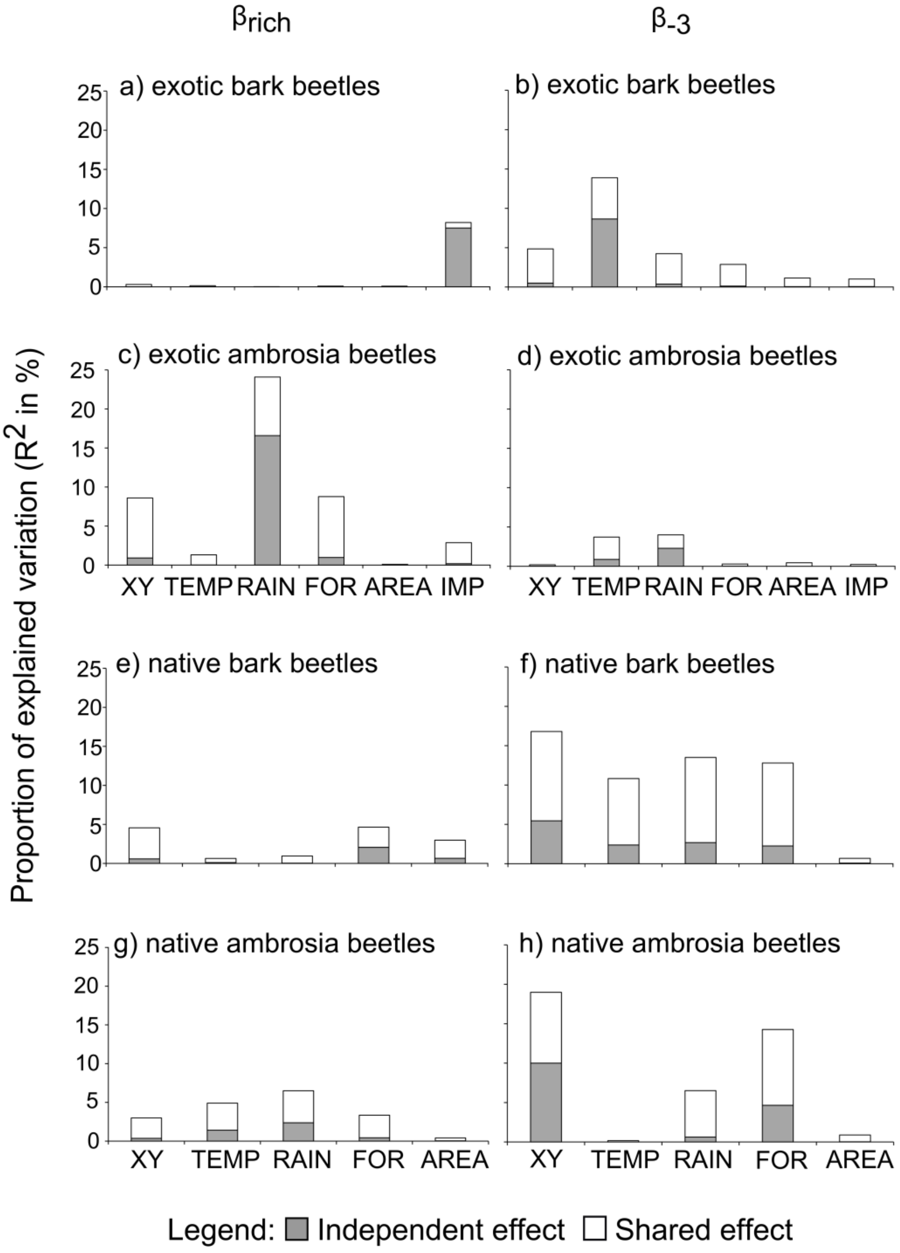

Fig. 2 The independent and shared contributions estimated from hierarchical partitioning of each explanatory variable for the species richness differences and species replacement of native and exotic Scolytinae in the USA, presented separately for bark and ambrosia beetles. XY: geographical distance; TEMP: difference in the mean annual temperature between states; RAIN: difference in the mean annual amount of rainfall between states; FOR: difference in forest vegetation between states; AREA: difference in area between states; IMP: difference in imports between states. AREA and IMP were log-transformed.

Concerning the relative importance of the drivers, the results showed that β_{rich} of exotic bark beetle species was mainly explained by the differences in the volume of imported commodities between states (Fig. 2a), whereas the difference in the mean annual temperature was the main explanatory variable for their β_{-3} (Fig. 2b). For exotic ambrosia beetles, β_{rich} was mainly explained by the difference in the mean annual amount of rainfall between states (Fig. 2c), while β_{-3} was influenced by both mean annual amount of rainfall and mean annual temperature differences (Fig. 2d). Concerning native bark beetle species, β_{rich} was explained by the combined effect of the geographical distance and the difference in forest composition between states (Fig. 2e), whereas the $\beta_{-3}$was strongly influenced by both environmental (differences in mean annual temperature, mean annual amount of rainfall and forest vegetation) and geographical distances (Fig. 2f). The same drivers were the main explanatory variables for native ambrosia beetle species β_{rich} (Fig. 2g), while their β_{-3} was mainly influenced by the geographical distance and the differences in forest vegetation (Fig. 2h).

Discussion

Various studies have demonstrated that the initial distribution of exotic forest pests is clearly not random, but often concentrated in areas with historically high rates of industrialization or volume of imports, where the insects become established and eventually spread (Haack 2001; Huang et al. 2012; Haack and Rabaglia 2013). In the USA, when considering all established exotic forest insects, there is a notable concentration in the northeast, with decreasing numbers to the west and south (Liebhold et al. 2013). This trend has been shown for different group of invasive organisms (Liebhold et al. 2013), but for Scolytinae, the drivers of assembly mechanisms of their communities are still largely understudied. Our large scale study elucidates some of these aspects, supporting the idea that differential establishment and spread of bark and ambrosia beetles have been strongly influenced by their feeding habit and climatic factors in the new environment.

Our results demonstrated that the communities of bark beetles at the USA state level are different in terms of both species richness and species composition. For the exotic bark beetles, species richness differences are primarily affected by the value of imports. Given that exotic bark beetles are commonly associated with wood packaging materials, it is logical that they are more numerous in states with higher import volumes

(Mack et al. 2000; Haack 2001; Marini et al. 2011; Huang et al. 2012; Liebhold et al. 2013). From these initial points of entry, exotic bark beetles can spread naturally to neighboring areas (Forsse and Solbreck 2009; Haack and Rabaglia 2013; Rassati et al. 2015) or they can jump to distant locations when dispersal is human mediated (Piel et al. 2008; Hulme 2009; Haack et al. 2010). In our analyses, the lack of a clear spatial trend in the species richness differences may reflect human-assisted dispersal of exotic bark beetles throughout the USA (Piel et al. 2008; Hulme 2009; Colunga-Garcia et al. 2013; Hu et al. 2013), mechanisms which is often related either to movement of imported goods and associated wood-packaging materials towards their final destinations (Rassati et al. 2014) or firewood carried by visitors to campgrounds or recreational facilities (Koch et al. 2012; Koch et al. 2014). Moreover, temperature was found to play a significant role in determining assemblages of exotic bark beetles, which may reflect similar temperatures among nearby states but often changing with increasing distance between them. This relationship may also be influenced by environmental requirements of bark beetles (Lombardero et al. 2000) and their associates, such as fungi, bacteria, nematodes, and mites that can significantly influence bark beetle fitness (Hofstetter et al. 2006; Cardoza et al. 2008). For example, each fungal associate often possesses different thermal optima for growth (Rice et al. 2008), and variation in seasonal temperatures can influence which fungal species are ultimately vectored by dispersing bark beetles (Six and Bentz 2007), thereby help to shape the communities of exotic bark beetles. Another important factor to help explain variation in species replacement between states is the origin of the imported goods given that different trading partners can lead to different exotic species being introduced (Haack 2006; Costello et al. 2007).

Regarding the exotic ambrosia beetles in the USA, our results showed that their communities at the scale of USA states differ more in terms of species richness than species replacement. Differences in species richness appeared to be primarily associated with differences in amounts of rainfall between states. Ambrosia beetles are strictly dependent on their symbiotic fungi, which needs certain conditions to grow and develop (Furniss and Carolin 1977; Hulcr et al. 2008; Reich et al. 2014). This dependency appears to limit the geographic distribution of many species of ambrosia beetles to wetter and warmer regions (Marini et al. 2011; Haack and Rabaglia 2013; Reich et al. 2014). In this regard, the analyses of interception frequency of exotic Scolytinae (Brockerhoff et al. 2006a) suggested that the great majority of introduced ambrosia beetles do not become established likely because of unsuitable climate at the point of arrival (Marini et al. 2011).

Overall, as shown by the spatial distribution of species richness for the exotic ambrosia beetles, which indicates gradual changes with geographical distance, the pool of exotic species arriving in the coastal areas tend to be filtered by the environment as they spread to interior portions of the continental USA (Haack and Rabaglia 2013). For example, much of the central and interior western parts of the USA are characterized by significantly lower rainfall as compared to the eastern USA (Hijmans et al. 2005), which appears to restrict establishment of many exotic ambrosia beetles (Reich et al. 2014). For these reasons, we also found low values of species replacement in exotic ambrosia beetle communities as well as the lack of clear spatial trends for this parameter.

Our results also indicated that the assembly of native Scolytinae communities is driven by different factors compared with exotic Scolytinae. For example, differences in species richness of native bark beetles were strongly influenced by forest composition, reflecting their relatively high host specificity (Wood 1982; Knížek and Beaver 2004). In cases where the species richness differences did not show a clear spatial pattern, the species replacement followed the rules of the distance-decay of similarity, with decreasing similarity between two communities with increasing distance between them (Nekola and White 1999). Scolytinae species that are native within a given area have co-evolved with their hosts through millions years of adaptation (Raffa and Berryman 1987) and both have characteristics that allow them to be adapted to local climatic conditions, thus, as the climatic conditions changes, the pool of host plants and native Scolytinae also change (Wood 1982). The significant role of geographical distance and environmental factors observed in our study in explaining the species replacement of native bark beetles supports this trend. Native ambrosia beetles generally followed the same trends as native bark beetles. For both species richness and species replacement, there was a tendency for the values to decrease as distance between states increased. If differences in climatic factors mainly explain differences in species richness, a fact related to the strong dependence that ambrosia beetles have on climate (Reich et al. 2014; Marini et al. 2011), then species replacement was primarily influenced by differences in forest composition. Although the latter result might be somewhat unexpected given that most ambrosia beetles are relatively polyphagous (Hulcr et al. 2007; Ranger et al. 2010), it is reasonable to think that ambrosia beetle species that are native within a given area have co-evolved with their hosts over time and are more adapted to certain habitats than others, thus, as the composition and characteristics of forest stands change, the beetles communities also change.

Our study demonstrated that the bark and ambrosia beetles differ in their spread pattern and in the composition of community structure within the invaded environment. The lower dependency that bark beetles have for strict climatic conditions may allow them to potentially colonize larger areas within the USA, and they are therefore more likely to become established in distant regions if moved by humans. Moreover, the strong effect of temperature on species replacement in bark beetles and their relatively high host specificity can be used to model or predict which species are likely to arrive and establish in certain areas, taking into account the most common sources of the imported goods at nearby ports. Regarding ambrosia beetles, our results showed that exotic species can easily spread through natural or human-mediated processes, but they will typically become established only in those areas where the climatic conditions are suitable for their reproduction and development. Although ambrosia beetles are generally highly polyphagous, conducting studies on host specificity of selected exotic ambrosia beetles could help identify which areas of a country are most at risk of ambrosia beetles invasion.

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Conclusions

Early detection is recognized worldwide of utmost importance to increase the possibility of alien species interception and eradication (Epanchin-Niell et al. 2014) as, if incoming wood-boring beetles are quickly detected, specific phytosanitary measures and timely action plans can be applied (Pluess et al. 2012). However, direct inspections of imported commodities and associated wood-packaging materials, as well as other preventive measures (such as the treatments detailed by ISPM 15) are not sufficient to prevent new invasions (Haack et al. 2014). For these reasons, the possibility to have reliable and efficient trapping strategies that can be used in high-risk areas, such as ports of entry, is essential to enhance the possibility of alien species interception. The first study reported in this thesis (Chapter II) highlighted the use of multi-funnel traps baited simultaneously with different attractive lures, both kairomones and pheromones, associated to traps set up in natural areas surrounding ports, as a monitoring protocol suitable for the surveillance of alien wood-boring beetles. However, the number of sites potentially at risk of new introductions is extremely high and the allocation of the resources by government, resource management agencies and other stakeholders must consider carefully which sites are at most risk to be invaded (Epanchin-Niell et al. 2014), both in coastal and continental areas.

In coastal areas, international ports are recognized to be the first points of entry for commodities and alien wood-boring beetles (Haack 2001), but both size and characteristics of the surrounding landscape can be clearly diverse among different ports. In particular, it was still unclear how the volume of imported commodities and the amount and type of forest cover in the first kilometers around ports can affect the early-detection of alien wood-boring beetles. Our results (Chapter III) highlighted that, in order to increase the possibility of detecting alien species soon after their arrival, extensive monitoring programmes should be concentrated in ports with large volumes of imported commodities and in the surrounding broadleaf forests, as the combination of these two characteristics is the most favorable for trapping alien wood-boring beetles. However, the difference among the communities of alien species trapped in ports and in the surrounding forests found in our study suggested that it is important to deploy traps simultaneously in both environments to catch also wood-boring beetles that emerging from wood-packaging materials stored in port areas fly towards surrounding forests searching for suitable habitats where to complete their life cycle. In fact, the presence of woody materials in ports is not constant over time and space, and this can affect the trapping performance within the latter habitat. In this respect, setting traps in both environments can increase the chances of detecting alien wood-boring beetles.

Beside the natural dispersal in the areas surrounding ports, alien species can also be transported by humans to disjunct sites located in continental areas dozens or even hundreds kilometers from the initial points of entry (Colunga-Garcia et al. 2013). Moreover, in the last 30 years, cargo has increasingly shipped in large containers (Stanaway et al. 2001) and only a fraction of these are opened and inspected within ports. Commodities are often transported directly to their final destination, such as industrial or commercial areas. In these cases, associated wood packaging materials are separated from goods and, when not still usable, sent to companies authorized to recycle or destroy the wood (Buehlmann et al. 2009), where can act as source of alien wood-boring beetles. Our results (Chapter IV) demonstrated that wood-waste landfills are useful sites for trapping alien wood-boring beetles in continental areas. Traps in such sites can both increase the possibility of alien species interceptions and add information on the distribution of already established ones, allowing for a timely response to implement eradication efforts.

Early-detection and monitoring of alien wood-boring beetles is strictly related to a better comprehension of the mechanisms driving the whole invasion process, from the arrival of a species to its establishment and spread. These patterns are, however, still largely under investigated, although the accidentally introduction of these insects, especially of bark and ambrosia beetles, will likely become more common in a next future (Haack and Rabaglia 2013). Our results elucidated some of these aspects both at the regional and continental scale, indicating the key effect of forest diversity and climate in affecting the assembly of alien species communities. Our study focused on alien ambrosia beetles shows that, at the regional scale (Chapter V), their establishment was strongly affected by forest composition, temperature and forest structure, although they are known to have poor habitat specificity in their native area (Hulcr et al. 2007). At the continental scale (Chapter VI), the mechanisms were instead slightly different, and the pattern of bark and ambrosia beetle spread was mainly depending on the feeding habit of these two main groups of scolytids. In particular, the lower dependency that bark beetles have for specific climatic conditions may allow them to potentially colonize larger areas, and they are therefore more likely to become established in distant regions if moved by humans. On the contrary, ambrosia beetles can more easily spread through natural or human-mediated processes, but they will typically become established only in those areas where the climatic conditions are suitable for their reproduction (i.e., growth of symbiotic fungi).

Overall, the five studies described in this thesis provide both applied and theoretical information that can be used for the improvement of alien wood-boring beetles early-detection at both national and international scale and as a starting point for future studies on the invasion process of this successful group of insects. At present, the availability of efficient trapping strategies for the interceptions of alien species is of crucial importance and it will become even more relevant in the upcoming future, as the climate change and the increase in importation and international trades will increase the probability of alien species arrival and establishment. At the same time, information on the mechanisms driving the invasion process of alien species can help in developing suitable management strategies and make the local detection programs more efficient individuating which habitats and host species are at most risk to be invaded.

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