

A first worldwide multispecies survey of invasive Mediterranean pine bark beetles (Coleoptera: Curculionidae: Scolytinae)

Massimo Faccoli¹, Diego Gallego², Manuela Branco³, Eckehard G. Brockerhoff^{4,5}, Juan Corley⁶, David R. Coyle⁷, Brett P. Hurley⁸, Hervé Jactel⁹, Ferenc Lakatos¹⁰, Victoria Lantschner⁶, Simon Lawson¹¹, Gonzalo Martínez¹², Demian F. Gómez¹², Dimitrios Avtzis¹³

- 1) Department of Agronomy, Food, Natural Resources, Animals and the Environment. University of Padua, Italy.
- 2) Department of Ecology, University of Alicante, Spain - Sanidad Agrícola Econex S.L., Spain
- 3) Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal
- 4) Scion (New Zealand Forest Research Institute), Christchurch, New Zealand
- 5) Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
- 6) Grupo de Ecología de Poblaciones de Insectos, Instituto de Investigaciones Forestales y Agropecuarias Bariloche, INTA - CONICET, Bariloche, Argentina.
- 7) Department of Forestry and Environmental Conservation, Clemson University, Clemson, SC, USA.
- 8) Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa
- 9) Biogeco, INRA, Univ. Bordeaux, Cestas, France
- 10) Institute of Silviculture and Forest Protection, University of Sopron, Hungary
- 11) Forest Industries Research Centre, University of the Sunshine Coast, Sippy Downs, Australia
- 12) Laboratory of Entomology, National Forestry Research Program, INIA, Tacuarembó, Uruguay
- 13) Forest Research Institute, Hellenic Agricultural Organization Demeter, Thessaloniki, Greece.

Corresponding author:

Massimo Faccoli

Department of Agronomy, Food, Natural Resources, Animals and the Environment

Viale dell'Università 16, 35020 Legnaro (PD), Italy.

e-mail: massimo.faccoli@unipd.it

Telephone number: +39. 049. 82 72 891

Abstract

Several European and Mediterranean species of pine bark beetles (Coleoptera: Curculionidae: Scolytinae) have become established in North America and the southern hemisphere, posing a novel threat to planted and naturally-occurring pine forests. Our objectives were to investigate (i) the occurrence and relative abundance of pine bark beetles in these regions, and (ii) the trapping performance of different blends of multispecies lures. In 2016–2017 a network of interception traps was installed in six non-European countries (Argentina, Australia, New Zealand, South Africa, the United States, and Uruguay), and in six European countries (France, Greece, Hungary, Italy, Portugal, and Spain) for comparison. Half of the traps were baited with alpha-pinene and ethanol, and the other half with alpha-pinene, ethanol, and a combination of bark beetle pheromones (ipsdienol, ipsenol, and Z-verbenol). Five Mediterranean scolytine species (*Hylurgus ligniperda*, *Hylastes ater*, *H. angustatus*, *Orthotomicus erosus*, and *O. laricis*) were found in non-European countries. *Hylurgus ligniperda* and *Hylastes ater* were the most widespread species found in several of the invaded regions, while *O. laricis* and *H. angustatus* occurred only in Argentina and South Africa, respectively. Despite large variation among species and countries, most species were trapped with the blend containing bark beetle pheromones, except *O. erosus*, which was more attracted to alpha-pinene and ethanol alone. This study represents the first step towards the development of an international monitoring protocol based on multi-lure traps for the survey and early-interception of invasive alien bark beetle species.

Keywords: Biological invasions, forest health, international monitoring protocol, invasive species, pest detection, pine pests, semiochemicals

Introduction

Wood-boring and bark beetles are among the most successful invasive alien species, causing significant economic and ecological damage to forests and urban/suburban areas worldwide (Brockerhoff et al. 2006a, 2014; Haack 2001, 2006; Kovacs et al. 2010). These insects are easily transported in almost all types of fresh or seasoned timber and woody material – particularly if bark is still present – such as timber, wood packaging material (i.e., pallets, crating, and dunnage), live woody plants, and other wood products (Meurisse et al. 2019). Hidden inside wood or under the bark, they can escape from phytosanitary detection and survive adverse climatic conditions that occur during intercontinental travel (Brockerhoff et al. 2006a; Liebhold et al. 2012; Rassati et al. 2015). Despite efforts to mitigate the pathways governing the introduction of alien insect species, there has been a global increase in the number of new invasive bark- and wood-boring beetles in the last few decades (Kirkendall and Faccoli, 2010; Roques 2010; Rassati et al. 2016), likely due to the increased global trade and acceleration of transport time (Seebens et al. 2017).

Mediterranean pine bark beetles (Coleoptera: Curculionidae: Scolytinae) belong to a group of species native to central and southern Europe, usually developing in the phloem of several European pine trees (*Pinus* spp.) and occasionally in other conifers. Many of these species – especially those in the genera *Orthotomicus*, *Hylurgus*, and *Hylastes* – are among the most abundant and common species infesting pines in the Mediterranean region. In spite of their abundance, these species are rarely considered pests in their native range. Outbreaks causing substantial damage to pine forests only occur after events that result in widespread tree stress, such as fires, storms, and periods of drought (Branco et al. 2014). At endemic levels, these insects usually breed and develop in fresh phloem of weakened, dying, recently cut or dead trees, or in pine stumps, roots, and logging waste (Raffa et al. 2015). Because they are generally non-aggressive, few studies have investigated the potential of these species to behave as forest pests in their native areas (but see Munro [1917] and Bevan [1987] for the UK).

Although many Mediterranean bark beetles are recorded as quarantine pests in the EPPO (European and Mediterranean Plant Protection Organization), COSAVE (Comité de Sanidad Vegetal, including countries of southern South America), and NAPPO (North American Plant Protection Organization) lists of alien species, their economic and ecological significance has been underestimated. Like many non-native species, increasing global trade has facilitated their introduction into new regions. Except for a few countries where some European and Mediterranean pine bark beetle species have been accidentally introduced since the beginning (e.g. New Zealand) or middle (e.g. Australia and Uruguay) of the 20th century, most of these species have successfully established only in the last decades in many temperate countries of the southern hemisphere, such as Argentina, Australia, Chile, South Africa, and Uruguay (Boomsma and Adams 1943; Ruffinelli 1967; Wingfield and Marasas 1980; Mausel et al. 2007; Tiranti 2010; Gómez and Martínez 2013; Brockerhoff et al. 2017). In these geographical regions, devoid of native pine species, extensive pine plantations have been intensively managed for timber and pulp production since the mid-20th century, using mainly North American pine species, such as ponderosa pine (*Pinus ponderosa* Douglas), lodgepole pine (*P. contorta* Douglas), Mexican weeping pine (*P. patula* Schiede), Monterey pine (*P. radiata* D. Don), slash pine (*P. elliottii* Engelman), Caribbean pine (*P. caribaea* Morelet) and loblolly pine (*P. taeda* L.). The introduction of any non-native plant species in new habitats often results in unexpected pest issues, and predictably this happened in this system as well. Mediterranean pine bark beetles are posing a constant and novel threat to the large plantations of highly susceptible, fast-growing, non-native pine tree species, which are growing in environments with no native pine pests (Sopow et al. 2015; Gómez et al. 2017) and associated native natural enemies able to provide biological control (Colautti et al. 2004).

The first step towards an efficient control strategy of quarantine species involves their rapid identification in newly invaded areas and knowledge of the biological traits expressed under the new environmental conditions. Complete and clear data are not available regarding which Mediterranean pine bark beetle species occur in the different pine-producing regions worldwide and how their populations behave in the invaded range. While the life-history of the European populations of these species has been well described (Raffa et al. 2015), several ecological and physiological features of the introduced populations in new areas, exposed to different climatic conditions, host trees and natural enemies, are only partly known. This information is of crucial importance for any management strategy, as the biological characteristics of an invasive alien species or population may determine its invasion potential and even the outcome of the entire invasion process (Brockerhoff and Liebhold 2017). The success of the invasion is greatly affected by the adaptation of the insect to the new environmental conditions. As bark beetles are known for adapting even to minute changes in environmental conditions by adjusting their breeding performance, phenology, and voltinism (Raffa et al. 2015), it can be expected that in areas beyond their native range these species will respond to the new local conditions, potentially creating strong interspecific competition with native species when they are present (Liebhold et al. 2017).

Early detection of alien species in new areas is extremely important, particularly to increase the success of eradication (Liebhold and Kean 2019), and successful methods have been developed in the USA and Europe (Rabaglia et al. 2008; Rassati et al. 2015). Because it is impossible to know which Mediterranean pine bark beetle species may be the next invader

in new countries, a generic yet effective monitoring tool is necessary to detect alien species arriving in new geographic areas. Monitoring protocols based on the use of traps baited with generic lures attractive for different bark beetle species have shown promising results in New Zealand (Brockerhoff et al. 2006b). The use of an international standard monitoring protocol (using the same traps and lures) would be extremely useful to survey the potential arrival of new species native to other geographic regions (especially the Mediterranean Basin, North-eastern Asia and North America), and it would allow data comparison among countries and continents. A comparison between international datasets obtained from monitoring both the invaded and the native regions would likely reveal differences in the behavior of these species between their natural range and newly invaded regions, which will be important for the development of effective strategies to reduce the risk of new introductions and to limit the species' spread. It is thus important to test different lure blends and concentrations to identify a formulation that allows the best monitoring performance in the context of early detection of alien pests. Interception traps could be then set up both in the core area of the invasion and along its borders, to facilitate the detection of the expanding front and the prompt application of control protocols.

Describing mechanisms of large-scale pine mortality in ecosystems in which the herbivore and tree are native to different geographic regions – and are thus devoid of co-evolutionary associations – will provide comparative future examples in a rapidly changing world. Given this perspective, our primary objectives were to investigate (i) the potential presence of Mediterranean pine bark beetle species belonging to the target genera *Orthotomicus*, *Hylurgus*, and *Hylastes* in non-European countries where pines are grown extensively, and (ii) the trapping performance of different blends of generic (multi-species) lures with the goal to develop the framework for an international standard monitoring protocol based on multi-lure traps. European populations of the same pine bark beetle genera occurring in the Mediterranean basin, *i.e.* in the native area, were also monitored for comparison of the trapping performance.

Materials and Methods

This study was conducted in twelve countries in the northern and southern hemispheres which included native and invaded ranges of Mediterranean pine bark beetle species (Fig. 1). The trapping protocol was similar in all study locations with minor deviations, which are detailed below.

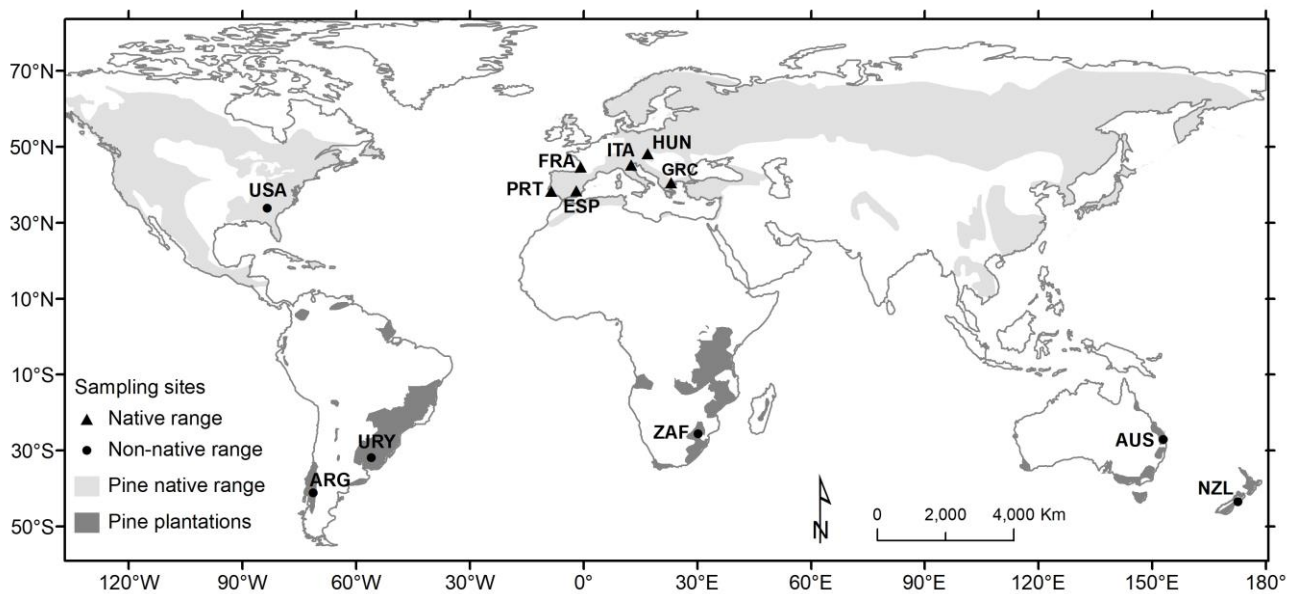


Fig. 1 Distribution of sampling sites in the native and non-native range of the target bark beetles species. Native range of pines (light grey) and distribution of pine plantations in the Southern Hemisphere (dark grey), are also shown. ITA: Italy, PRT: Portugal, ESP: Spain, FRA: France, GRC: Greece, HUN: Hungary, ARG: Argentina, AUS: Australia, NZL: New Zealand, ZAF: South Africa, USA: United States, URY: Uruguay.

Sampled sites

A network of interception traps was installed in six European countries (France, Greece, Hungary, Italy, Portugal, Spain) where the target species are native, and in six newly invaded non-European countries (Argentina, Australia, New Zealand,

South Africa, United States, and Uruguay) (Fig. 1, Table 1). European study sites were characterized by the presence of Mediterranean pine forests composed of maritime pine (*P. pinaster* Aiton), Aleppo pine (*P. halepensis* Miller), Turkish pine (*P. brutia* Tenore) and stone pine (*P. pinea* L.), except in Hungary where Scots pine (*P. sylvestris* L.) and Austrian pine (*P. nigra* Arnold) forests were investigated. In contrast, natural forests or plantations of North American pine species, such as ponderosa pine, lodgepole pine, loblolly pine, Mexican weeping pine, slash pine, Caribbean pine and Monterey pine occurred in non-European countries (Fig. 1, Table 1).

Lures and traps

In each country, six black cross-vane traps for wet collection (Crosstrap® mini traps, ECONEX, Spain) were set up and activated with two different blends of generic lures attractive for conifer bark beetles. The first was composed of a dispenser (A) of (-) alpha-pinene (20 g with a release rate of 30 mg per day at 20 °C), which is one of the main components of pine resin thus potentially attractive to pine beetles, and an ethanol dispenser (B, containing 100 g with an ultra-high release rate of 1.5 g per day at 20 °C), which is a common volatile released by decaying tress and thus attractive to bark beetles. The second blend was composed of (-) alpha-pinene and ethanol, plus a third dispenser (C) releasing a blend of the most common pheromones of conifer bark beetles ipsdienol, ipsenol, and Z-verbenol (dispensers containing 300 mg of each component, with 1.5 mg/day of release rate). All dispensers were in poly laminated blister form (sleeve dispensers), with different volumes. Dispensers A and C were hermetically enclosed with a polyolefin layer permitting a controlled release of attractants, while this layer was microperforated to increase ethanol release from dispenser B.

Traps were set up in summer in each sampling location: May-September 2016 for countries in the northern hemisphere and October 2016 - March 2017 for the countries in the southern hemisphere. After approximately 60 days, corresponding to the operating time of the dispensers, all dispensers were changed to cover the main flying activity of the adults (120 days). All traps and lures were provided by ECONEX (Spain).

Trap setting and checking

In each sampled country, two treatments with three replicates (i.e., three traps) per treatment were tested: treatment 1 with traps baited with dispensers A+B (*AB blend*), and treatment 2 with traps baited with dispensers A+B+C (*ABC blend*). Three traps were set up per each country and treatment, for a total of six traps per country. Traps were installed singly in forest gaps of pine stands at a height of about 2 m. In each country, a pair of traps (one per treatment) was set up in three sites in pine forests or pine plantations (Table 1). In each country, the three selected sites had the same relative composition, i.e., same pine species, and similar age, silvicultural characteristics and management. The distance between traps occurring in the same site ranged from 100 to 200 m, while the distance between sites ranged from 2 to 8 km. Wet collection cups were filled with 100 ml of pure propylene glycol to preserve trapped insects. Insects collected were filtered with a household strainer, gathering up the liquid in another jar, and refilling the collector jar with the same liquid, if it was not diluted. When the propylene glycol of the collecting jar was diluted by rain, it was discarded and the jars refilled with fresh liquid. Traps were emptied weekly or every second week and the collected insects transferred into a vial filled with 95% ethanol; samples were stored at -20° C to reduce DNA degradation of the trapped beetles, so the samples could be used for future genetic studies.

Sample handling and insect identification

All trapped bark beetles were identified to species level and counted. Insect identification was carried out using international identification keys based on the morphological features of the target taxonomic groups (Balachowsky, 1949; Wood, 1982, 2007; Pfeffer, 1995). This protocol allowed having an insect identification protocol carried out with the same morphological parameters for all sampled populations.

Data analysis

Recorded catches corresponding to the different species were compared by generalized linear models (GLM), with a Negative Binomial distribution function (which provided the best fit according to assessment of deviance) and a log link function, testing for differences among countries and pheromone lure types (representing the fixed factors) used to bait the traps (*AB blend* vs. *ABC blend*). The data comparison was performed either for each country and species separately or nested for species using countries as replicates. For species where the GLM did not converge, the non-parametric Mann-Whitney U test was used. Additionally, for the species with broader distribution the overall data were compared in both the native range and non-native range (using countries as replicates). Differences at a 0.05 level of confidence were considered significant. Analyses were performed using IBM SPSS® statistics 25.

Table 1 Description of study sites in the European (native) and non-European countries.

Area	Country	Site	Lat.	Long.	Elevation (m a.s.l.)	Mean T	Pine species	Tree age (years)	Stand density (trees per ha)
European	France	Cestas	44° 73' N	00° 76' W	60	16–27 °C	<i>P. pinaster</i>	30	300
	Greece	Vassilika, Thessaloniki, Suburban Forest	40° 30' N	23° 05' E	67	15–16 °C	<i>P. brutia</i>	46–50	500
			40° 37' N	22° 58' E	108			65	
			40° 37' N	22° 59' E	130			65	
	Hungary	Sopron – Dudlesz Sopron – Szárhalom Sopron – Hegy	47° 75' N	16° 59' E	320	8–11 °C	<i>P. nigra</i> <i>P. silvestris</i>	60	1080
			47° 69' N	16° 63' E	320			60	920
47° 64' N			16° 55' E	390	80			320	
Italy	Veneto, Rosolina Mare	45° 11' N	12° 32' E	3	13–24 °C	<i>P. pinea</i> <i>P. pinaster</i>	70	150	
									Portugal
38° 98' N	08° 38' W	65	30	150					
Spain	Sierra Espuña Murcia	37° 82' N	01° 55' W	600	15–27 °C	<i>P. halepensis</i>	80	300	
Non European	Argentina	INTA Bariloche La Veranda Ranch Talata Ranch	41° 07' S	71° 14' W	770	8–22 °C	<i>P. ponderosa/radiata</i> <i>P. ponderosa</i> <i>P. ponderosa /contorta</i>	20–25	300
			41° 13' S	71° 11' W	1010			30	700
			41° 14' S	71° 11' W	980			29	500
	Australia	Queensland, Beerburrum State Forest	27° 00' S	153° 00' E	30	15–25 °C	<i>P. caribaea</i> x <i>P. elliotii</i>	0 (clearfelled stand next to older stand)	0 (clearfelled stand next to older stand)
			26° 56' S	152° 58' E	25				
	26° 54' S	153° 01' E	15						
	New Zealand	Canterbury, Chaney's Forest	43° 25' S	172° 39' E	10	12–17 °C	<i>P. radiata</i>	0 (clearfelled stand next to older stand)	0 (stand was at ca. 270 before felling)
South Africa	Sappi Helvetia plantation	25° 32' S	30° 17' E	1661	14–28 °C	<i>P. patula</i>	11	1211	
		25° 35' S	30° 19' E	1626			15	1110	
		25° 34' S	30° 22' E	1783			9	1160	
Uruguay	GMO, Tacuarembó Weyerhaeuser, Tacuarembó Terena, Tacuarembó	31° 48' S	55° 56' W	130	13–27 °C	<i>P. taeda</i>	17	376	
		31° 42' S	55° 52' W	160			17	306	
		31° 49' S	56° 03' W	190			13	350	
USA (Georgia)	Orchard PMRC Tower	33° 53' N	83° 22' W	210	14–28 °C	<i>P. taeda</i>	31–33	210	
		33° 52' N	83° 21' W	180			40	153	
		33° 53' N	83° 21' W	210			40	134	

* Range of mean T during the sampled months.

Results

Trapped species

More than 36,000 beetles belonging to 10 species of the three target genera (*Orthotomicus*, *Hylurgus*, and *Hylastes*) (Table 2) and more than 34,000 belonging to 16 species of non-target genera (Table 3) were trapped in the monitored countries. Apart from two species trapped only in the USA (*Orthotomicus caelatus* and *Hylastes salebrosus*, both native to North America), all other collected scolytines of the target genera were native to Europe (Table 2). In particular, two species of *Hylurgus* (*H. ligniperda* and *H. micklitzi*), two *Orthotomicus* (*O. erosus* and *O. laricis*) and four *Hylastes* species (*H. ater*, *H. linearis*, *H. angustatus*, *H. attenuatus*) were found during the whole monitoring program. *H. ligniperda* and *O. erosus* were the most commonly trapped species with more than 12,000 adults per species followed by *H. attenuatus*, and *H. micklitzi* with more than 2,000 each (Table 2). The Mediterranean pine engraver beetle *O. erosus* was the only species found in all six European countries monitored, followed by *H. ligniperda* found in five countries, and *H. ater* and *H. attenuatus* in four (Table 2). *Hylurgus micklitzi* and *Hylastes linearis* were found only in two and one country, respectively. Interestingly, the European species *O. laricis* and *Hylastes angustatus* were found only in two non-European countries: Argentina and South Africa, respectively.

In the southern hemisphere, Mediterranean pine bark beetles were found in all monitored countries. *H. ligniperda* and *H. ater* were the two most common species, having been found in five and three countries, respectively (Table 2), followed by *O. erosus* (two countries), while *O. laricis* was found only in Argentina and *H. angustatus* was found only South Africa. Therefore, five European species of pine bark beetles were identified as established alien species in the southern hemisphere, while the other three species found in Europe (*H. micklitzi*, *H. linearis*, and *H. attenuatus*) were not. Argentina and South Africa were the countries of the southern hemisphere with the highest number of alien Mediterranean pine bark beetle species (three species, although with a suite of different species) followed by Australia, Uruguay, and New Zealand with only 2 species each (Table 2).

Overall, 16 species belonging to 11 non-target genera (Table 3) were trapped during the whole monitoring experiment. Both bark (10 species) and ambrosia (6 species) beetles were trapped. Bark beetles, which include species mainly infesting pines, were represented mainly by *Ips sexdentatus* (> 19,000 adults) and *I. grandicollis* (> 10,000 adults). *Ips sexdentatus* was collected in all monitored European countries except in Spain and Italy where both the species are known to occur, although the large monospecific *P. halepensis* forests – not recorded among the preferred hosts of *I. sexdentatus* – occurring in Spain reduce the presence of this species. Instead, the Eastern five-spined engraver beetle *I. grandicollis*, native to the Americas but accidentally introduced and established in Australia, and the largely spread *Hypothenemus seriatus* were the only two bark beetle species trapped in non-European countries, and both found only in Australia (Table 3). Ambrosia beetles included six species largely polyphagous on conifers (*Xyleborus eurygraphus* and *Gnathotrychus materiarius*) or broadleaves (*Xylosandrus crassiusculus*, *Xyleborus perforans*, *Xyleborus ferrugineus*) or both (*Xyleborinus saxesenii*). The latter was the most common trapped ambrosia beetle, both quantitatively (more than 3,700 adults trapped mainly in Australia) and in term of number of countries where it was found (5). Non-target bark and ambrosia beetle species were found in all European countries, and especially in Spain with 6 species, but only in 2 non-European countries (Uruguay and Australia).

Trapping performance of different lures

Overall, the most insects were captured with the ABC blend (Fig. 2) with the exception of *O. laricis*, although the captures of this species were extremely low and recorded only in Argentina (Table 2). Greater numbers of *H. attenuatus* and *H. linearis* were captured with the ABC lures (GLM, $P < 0.001$) (Fig. 2). The two tested blends of lures (AB blend and ABC blend) showed significantly different results between species according to their different populations, *i.e.* country-by-country (Fig. 3). Specifically, captures of *H. ligniperda* in France (GLM, $P < 0.001$) and Greece (GLM, $P < 0.05$), of *H. micklitzi* in Greece (GLM, $P = 0.058$), and of *H. attenuatus* in France (GLM, $P < 0.01$) were significantly greater with the ABC blend than the AB blend, whereas in the other countries the captures of these species show no significant difference. No *H. ligniperda* was trapped in Hungary.

By contrast, the effect of the two tested blends on captures of *H. ater* and *O. erosus* varied by country. In France *H. ater* was trapped primarily with the ABC blend (GLM, $P < 0.01$), in New Zealand with the AB blend (GLM, $P < 0.05$), while in Hungary, Argentina and Australia there were no differences between lures (Fig. 3). For *O. erosus*, the ABC blend yielded greater catches than the AB blend in France (GLM, $P < 0.05$) and Hungary (GLM, $P = 0.07$), but lower captures in Italy (GLM, $P < 0.01$), Spain (GLM, $P < 0.05$), South Africa (GLM, $P < 0.05$), and Uruguay (GLM, $P < 0.05$); while in Greece and Portugal there were no significant differences (Fig. 3). Finally, captures of *H. angustatus* in South Africa and *H. linearis* in Portugal – the only countries in which these two species were trapped – did not differ between the two tested blends.

For *H. ligniperda* and *O. erosus*, species with a broad distribution covering many countries, the overall data were compared in both the native and non-native range, using countries as replicates. In this respect, a significant effect was found in the native region where the ABC blend trapped more *H. ligniperda* than AB blend (GLM, P<0.01), probably affected by the high ABC blend values recorded in Greece and France (Fig. 3). The same effect, however, was observed neither for *H. ligniperda* nor for *O. erosus* when they were found in the non-native range at very low catches (South Africa and Argentina).

Table 2 Number of insects per species belonging to the target genera (*Orthotomicus*, *Hylastes*, and *Hylurgus*) trapped in the monitored countries.

Area	Country	Trapped species										
		<i>Hylurgus ligniperda</i>	<i>Hylurgus micklitzi</i>	<i>O. erosus</i>	<i>O. laricis</i>	<i>O. caelatus</i>	<i>Hylastes ater</i>	<i>Hylastes linearis</i>	<i>Hylastes angustatus</i>	<i>Hylastes attenuatus</i>	<i>Hylastes salebrosus</i>	Total
European	France	2154		2129			162			808		5253
	Greece	451	673	285								1409
	Italy	142		634			2					778
	Hungary	9		101			138			2120		2368
	Portugal	1173		8133			1	11		24		9342
	Spain		1448	781						1		2230
Non European	Argentina	195			5		1					201
	Australia	436					1					437
	New Zealand	11312					50					11362
	South Africa	1		288					13			302
	Uruguay	2600		117								2717
	USA					9					50	59
Total insects per species		18473	2121	12468	5	9	355	11	13	2953	50	36458
Countries per species		10	2	8	1	1	7	1	1	4	1	-

Table 3 Number of insects per species belonging to non-target genera trapped in the monitored countries. Countries not listed did not report any non-target species.

Trapped species	Sampled countries									
	Portugal	Spain	France	Greece	Italy	Hungary	Uruguay	Australia	New Zealand	Total
<i>Xyleborinus saxesenii</i>	311	4				597	1	2828	51	3792
<i>Xyleborus perforans</i>								623		623
<i>Xyleborus ferrugineus</i>								263		263
<i>Xyleborus eurygraphus</i>	19	8								27
<i>Xylosandrus crassiusculus</i>					4					4
<i>Gnathotrychus materiarius</i>			34							34
<i>Ips grandicollis</i>								>10000		>10000
<i>Ips sexdentatus</i>	7472		7000	130		4438				19040
<i>Pityogenes calcaratus</i>		10								10
<i>Hylurgops palliatus</i>			3							3
<i>Tomicus destruens</i>	90		1							91
<i>Crypturgus numidicus</i>		2			13					15
<i>Crypturgus mediterraneus</i>		122								122
<i>Carphoborus pini</i>		1								1
<i>Hypothenemus seriatus</i>								49		49
<i>Hypothenemus sp.</i>							6			6
Species per country	4	6	4	1	2	2	2	5	1	16

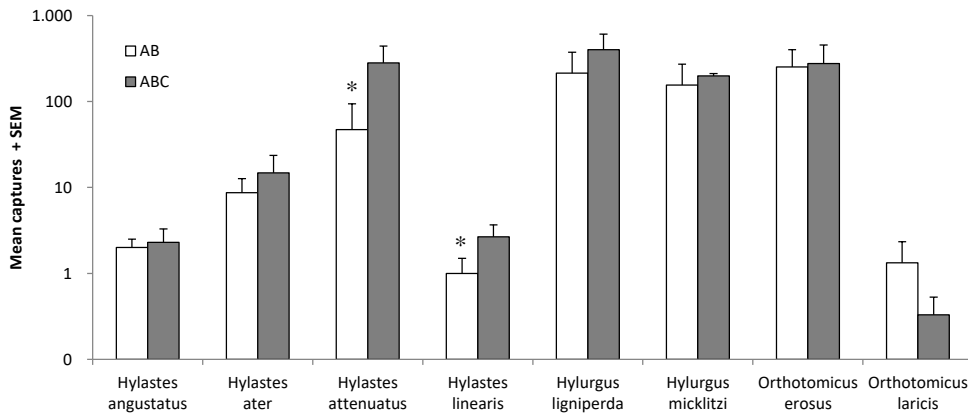


Fig. 2 Captures of the target species according to the different tested blends AB (alpha-pinene and ethanol) and ABC (alpha-pinene, ethanol, ipsdienol, ipsenol and Z-verbenol). *Significant differences ($P < 0.05$) between AB and ABC captures.

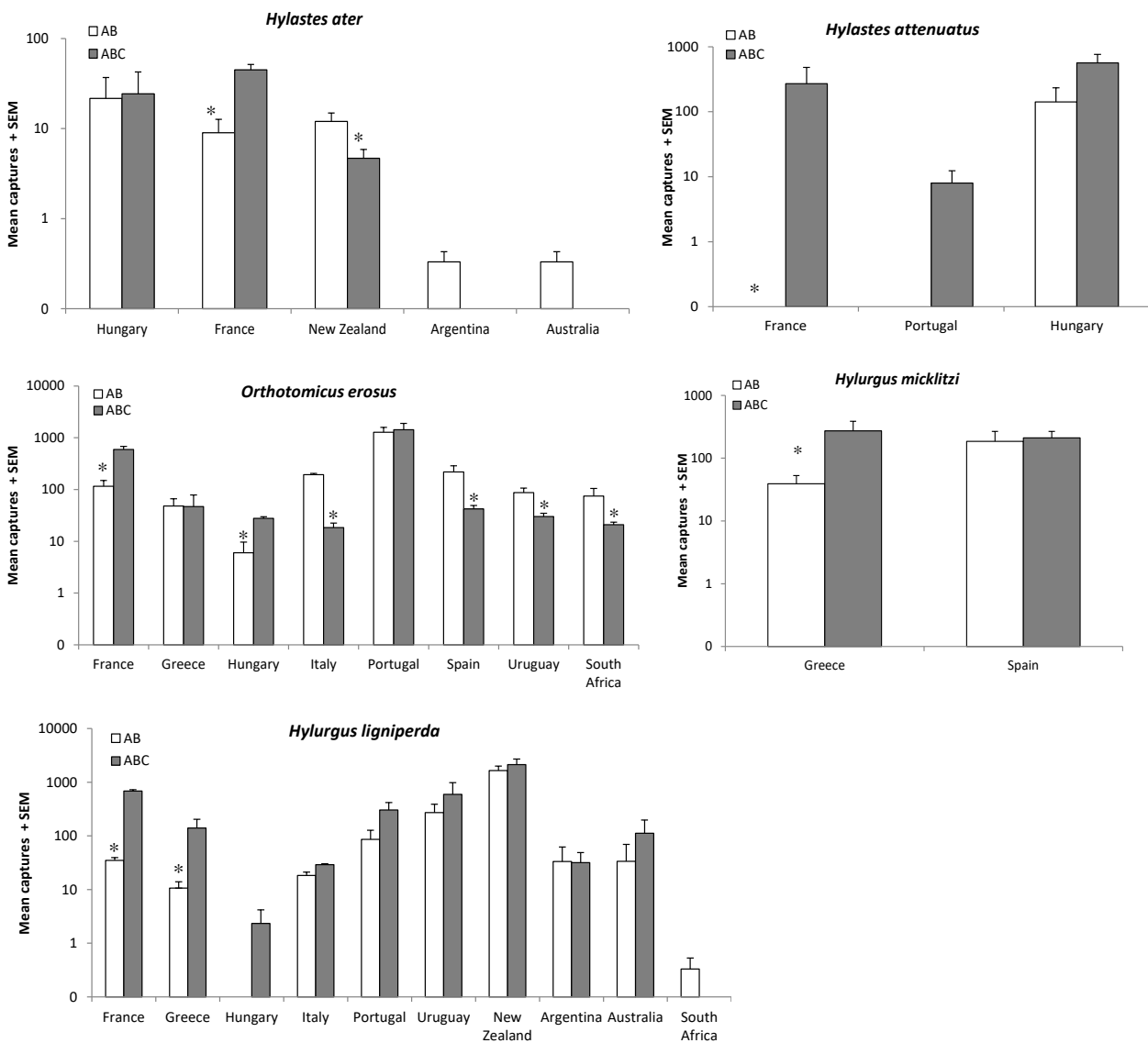


Fig. 3 Captures of *Hylastes ater*, *Hylastes attenuatus*, *Orthotomicus erosus*, *Hylurgus micklitzi* and *Hylurgus ligniperda* in different countries from the different tested blends AB (alpha-pinene and ethanol) and ABC (alpha-pinene, ethanol, Ipsdienol, Ipsenol and Z-verbenol). *Significant differences ($P < 0.05$) between AB and ABC captures recorded in the same country.

Discussion

The present study represents the first effort for a multi-continental coordinated monitoring of invasive alien bark beetle species. Trapping and monitoring of specific pine pests belonging to the genera *Orthotomicus*, *Hylurgus*, and *Hylastes* were successfully carried out in twelve countries using the same lure blends and trap model. Specifically, the survey performed in the native areas of the target pests has been of crucial importance to validate the trapping protocol that was also applied in the non-native countries of North America and the southern hemisphere. Moreover, many species other than the target European genera were trapped, including both bark beetles and polyphagous ambrosia beetles. Given that one of the aims of the study was to develop a multi-species lure system, the presence of these other species in traps is very important and provides the opportunity to apply the present monitoring protocol also to a greater number of pests. Although none of the trapped species represented new records for the monitored country, the trap captures confirmed the occurrence of a given species in the regions, and assembled data on the biological features of the local populations, allowing comparison among countries about population density.

Ten species belonging to the three target genera were found across the five continents where trapping occurred. These genera have Palearctic (*Hylurgus* spp.) or Holarctic (*Orthotomicus* and *Hylastes* spp.) natural distributions, which likely explains – together with the lack of native pine hosts – why only alien species and no native species were found in the southern hemisphere (*i.e.*, in South America, South Africa, Australia and New Zealand). *Orthotomicus caelatus* and *H. salebrosus* were only captured in the USA; these two species are native to North America, and they are morphologically and ecologically similar to the European *O. erosus* and *H. ater*. Although *H. ater* is not established in North America, it has been intercepted with imports multiple times (Brockerhoff et al. 2006a). However, invasive populations of *O. erosus* now occur on the west coast (California) of the USA (Haack 2004) where the climate is similar to the dry climate and forest types of the Mediterranean region. This may explain why *O. erosus* is established there and not where we carried out our sampling (Georgia), which has humid subtropical climate conditions and forest types more similar to southeastern China from where the main biological invasions recorded in this area originate (Haack 2004).

Most of the collected scolytines were bark beetle species infesting pines, and 60% of the species captured are now invasive in other countries around the world, with several others having been intercepted at borders (Brockerhoff et al. 2006a). Although the species studied here are rather common and widespread in Europe (Pfeffer 1995), their occurrence and distribution within their native range is not uniform. The Mediterranean pine engraver *O. erosus* was the most commonly trapped species and found in all six European countries monitored, followed by *H. ligniperda* which was found in five countries. Overall, considering also the invaded regions, *H. ligniperda* was the most abundant and widespread species, followed in total numbers by *O. erosus* and *H. attenuatus* (Table 2). The latter represented about 85% of trap catches in Hungary and it was the third most abundant across all countries (nearly 10% of all catches). In Portugal, *O. erosus* was the most captured species (8,133 catches), representing 87% of all the target bark beetles collected, followed by *H. ligniperda* (12.7%). These results suggest that *O. erosus* and *H. ligniperda* can be very abundant and they are rather successful invaders. They are also known to be important quarantine pests, potentially causing economic and ecological impacts and thus necessitating the use of phytosanitary treatments of log exports in the invaded range. In particular, *O. erosus* is considered an economically important bark beetle in many native regions in the Mediterranean basin (Mendel et al. 1988; Paiva 1995). Although considered by many as a secondary pest (Dajoz 2000), this bark beetle may attain high population densities killing living pines and causing high tree mortality. Trees subject to drought stress and following forest fires are particular cases for which severe outbreaks of *O. erosus* in its native range have been observed. In such situations, high population densities can then lead to damage of healthy stands (Paiva and Pessoa 1987; Ferreira and Ferreira 1990). *Hylurgus ligniperda*, on the other hand, was the most common species in the invaded regions, in particular high numbers were captured in New Zealand where *O. erosus* was absent, and in Uruguay where catches of *H. ligniperda* were more than 20 times greater than those of *O. erosus*. Although *H. ligniperda* usually does not cause any noticeable direct economic impacts as it does not attack live trees or seedlings, it is a quarantine pest that is undesirable on timber exports. Although both *H. ligniperda* and *O. erosus* have been intercepted with similar frequency at United States and New Zealand borders, representing about 6% and 8% of all bark beetle interceptions in these countries, respectively (Brockerhoff et al. 2006a), *H. ligniperda* has been the more successful invader by far, invading many more non-native countries. This suggests that *H. ligniperda* has a greater ability to invade new regions with suitable host plants. Pathways of arrival also play a major role in biological invasions. For instance, the absence of North American bark beetle species in South America, despite the fact that they could establish there, is likely because of the low wood trade from USA to Argentina (Lantschner et al. 2017). Instead, the North American species *Ips grandicollis* established in Australia, but apparently due to dunnage moved by the US into Australia during the Second World War when quarantine protocols were not yet applied.

Some European species were captured only in a few of their native countries, such as *Hylastes linearis* in Portugal and *Hylurgus micklitzii* in Spain and Greece. This may be due to a local absence or a low population density of the species rather

than a reduced response of these species to the tested pheromone blends. This is particularly true for *H. micklitzi*, which was trapped only in two countries (Spain and Greece) but in large numbers (1,448 and 673 adults, respectively), suggesting that the species is highly attracted to the lures we used. Moreover, *H. micklitzi* is highly specialized on its main host species, *P. halepensis* and *P. brutia*, and this explains why this insect was captured only in Spain and Greece, the only countries where the study was carried out in *P. halepensis* and *P. brutia* pine forests, whereas *H. ligniperda* was trapped mainly in the European countries where the monitoring was set up in forests with other pine species.

Interestingly, two European species were trapped only in the invaded countries: *Hylastes angustatus* was found only in South Africa, and *O. laricis* was collected only in Argentina. Both these species have a large European distribution and they are very well known in most Mediterranean countries, although they are not considered pests producing large infestations and damage. In their native area, populations of these species remain at very low density, and hence they are rarely trapped with generic lures. The lack of specific natural enemies and competition with other species in the invaded areas may explain why species of secondary importance in their native area may become a pest in invaded countries.

As reported in the results, *ABC* was the best “overall” blend across all species and countries, *i.e.* the blend allowing the highest captures for the highest number of species, although with performance statistically higher only for *H. attenuatus* and *H. linearis*. However, according to our results, the blend *AB* also managed to catch all target species (except *H. attenuatus*). Nonetheless, the effect of the two tested blends (*AB* and *ABC*) on each species varied among the monitored populations. The *ABC* blend was generally more effective than the *AB* blend for *H. ligniperda*, *H. micklitzi* and *H. attenuatus*. For the other species, including *H. ater*, *H. angustatus*, *H. linearis* and *O. erosus*, the trapping performance of the two tested blends varied among the monitored populations in the different countries, with greater catches for one or the other blend, or no differences between lures. This variation may be explained by differences in the attractants and pheromones characteristic of each species. *O. erosus*, for example, is attracted by alpha-pinene and ethanol as a primary signal of host decline. Secondly, an aggregation pheromone is released, composed of ipsdienol, *Z*-verbenol and methyl-butenol (Giesen et al. 1984). The *ABC* blend we tested lacks methyl-butenol, which is likely to have reduced the differences in catches we would have expected to occur between the tested blends. The same mechanism may occur for other species, such as *H. ater* for which alpha-pinene and ethanol are the only known major attractant (Perttunen 1957; Brockerhoff et al. 2006b).

Our results represent the first step towards the development of an international and coordinated monitoring system based on multi-lure traps for alien bark beetle species to improve pest surveillance and monitoring in pine forests and plantations worldwide. The proposed protocol based on cross-vane traps baited with the *ABC* blend is affordable, user-friendly, generic and effective against a large number of bark beetle species belonging to different genera. The understanding of the direct and indirect transport pathways and the possible invasion mechanisms of alien species in new regions of the planet is a point of crucial importance to address the processes of biological invasions. Future research will include molecular analyses of the insect samples that will contribute to our understanding of genetic affinities among the different populations and is likely to identify the infestation origins in each country.

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