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Bark Beetles Control in Forests of Northern Spain

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1. Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are an insect group that contains at least 6000 species from 181 genera around all the world (Wood, 1982). It is well known that some species are among the most destructive insects of coniferous forests representing a continuous threat (Ayres & Lombardero, 2000). The knowledge of these insects in Spain is very far away of desirable. Some studies have advanced in the knowledge of the taxonomic and faunistic composition of diverse forest types (Plaza & Gil, 1982; Plaza, 1983; Gil & Pajares, 1986; Pajares, 1987; González, 1990; Lombardero, 1994; Lombardero & Novoa, 1994; Riba, 1994; Fernández, 1996; López et al., 2007a), but the understanding of their dynamics and interrelationship biotic factors is very low. The main objective of the present work is to present a synthesis of the research works that have been performed so far in Spanish mainland, particularly in its northern plateau. First, we will introduce the situation of the conifer species Pinus radiata D.Don, the most widely species planted in this geographic zone, and its relationship with both bark beetles and associated phytopathogenic fungi (mainly ophiostomatoid fungi and Fusarium circinatum). Secondly, a brief and overall approach to the chemical ecology of bark beetles is given and finally there will be exposed some particular cases of species considered as serious threats for spanish forestry, focusing on studies aimed to the development of integrated pest management strategies.

2. The status of *Pinus radiata* D.Don in Northern Spain and its association with bark beetles (Coleoptera: Scolytinae) and ophiostomatoid fungi (Sordariomycetes: Ophiostomatales) and *Fusarium circinatum* (Sordariomycetes: Hypocreales)

The commonly known as Monterey pine (*Pinus radiata*), arising from Mexico, was introduced in Spain during the first half of the XIX century, initiating its plantation in the Basque Country. The first introduction was carried out by Mr. Carlos Adán de Yarza in his botanical garden of Zubieta (Lekeitio, Vizcaya), planting the first stands in 1840 near Amoroto (Vizcaya). The common name "insignis" became popular, both in Spanish and Basque language, due to be more frequent at that time the scientific synonymy *Pinus insignis*. Monterey pine has been planted in Spain in the North Atlantic orle between the

parallels 42 and 44 ° N in altitudes below 800 m (Autonomic Communities of Galicia, Asturias, Cantabria, Basque Country and Navarra), showing up also in the Mediterranean province of Gerona (Cataluña), with similar latitude, and promptly in the botanic grounds of the laurisilva and the fayal-brezal (*Myrica-Erica*) of the Canary islands (28-29 ° N). The worldwide and national areas occupied with commercial plantations of *P. radiata* are indicated in **Table 1**.

Country	Area	Autonomic Community	Area
Chile	1,400,000 ha.	Basque Country	160,000 ha.
New Zealand	1,200,000 ha.	Galicia	60,000 ha.
Australia	650,000 ha.	Asturias	26,000 ha.
Spain	270,000 ha.	Cantabria	15,000 ha.
South Africa	55,000 ha.	Navarra	5,500 ha.
Others	30,000 ha.	Canarias	3,000 ha.
		Gerona	500 ha.
Worldwide total	3,605,000 ha.	National total	270,000 ha.

Table 1. Worldwide and national areas (hectares) intended to the lumber exploitation of Monterey pine (adapted from Michel 2004).

This area of artificial plantation gathers in its whole the characteristics that favour the appearing of pests and diseases. Thus, all of them form (1) pure masses of more or less extension where the nutrient abundance and the facility of dispersion support the population increase of the harmful species; (2) contemporary masses that favour the appearance of pests and diseases related to different ages intervals; and (3) exotic masses which are submitted to intercontinental transports with high risk of introduction of exotic pests (Alonso Zarazaga & Goldarazena, 2005; López et al., 2007b) and diseases (Landeras et al., 2005) of greater potential virulence due to be free of natural enemies or under favourable environmental conditions. Besides, the economic profitability of Monterey pine, at first like cellulose pulp and now also as saw wood (50% for each intention nowadays in Northern Spain), has sponsored its plantation in geographic areas that are far from the optimum conditions. This has caused physiologically weak forestry masses, favouring the attack of pests and diseases due to a higher incidence of abiotic noxious agents such as frosts and droughts.

Bark beetles can cause damage both by the direct boring action and by the inoculation of phytopathogenic fungi. Once the host tree has been localized, pioneer specimens produce aggregation pheromones, which joined with the volatile kairomones of the recently attacked tree, attract other members of the same species. While they feed and build their galleries under the bark, some species cut the vascular flow causing the dead of the tree. At the same time, they inoculate spores and fragments of mycelium into the phloem, playing an important role in the transmission of some phytopathogenic fungi such as the causal agents of Dutch elm disease and blue-staining fungi that alter the structure of the wood and reduce its worth (Pajares, 1987).

Some species of bark beetles are able to cause significant damage to forests. For example, during the 1970's, outbreaks of *Ips typographus* destroyed 2 million m³ of timber in

Scandinavia (Bakke, 1983). Regarding to United States, *Dendroctonus ponderosae* has caused an average annual loss of about 1.5 billion board feet particularly in *Pinus contorta* in western North America since 1895 (Wood, 1982). According to Carter et al. (1991), outbreaks of *Dendroctonus frontalis* in southeastern Texas covered 3,200,000 hectares with heavy economic losses from 1974 to 1980. Furthermore, from 1999 to 2003, *Dendroctonus frontalis* caused losses of about USD \$1.5 billion in the southeastern U.S.A. (Nowak, 2005). The current outbreak of *Dendroctonus ponderosae* in Canada has impacted about 12 of the 14.3 million ha of *Pinus contorta* in the British Columbia (Westfall, 2006). In Spain, the most damaging species is *Tomicus piniperda*, which can cause annual losses of up to 72 million € in the Basque Country region (northern Spain) (Amezaga, 1993). Among the scolytid species present in the Iberian Peninsula, *Tomicus piniperda* and *Ips sexdentatus* represent the most relevant directly damaging species, as it will be explained later (see section 4).

Many sapstain fungi, especially ophiostomatoid fungi (Sordariomycetes: Ophiostomatales), are associated with phloeophagous bark beetles and might help to overcome the defences of attacked trees (Kirisits, 2004). They are commonly called "blue-stain fungi" because the discoloration they cause, namely blue, gray, brown or even black on the sapwood of trees, mostly on conifers (Kirisits, 2004). This sap stain is caused by the growth of the hyphae in the ray parenchima cells and resin ducts, disrupting the sap flow, and tracheids are also colonized in later stages of infection. As a consequence of this discoloration, the lumber defect is largely cosmetic. Most ophiostomatoid fungi that cause sapstain are moderately to weakly virulent pathogens, but some species can display relative high virulent levels and cause the death of infected trees when they are inoculated in high doses (Kirisits, 2004). Although some blue-staining fungi can cause strong damage on the strength properties of the wood assigned to furniture industry (Seifert, 1993) and these losses can amount to a 50% price reduction in the Basque Country (Maderas Elorriaga Company, Muxika, Vizcaya, personal communication, from about 180 €/m³ to 85 €/m³), the knowledge of conifer bark beetle-associated blue-staining fungi in the Iberian Peninsula is very limited (Fernández et al., 2004; Villareal et al., 2005). Romón et al. (2007a) isolated 16 species of Ophiostoma sensu lato or their asexual states from 13 bark beetle species and the root weevil Brachyderes incanus (Coleoptera: Curculionidae: Entiminae). Among the isolated taxa, species such as Ophiostoma ips, O. minus, O. piceae and O. pluriannulatum are important agents of bluestaining (Seifert, 1993), whereas O. ips, O. minus and L. wingfieldii pathogenicity is well recognized (Raffa & Smalley, 1988; Lieutier et al., 1989; Yamaoka et al., 1990; Fernández et al., 2004). Sixty-nine of the reported associations by Romón et al. (2007a) had not been previously recorded until them. Studies based on fungus-conifer scolytids association have not been only carried out in P. radiata inhabiting bark beetle populations, but also in P. pinaster (Bueno et al., 2010). They identified twenty-five taxa belonging to the Eumycotina group from isolations of I. sexdentatus adults and naturally colonized tissues (sapwood and phloem).

On the other side, pitch canker disease, caused by the fungus *Fusarium circinatum*, is one of the most important pathogens of *Pinus radiata*. This fungus species is endemic to southeastern United States (Dwinell et al., 1985). Pitch canker disease was recently identified and reported in California, predominately in planted urban *P. radiata* and in native Monterey pine forest (Correll et al., 1991). Since being first reported in the United States, it has been also found in different countries around the world: Japan (Muramoto et al., 1989),

Mexico (Rodriquez, 1989), South Africa (Viljoen et al., 1994) and Spain (Landeras et al., 2005). Its accidental introduction in these areas is probably due to softwood lumber, seedling and seed exports. In California, wounding has not resulted. The transmission of the pathogen to cones seems to be not (Correll et al., 1991) or branches (Fox et al., 1991), despite the presence of significant airborne inoculum. In contrast, a complex of insects have been demonstrated to be able to transmit *F. circinatum* (Fox et al., 1991; Hoover et al., 1995; Storer et al., 2004). Within this species-complex there are included some bark beetles species, like Ips paraconfusus, I. mexicanus, I. plastographus, Pityophthorus carmeli, P. nitidulus, P. setosus and Conophthorus radiatae and the anobid beetle Ernobius punctulatus (Coleoptera: Anobiidae). All of them appear to be phoretically associated with the fungus, and are known to visit and infest non-diseased trees. Romón et al. (2007b) isolated F. circinatum from adult bark beetles collected from baiting logs in two stands of *P. radiata* located in two plots of Biscay province (Morga and Muxika). Five conifer bark beetles were found to carry the inoculum: Pityophthorus pubescens (25.00%), Hylurgops palliatus (11.96%), Ips sexdentatus (8.57%), Hypothenemus eruditus (7.89%), Hylastes attenuatus (7.40%) and Orthotomicus erosus (2.73%) (Table 2). Frecuency of occurrence of *F. circinatum* is given within parentheses. In addition, Brachyderes incanus (14.28%) had the second highest frequency of occurrence of the fungus. Frequencies of occurrence of fungi were computed using the formula of Yamaoka where F = (NF / NT) 100 (%) and F represents the frequency of occurrence (%) of the fungus, NT represents the total number of samples from which isolation attempts were made and NF represents the number of samples from which the fungus was isolated.

Insect species	Total no. samples	NF	F (%)*
Pityophthorus pubescens	32	8	25.00
Brachyderes incanus	42	6	14.28
Hylurgops palliatus	117	14	11.96
lps sexdentatus	35	3	8.57
Hypothenemus eruditus	38	3	7.89
Hylastes attenuatus	54	4	7.40
Orthotomicus erosus	73	2	2.73
Dryocoetes autographus	45	-	-
Hylastes ater	32	-	-
Tomicus piniperda	18	-	-
Xyleborus dryographus	9	-	-
Hylurgus ligniperda	5	-	-

^{*} Frequency of occurrence F = (NF / NT) 100 (%), where NT represents the total number of samples from which isolations attempts were made, and NF represents the number of samples from which F. circinatum was isolated.

Table 2. Variation about frecuency of occurrence of *Fusarium circinatum* of several bark beetles (Coleoptera: Curculionidae: Scolytinae) and weevils (Coleoptera: Curculionidae: Entiminae) species in northern Spain.

Table 3 includes the relationship of different fungi species, belonging to the Orders Ophiostomatales, Sphaeropsidales and Hypocreales associated with conifer inhabiting bark beetles (and the weevil *Brachyderes incanus*), with special emphasis to isolations detected in the Basque Country (northern Spain).

Bark beetles	Order	Order	Order Hypocreales
species	Ophiostomatales	Sphaeropsidales	, <u>, , , , , , , , , , , , , , , , , , </u>
Hylastes attenuatus	Ophiostoma ips** O. olivaceum O. piceae** O. piliferum-like O. quercus O. stenoceras Leptographium guttulatum** L. truncatum-like L. wingfieldii*** Pesotum fragans	Diplodia pinea**	Fusarium circinatum*** F. oxysporum** F. lateritium*
Hylastes ater	Ophiostoma ips** O. minus O. penicillatum O. piceae** O. piliferum O. floccosum* O. olivaceum* O. piliferum-like* O. piliferum-like* O. pluriannulatum** O. quercus* O. rectangulosporium-like* O. stenoceras* Leptographium stenoceras L. guttulatum** L. lundbergii L. serpens L. wingfieldii***		Fusarium moniliformis**
Hylurgops palliatus	Ophiostoma ainoae O. bicolor O. cucullatum O. galeiformis O. japonicum O.neglectum O. penicillatum O. piceae** O. piceaperdum O. simplex	Diplodia pinea**	Fusarium circinatum*** F. proliferatum**

Bark beetles	Order	Order	Order Hypocreales
species	Ophiostomatales	Sphaeropsidales	<i>J</i> 1
Hylurgops palliatus	O. stenoceras O. ips** O. olivaceum* O. piliferum-like* O. pluriannulatum** O. quercus* O. rectangulosporium-like* Ceratocystiopsis alba C. minuta Leptographium guttulatum** L. lundbergii L. procerum L. wingfieldii*** L. truncatum-like* Pesotum fragans*		
Ips sexdentatus	Ophiostoma ainoae O. araucariae O. brunneo-ciliatum O. clavatum O. ips** O. japonicum O. minus* O. obscura O. piceae O. piceaperdum O. olivaceum* O. pluriannulatum** O. rectangulosporium-like* Ceratocystiopsis minuta Leptographium guttulatum** L. truncatum-like* Pesotum fragans		Fusarium circinatum*** F. moniliformis**
Dryocoetes autographus	Ophiostoma ainoae O. araucariae O. cucullatum O. galeiformis O. japonicum O. obscura O. neglectum O. piceae		

Bark beetles	Order	Order	Order Hypocreales
species	Ophiostomatales	Sphaeropsidales	Jr
Dryocoetes autographus	O. piceaperdum O. simplex O. ips** O. minus* O. olivaceum* O. piliferum-like* O. rectangulosporium-like* Ceratocystiopsis alba C. minuta Leptographium	Spriaeropsidales	
Orthotomicus erosus	guttulatum** Ophiostoma ips** O. piceae** O. pluriannulatum** O. canum-like O. floccosum O. olivaceum O. stenoceras O. rectangulosporium-like Leptographium guttulatum** L. wingfieldii*** Pesotum fragans		Fusarium circinatum*** F. moniliformis** F. culmorum* F. lateritium*
Tomicus piniperda	Ophiostoma canum O. clavatum O. floccosum O. galeiformis O. huntii O. ips** O. minus O. piceae** O. piceaperdum O. piliferum O. pluriannulatum** Ceratocystiopsis minuta Leptographium euphyes L. guttulatum ** L. lundbergii L. procerum L. wingfieldii***	Diplodia pinea**	
Pityophthorus pubescens	Ophiostoma ips** O. piliferum-like* Leptographium guttulatum**		Fusarium circinatum*** F. lateritium*

Bark beetles species	Order Ophiostomatales	Order Sphaeropsidales	Order Hypocreales
Hypothenemus eruditus	Ophiostoma pluriannulatum** O. quercus* Pesotum fragans*		Fusarium circinatum*** F. culmorum* F. lateritium*
Xyleborus dispar	Ophiostoma pluriannulatum	Diplodia pinea	
Xyleborus dryographus	Ophiostoma olivaceum <u>O. piceae</u> ** Sporothrix schenckii-like		Fusarium moniliformis**
Hylurgus ligniperda	Leptographium guttulatum		
Pityogenes calcaratus	Ophiostoma ips <u>Leptographium</u> guttulatum**		
Brachyderes incanus	Ophiostoma piceae** O. pluriannulatum** O. quercus Leptographium guttulatum		Fusarium circinatum*** Fusarium moniliformis

Table 3. Fungal species frequently associated with bark beetles colonizing conifers. Fungal taxa underlined indicate high percentage of isolation with the corresponding insect within a row. Fungal species with an upper symbol are present in the Basque Country, as follows: Saprophytic species (*), facultative pathogens (**) and strictly pathogens (***)(Adapted from Kirisits, 2004; Romón et al., 2007a, 2007b).

3. Chemical ecology of the host tree colonization by bark beetles: Basis for the development of a sustainable strategy for the protection of forestry masses

Each species of bark beetles is adapted to only one or a few host tree species probably due to natural selection driven by trees biochemicals. It is likely that each species of tree has coevolved chemicals to defend against the selection pressures of bark beetles and other insects (Berryman et al., 1985; Byers, 1995). Plant chemicals can be attractive, repellent, toxic or nutritious to bark beetles and have effects on: (1) finding and accepting the host tree (selection and suitability); (2) feeding stimulation and deterrence; (3) host resistance; (4) pheromone biosynthesis and communication; and (5) attraction of predators, parasites and competitors of bark beetles (Byers, 2004).

Bark beetles must locate a suitable host from among a relatively few that are widely scattered in the forest. During dispersal flight, insect must discriminate between potential conifer hosts and avoid any unsuitable host and non-hosts decidious trees, so the ability to detect and recognize different olfactory signals in a complex olfactory landscape represents an important cue for bark beetles colonization processes (Raffa, 2001; Zhang & Schlyter,

2004). The host tree is restricted usually to one or a few species and in most cases the insects seek weakened, less resistant trees. It is expected that insects can detect certain volatile host plant chemicals that indicate its suitability [see Fig. 1 for massive attack of *Tomicus piniperda*, the aggregation of this bark beetle species is considered to be predominantly mediated by host tree kairomonal compounds blend (Vité et al., 1986) although *trans*-verbenol has been recently suggested as its potential pheromone (Poland et al., 2003)]. Pheromones and/or kairomones involved in the semiochemical communication of bark beetle species most commonly colonizing *Pinus radiata* in Spain are indicated in Table 4.

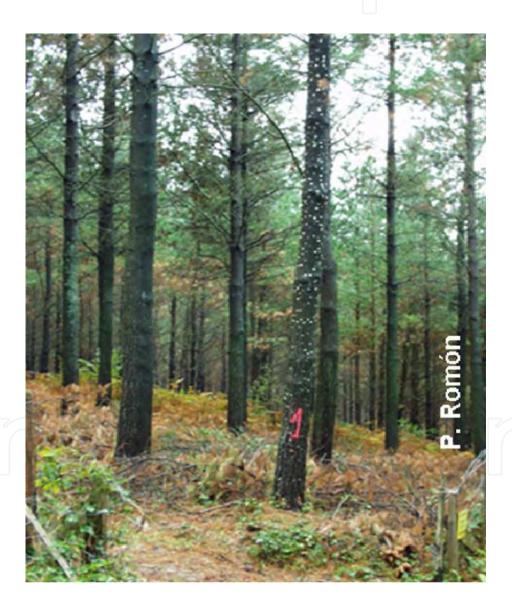


Fig. 1. Massive attack of *Tomicus piniperda*, causing, after one year since the population outbreak, a scattered distribution pattern of nearly dead trees within a *Pinus radiata* plantation near Morga, Biscay province, Basque Country (Spain).

Bark beetle species	Pheromone	Kairomone
Hylurgops palliatus	-	Alpha-pinene (Perttunen,
		1957)
		Beta-pinene (Volz, 1988;
		Byers, 1992)
		Terpinolene
		Myrtenol
		Ethanol
		3-carene
		cis-verbenol
		trans-verbenol
Ips sexdentatus	Ipsdienol (Vité et al.,	Myrcene (Hughes, 1974)
	1972)	2-methyl-3-buten-2-ol
	Ipsenol	(Serez, 1987)
		cis-verbenol
Tomicus piniperda	trans-verbenol (Poland et	Alpha-terpineol (Kangas et
	al., 2003)	al., 1970)
		cis-carveol
		trans-carveol
		Beta-pinene (Volz, 1988)
		Myrtenol
		Ethanol (Byers, 1992)
		Alpha-terpinolene
		Delta-3-carene
		Alpha-pineneoxide
		(Czokajlo, 1998)
		Alpha-pinene
		(Song et al., 2005)

Table 4. Pheromonal and kairomonal components of most common conifer bark beetles distributed among *Pinus radiata* plantations in northern Spain, compendium revision.

Host plant's suitability to bark beetles varies with its nutritional quality and composition of anti-insect toxins (Scriber, 1984). Nonhost trees are probably less nutritional, and the beetle would not be adapted to detoxifying some of the nonhost toxins that have evolved for use against other herbivorous insects. A beetle would save much time and energy if it can discriminate the host from the nonhost and determine the intraspecific suitability of a host by olfactory means, deciding not to land due to the detection of volatile compounds called allomones (Byers, 1995). It is more difficult to isolate repellents and inhibitors used in avoidance behavior than to isolate attractants since tests of avoidance require one to first isolate the attractive host odors and then present these with and without the possibly inhibitory odor compounds. Several studies indicate that at least some species of bark beetles avoid nonhost volatiles during their search for host trees. For example, the attraction of both *T. piniperda* and *Hylurgops palliatus* to ethanol (1-6 g/day) was reduced by odors from cut logs of *Betula pendula* and *Populus tremula* (Schroeder, 1992). In this way, one monoterpenic compound called verbenone has shown relatively promising results as an anti-aggregant pheromone (Lindgren et al., 1989). Verbenone (4,6,6-trimethylbicyclo[3.1.1]-

hept-3-en-2-one) is a simple oxidation product of trans-verbenol, which in turn is a biological oxidation product of α-pinene (Birgersson & Leufvén, 1988), one of the most ubiquitous monoterpenes in the Pinaceae. a-Pinene is quite toxic to a number of coniferophagous insects, whereas trans-verbenol and verbenone appear to be less toxic (Lindgren et al., 1996). Thus, insects inhabiting environments high in a-pinene could be expected to have either a high tolerance or an effective detoxification system. Verbenone has been found in relatively large amounts (mg) in hindguts of Dendroctonus ponderosae (Pierce et al., 1987), Dendroctonus frontalis (Renwick & Vité, 1968), Dendroctonus brevicomis (Byers et al., 1984) and Dendroctonus pseudotsugae (Rudinsky et al., 1974), and in low amounts (ng) in T. piniperda (Lanne et al., 1987), but it appears to be absent in I. paraconfusus, I. typographus and Pityogenes chalcographus (Byers, 1983; Birgersson et al. 1984, 1990). During the last two decades, verbenone has been demonstrated as a good natural repellent for the control of damages caused by several insect species such as Hylobius pales (Salom et al., 1994), Hylobius abietis (Lindgren et al., 1996), D. ponderosae (Lindgren et al., 1989), Ips pini and Ips latidens (Lindgren & Miller, 2002). It has been hypothesised that bark beetles species which required relatively fresh host tissue would be more affected by the presence of verbenone, whereas species inhabiting aged tissues would have higher tolerance to verbenone and/or a more efficient detoxifying system (Lindgren, 1994; Lindgren & Miller 2002; Romón et al., 2007b).

Bark beetle pheromones used in aggregation and for avoidance of competition consist of many varied structures. Plant compounds, predominantly the monoterpenes α -pinene or myrcene, are used as kairomonal precursors for their pheromonal components (Hendry et al., 1980). Many of the same pheromonal compounds are used by species in throme same genus, such as ipsenol, ipsdienol, and *cis*-verbenol in the genus *Ips* (Byers, 1995) or *exo*-brevicomin, frontalin, *trans*-verbenol and verbenone in the genus *Dendroctonus* (tribe Tomicini) (Borden, 1982). Some compounds such as *cis*- and *trans*-verbenol may be found in *Ips* (Tribe Ipini) as well as *Tomicus* (Tribe Tomicini). However, *cis*-verbenol has so far only been proven as an aggregation pheromone component for species in the tribe Ipini, whereas *trans*-verbenol has semiochemical activity only in the tribe Tomicini. The base structure of ipsenol, ipsdienol, and myrcenol resembles the plant monoterpene myrcene; likewise, *cis*-and *trans*-verbenol resemble α -pinene. These structural similarities support the hypothesis that in many cases bark beetles use plant compounds as precursors for their pheromone components (Byers, 1995).

Aggregation pheromone components were first identified in bark beetles from males of *Ips paraconfusus* as a synergistic blend of (*S*)-(-)-ipsenol, (*S*)-(+)-ipsdienol, and (4*S*)-*cis*-verbenol (Wood et al., 1968). Several other *Ips* species were soon discovered to produce and respond to various blends of these compounds (Vité et al., 1972). The similarity of chemical structure between myrcene and ipsenol and ipsdienol led to propose, and demonstrate by gas chromatography and mass spectrometry (GC-MS), that myrcene is precursor of pheromones in *Ips* spp. (Hughes, 1974; Byers et al., 1979).

More resistant tree genotypes may have evolved through natural selection lower levels of pheromonal precursor terpenes or attractive kairomones and/or higher concentrations of other toxic monoterpenes. Evolution of plant chemicals that increase tree's resistance to colonization by bark beetles requires that (1) the plant chemicals are detrimental to the beetle; (2) the host chemistry is genetically driven; (3) population variation in genotypes of these trees exists; and (4) the bark beetle exerts selection pressure on the tree by killing or

reducing fertility. The beetle population should coevolve, if possible, by shifting their genotype frequencies to those that offer more protection against the plant chemicals.

Host tree chemistry affects most aspects of bark beetle biology, moreover, bark beetles probably differentially affect survival of host trees and alter genotypic frequencies and host chemistry (Byers, 1995). Geographic and intraspecific variation in toxicity of host compounds has been little studied. Thus, more studies are needed in stands with ongoing outbreaks of bark beetles to determine if natural selection can slant trees intraspecific variation that will determine their monoterpene properties.

4. An overview to conifer-inhabiting bark beetle species with most forestry importance in spanish mainland, particularly northern Spain: Concrete cases

Alonso-Zarazaga (2002) listed 128 bark beetles species present across iberian and balearic area, including both conifer and decidious-inhabiting species. Two more alien species to Iberian Peninsula should be added to this checklist, i.e. *Gnathotrichus materiarius* and *Xylosandrus germanus* (López et al., 2007b). *Gnathotrichus materiarius* is considered as a nearctic native species, whereas the origin of *Xylosandrus germanus* is asiatic. According to Kirkendall and Faccoli (2010) and references therein, owing to commercial trading, bark beetles mainly travel in wood and in wooden packing materials such as crating, dunnage and pallets. Both alien species were found in sawmills and wood-proccesing companies of Basque Country that use imported lumber from France. Nowdays, their establishment to different *P. radiata* stands in the Basque Country is fully confirmed (Goldarazena et al., personnal observation). Although these two species are not considered as highly dangerous species, the prevention of the entry and early detection of invasive species, through different pathways including treatment of imported commodities, should be a priority task, in order to avoid potential negative environmental impacts that would be generated.

Within all of these species present in spanish mainland, few species are capable of killing healthy trees (Gil & Pajares, 1986). The register of produced damage data are very scarce, because there have not been studies focused on tracing the economical incidence of these insects in contrast with other countries. First reports were produced in 1907, related to some attacks of Tomicus piniperda and other decidious bark beetle species (Gil & Pajares, 1986). During mid-1950's and mid-1970's several attacks of Ips acuminatus (Fig. 2) were registered in P. sylvestris stands of Guadalajara province (1954-57) and Cuenca province (1972-1973) (Gil & Pajares, 1986) but there are no economic data available for these events. In addition, less important sporadic attacks of I. sexdentatus and O. erosus had occurred in north and central Spain in last decades (Gil & Pajares, 1986). According to Grégoire & Evans (2004), three species of conifer-inhabiting bark beetles species, i.e. Ips sexdentatus, Ips acuminatus and Tomicus piniperda should be considered as significant pests for Spanish mainland. Not only these species, but also Pityophthorus pubescens is a serious candidate to be considered as potential forest pest, taking into account its importance as vector of Fusarium circinatum, fact that has been previously mentioned in text (Romón et al, 2007b). Following sub-sections will deepen about different studies carried out with these species in Spanish mainland so far. Although quantitative data of economical damage produced by bark beetles are difficult to estimate, some data are provided.



Fig. 2. *Ips acuminatus*, dorsal and lateral view. Photographs taken from López et al., 2007a.

4.1 The six-toothed bark beetle *lps sexdentatus* (Börner) (Fig. 3)

This species is considered as one of the forest pests that higher damages cause in conifer stands of Iberian Peninsula (Gil & Pajares, 1986). Although endemic populations of I. sexdentatus tend to colonize weakened or dead trees, it is well reported that healthy trees can be attacked under epidemic conditions. Frecuently, improper management of logs, for example storaging them for long time, adverse abiotic and climatic conditions (storms, fires, droughts) generate breeding resources for I. sexdentatus and favor the generation of these population outbreaks. As a significant example, 11,997 ha of a mixed forest with predominant presence of P. pinaster were affected by a fire during 2005 in Gualadajara province (Sánchez et al., 2008). Due to this fact, Ips sexdentatus populations significantly increased causing severe damages in some zones. Thus, a massive trapping program was carried out in following years, setting 99 and 237 Theysohn traps in 2006 and 2007 respectively. This trapping methodology led to the capture of 4,928,270 beetles. Moreover, different silvicultural techniques (extraction of affected timber) were applied in parallel. All of these measurements contributed to reduce the negative effects of this population outbreak. On the other hand, 25,000 ca. trees were killed by I. sexdentatus in Castilla y León province in 2000 (Consejería de Medio Ambiente, Junta de Castilla y Léon 2001, as cited in Bueno et al., 2010).



Fig. 3. Ips sexdentatus, dorsal and lateral view. Photographs taken from López et al., 2007a.

In addition, it is remarkable that the incidence of "Klaus" named windstorm during January 2009 affected 37.9 million m³ of maritime pine (*P. pinaster*) in Aquitanie (southern France) (Inventaire Forestier Nationale, 2009). As a consequence a great amount of timber was left as suitable breeding material for *I. sexdentatus*. The importation of those *P. pinaster* logs to different sawmills and timber-processing industries located at the Basque Country is a common commercial activity. So, it must be taken into account that the importation and storage of such infested logs for a long time would have consequences for the forest management and put into risk the adjacent *P. radiata* stands (Goldarazena et al., personnal observations).

Some studies focusing on verbenone have been carried out in order to test it as a potential component of IPM strategies for the protection of different pine species stands against this species. Two compounds, verbenone and trans-conophthorin, have been mainly considered as the most potential anti-aggregative semiochemicals. Biological implication of verbenone in bark beetles has been previously mentioned (see subsection 3), so it is worthwhile to remark the bioactivity of the second compound. The spyroketal conophthorin [5S,7S-(-)-7-methyl-1,6dioxaspiro(4.5)decane] is a non-host bark volatile found in angiosperm trees, such as Betula pubescens and B. pendula (Betulaceae) in Europe (Byers et al., 1998), and Populus tremuloides, P. trichocarpa (Salicaceae), B. papyrifera and Acer macrophyllum (Aceraceae) in North America (Huber et al., 1999). On insects, it was first identified from the abdomina of workers of some Hymenoptera: Vespidae, i.e. Paravespula vulgaris (Francke et al., 1978), and P. germanica and Dolichovespula saxonica, together with males of the bark beetle species Leperisinus fraxini (Francke et al., 1979). Later, Kohnle et al. (1992) found it in the frass of the fir bark beetle Cryphalus piceae, reducing field response of the insect to attractants. The name conophthorin comes from the genus Conophthorus, that includes species known to produce it with an inhibitor effect to aggregation pheromones or host kairomones, that is, Conophthorus coniperda

(Birgersson et al. 1995; de Groot et al., 1998, Rappaport et al., 2000) and *C. resinosae* (Pierce et al., 1995; de Groot & DeBarr, 2000; Rappaport et al., 2000). In addition, there are several studies about the repellent effect of (*E*)-(-)-conophthorin and racemic conophthorin to pheromone baited traps in bark beetles species that are not known to produce it, as seen in *Xylosandrus germanus* (Kohnle et al. 1992), *Dendroctonus ponderosae* (Huber et al., 1999), *D. pseudotsugae* (Huber et al., 1999, 2000, 2001), *Dryocoetes confusus* (Huber et al., 2000) *Pityophthorus setosus* (Dallara et al., 2000), *C. cornicolens* and *C. teocotum* (Rappaport et al. 2000), *I. pini* (Huber et al., 2000, 2001), *I. duplicatus* (Zhang et al., 2001), *I. sexdentatus* (in France) (Jactel et al., 2001) and *I. typographus* (Zhang & Schlyter, 2003). In spite of this fact, there are reports referring to conophthorin as attractant as well, according to results observed in *I. mexicanus*, *Lasconotus pertenuis* (Coleoptera: Colydiidae) and *P. carmeli* (Dallara et al. 2000) and in *Epuraea thoracica* (Coleoptera: Nitidulidae) (Kohnle et al., 1992).

Romón et al. (2007b) detected a significant negative dose-dependent relationship between verbenone release rate and catches of *I. sexdentatus* in *P. radiata* stands with traps baited with an specific *Ips sexdentatus* attractant blend (Myrcene 250 mg/day + Ipsdienol 0.20 mg/day + Ipsenol 0.40 mg/day) (Fig. 4). Four verbenone release rate were used, as follows: 0.01, 0.2, 1.8 and 3.1 mg/24h (at 22-24°C). It has been previously mentioned that bark beetles species which requiere relative fresh host tissues would have a less tolerance to verbenone. So, if we asume that *I. sexdentatus* requires relatively fresh phloem and may attack healthy and live trees when endemic populations outbreak, it is feasible to show a negative response to the presence of verbenone, as these results show. In contrast with these results, bark beetle species (*Tomicus piniperda*, *Orthotomicus erosus*, *Dryocoetes autographus*, *Hypothenemus eruditus*, *Xyleborus dryographus*, *Hylastes ater*, *H. attenuatus* and *Hylurgus ligniperda*), trapped accidentally while carrying out that bioassay, have been shown to be not affected significantly by verbenone.

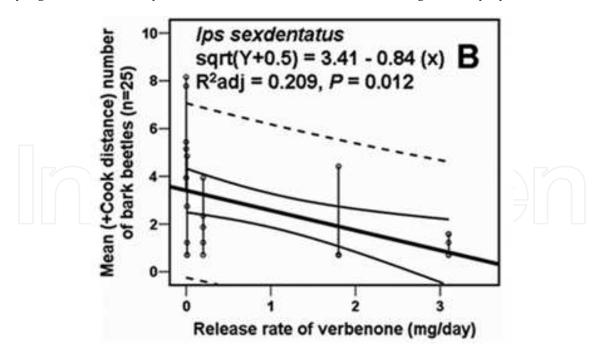


Fig. 4. Effect of four different release rates of verbenone on the attraction of *Ips sexdentatus*. Slope of regression line is significantly different from zero (t-test, P < 0.001). Confidence limits (95%) (thin solid lines) are associated with the regression line (thick solid line). Dashed lines represent confidence limits (95%) for catches in control trap.

Moreover, Etxebeste and Pajares (2011) tested verbenone and *trans*-conophthorin against *I. sexdentatus* populations present in mixed pine stands (*P. sylvestris, P. nigra* and *P. pinaster*). Two verbenone release rate (2, 40 and 60 mg/day) and one *trans*-conophthorin release rate (0.3 mg/day) were tested in two different field trapping bioassays with Ipsdienol 2.35 mg/day as attractant. Both verbenone and conophthorin and their combination significantly elicited a reduction of trap catches. Verbenone at 2 and 40 mg/day reduced the catches in a similar percentage (73% and 82% respectively) whereas *trans*-conophthorin reduced them by 45-49%. The strongest effect was showed by the blend of both compounds (verbenone at 40 mg/day plus *trans*-conophthorin at 0.3 mg/day) with a trap reduction rate of 90%. Another experiment was conducted to determine the potential of verbenone (at 60 mg/day) as a tool for tree protection. All control considered trees, that is, with no verbenone releasing device, were attacked by *I. sexdentatus*, whereas verbenone treated trees were less attacked.

4.2 The pine shoot beetle *Tomicus piniperda* L. (Fig. 5A) and Mediterranean pine shoot beetle *Tomicus destruens* (Wollaston) (Fig. 5B)

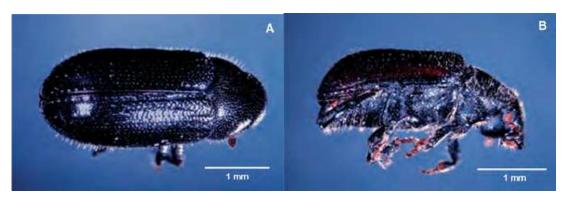


Fig. 5. (A) *Tomicus piniperda*, dorsal view; (B) *Tomicus destruens*, lateral view. Photographs taken from López et al., 2007a.

Genus Tomicus is represented by Tomicus piniperda, Tomicus minor and Tomicus destruens (Mediterranean pine shoot beetle) in Spain, since the latter taxa was definitively distinguished from *T. piniperda* and considered as a distinct species by the use of molecular techniques (Gallego & Galián, 2001; Kerdelhué et al., 2002). Morphologically, the colour of the elytra, colour of the antennal club, distribution of the antennal setae and distribution of the punctures along the elytral declivity seem to be the most useful diagnostic characters to differ both species, but only between mature exemplars (Faccoli, 2006). Concerning their distribution, T. destruens seems to be the predominant species in Spanish mainland, living in low an hot areas, whereas T. piniperda inhabit wet and cold areas of north-central Spain (Northern Plateau, the Pyrenees and perphaps in the Betic Mountains, at the South) (Gallego et al., 2004). However, there would be an overlapping of both species in the Atlantic Coast and the Bay of Biscay Coast, where they apparently coexist in sympatry. On the other hand, these authors consider T. minor as a less abundant species, with a fragmented distribution through high and wet areas. These potential distributions were suggested after applying predictive General Additive Models and Ecological Niche Factor Analysis models from 254 specimens of 81 different plots of Spanish mainland.

Although *P. sylvestris* is considered as the natural host of *T. piniperda*, its development in *P. radiata* (including maturation feeding in Monterrey pine shoots) is well reported. Host preference between both pine species has been tested in northern Spain. Amezaga (1996) observed that *T. piniperda* is able to exploit *P. radiata* as well as *P. sylvestris*. Even though brood production (number of progeny adults per gallery) was not significantly affected by tree species, the development was slowlier in *P. radiata*, and callows weighed less. In addition, after sampling two study areas at different altitudes (at 250 and 650 m) no sister generation was detected, so Amezaga (1996) hypothesized with the small chance of ocurring a second generation in northern Spain. She observed that attacks of *T. piniperda* began in March. Thus, according to her results *T. piniperda* might start its swarmming flight approximately in January over the entire altitudinal range in which pine stands are present in Northern Plateau.

Regardig to damage data, in 1989 massive outbreaks of Tomicus piniperda caused losses of 72 million € in Pinus radiata in Basque Country region (Northern Spain) (Amezaga, 1993). It has been estimated that a total of 200,000 ha have been affected by T. piniperda from 1990 to 1999 in Spain (Grégoire & Evans, 2004). The monitoring of *T. piniperda* populations is a common task carried out in different spanish provinces, but to our knowledge there has not been any control program with potential antiaggregant compounds until now. In contrast, researches aimed to T. destruens have been conducted in order to test the role of non host volatiles in its behaviour. Guerrero et al. (1997) showed by single-cell electrophysiological technique that *T*. destruens antennae possess specific olfactory cells capable of detecting benzyl alcohol. Furthermore, a ca. 700 mg/day release rate of this compound can significantly reduce the attraction of T. destruens to host logs. Besides, the development of an effective lure for monitoring populations of *T. destruens* has been another objective. Gallego et al. (2008) tested different releases rates of ethanol and a-pinene, alone or in combination (with an upper threshold of 1800 and 900 mg/day respectively) in monospecific P. halepensis stands of southern Spain. The addition of trans-verbenol was also tested, but it did not affect the response. a-pinene alone did not showed a strong attraction effect, but a synergistic effect when adding it to ethanol. The most attractive blend appeared to when releasing 300 mg/day of a-pinene and 900 mg/day of ethanol.

4.3 Twig beetle *Pityophthorus pubescens* (Marsham) (Fig. 6)

Genus *Pityophthorus* has been typically not considered as a major pest species-complex, due to its life habits and development in branches of mainly dead and decaying trees (Bright, 1981; Wood, 1982).



Fig. 6. *Pityophthorus pubescens*, dorsal and lateral view. Photographs taken from López et al., 2007a.

However, Pityophthorus setosus and P. carmeli has been associated with the causal agent of pitch canker Fusarium circinatum (Storer et al., 2004; Sakamoto et al., 2007). In Spanish mainland, it has been previously reported that Pityophthorus pubescens is also associated with F. circinatum in P. radiata stands of the Basque Country (northern Spain) (Romón et al., 2007b). Regarding the chemical ecology of *P. pubescens*, López et al. (2011) showed that both sexes emit (2R,5S)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran, also known as (E)pityol, through different techniques of volatile collection (PORAPAK-Q and Solid Phase Microextraction/SPME). Positive enantiomer of this compound is also a component of the aggregation pheromone of other species of the genus, such as P. pityographus (Francke et al., 1987) and P. carmeli, P. nitidulus and P. setosus (Dallara et al., 2000), and the female-produced aggregation pheromone of the cone beetles Conophthorus resinosae, C. coniperda and C. ponderosae (Pierce et al. 1995; Birgersson et al. 1995; Miller et al., 2000). However, in contrast with P. pubescens only one of the sexes of these species seems to emit (E)-(+)-pityol, as follows: males of P. pityographus and P. carmeli and females of P. nitidulus and P. setosus. Electroantennographic assays has revealed that both males and females of *P. pubescens* are able to detect (*E*)-(+)-pityol (López et al., 2011) (Fig. 7).

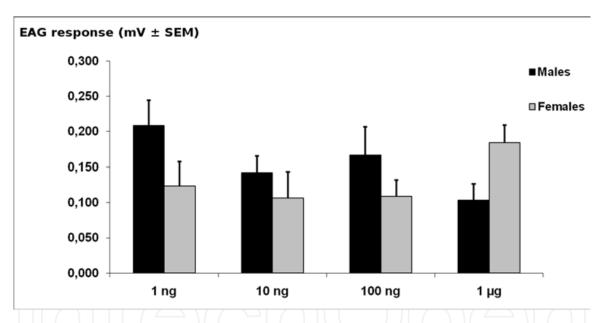


Fig. 7. Absolute EAG (mV± SE) responses of P. pubescens males (dark grey) and females (light grey) to serial dilutions containing 1 ng, 10 ng, 100 ng and 1 μ g of (E)-(+)-pityol. Means followed by different letters were significantly different (Two-way ANOVA followed by Tukey multiple range test (P≤0.05), n = 8).

Moreover males were more attracted to (E)-(+)-pityol and (E)-(±)-pityol in olfactometric bioassays when testing three different doses (from 1 to 100 ng in decadeic steps) (López et al., 2011) (Fig. 8). In addition, sex-ratio appears to be male-biased in field trapping performed in different *Pinus* spp. stands of the Basque Country with multiple funnel traps baited with (E)-(+)-pityol and racemic pityol (López et al., unpublished data). Thus, the use of (E)-(+)-pityol or its cheaper racemate form might be an useful tool for monitoring P. pubescens populations and even to trap out male beetles in P. radiata stands.

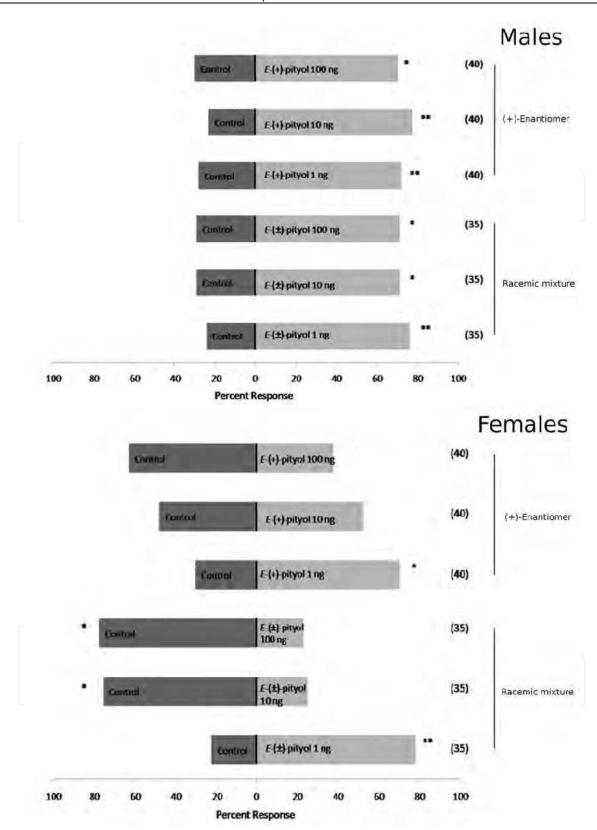


Fig. 8. Response of *P. pubescens* males (upper panel) and females (lower panel) at different doses of (*E*)-(+) and racemic (*E*)-pityol in Y-tube olfactometer trials. One and two asterisks indicate significant differences at P<0.05 and P<0.01, respectively (Chi-square test, with a significance level of a = 0.05). Number in parentheses indicates number of beetles responding.

On the other hand, verbenone shows promise as a disruptant of the aggregation of *P. pubescens*. Field studies has been undertaken in *P. radiata* stands testing four different release rates (0.01, 0.20, 1.80 and 3.10 mg/day) (at 22-24°C) of this compound (Romón et al., 2007b). This work revealed a significant negative dose-dependent relationship in captured insects when comparing with control traps baited with a racemic pityol-releasing device at 0.14 mg/day (Fig. 9).

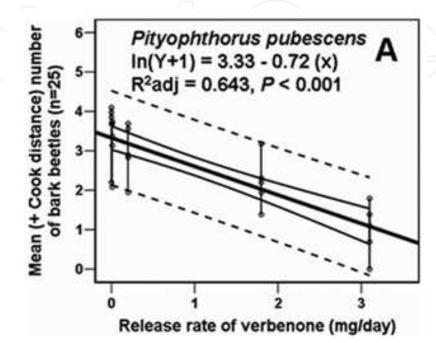


Fig. 9. Effect of four different release rates of verbenone on the attraction of *Pityophthorus pubescens*. Slope of regression line is significantly different from zero (t-test, P < 0.001). Confidence limits (95%) (thin solid lines) are associated with the regression line (thick solid line). Dashed lines represent confidence limits (95%) for catches in control trap.

4.4 Hylurgops palliatus (Gyllenhal) and Hylaster ater (Paykull)

Even though these two species (Fig 10 & 11) are considered as secondary, due to their colonization of dying or decaying trees, it is remarkable to mention their association with different species of pathogenic fungi (see subsection 2, table 3).

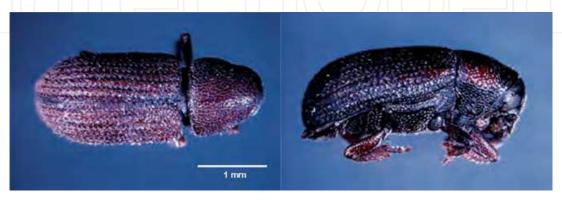


Fig. 10. *Hylurgops palliatus*, dorsal and lateral view. Photographs taken from López et al., 2007a.



Fig. 11. Hylastes ater, dorsal and lateral view. Photographs taken from López et al., 2007a.

5. Conclusions

At the light of what has been exposed, it is apparent that further extensive studies are needed to determine many gaps that still remain unclear. Excluding taxonomic and faunistic composition, which have been widely studied, little is known about the chemical ecology (including insect-host and insect-non host detailed interactions) of the species perceived as pest, although substantial progresses have been made. A deeper study of populations dynamics would be neccesary, in order to characterize better the brood production, flight periods and number of generations per year. This information would aid to a major understanding of which control methods should be applied. In addition, the development of useful IPM strategies, especially in the field of semiochemicals which might act as effective anti-aggregants, represents an important research line with many questions to be responded. Moreover, proper forestry management should be also recommended to be combined with, due to its relative influence on favoring the generation of populations outbreaks.

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Integrated Pest Management is an effective and environmentally sensitive approach that relies on a combination of common-sense practices. Its programs use current and comprehensive information on the life cycles of pests and their interactions with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means and with the least possible hazard to people, property, and the environment.

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