Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

On the multitrophic interactions between *Ips typographus* their tree host, associated microorganisms, and a predatory *Medetera* fly

Maria Sousa

Introductory Research Paper

Department of Plant Protection Biology, SLU Alnarp

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1. Introduction

Bark beetles (Coleoptera: Scolytidae) have an important role in forest ecosystems as pioneers in the recycling of nutrients in dead or dying trees (Edmonds and Eglitis 1989). However, some species from the genus *Dendroctonus (e.g D. brevicomis, D. frontalis* and *D. ponderosae*) or *Ips (e.g I. typographus*) are also able to attack healthy living trees, and kill them as result of mass colonization . When colonizing living trees, the beetles use an aggregation pheromone for a mass attack and introduce an associated blue-staining fungi into the tree tissue . Additionally, the fungus reduces the water uptake by the tree and disseminates through the wood causing discoloration and reducing the quality of the timber, making it unsuitable for the wood industry (Gitau et al. 2013; Paine et al. 1997).When the bark beetle populations are at high densities they can rapidly destroy millions of hectares of living forest, causing significant economic and ecological impact (Goheen and Hansen 1993; Kärvemo and Schroeder 2010a; Williams et al. 2018) The outbreaks are often triggered after wind damage, fire or drought (Aukema et al. 2008; Kausrud et al. 2012; Wermelinger 2004).

In order to reduce beetles outbreaks, different control strategies have been used (Wermelinger 2004). A common practice in the control of beetles, such as *I. typographus*, is the use of synthetic versions of the bark beetle pheromones (Jakuš 1998). Here, pheromone traps are developed to catch as many flying beetles as possible and reduce the population density in the area under attack. One negative aspect of using pheromone traps is that not only the beetles are caught, but also their natural enemies are being, as well, attracted and killed by the same traps (Aukema et al. 2000b), since a significant number of natural enemies among parasitoids and predators are known to respond to beetles pheromone components as kairomones, especially clerid beetles, such as *Enoclerus* and *Thanasimus* are attracted and traped (Erbilgin and Raffa 2001; Pettersson 2000; Pettersson and Boland 2003).

Over the years, the use of natural enemies for biological control of beetles, has increased (Aukema et al. 2000b, 2000a). One group of potentially important, but currently not used, natural enemies of Scolytidae are the long legged flies (Dolichopodidae) of the *Medetera* genus (Aukema and Raffa 2004; Beaver 1966). The biological control process is as follows: the flies are attracted to bark beetle attacked trees, where they lay their eggs on the bark surface, and the enclosed larvae migrate into the beetle galleries, where they feed on the beetle larvae. Larvae from this genus can consume between 5 to 20 individuals depending on bark beetle density (Beaver 1966) and more than ten *Medetera* larvae can be found per 100 cm^2 of attacked bark (Dippel et al. 1997). Together with other dolichopodidadae predators, they can account for more than 80% of the bark beetle mortality (Wermelinger 2002).

Although the importance of these predators for biological control of bark beetles have long time ago been identified, little information is available regarding *Medetera* biology and ecology. This lack of information despite the fact that the these flies are widespread in the forest, their life cycle is concealed and occurs under the bark of attacked trees. In additional, the available keys used for identification are incomplete and do not include all the species found. Therefore, a thorough compilation of *Medetera* biology is important to synthetize the existing knowledge, which is the first step for later understanding how these predators can be used to prevent or reduce future epidemic bark beetles attacks.

In this introductory essay, I start by providing an individual survey on the spruce bark beetle *I. typographus* and *Medetera spp*. in Sections II and III. Then, Section IV addresses several state-of-the-art contributions that study relevant aspects of their behavioral interplays. Finally, Section V wraps up the paper and suggests directions to future work that, based on the discussion in this review, which is believed to be valid hypotheses when it comes to understanding the use the *Medetera* as controling agents of *I. typographus*.

2. Bark beetles (Coleoptera: Scolytidae)

Bark beetles occur in different regions around the world and are associated with most major groups of terrestrial plants (Raffa et al. 2015). They spend a significant part of their life, to feed and reproduce, under the bark surface, in the inner bark or phloem of dead or dying trees and contribute among others to nutrient recycling, soil structure, forest regeneration and biodiversity (Edmonds and Eglitis 1989). Some beetles have a relative narrow host range and may utilize only one species of host tree, while others may utilize several species within a genus and sometimes more than one genera (Raffa et al. 2015).

Several bark beetles species can also colonize healthy mature standing trees and, when the weather and local forest conditions are favorable, their population can increase and infest large areas of conifer forests (Gitau et al. 2013; Kärvemo and Schroeder 2010a). These species are described as "aggressive bark beetles" where some of them, like *D. frontalis,* are "obligate tree killers", only attacking living, healty, standing (pine) trees, while others, like *I. typographus*, are "facultative tree killers", since they also attack wind fallen and weakened or dying (spruce) trees.

The most "aggressive" tree-killing species of bark beetles are from the genus *Dendroctonus* (e.g *D. ponderosae*, *D. frontalis* and *D. brevicomis*) in North America (Klutsch et al. 2009; Williams and Liebhold 2002) and the spruce bark beetle *I. typographus* in Europe (Christiansen and Bakke 1988). Beetles from the *Dendroctonus* genus, are usually more damaging compared to the beetles from the *Ips* genus. The most destructive one ~~is~~ used to be the Southern Pine Beetle (SPB, *D. frontalis*) attacking different species of pines in southeastern USA. But nowadays, the Mountain Pine Beetle (MPB, *D. ponderosae*) has an enormous outbreak in the district of British Columbia (B.C.) in Canada, where it is generally associated with the lodgepole pine (*Pinus contorta* Dougl.). However, the MPB can also attack and reproduce in a dozen others species of *Pinus* in North America. This beetle caused tree mortality of more than 600 million m^3 of logopole pine (*Pinus contorta*) in British Columbia between 1960 and 2009. During the same period in Sweden, three major outbreaks with *I. typographus* have occurred resulting in a 9 million m^3 of killed Norway spruce trees (*Picea abies*) (KärvEmo and Schroeder 2010b). And in continental Europe, bark beetles are estimated to have attacked more than 3 million ha of spruce forest, resulting in more than 32 million m^3 of killed trees, between 1990 and 2001 (Grégoire and Evans 2007).

The colonization and establishment of the so called "aggressive" bark beetles in alive and healthy host conifer trees always occur via a characteristic two step sequence of behavior: *(i)* first, a pioneer beetle (male or female depending on the species) identifies a suitable host tree and releases aggregation pheromones to attract more members of same species; *(ii)* second, when the beetles start excavating galleries, they introduce an associated fungi into the host tree.

In the next sections of this chapter we will focus on the biology of "aggressive" bark beetles giving special attention to the European spruce bark beetle *I. typographus*.

2.1 Life cycle of the European spruce *I. typographus*

During spring, when temperature exceeds 20 °C, adults disperse in the forest searching for a suitable host to mate and breed in (Wermelinger 2004). When a host tree is found the male starts excavating a nuptial chamber in the phloem under the bark (Figure 1). At the same time, an aggregation pheromone is released to attract conspecifics from both sexes. The *I. typographus* is a polygamous species, and each male usually mates with two or more females. After mating, females construct vertical maternal galleries for oviposition and the eggs are laid alternately along both sides of the maternal galleries (Mills 1986). The number of eggs laid per gallery is proportional to the gallery length (Anderbrant 1990) and each female can lay up to 80 eggs. The eclosed larvae feed on both cambium and phloem tissue, and symbiotic microbes (e.g. a blue staining fungus which they are associated with and which were introduced by the parental beetles during the galleries construction (Ayres et al. 2000; Graham 1967; Six 2003). Larval galleries radiate progressively from the maternal galleries during the four consecutive larval instars. At the end of each larval tunnel a pupal chamber is constructed where the larvae pupate.

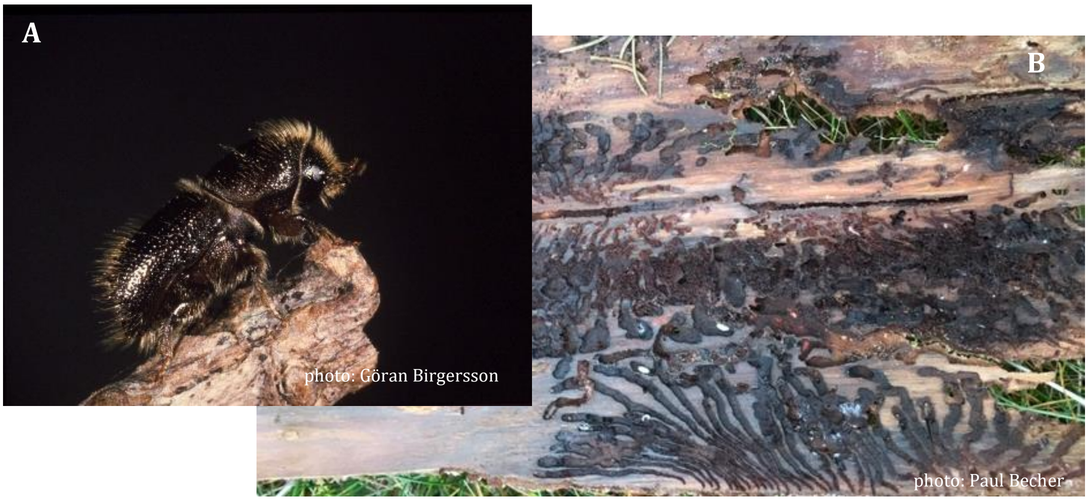


Figure 1: A female *Ips typographus* bark beetle; B bark beetle larval galleries under the bark of Norway spruce (*Picea abies*)

Environmental conditions play an important role for beetles' metabolism and development. If both the spring and the summer is hot and dry, early emerging brood adults might attack new trees, otherwise, they overwinter on the soil or less common under the bark of the host (Annila 1969). In Sweden, according to Weslien (1992) less than 10% of the *I. typographus* populations overwinter in logs and the flight period generally starts in May (Hedgren and Schroeder 2004). During warm springs the first flight period can occur already in April, and if the warm weather continues, as in 2006 and 2018, they can have a true second generation flight period during the summer. However, in general, the parental beetles will re-emerge and make "sister brood flights" once, or even twice, during the summer, depending on the weather.

According to Forsse (1985) when beetles colonize trees and begin to construct their galleries, flight ability is lost because flight muscles are considerable reduced in both sexes during this period. Females break down their flight muscles to produce their eggs while, males break down their flight muscles during the pheromone production. Both have to regenerate their muscles before the sister brood flight which may take days or weeks, depending on the weather conditions.

2.2 Beetles' associated microorganisms

Like many other insects, bark beetles have shown complex associations with microorganisms that are important for their survival under the bark. In general, associated microorganisms can provide nutrients for the developing larvae, protection against pathogens and tree defenses or assist in the bark beetle pheromone/semiochemical production (Morales-Jiménez et al. 2012; Vega and Hofstetter 2014). Different species of fungi, yeasts and bacteria have been associated with bark beetles.

2.2.1 Fungi

Bark beetles are tightly associated with different genera of fungi from Ascomycetes (e.g. *Ophiostama*, *Grosmannia*, *Ceratocystiopsis* and *Endoconidiophora*) or Basidiomycetes (e.g. *Entomocorticium*) (Evans et al. 2011; Kandasamy et al. 2016). Depending on the bark beetle species, the fungi can be transported to the host trees, in the guts, exoskeleton, in special invaginations of the cuticle called mycangia (Six 2003) or within nematode-containing structures called nematangia , when they start excavating galleries (Cardoza et al. 2006). The *I. typographus*, for example, has no mycangia and their associated fungi are carried in non-glandular pit-like structures on the exoskeleton, on pits and punctures of the head and pronotum on the elytra and in the gut (Furniss et al. 1990).

When the fungi is introduced into the host tree, it colonizes the beetle galleries and tree phloem tissues in close proximity to broods during larval feeding and help the beetle fitness by *(i)* detoxifying the terpene rich phloem and providing nutrients for the bark beetle larvae (Adams et al. 2009), *(ii)* exhausting tree defenses by over-stimulating the production of oleoresins or phenolic compounds (Hammerbacher et al. 2013; Zhao et al. 2011) and *(iii)* accelerate tree death by reducing or disrupting transpiration and vital tree processes that are important for the living trees (Furniss et al. 1990; Krokene and Solheim 1998). Some filamentous fungi can also give origin to a blue, grey or black discoloration of the wood reducing the quality of the timber, with high economic impact on the wood industry (Gitau et al. 2013).

The *I. typographus* associated fungi are reviewed in Table 1. According to Kandasamy et al. (2006) the details of these associations are not well understood. The fungi isolates found in the *I. typographus* galleries changed according to the stage of the attack, but also between the different geographic areas. The different ophiostomatoid *spp* found associated with *I. typographus*, for example, might be due to differences in optimal temperature required by the fungi to grow, or due to local adaptations to climate, phloem moisture levels, host phloem chemistry, which may change according to tree species (Lindström et al. 1989).

2.2.2 Yeast

Yeasts are the most abundant fungi found associated with bark beetles. They have been found associated with all developmental stages of the beetles, gallery walls, pupal chambers and xylem tissues of the host tree (Hofstetter et al. 2015; Lewinsohn et al. 1994). Each individual adult often carry two or more yeast species and unlike the filamentous fungi, which seems to have species – specific associations with beetles, several species of yeasts were seen to be commonly carried by several bark beetle species (Six 2003). So far, many yeast species such as *Pichia holstii, Pichia pinus, Hansenula capsulata, Candida diddensii, Candida nitratophila, Cryptococcus spp.* and *Metschnikowia spp*. have been found associated with several *Ips* species in Europe (Händel et al. 2003; Leufvén et al. 1984; Lukášová et al. 2013; Vega and Hofstetter 2014). The *I. typographus* associated gut yeasts are reviewed in Table 1.

Bark beetle associated yeasts are known to produce volatile compounds that can have positive effects on beetle performance by attracting or repelling bark beetles to or from the host tree (Hunt and Borden 1990; Leufvén et al. 1984) or by promoting the growth of mutualistic fungi and inhibiting or delaying the pathogenic fungus establishment in the gallery environment (Adams et al. 2008). Moreover, some yeast species metabolize toxic tree chemicals such as terpenoids that are present on phloem resins, into less toxic compounds that may be important for the beetle tolerance or survival on the host tree (Sutherland 2004). In addition, yeasts that are inoculated by the adult bark beetles into the host phloem can be used as food source for brood bark beetle larvae (Graham 1967).

2.2.3 Bacteria

Associated bacteria have mainly been identified from the beetle guts. However, a few studies have also showed the presence of bacteria in the exoskeleton, mycangia and gallery walls (Hulcr et al. 2011; Morales-Jiménez et al. 2012; Scott et al. 2008). The bark beetle adult guts included bacterial species from the genera *Rahnella*, *Bacillus*, *Chryseobacterium, Acinetobacter, Enterobacter, Klebsiella, Pantoea, Pseudomonas* and *Serratia* (Adams et al. 2009; Bridges 1981; Moore 1971; Muratoglu et al. 2011; Sevim et al. 2012; Vasanthakumar et al. 2006; Vega and Hofstetter 2014). The *I. typographus* associated bacteria are reviewed in Table 2 It was also shown that adults, larvae and pupae guts differ in bacterial diversity, which could be related to the different metabolic activities and may indicate that some bacteria are essential only during a specific developmental stage of bark beetles life cycle (Morales-Jiménez et al. 2012).

The bark beetle associated bacteria are important for the bark beetle development, survival and colonization of host trees. Some associated bacteria can fix nitrogen (Bridges 1981) or recycle uric acid increasing the nitrogen or carbon content on the bark beetles diet, which is crucial for their survival (Morales-Jiménez et al. 2013). Others influence the growth and reproduction of bark beetles associated fungi by producing volatile compounds (Adams et al. 2009) or antibiotics that can inhibit the growth of an antagonistic fungus (Cardoza et al. 2006; Scott et al. 2008). In addition, some of the associated bacteria help the bark beetle overcoming host defense by metabolizing toxic monoterpene hydrocarbons and diterpenes acids (Boone et al. 2013; Howe et al. 2018)

2.3 Bark beetle chemical communication

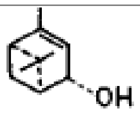
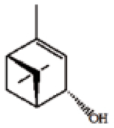
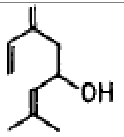
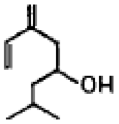
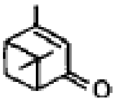
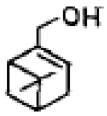
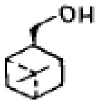
Bark beetles often use olfactory signals to communicate and interact with their hosts and among individuals of the same or different species. The communication is mediated by semiochemicals that can be divided into pheromones and allelochemicals. Pheromones are intraspecific signals used for communication within species while allelochemicals are interspecific signals used for communication between species.

Table 1: List of *I. typographus* associated microorganisms and their volatile compounds.

Microorganism	**VOC´s emitted**	**Presence on Beetles**			**Ref**
		Phloem/Sapwood	**Adults**	**Galleries**	
Fungi					
Endoconidiophora polonica	isoamyl alcohol, isoamyl acetate, 2-phenylethanol, 2-phenylethyl acetate, geranyl acetone		x		(Kandasamy et al. 2016)
Grosmannia europhioides	isoamyl alcohol, isoamyl acetate, 2-phenylethanol, 2-phenylethyl acetate, 1-hexenol,		X		(Kandasamy et al. 2016)
Grosmannia penicillata	isoamyl alcohol, isoamyl acetate, 2-phenylethanol, 2-phenylethyl acetate, benzyl alcohol, (*E*)-β-caryophyllene		X		(Kandasamy et al. 2016)
Ophiostoma bicolor	isoamyl alcohol, isoamyl acetate, 2-phenylethanol, 1-hexenol, geranyl acetone	x	X		(Kandasamy et al. 2016)
Ophiostoma ainoae		x	X		(Kandasamy et al. 2016)
Ophiostoma piceae	isoamyl alcohol, isoamyl acetate, 2-phenylethanol, 1-hexenol, 1-octanol, 1-nonanol, benzyl alcoho	x	X		(Kandasamy et al. 2016)
Yeast					
Hansenula holstii	α-terpineol, 2-phenylethanol, isoamyl alcohol,, isoamyl acetate, 2-phenylethyl acetate		X	x	(Brand et al. 1977; Leufvén and Nehls 1986; Leufvén et al. 1988)
Hansenula capsulata	borneol, α-terpineol, terpinene-4-ol, *trans*-pinocarvol, myrtenol, 2-phenylethanol, verbenone		X	x	(Hunt and Borden 1990; Leufvén and Nehls 1986; Leufvén et al. 1988)
Candida diddensii	borneol, α-terpineol, terpinene-4-ol, verbenone		X	x	(Brand et al. 1977; Leufvén and Nehls 1986; Leufvén et al. 1988)
Candida nitratophila	borneol, α-terpineol, terpinene-4-ol, myrtenol, 2-phenylethanol, verbenone		X	x	(Brand et al. 1977; Leufvén and Nehls 1986; Leufvén et al. 1988)
Cryptococcus albidus	borneol, α-terpineol, terpinene-4-ol		x	x	(Leufvén and Nehls 1986; Leufvén et al. 1988)
Cryptococcus laurentii	borneol, α-terpineol, terpinene-4-ol		x	x	(Leufvén and Nehls 1986; Leufvén et al. 1988)
Pichia pinus	borneol, α-terpineol, terpinene-4-ol, verbenone, isoamyl alcohol, isoamyl acetate, 2-phenylethyl acetate		x		(Brand et al. 1977; Hunt and Borden 1990; Leufvén and Nehls 1986)
Bacteria					
Bacillus sphaericus			x		(Muratoglu et al. 2011)
Bacillus fusiformis			x		(Muratoglu et al. 2011)
Acinetobacter lwoffii			x		(Muratoglu et al. 2011)
Acinetobacter junii			x		(Muratoglu et al. 2011)
Acinetobacter calcoaceticus	sulfoacetaldehyde		x		(Muratoglu et al. 2011), http://bioinformatics.charite.de/mvoc/#
Acinetobacter baumannii			x		(Muratoglu et al. 2011)
Kluyvera sp.			x		(Muratoglu et al. 2011)
Proteus vulgaris			x		(Muratoglu et al. 2011)
Serratia liquefaciens			x		(Muratoglu et al. 2011)
Vagococcus sp			x		(Muratoglu et al. 2011)

2.3.1 Pheromones

Table 2: Pheromone candidates of *I. typographus* found in the hindguts of males attacking *Picea abies* according to Birgersson (1984) and Schlyter (1987).

Compounds	Structure	Activity
2-methyl-3-buten-2-ol		Aggregation pheromone component (promote landing)
(–)-*cis*-verbenol		Aggregation pheromone component (long distance attraction)
(+) - *trans*-verbenol		No effect
Ipsdienol		Not clear. It has been shown that it increases the trap catches but not significantly
Ipsenol		Regulates density of attack
Verbenone		Only minor amounts detected in beetles hindguts. Mainly produced by associated microorganisms as anti-aggregation/inhibition pheromone
Myrtenol		No pheromone effect; "only" a detoxification product from α-pinene
***trans*-myrtanol**		No pheromone effect; "only" a detoxification from β-pinene
2-phenylethanol		Maybe density regulation on bark surface

Bark beetle pheromones are usually made up of a mixture of compounds that can either be synthesized from host precursors or by *de novo* pathways (Ivarsson and Birgersson 1995; Ivarsson 1998; Lanne et al. 1989). The quantitative and qualitative composition of pheromones is unique for each bark beetle species. Bark beetles are known to produce at least two different types of pheromones: the sex pheromone and the aggregation pheromone. The sex pheromone is produced by males to attract and orient females for the mating site.

The aggregation is an essential behavior in the life of “agressive” bark beetles, such as *I. typographus* and several species of *Dendroctous*, as it helps beetles to colonize living host trees and coordinate their attack. The aggregation pheromone is released by a male or a female, depending on the species, after a suitable host tree has been identified and attracts conspecifics of both sexes for mating and a coordinated mass attack on a selected host tree (Kandasamy et al. 2016). Additionally, the aggregation pheromone from one bark beetle species may also work as inhibitory signal for other species indicating that this specific tree has already been occupied (Birgersson et al. 1994).

The aggregation pheromones from bark beetles generally include a mixture of oxygenated hemi- and monoterpenes, cyclic acetals and often in combination with host tree produced monoterpene hydrocarbons. The monoterpene hydrocarbons are usually host tree produced kairomones that enhance/synergize the bark beetle produced compounds. In some *Pityogenes* species, fatty acid derivatives can also be part of the aggregation pheromones (Birgersson et al. 1990; Birgersson et al. 2000; Byers et al. 1990).

Pheromone candidates found in the *I. typographus* males’ hindguts are shown in Table 2. The *I. typographus* has an aggregation pheromone composed by (–)-*cis*-verbenol and 2-methyl-3-buten-2-ol (Birgersson et al. 1984; Krawielitzki et al. 1977). While methylbutenol is a short range attractant and promotes landing, *cis*-verbenol is a heavier and less volatile compound that acts at longer distance (Schlyter et al. 1987). The 2-methyl-3-buten-2-ol is *de novo* produced (Lanne et al. 1989), but the (–)-*cis*-verbenol is one of very few bark beetle produced pheromone components that are detoxification/hydroxylation products from host tree defense toxic monoterpene hydrocarbons: in this case from (–)-α-pinene; the other is (+)- and (–)-*trans*-verbenol, in several *Dendroctonus* species, from (+)- and (–)-α-pinene, respectively. *I. typographus*, on the other hand, detoxify (+)-α-pinene to (+)-*trans*-verbenol, but do not use the compound in the pheromone blend (Birgersson 1989; Per Ivarsson and Birgersson 1995; PRJ Ivarsson 1998; Renwick et al. 1976).

The corresponding keton, verbenone, is produced also by males of some *Dendroctonus* species as an anti-aggregation compound. "All other" monoterpene alcohols used by bark beetles are *de novo* produced (Blomquist et al. 2010).

The pheromone compounds accumulate in the male hindguts right after they start boring a host tree and the pheromone blend is exuded or excreted with the beetle fecal pellets (Evans et al. 1985). According to Birgersson et al. (1984), the proportion of these pheromone candidates varies in relation to the attack sequence of the bark beetle. For example, the maximal amounts of methylbutenol, the pinene alcohols, and the aromatic alcohol 2-phenylethanol were detected when the males were excavating their nuptial chambers, before accepting the females while ipsenol and ipsdienol were only detected in male's hindguts after the acceptance of females and the amounts increased when the females started laying their eggs.

2.3.2 Microbial produced compounds

Bark beetle associated microorganisms are also known to produce compounds that may be used as subtracts by other associated microorganisms or directly by the beetles for the production and regulation of pheromones and other allelochemicals. For example, when *I. typographus* associated yeasts are established in their host tree, this indicates that this spot of the host tree is "taken"/"concurred" by the beetles, and they do not need any more attacks on the host tree to kill it. Therefore, the male beetles reduce their production of aggregation pheromone, and instead the yeasts produce *trans*-verbenol and verbenone, which prevents new attacks nearby on the tree surface, and reduces larval competition (Bakke 1981; Leufvén et al. 1984).

In Table 1 I have summarized the associated *I. typographus* microorganisms and some of their volatile products described in literature. Most of the compounds produced by the fungi and the yeast are oxygenated hemi- and monoterpenes or low molecular weight aliphatic and aromatic alcohols and ketones. Many of these compounds have already been described to influence bark beetle behavior. For example, isoamyl alcohol, isoamyl acetate and 2-phenylethyl acetate are synergic attractants thought to play a role in bark beetles' attraction to their symbionts or to symbiotic habitats (Brand et al. 1977). The 1-hexenol produced by *Ophiostoma* spp. and *Grosmannia* spp. is known to interrupt pheromone response of *I. typographus* (Zhang et al. 1999). Although the benzyl alcohol has no antennal or behavioral response on *I. typographus* it has been shown to disrupt aggregation pheromone of *D. ponderosae* (Borden et al. 1998). And the verbenone produced by both *Candida* spp. and *Hansenula capsulate* has shown to inhibit attraction of *I. typographus* to its aggregation pheromone (Schlyter et al. 1989).

Compounds such as α-terpineol, terpinen-4-ol, *trans*-pinocarvol and borneol are oxygenated monoterpenes thought to be produced by the tree as defensive response against bark beetles. However, it has been observed that, the production of these compounds starts at the beginning of the bark beetle attack but increases while females elongate their galleries, especially when the gallery walls are brown stained, which might be an indicator of the establishment of associated microorganism and a sign that these microorganisms are participating in the production of these compounds (Birgersson and Bergström 1989). However, the exact effect of these four compounds on the beetles is not yet known.

2.4 Bark beetle natural enemies

Natural enemies such as predators and parasitoids have an important role in the population dynamics of bark beetles (Vega and Hofstetter 2014). Parasitoids are organisms that spend a significant part of their larval development by feeding on arthropod hosts. One single arthropod host can sustain the development of one or more parasitoids and it is usually killed during the development of the parasitoids (Pettersson 2000). Most of Scolytids parasitoid species belong to the Hemynoptera order (Braconidae and Pteromalidae) and can attack various developmental stages of bark beetles. One parasitod larvae consume only one bark beetle larvae or pupae. The *Coeliodes bostrichorum* was the most efficient parasitoid of *I. typographus* reported, and the species seems to be entirely confined to the bark beetles breeding in spruce (Feicht 2006; Kenis et al. 2007).

Predators, on the other hand, are carnivorous species that normally feed on more than one prey during their development or during their adult life stage. Most predators, like clerid beetles, arrive shortly after the bark beetle attack started and often colonize the lower parts of the boles/trunks, while parasitoids come a bit later and often prefer the upper parts of the tree where the bark is thinner (Wermelinger 2002).

Many species of predators are associated with the bark beetle galleries. However, only a few are known to forage on eggs, larvae, pupae or adults bark beetles. Most Scolytids predators belong to the Coleoptera (Cleridae, Trogossitidae and Rhizophagidae) (Billings and Cameron 1984; Grégoire et al. 1991; Mills 1985; Weslien 1992) and Diptera (Dolichopodidae and Lonchaeidae) (Hopping 1947; Mills 1985; Wermelinger 2002). The Coleoptera (beetles), such as *Thanasimus formicarius (L.)*, are so far the most investigated predators of *I. typographus* (Kenis et al. 2007); the adults can prey on up to three bark beetle individuls per day, while larvae can prey on approximately fifty bark beetle larva during their whole larval stages/development (Dippel et al. 1997; Mills 1985).

Both predators and parasitoids are known to exploit bark beetle pheromones (Erbilgin and Raffa 2001; Grégoire et al. 1992), bark beetle larval frass odours (Grégoire et al. 1991), volatiles emitted by the tree under attack (Gijzen et al. 1993), or the volatiles produced by the introduced microorganisms on the surrounding bark tissue (Boone et al. 2008).

3. *Medetera* long-legged flies (Diptera: Dolichopodidae)

The long legged flies (Dolichopodidae) is one of the largest families of Diptera, where most of the species are predators and play an important ecological role as natural enemies of a wide variety of organisms. Many species from the *Medetera* genus, for example, are known to prey on Scolytidae brood at early developmental stages (Fisher von Waldhem, 1819). In this chapter I will describe the most important aspects of *Medetera* life, known so far.

3.1 Biology of *Medetera* genus

Species of the *Medetera* genus are among the less studied and most difficult dolichopodidae flies to identify. So far, the genus includes more than 250 described species, however new species are frequently discovered and none of the current available keys seems to be enough for identification (Bickel 1985; Pollet et al. 2011).

In general, the adults from this genus are described as relatively small flies (length 1.2 to 4.4 mm) with a body coloration usually from dark, metallic green to black. The head is strongly concaved dorsally with adjacent antennae covered in small sensillas, the sacape and pedicel are usually yellow and short. They have a long arista almost at the tip of the antenna. The eyes are large, oval, widely separated, usually dark green, appearing dark red in dried specimens. The palpi are typically dark brown with strong apical seta and short hairs. The proboscis is normally large and massive. They have a concaved thorax covered with heavy pruinosity. The abdomen is cylindrical covered with short setulae, female sterna abdomen presents color like bands, while, the male sterna is modified to receive the hypopygium, which is bended and tucked up against and slightly enfolded by the abdomen. The male hypopygium is a very complex structure that contains a range of different characters used not only to differentiate between males and females but also for species recognition or identification. The legs are elongated with poorly developed bristles and the wings usually hyaline (Figure 2) (Bickel 1985; Teskey et al. 1981).

<u>Distribution and hosts</u>

Species from the *Medetera* genus are globally distributed (Pollet et al. 2011). The geographic-ecological variation seems to be related to their habitat (Bickel 1985). Most of *Medetera spp.*

are sensible to cold and have been mainly found from early spring until late summer. Adults are typically found in largest numbers at vertical surfaces such as tree trunks (Beaver 1966; Bickel 1985). During field experiments, Bikel (1985) observed that smooth barked trees are preferred over trees with rough, gnarled bark. In Scandinavia for example, it has been observed that among conifers, Norway spruce trees are preferred over Scots pine. Besides the bark texture, the size and position of the tree seems to be important criteria used by adults.

Adults concentrate at the lower parts of trunks (Kenis et al. 2007; Nicolai 1995; Wermelinger 2002). For example, adults of *M. jacula* were observed to mostly concentrate on the trunks between 0 – 3 m high or on the grass around the tree (https://diptera.info/articles.php?article_id=12). While landing on the tree bark, *Medetera* flies keep their head always facing upward and the forelegs positioned so that the body is inclined from the surface. If disturbed, adults fly off immediately and land in a short distance nearby in the same tree always maintaining the upright position (Bickel 1985).

According to Bickel (1985) *Medetera spp* can also be found in very dry habitats or in non-wooded areas such as grasslands, deserts and/or beaches.


photo: Maria Sousa
photo: Göran Birgersson
photo: Göran Birgersson

Figure 2: Adults and larvae from *Medetera* genus. To the left a male and in the middle an ovipositing female. To the left a *Medetera* larvae which is recognized by the V-shape mandibular. The larvae is in a cocoon shape, normally formed before pupation. The samples were collected from a bark beetle attacked spruce tree at Asa, Sweden.

Feeding

Medetera adults possess a massive and powerful proboscis used to predate on small arthropods with a soft integument (Kenis et al. 2007; Nicolai 1995). Feeding has been reported on spiders, mites, small chilopods, Collembola, Psocoptera, Thysanoptera, small Diptera (Sciaridae, Psychodidae, Cecidomyiidae), Homoptera, especially aphids, and early instar caterpillars (Bickel 1985).

The prey is detected by their movement from a distance of 0.5 - 7 cm and usually followed before the attack. Dead or inactive preys are not attractive. Preys that are too large or to active are usually abandoned. The fly strikes rapidly capturing the prey between the extended labella. Small preys are completely swallowed by the flies. Depending on the prey size, the swallowing process can take from five seconds to ten minutes. Large preys are held by their abdomen and slowly ingested. The cuticle or appendages of the prey are discarded (Fitzgerald 1968) (https://diptera.info/articles.php?article_id=12).

The predatious larvae of *Medetera* live under the bark of dead or dying trees (Bickel 1985), where they prey on Scolytidae larvae, pupae and new emerged adults (Kenis et al., 2007; Vega and Hofstetter, 2014). However, *Medetera* larvae are not entirely confined to a diet of Scolytidae but was also observed to attack Diptera, Hymenoptera and even penetrate Braconid coccons (Beaver 1966). According to Aukema (2004), which has observed the killing action of *M. bistriata*. *Medetea* larvae uses a toxin to first immobilize or kill the prey before start feeding. Moreover, morphological observations suggest a structure of a peptide neurotoxin.

Courtship and mating

Courtship and mating among *Medetera spp* is not well described and no available information was found regarding sound and scent. Mating has been observed to occur only after a serious of unsuccessful attempts by the male to copulate (Fitzgerald 1968). During mating, the male approaches the female from behind, without face to face courtship, and arches over the receptive female, he curls his abdomen forward so that hypopygium gets in contact with the distal end of female abdomen. While copulating males thrust repeatedly forward with their abdomen and use their forelegs on either side of the female abdomen to help stabilizing the coupling. Copulation usually lasts for several minutes (Bickel 1985; Schmid 1970) or 10-25 seconds as observed for *M. jacula* (Bickel 1985).

Until now, it is not clear if mating occurs in the same tree where oviposition occurs. According to Hopping (1947) mating may occur on infested bark beetle trunks. However, according to Bikel (1985) the aggregation trees for mating are really those on which females oviposit and only one *Medetera* species has been found at a given mating site. In my field experiments, I have observed the presence of both males and females on recently bark beetle attacked trees, however, the number of females seems to be much more high compare to the number of males and althrough not so frequent, I have also observed matings on the same trees where oviposition occurs.

Moreover, according to Bikel (1985) *Medetera* aggregates for mating in small groups or “leks”. In these “leks” the number of males are usually more abundant than females (ratio 2:1 or 3:1) and this is because, while the females apparently leave the tree shortly after mating to start searching for suitable oviposition sites, the males remain in the same tree waiting for further females.

__Oviposition__

Gravid *Medetera* females has been seen searching for an oviposition site by exposing the tip of the long ovipositor over the tree bark (Birgersson pers. comm.). According to Wermelinger (2002) althrought, the bark thickness seems to not influence oviposition, females typically prefer to oviposit in the lower parts of the trunks. However, according to Bikel (1985) females have broad tastes and will deposit their eggs in various subcortical environments.

4. Interaction between *Medetera* and *I. typographus*

Medetera is one of the most abundant predators found on *I. typographus* attacked logs that seem to contribute the most to beetle mortality (Hedgren and Schroeder 2004; Wermelinger 2002). In a recent report (Schroeder 2009), it was found that the number of successful bark beetle attacks, measured as number of broods per female gallery, a few years after the Swedish hurricanes Gudrun and Per, have decreased dramatically when the number of *Medetera* predators have increased, while the number of hymenopteran parasitoids remained at the same level during the last three years, see Figure 3.

According to Weslien and Regnander (1992) each *Medetera* larvae was estimated to consume around five *I. typographus* individuals and the density of 38 *Medetera* larvae found per m^2 of attacked bolts were assessed to eat in around 100-200 *I. typographus* offspring. Wermelinger (2002) also found that together with other dolichopodidadae predators, they accounted for more than 80% of the bark beetle mortality.

Throughout the whole summer, the females of *Medetera* are able to lay eggs in newly infested trunks very soon after an infestation of bark beetles (Nicolai 1995). I have in my own fieldwork seen *Medetera* females ovipositing in mid-June, on spruce trees that were attacked by *I. typographus* in mid-May. These findings are supported by the emergence dynamics of insects from bark beetles infested spruce trees, see Figure 4 from Wermelinger, (2004) which shows a lag of about one week after the *I. typographus* are peaking.

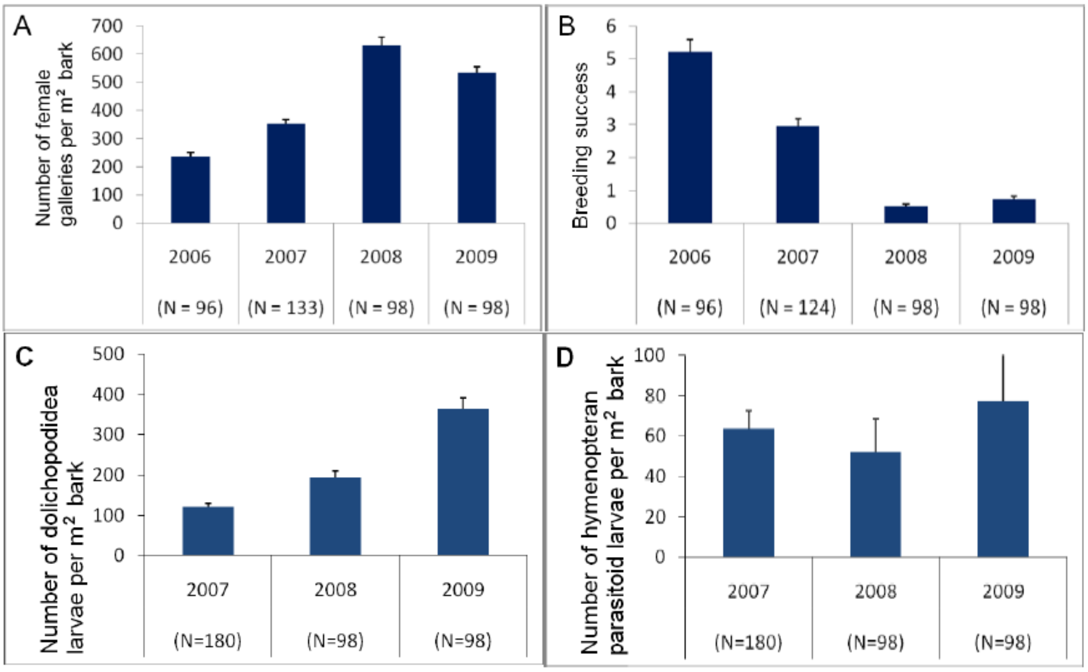


Figure 3: Emergence quantification of spruce bark beetles and associated natural enemines. Although the number of female galleries remains high (A), the number of successful brood production, as number of female brood per female gallery, dramatically decreased (B) due the pronounced increase in the number of predacious dolichopodid larvae (C). The number of hymenopteran parasitoids remain constant (D) (*all figures from Schroeder, 2009*)

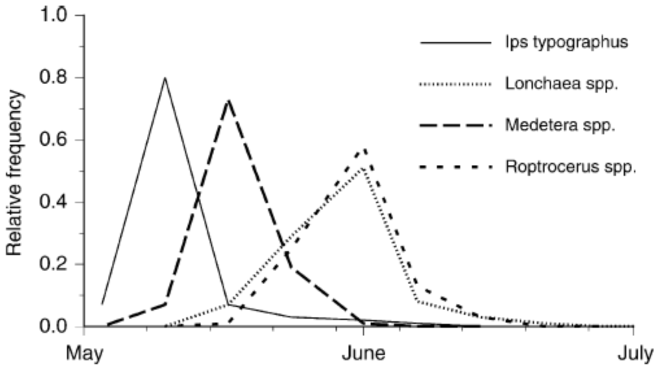


Figure 4: Emergence dynamics of bark beetles and associated natural enemines from logs of bark beetle-infected spruce trees: *I. typographus* (Scolytidae) (*figure from Wermelinger 2004*).

4.1 Predator-prey relationships

When a suitable host is found, the females deposit their eggs in bark cervices and under scales always near or at the entrances of bark beetle galleries (Bickel 1985). Usually, 1- 4 eggs are laid at a time, and one gravid female can lay over 100 eggs (Beaver 1966) i.e. *M. dendrobaena* was observed to produce up to 120 eggs (Dippel et al. 1997). The eggs hatch after about ten days and the newly eclosed larvae penetrates into the bark beetle larvae galleries, where they seemed to orient toward mining prey (Beaver 1966). The larvae presents a well-developed pseudopodia that allows the movement over dry bark surfaces from the oviposition sites to the gallery entrance (Fitzgerald 1968). According to Nagel and Fitzgerald (1975) predatory larvae are unable to penetrate unmined phloem but they can slowly move in a phloem through the prey galleries even if tightly packed with frass and the movement seems to be facilitated after the mined inner bark began to dry out/up.

The initial attacks of the first instar larvae *M. aldrichii* occurred within 2 or 3 days of host eclosion. Larvae seems to be attracted by the activity of the feeding host, prior to stablishing physical contact. *Medetera* larvae are known to strike the bark beetle larvae with their tentorial rods and inject a toxin before they are paralyzed and consumed (Aukema and Raffa 2004). Beetle larvae are completely consumed with the exception of the head capsule and cuticle (Nagel and Fitzgerald 1975). Larvae can consume five to 20 individuals during their larval development stage (Beaver 1966) but the consumption rates seems to increase with an increasing bark beetle density (Nicolai 1995). When the number of abundant prey is high, *Medetera* larvae can kill more than necessary; but if food is scarce, they can be cannibalistic (Beaver, 1966). According to Dippet et al. (1997) more than ten *Medetera*, larvae can be found per 100 cm^2 of attacked bark.

Under laboratory conditions four larval stages of *M. dendrobaena* can be distinguished and the larval stages are well separated from each other (Nicolai 1995). When the larvae reach the mature stage, they pupate close to places where the adults can easily exit. According to Fitzgerald (1968), prepupal *M. aldrichii* larvae are photo-sensible and usually follow the light entering the bark beetle ventilation holes to locate potential pupation and exit sites. When a suitable site for pupation is found, a coccon is first constructed, in which the larva lies in a “U” shape for a week or longer (Figure 1). The pupal phase lasts for about 18-21 days (Beaver 1966).

The number of generations per year is not fixed, adults can be found on infested trunks lying their eggs during the whole summer, until the first periods of frost in autumn (Nicolai 1995; Stephen and Dahlsten 1976). However, until now, it is not clear how *Medetera* spp. overwinter.

According to Dr. Heino Öunap (University of Tartu, pers. comm), the *Medetera* larvae overwinter under the bark of bark beetle attacked trees, and pupate and emerge the next summer, not published results. Moreover, according to Wermelinger (2012) a substantial mortality of these maggots has been detected over winter.

4.2 Predator-prey specificity

So far, it is hard to determine/discusse host specificity of *Medetera*. According to Beaver (1966) the different *Medetera* species seemed to be confined to tree species or group of species, to the bark texture and diameter and to specific environmental conditions. However, according to Bicker (1985) almost all *Medetera* species associated to conifer attacking scolytidae, belong to the *M. signaticornis-M. pinacola* group (for more details of the species see Table 3) and although some of these species appear to have a principal association with their prey/host (e.g. *M. aldrich* with the bark beetle species *Dendroctonus* and *M. bistriata* with tree genus *Pinus*), others have been found in different tree hosts, bark beetle hosts and sometimes have been found emerging from the same log.

4.3 Predator-prey location mechanisms

How the different *Medetera spp* locate attacked trees or how they locate their prey underneath the bark is still not understood. But olfaction and acoustics, as well as other cues, may play an important role during host/prey location and capture.

4.3.1 Olfactory cues

The odor surrounding a bark beetle attacked spruce tree consist of a large number of volatiles, in which, the sequence and the concentration variates between the different stages of the bark beetle attack (Birgersson et al. 1984; Birgersson and Bergström 1989; Pettersson and Boland 2003). Until now, it is thought that multiple semiochemicals may be involved in the host location of *Medetera*, since the flies start arriving to infested logs shortly after colonization by bark beetles but their presence and ovipostion extends through the whole bark beetle brood development, even after pheromone emission is ceased. Some of the semiochemicals that may be involved in the host location of *Medetera* are e.g. *(i)* compounds from bark beetle pheromones, *(ii)* compounds produced by the tree in response to the bark beetle attack or *(iii)* compounds from bark beetle associated microorganisms. The semiochemicals already tested on *Medetera* are summarized in Table 4.

(i) Compounds from bark beetle pheromone

Many species of parasitoids and predators are attracted to the prey pheromone compounds and others signals, such as interspecific repellents (Hulcr et al. 2005). The insect natural enemies can also respond to individual compounds or only to the exact proportions of the whole blend. Although little information in known, studies have shown that *M. bistriata* adults do not respond to the individual compounds of the host bark beetle pheromone, but are strongly attracted by the blend of (*S*)-*cis*-verbenol and 2-methyl-3-buten-2-ol (Williamson 1971). In another study, the attraction of adults of *M. setiventris* and *M. melancholica* was considerably higher when the aggregation pheromone was combined with a mixture of host tree volatiles such as, α-pinene and limonene (Hulcr et al. 2005; Hulcr et al. 2006).

(ii) Compounds produced by the tree in response to the bark beetle attack

Spruce host trees produce several monoterpene compounds in response to the bark beetle attack. These volatile products may serve as attractants for beetle predators and parasitoids (Gijzen et al. 1993). According to Hulcr et al. (2005) *M. setiventris* adults prefer freshly attacked trees which produce mainly unoxidized monoterpenes (e.g. α-pinene, β-pinene, limonene). Similarly, *M. signaticornis* was attracted by a solution of the spruce monoterpenes on ethanol (Rudinsky et al. 1971). The α-pinene and β-pinene, are the main constituents of attacked spruce tree and have stimulated ovipositon of gravid females and attracted the newly eclosed larva from the oviposition sites toward prey gallery openings (T. Fitzgerald and Nagel 1972). According to Fitzgerald (1962) gravid *M. aldrichii* females were observed to respond to volatilized 95% α-pinene by exerting their ovipositor and depositing their eggs.

(iii) Compounds from bark beetle associated microorganisms

Bark beetle associated microorganisms are also known to produce a range of volatiles (Table 1) that may be used by predators to locate their prey. *Medetera* spp. were highly attracted to colonized logs, wither with the fungus *Ophiostoma ips* or with a bacteria strain *Burkholderia sp.*, which are *Ips pini* associated microorganisms, compare to the non-colonized logs (Boone et al. 2008). However, the volatile constituents from these microorganisms that mediate *Medetera* attraction are not yet identified.

4.3.2 Other cues

In combination to olfaction, *Medetera* may also use other cues such as color, texture, form and contrast to orient and land on a suitable host. According to Goyer et al. (2004), *M. bistriata* orientation was strongly affected by color and season. In their study, it was observed that white logs caught 50-56% fewer flies compare to the unpainted or black logs and that the fly orientation was dramatically affected by season: in spring, flies showed preference for vertical logs compared to the horizontal logs. However, this preference decreased during summer and switched during fall. Also, according to Hedgren et al. (2004) *Medetera* flies were ten times more abundant on standing living trees compared to cut or dead trees.

Both adults and larvae of *Medetera* genus, may also use acoustic signals to detect their prey under the bark. Bark beetles are known to produce acoustic signals that have been implicated in defense, courtship, aggression, species location and recognition (Ryker and Rudinsky 1976). On *I. typographus,* a sexual dimorphism was observed on the stridulatory organs (Rudinsky 1979). According to this author, females produce two different acoustic signals to respond to male attraction while entering the gallery through the boring dust. These signals can be heard and distinguished with an unmagnified trained observer close to the source of the sound, however, their exact role is still unknown. In addition, the author also observed a single pulse that may be produced by the males as phonoresponse to the females as observed in others scolytids (Ryker and Rudinsky 1976). Stress sounds, similar to brief clicks, were also produced by males and females of *I. typographus* under stress conditions (Rudinsky 1979). Although it is thought that scolytids predators and parasitoids use these acoustic signals to hunt their preys little information is available in relation to how these signals are received and transmitted through the air or through the wood (Gitau et al. 2013). In addition: standing by a bark bettle attacked spruce tree in the spring is possible to hear them chewing the phoem. And also the bark beetle larvae can be heard when they chew into the phloem. If we can hear them, the the flies might be able to hear them (G. Birgersson pers. comm.).

Table 3: List of conifer-scolitidae associated *Medetera* and list of the species recorded in Sweden.

Species	**Conifer-scolitidae associated *Medetera* according to Bickel (1985) (*M. signaticornis-M. pinacola* group)**	**Recorded in Sweden***	**Emerged from spruce trees attacked by *Ips typographus***	**Ref.**
Medetera abstruse, Thuneberg, 1955		x		
Medetera aldrichii, Wheeler, 1899	x			
Medetera ambigua, Zetterstedt, 1843		x	x	(Wermelinger et al. 2012)
Medetera apicalis, Zetterstedt, 1843		x		
Medetera betulae, Ringdahl, 1949		x		
Medetera bistriata, Parent, 1929	x			
Medetera bispinosa, Negrobov, 1967	x			
Medetera borealis, Thuneberg, 1955		x		
Medetera breviseta, Parent, 1927	x	x	x	(Hedgren and Schroeder 2004; Wermelinger 2002)
Medetera collart,i Negrobov, 1967	x			
Medetera complicata, Negrobov, 1967	x			
Medetera cuspidata, Collin, 1941		x		
Medetera diadema, Linnaeus, 1767		x		
Medetera dichrocera, Kowarz, 1877	x	x		
Medetera excellens, Frey, 1909		x	x	(Hedgren and Schroeder 2004; Wermelinger 2002; B Wermelinger et al. 2012)
Medetera fasciata, Frey 1915	x			
Medetera fascinator, Negrobov & Saigusa 1998	x			
Medetera flavirostris, Negrobov, 1967	x			
Medetera flinflon, Bickel 1987	x			
Medetera fumida , Negrobov, 1967	x	x	x	(Hedgren and Schroeder 2004)
Medetera gaspensis, Bickel 1987	x			
Medetera impigra, Collin, 1941		x		
Medetera infumata, Loew, 1857		x		
Medetera jacula, Fallen, 1823		x		
Medetera japonica, Negrobov, 1970	x			
Medetera maura, Wheeler 1899	x			
Medetera melancholica, Lundbeck, 1912	x	x		
Medetera micacea, Loew, 1857		x		
Medetera muralis, Meigen, 1824		x		
Medetera neomelancholia, Bickel 1985	x			

Medetera nitida, Macquart, 1834	x	x		
Medetera obscura, Zetterstedt, 1838	x	x		
Medetera occultan, Negrobov, 1970	x			
Medetera pallipes, Zetterstedt, 1843		x		
Medetera parenti, Stackelberg, 1925		x		
Medetera penicillata, Negrobov, 1970	x			
Medetera petrophila, Kowartz, 1877		x		
Medetera piceae, Ounap, 1997		x	x	(Hedgren and Schroeder 2004; Õunap 1997)
Medetera pinicola , Kowarz,1877	x	x	x	(Hedgren and Schroeder 2004; Hulcr et al. 2005; Wermelinger 2002; Wermelinger et al. 2012)
Medetera plumbella, Meigen, 1824		x		
Medetera polonica, Negrobov & Capecki 1977	x			
Medetera prjachinae, Negrobov, 1974		x	x	(Hedgren and Schroeder 2004)
Medetera pseudoapicalis, Thuneberg, 1955		x		
Medetera ravida, Negrobov, 1970	x			
Medetera robusta, Ounap 1997		x		(Õunap 1997)
Medetera stackelbergiana, Bickel 1987	x			
Medetera senicula, Kowarz, 1877		x		
Medetera setiventris, Thuneberg, 1955	x	x	x	(Hedgren and Schroeder 2004; Hulcr et al. 2005)
Medetera signaticornis, Loew, 1857	x	x	x	(Hedgren and Schroeder 2004; Hulcr et al. 2005; Wermelinger 2002; Wermelinger et al. 2012)
Medetera striata, Parent, 1927	x	x		
Medetera sutshanica, Negrobov & Stackelberg 1974	x			
Medetera tenuicauda, Loew, 1857		x		
Medetera tristis, Zetterstedt, 1838		x		
Medetera truncorum, Meigen, 1824		x		
Medetera vales, Loew, 1861		x		
Medetera vidua, Wheeler 1899	x			
Medetera zinojevi, Negrobov, 1967	x	x	x	(Hedgren and Schroeder 2004)

*Data was collected from Lund museum (http://www.botmus.lu.se/ent/search_new2.php?taxa=Medetera&sort=1&country=Sweden

Table 4: Review of the bark beetle associated compounds tested on different *Medetera* spp, their effect and occurrence.

Compounds	Bark beetles associated			Effect	Species tested	Ref.
	Pheromone	Host tree	Microorganisms			
(*S*)-*cis*-verbenol + 2-methyl-3-buten-2-ol	x			attractant	*M. setiventris,* *M. bistriata*	Hulcr et al. 2005; Williamson 1971
(*S*)-*cis*-verbenol + 2-methyl-3-buten-2-ol + (±)-α-pinene + limonene	x	x		attractant	*M. setiventris,* *M. melancholica*	Hulcr et al. 2005
Ipsdienol	x			attractant	*M. setiventris*	Hulcr et al. 2006
Chalcogran	x			attractant	*M. setiventris*	Hulcr et al. 2005
Bricomin	x			attractant	*Medetera spp.*	Vité et al. 1969
Frontalin	x			attractant	*Medetera spp.*	Vité et al. 1969
α-pinene		x		attractant	*M. signaticornis,*	Rudinsky et al. 1971
				ovipositon stimulus for gravid female and orientation guide of the newly eclosed larva to oviposition sites toward prey gallery openings	*M. aldrichii,*	Fitzgerald and Nagel 1972
				increases attractiveness of bark beetle kairomones	*M. bistriata*	Williamson 1971
β-pinene		x		attractant, orientation guide of the newly eclosed larva from oviposition sites	*M. signaticornis* *M. aldrichii*	Rudinsky et al. 1971; Fitzgerald and Nagel 1972
camphene		x		attractant	*M. signaticornis*	Rudinsky et al. 1971
camphor		x	x	no effect	*Medetera spp.*	
limonene		x		attractant	*M. signaticornis*	Rudinsky et al. 1971
methyl (*E*, *Z*)-2,4-decadienoate	x			no effect	*M. setiventris*	Hulcr et al. 2005

5. Remarks and future investigations

In this introductory essay I provided a literature review and summarized what I consider to be the most relevant aspects on the interaction between bark beetles and *Medetera* spp. From my point of view, a compilation of such information is needed to understand how these natural enemies, and their interactions, can be used to prevent or reduce future epidemic bark beetles attacks.

While revising the literature I verified that over the past few decades only a few studies have been completely dedicated to the biology of *Medetera*, even though their potential as biological agents of bark beetles was noticed already in the early 1950´s, if not before. I think that the very limited information available is mainly because the distinction between species is difficult to perform with the naked eye. Moreoever, the available keys used for species identification is often incomplete and most of the times do not include all the species found. Therefore, the biology of the *Medetera* genus described in literature appears to be scarse and many ecological aspects are still unknown. Information regarding courtship, sound and scent was not found. Features such as number of generations per year and where adults overwinter are not clear and need to be considered in future studies.

Host selection and specificity are other important aspects that needs further consideration. It is not proved if the different *Medetera spp* are selective or specialized to host trees or to scolytidae prey. *Medetera* adults have mainly been found on attacked trees for oviposition, but it is not clear if the reason is because the predator instinctively knows that their offspring has higher change of survival because they can feed on the bark beetles offspring, or because the tree's natural defense is reduced due to the bark beetle attacks and therefore are more susceptibel to other plagues. Bark beetle attacked trees can have a variety of other insects which both adult and larval *Medetra* can feed on, and thereby increasing their survival rate. Further investigations on this matter needs to be carried out. Understanding host selection and specificity is a crucial step to see how, when, or which of these predators can be used as efficient bioregulators of a bark beetles population.

When it comes to the subject on how *Medetera* detects bark beetle attacked trees, I believe that further investigations also need to be carried out. It is known that *Medetera* adults arrive to infested logs shortly after colonization by bark beetles, and the presence and oviposition of the *Medetera* has been noted throughout the entire bark beetle brood development. However, it is not clear exactly how they locate a tree under attack, or how they locate their prey underneath the bark. From my point of view, it is important to address this issue while considering that multiple sensory cues (e.g., olfaction, vision, sound) may be involved in the host/prey location. Moreover, the odour surrounding a bark beetle attacked spruce tree varies qualitatively and quantitatively between the different stages of the bark beetle attack and maybe a result not only of the beetle itself but also its associated microoranisms. For this reason, it is possible that *Medetera* might be using a combination of multiple semiochemicals, and the multitrophic interaction (tree, beetles, microorganisms, flies) needs to be considered in future studies.

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