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Review article

Stimulation of tree defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers

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Abstract

- Our aim is to present why the hypothesis, that Ophiostomatoid fungi play an important role in the establishment of most bark beetle species on living conifers, is valuable.
- After summarizing knowledge about the relationships of bark beetles with conifers and fungi, we conclude that controversy results from misinterpretations when using fungal pathogenicity to demonstrate the role of Ophiostomatoid fungi in beetle establishment on host trees.
- We demonstrate that fungal pathogenicity is not the right parameter to appreciate the role of fungus in beetle establishment on host trees. We argue that artificial low density inoculations that allow the appreciation of fungus ability to stimulate tree defenses and thus to help beetles in overcoming tree resistance must be used in complement to mass inoculations. In both cases, results must be expressed in terms of tree defense stimulation rather than in terms of tree killing.

(i) Fungal species stimulating tree defenses are generally not those that grow the best in the sapwood.
(ii) We argue that beetle development in the phloem, fungal invasion of the sapwood and phloem, and tree death, occur after tree defenses are exhausted, and that any fungus present in the beetle gallery could thus potentially invade the sapwood after defense exhaustion.

- We conclude that stimulation of the tree defense reactions in both the phloem and the superficial sapwood is a real benefit brought by fungi to the beetles during the first phase of establishment (overcoming tree resistance).
- Considering the origin of the bark beetle fungus associations attacking living trees and their general functioning based on stimulation of tree defenses, we develop three hypotheses:

(i) any beetle species would be helped in its establishment in a given tree species by developing an association, even loosely, with a fungus species belonging to the Ophiostomatoid flora of that tree species;
(ii) the necessity of a considerably low level of tree resistance for fungus extension into the tree is the selection pressure that has led fungi to develop their intrinsic ability to stimulate tree defenses, through their ability to grow into the phloem. This association can be completed by antagonistic fungal species controlling extension of the previous fungal species in the tree tissues;
(iii) Beetle species using the strategy of overcoming tree resistance are associated with a fungal complex, of which species could assume three roles regarding relationships between beetles and trees: 1- to stimulate tree defenses in the phloem and superficial sapwood, 2- to grow into the sapwood after tree resistance is overcome, and 3- to control phloem extension of the first other two categories. Bringing nutrients to the beetle progeny can be a fourth role.

- We propose that bark beetle – Ophiostomatoid associations can be categorized, based on associations' frequency and complexity while taking into account beetle aggressiveness. We show that a close correspondence exists between beetles' aggressiveness and the ability of their main associated fungal species to stimulate the defenses of their host tree.
- We conclude with suggesting that most sapwood invading fungi might be "cheaters" which have taken advantage of the efficiency of the relationship between beetles and fungi that stimulate tree defenses.

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Résumé – La stimulation des défenses de l'arbre par les champignons Ophiostomatoïdes peut expliquer le succès des attaques de Scolytes sur conifères.

• Notre objectif est de présenter les raisons de la validité de l'hypothèse selon laquelle les champignons jouent un rôle important dans l'installation de la plupart des espèces de Scolytes sur conifères vivants.

• Après avoir résumé les connaissances sur les relations des Scolytes avec les conifères et les champignons, nous concluons que la controverse résulte d'interprétations erronées lorsque l'on utilise le pouvoir pathogène des champignons pour démontrer le rôle des Ophiostomatoïdes dans l'installation des insectes sur les arbres hôtes.

• Nous démontrons que le pouvoir pathogène n'est pas le paramètre correct pour apprécier le rôle du champignon dans l'installation des Scolytes sur les arbres hôtes. Nous soutenons que des inoculations artificielles à faible densité, qui permettent d'apprécier la capacité du champignon à stimuler les défenses de l'arbre et à ainsi aider le Scolyte à surmonter la résistance de celui-ci, doivent être utilisées en complément des inoculations massives. Dans les deux cas, les résultats doivent être exprimés en termes de stimulation des défenses de l'arbre plutôt qu'en termes de mortalité de l'arbre.

(i) les espèces de champignons qui stimulent les défenses de l'arbre ne sont généralement pas celles qui présentent la meilleure croissance dans l'aubier.
(ii) nous soutenons que le développement de l'insecte dans le phloème, l'invasion de l'aubier et du phloème par le champignon, et la mort de l'arbre, interviennent après épuisement des défenses de l'arbre, et que tout champignon présent dans les galeries de l'insecte pourrait donc potentiellement envahir l'aubier après épuisement de ces défenses.

• Nous concluons que la stimulation des réactions de défense de l'arbre à la fois dans le phloème et l'aubier superficiel représente un bénéfice réel apporté par les champignons aux Scolytes pendant la première phase de leur installation (surmonter la résistance de l'arbre).

• En ce qui concerne l'origine des associations Scolytes – champignons attaquant les arbres vivants et considérant leur fonctionnement général basé sur une stimulation des défenses de l'arbre, nous développons trois hypothèses :

(i) toute espèce de Scolyte serait aidée dans son installation sur une espèce d'arbre donnée en développant une association, même lâche, avec une espèce de champignon appartenant à la flore Ophiostomatoïde de cette espèce d'arbre ;
(ii) la nécessité d'un très faible niveau de résistance de l'arbre pour autoriser l'extension fongique dans le végétal est la pression de sélection qui a conduit les champignons à développer leur capacité intrinsèque de stimulation des défenses de l'arbre, à travers leur capacité à croître dans le phloème. Cette association peut être complétée par des espèces fongiques antagonistes contrôlant l'extension des espèces précédentes dans les tissus de l'arbre ;
(iii) les espèces de Scolytes utilisant la stratégie de surmonter la résistance de l'arbre sont associées à un complexe fongique dont les espèces assurent trois fonctions eu égard aux relations entre les Scolytes et les arbres : 1– stimuler les défenses de l'arbre dans le phloème et l'aubier superficiel, 2– croître dans l'aubier après que la résistance de l'arbre ait été vaincue, and 3– contrôler l'extension des deux catégories précédentes dans le phloème. L'apport de nutriments à la progéniture du Scolyte peut représenter une quatrième fonction.

• Nous proposons que les associations Scolytes – Ophiostomatoïdes puissent être classées, en se basant sur la fréquence et la complexité de l'association et en prenant en compte l'agressivité de l'insecte. Nous montrons qu'il existe une étroite correspondance entre l'agressivité des insectes et la capacité de leur principale espèce fongique associée à stimuler les défenses de l'arbre hôte.

• Nous concluons en suggérant que la plupart des espèces de champignons envahissant l'aubier pourraient être des "tricheurs" qui ont profité de l'efficacité des relations entre les Scolytes et les espèces fongiques stimulatrices des défenses de l'arbre.

1. INTRODUCTION

Bark beetles (Coleoptera: Scolytinae, Curculionidae) represent a diversified group of insects gathering a little more than 6 000 described species worldwide and at least 225 genera (Knizek and Beaver, 2004), specialized in exploiting woody plants (hardwood trees and shrubs, and conifers) and exhibiting a high diversity of associations with micro-organisms (Harrington, 2005; Kirisits, 2004; Paine et al., 1997; Six, 2003; Whitney, 1982, among others). All species bore galleries in their host, where larval development and most often adult maturation take place. However, with the exception of the species involved in Dutch elm disease, bark beetles of hardwood trees do not generally cause much damage. Conifer bark beetles on the contrary are directly responsible for frequent extensive and dramatic damage to forests, making them the most important forest pests in the temperate zones (Grégoire and Evans, 2004). Understanding how their attacks succeed is thus essential, and their relationships with host trees have been documented in a variety of situations. Moreover, their frequent relationships with fungi have also stirred up many studies aiming at determining the role of these micro-organisms in the interactions between trees and bark beetles.

We believe that fungi play an important role in bark beetle establishment on conifers, a hypothesis first proposed by Reid et al. (1967) and Berryman (1972) that has raised a lot of controversy, and our objective in this paper is to present and justify our positioning and our views regarding this hypothesis. We re-examine the relationships between trees and bark beetle – associated fungi, to propose that fungi effectively play a role in beetle establishment on trees, based on the stimulation of tree defenses and not on tree killing. We then propose hypotheses to explain why and how the beetle-fungi relationships interfere in this context. We finally propose different types of beetle-fungus associations based on our hypotheses.

Before proceeding however, we will first summarize what is known about relationships between bark beetles and conifers on the one hand, and between bark beetles and their associated micro-organisms on the other.

2. RELATIONSHIPS OF BARK BEETLES WITH CONIFERS AND FUNGI

2.1. Bark beetles and conifers

Although some conifer bark beetles species develop inside the sapwood, the vast majority are phloem borers. Four groups of conifer bark beetle species can be distinguished based on the type of relationship they maintain with their hosts. The "true parasites" can develop and reproduce on healthy trees without killing them and this group seems to contain only two species, which both live on spruces, the European *Dendroctonus micans* and the North American *Dendroctonus punctatus*. The "near-obligate parasites" (Raffa et al., 1993) are regular tree-killers able to kill healthy trees over large areas during periods of outbreaks and which survive on subdued or dying trees when their population is at a low level. The group is composed of a small number of species, amongst which are *Dendroctonus ponderosae*, *Dendroctonus brevicomis*, *Dendroctonus frontalis*, attacking North American pines and *Ips typographus* attacking spruces in Europe. Following its massive outbreaks in extended areas of spruces in North-West America in the 1990's, *Dendroctonus rufipennis* can also be considered a near-obligate parasite. The "facultative parasites" (Raffa et al., 1993) usually colonize fallen or weakened trees but are occasionally able to colonize healthy trees and to cause significant damage, especially when there are circumstances favorable to a considerable increase of their populations on weakened trees (important storm felling for example). Species such as *Ips pini* on pines in North America, or *Ips acuminatus* and *Ips sexdentatus* on pines and *Pityogenes chalcographus* on spruces in Europe, belong to this group. The "saprophyte" group (Raffa et al., 1993) comprises the large majority of species which never colonize and reproduce on healthy trees. They usually develop on logs and dead trees, eventually killed by species of the other groups, but a few species are able to attack very weakened living trees. Although this latter group represents around 95% of the total bark beetles species in the temperate zones and plays an important ecological role, few studies have been devoted to them because of their very low economical interest outside of those vectoring tree pathogens. The near-obligate and the facultative parasites together make up the quasi-totality of species directly causing damage to trees.

Besides the saprophytic species, all groups must face a certain level of tree resistance before succeeding in establishing in their host. Conifer resistance to bark beetle attacks involves multiple passive or active defense mechanisms which overlap and complete each other (Berryman, 1972; Christiansen et al., 1987; Franceschi et al., 2005; Lieutier, 2002; 2004; Paine et al., 1997). Among these mechanisms, the hypersensitive reaction which develops in response to beetle attacks has been demonstrated to play an essential role in the resistance of many conifer species to many bark beetle species (Christiansen et al., 1987; Lieutier, 2002; Paine et al., 1997; Raffa, 1991; Raffa and Berryman, 1982a; 1982b; Safranyik et al., 1975). It develops around each point of attack in both the phloem and the superficial sapwood and is visible as a longitudinal elliptical resin impregnated zone associated with more or less extended cell necrosis (Berryman, 1972; Reid et al., 1967). Such a zone is considerably enriched with terpenes and neosynthesized phenolic compounds (Bois and Lieutier, 1997; Brignolas et al., 1995; Delorme and Lieutier, 1990; Långström et al., 1992; Lieutier et al., 1991a; Paine et al., 1987; Raffa and Berryman, 1982a; Russell and Berryman, 1976) contributing to stop the aggressors. Resin flow released by a section of resin ducts can constitute another important resistance mechanism, at least for beetles of which the females bore transversal egg galleries (Berryman, 1972; Lieutier, 2002).

Depending on how they cope with tree defense mechanisms when they establish on their host, it has been suggested that bark beetle species attacking living conifers fit in with one or the other of two strategies (Lieutier, 1992; 2002). The near-obligate and facultative parasites would use the strategy of overcoming host resistance (or exhausting host defenses). The insects stimulate the syntheses of defensive compounds and thus increase the energy demand until the tree becomes unable to mobilize energy rapidly enough (Christiansen et al., 1987). At this moment, the tree's defenses are exhausted and beetle establishment succeeds. The stimulation of these syntheses is achieved basically through beetle aggregation on the target trees (Berryman, 1972, 1976; Raffa, 2001; Raffa and Berryman, 1983a; Wood, 1982), which multiplies the points of aggression and thus increases the mechanical stress inducing and stimulating the tree's hypersensitive response (Lieutier et al., 1995). Above a critical threshold of attack density (Berryman, 1976; Raffa, 2001; Raffa and Berryman, 1983a; Safranyik et al., 1975; Thalenhorst, 1958), tree resistance is depleted to such a low level that it is not able to stop aggressors anymore, finally resulting in beetle establishment and tree death. If it allows limiting interspecific competition because a low number of beetle species are able to attack living trees, such a strategy however can lead to intraspecific competition (Raffa, 2001; Raffa et al., 1993). The opposite is true for the strategy of avoiding host defenses, which would be used by the true parasites. Beetles behave in order to minimize the development of the tree's hypersensitive reaction. They thus do not need to aggregate and they attack their hosts individually. They also bore transversal galleries to avoid stimulating the hypersensitive reaction too much, but this behavior causes the cutting of numerous resin ducts, which implies high beetle tolerance to constitutive resin (Lieutier, 2002). In such a strategy which allows avoiding both interspecific and intraspecific competition, tree resistance is not depleted and the tree stays alive after attack success and during beetle brood development.

2.2. Bark beetles and their associated micro-organisms

Among the numerous species of micro-organisms (nematodes, fungi, yeasts, bacteria) described as associated with bark beetles, fungi have been the subject of most studies (Six, 2003; Whitney, 1982). Associations with Basidiomycetes, mainly from the genus *Entomocorticium*, have been described in *D. ponderosae*, *Dendroctonus jeffreyi*, *D. brevicomis*, *D. frontalis*, *I. typographus* and *Ips avulsus* (references in Six, 2003; and Kirisits, 2004) but most bark beetle associated fungi are Ascomycetes, which have indeed been isolated from practically all species where they have been looked for. Bark beetle associated Ascomycetes mostly belong to the genera *Ophiostoma*, *Ceratocystis* and *Ceratocystiopsis* and their related asexual form *Leptographium*. These genera form, with few other genera, the morphologically homogenous group of Ophiostomatoid fungi (Kirisits, 2004; Six, 2003; Wingfield et al., 1993), which are often referred to as "blue-stain", "black-stain" or "sap-stain" fungi, because the melanized hyphae of most species give a bluish-grey color to the sapwood they colonize, mostly in conifers (Harrington, 2005; Kirisits, 2004).

Functional relations between bark beetles and Ophiostomatoid fungi correspond to various degrees of interactions including antagonism, commensalism and mutualism, facultative or obligatory (Klepzig and Six, 2004; Raffa and Klepzig, 1992; Six, 2003; Six and Klepzig, 2004). Considering bark beetle species attacking living trees, the most important benefit that Ophiostomatoid fungi usually gain from the associations is transport by the insect, on which they completely depend for their dissemination (Klepzig and Six, 2004; Six, 2003). Facilitation in entering the available hosts through wounding made by the beetles and limited interspecific competition is also an advantage for fungi associated with beetles attacking living trees, compared to fungi developing on dead substrates. Physical relationships are also very diverse. Fungal spores can be carried by beetles externally on the cuticle or inside more or less complex mycangia (Harrington, 2005; Six, 2003; Six and Klepzig, 2004) which, depending on beetle species and according to the classification of Six (2003), can be simple cuticular pits, pocket or tube sacs of varying depth in the integument, or setal brush, each of these structures being eventually associated with glandular cells. Transportation within the mycangia would also give fungi a protection from UV light and desiccation (Klepzig and Six, 2004; Six, 2003).

The existence of mycangia, which has arisen several times independently in the Scolytinae (Six, 2003), demonstrates that bark beetles also have real benefits from the association. The nature of the benefits however is not always very clear. Their associations with Ophiostomatoid fungi seem to bring bark beetles diverse benefits depending on both beetle and fungal species (Harrington, 2005; Paine et al., 1997; Six, 2003). Nutrition is certainly important since, except for the true parasitic species, bark beetles larvae feed on dying or dead trees, that is on nutritionally poor substrates. Considering the near-obligate and facultative parasitic species, it is thought that fungi can improve larval diet through modifying the substrate or providing complementary nutrients such as vitamins, proteins or sterols (Klepzig and Six, 2004; Six, 2003). Evidence for a role of mycangial basidiomycetes in helping *D. frontalis* to meet its nitrogen needs has been found (Ayres et al., 2000). Similarly, the presence of the Ophiostomatoid species *Grosmannia clavigera* and *Ophiostoma montium* can increase the nitrogen levels in the phloem of trees attacked by *D. ponderosae* by 40%, probably through their ability to restitute nitrogen from the sapwood to the phloem (Bleiker and Six, 2007), and all larval stages and the adults of the insect can feed on the fungi (Adams and Six, 2007). More generally, mycophagy on Ophiostomatoid fungi, or improvement of larvae development in relation to the presence of such fungal species, has been reported on several occasions (references in Six, 2003; Six and Klepzig, 2004; and Harrington, 2005), and Six and Klepzig (2004) have speculated that all *Dendroctonus* species, except the true parasitic ones, would feed on fungi. According to Harrington (2005) however, fungal feeding does not seem obligatory for the completion of the life cycle of bark beetles, and fungi would only supplement larval diet, leading to shorter larval galleries and thus reducing both intraspecific and interspecific competition. Based on their nutritional relations with fungi and the localization of their galleries, bark beetles species have been traditionally divided into three groups, the xylomycetophagous species which bore galleries in the sapwood and feed exclusively on symbiotic fungi, the phloeomycetophagous species whose galleries are located in the phloem and which feed on both phloem and fungi, and the phloeophagous species which feed only on the phloem (Francke-Grosmann, 1967; Kirisits, 2004).

Apart from the nutritional aspect, another important benefit that conifer bark beetles could gain from the association would be the help brought by Ophiostomatoid fungi to beetle species attacking living trees, in the success of their attacks and establishment on trees. The reality of this benefit is the subject of the present paper.

2.3. Controversies

Following the pioneer work of Reid et al. (1967) and the first model of conifer-bark beetle-fungus interactions proposed by Berryman (1972), blue stain fungi have been said to be responsible for the development of the hypersensitive reaction, and to be essential for beetle establishment on conifers through helping to overcome host resistance and killing the tree, both beetles and fungi contributing simultaneously to tree death (Berryman, 1972). Establishment is said to begin (i.e. beetles bore galleries and begin to lay eggs) when host resistance stops and death is assured (Berryman, 1982; Coulson, 1979; Wood, 1982). However, these early propositions have often been misinterpreted as it is necessary for the tree to be already killed by fungi for beetle establishment to begin. Then, studies developed to prove the essential role of associated fungi in beetle establishment on trees aimed at demonstrating the pathogenicity of these fungi for the beetle host trees, mainly by using artificial mass inoculations supposed to mimic bark beetle attacks (Christiansen, 1985a; Guérard et al., 2000; Horntvedt et al., 1983; Kim et al., 2008; Kirisits, 1998; Krokene, 1996; Krokene and Solheim, 1998; Neal and Ross, 1999; Ross and Solheim, 1997; Solheim et al., 1993; Solheim and Långström, 1991; Yamaoka et al., 1995), intrinsically supposing that a high beetle aggressiveness could be due to a high fungal pathogenicity. However, as results accumulated it soon became appearant that no correlation existed between fungal pathogenicity and beetle aggressiveness (Harrington, 1993a,b, 2005; Lieutier, 2002; Paine et al., 1997). This has led several authors to deny any role of Ophiostomatoid fungi in beetle establishment on conifers (Harrington, 1993b, 2005; Klepzig and Six, 2004; Six, 2003).

The reasons for controversy seem to result from misinterpretations. Indeed, studies using fungal pathogenicity to demonstrate the role of Ophiostomatoid fungi in beetle establishment have been based on three kinds of arguments:

- assertion: tree death is a prerequisite for beetle establishment;
- observations: most bark beetle species vector staining fungi and the sapwood of bark beetle killed trees is stained;
- experiments: experimental demonstrations that fungi isolated from aggressive beetles are able to kill trees after artificial mass inoculations supposed to mimic bark beetle attacks.

However, all these arguments do not lead to the conclusion that fungi are responsible for killing the bark beetle attacked trees before beetle attacks succeed. They do not even prove that fungi are involved in the tree killing process at all (Lieutier, 2002). Indeed it is possible that fungi invade the sapwood after the tree has been killed by other factors. Moreover, artificial mass inoculations certainly mimic natural bark beetle attack very badly, as the conditions of fungal introductions differ largely between the two situations (see below). In addition, it has already been reported that trees can be successfully colonized and killed by bark beetles without blue stain fungi (Bridges et al., 1985; Whitney and Cobb, 1972). Nevertheless, that fungi would not be responsible for killing trees attacked by bark beetles would not prove either that they do not play any role in beetle establishment. One may even question the necessity that the trees be killed for beetle establishment to begin.

3. RELATIONSHIPS BETWEEN TREES AND BARK BEETLE – ASSOCIATED FUNGI AND REALITY OF THE ROLE OF THE FUNGI IN BEETLE ESTABLISHMENT ON TREES

3.1. Definition and quantification of beetle aggressiveness and fungal pathogenicity

According to Raffa and Smalley (1988), aggressiveness is "used to denote the relative degree of vigor characterizing trees that can be colonized by a Scolytid species". Thus aggressiveness is synonymous with the ability of the beetle to become established on a living tree. Consequently, bark beetle aggressiveness can be considered to be highest for the true parasitic species living permanently on healthy trees, intermediate for near obligate and facultative parasites, and very low to inexistent for the saprophytic species. For the aggregative species using the strategy of overcoming host resistance, the level of aggressiveness can be more precisely quantified by the attack density above which trees are killed, i.e. the level of the critical threshold of attack density (Berryman, 1976; Christiansen et al., 1987; Lieutier, 2004; Paine et al., 1997; Raffa and Berryman, 1983a), aggressiveness being inversely related to threshold level. Such a threshold has been quantified for some bark beetle species and, although it is influenced by genetic and environmental factors affecting the trees' level of resistance, it can be used to rank these species according to their aggressiveness. For example, the critical threshold of attack density, expressed in number of attacks per m^2 of bark, was estimated, depending on tree vigor, between 50 and 120 for *D. ponderosae* on lodgepole pine (Raffa and Berryman, 1983a; Raffa, 2001; Waring and Pitman, 1983), around 75 for *D. rufipennis* on *Picea engelmanii* (Raffa, 2001), around 45 for *S. ventralis* on *Abies grandis* (Raffa and Berryman, 1982a) and between 200 and 400 for *I. typographus* on Norway spruce (Mulock and Christiansen, 1986), while on Scots pine, that of *I. acuminatus* was around 850 (Guérard et al., 2000) and that of *Tomicus piniperda* during its trunk attacks around 400 (Långström and Hellqvist, 1993; Långström et al., 1992).

Fungal pathogenicity is the ability to kill a tree, and the level of pathogenicity corresponds to the fungal virulence. In the case of bark beetle associated fungi, it has often been measured by the critical threshold of inoculation density, which is the density of artificial inoculations above which trees are killed (Christiansen et al., 1987; Lieutier, 2004; Paine et al., 1997; Raffa and Berryman, 1983a). A low threshold indicates a high level of pathogenicity. In such artificial mass inoculations, fungal cultures have generally been introduced at the cambium level, in holes previously bored with a puncher. As tree death is generally thought to result from disruption of water transport in the sapwood, tree death caused by fungal mass inoculations has been estimated by the fading of the foliage or, most often, by measuring sapwood occlusion or sapwood invasion by the fungus (percentage of occluded or blue stained sapwood in transversal bole sections), after harvesting the trees a few months after inoculations (Christiansen, 1985a; 1985b; Christiansen et al., 1987; Croisé et al., 1998a; Solheim et al., 1993, among others). In some cases, tree death has been determined by measuring sapwood specific hydraulic conductivity of bole sections (Guérard et al., 2000). Sometimes, the percentage of dead phloem or cambium was also used (Krokene and Solheim, 1998). The same methods have also often been used to compare the resistance level of different trees to the same fungus or the effect of a treatment on tree resistance (Brignolas et al., 1998; Christiansen and Glosli, 1996; Sallé et al., 2008; Solheim et al., 1993, among others) since the critical threshold of inoculation density depends on genetic and environmental factors affecting the trees' level of resistance. Critical thresholds of inoculation density have been determined for various fungi. Expressed in the number of inoculations per m^2 of bark, it is for example 100 to 200 for *Ceratocystis polonica* on Norway spruce (Christiansen, 1985b). It is 300 to 400 for *Leptographium wingfieldii* (Croisé et al., 1998a; Langström et al., 1993; Solheim et al., 1993), 400 to 800 for *Ophistoma minus* (Langström et al., 1993; Solheim et al., 1993), and more than 1000 for *Ophiostoma brunneo-ciliatum* (Guérard et al., 2000), all on Scots pine. In many cases however, fungal pathogenicity has been tested on seedlings with few inoculations, which is difficult to compare with mass inoculations on adult trees.

3.2. Relationships between beetle aggressiveness and fungal pathogenicity

Fungi associated with conifer bark beetles appear to have diversified levels of pathogenicity but all of them, even those associated with aggressive tree killing bark beetles, need high inoculation densities to kill a tree. In contrast, classical Ophiostomatoid pests not specifically associated with conifer bark beetles, such as *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* in elms, *Ceratocystis fagacearum* in oaks, *Ceratocystis fimbriata* in various woody plants, *Leptographium wageneri* in conifers, can kill a tree with only a few inoculations. With this in mind, it may be of interest to mention that most of these fungi are invasive species, thus corresponding to recent tree-fungus associations. Such a comparison leads to the statement that conifer bark beetles are associated with moderately pathogenic fungi that have a very little capacity to colonize living trees, as already concluded by several authors (Harrington, 1993a; 2005; Lieutier, 2002; Paine et al., 1997; Raffa and Klepzig, 1992, among others).

In these conditions, one may wonder if a relationship between beetle aggressiveness and fungus pathogenicity exists among blue stain fungi associated with conifer bark beetles. Table I presents the relationships between conifer beetle species and Ophiostomatoid fungal species supposed, for most of them, to play a role in beetle establishment. Beetle species are grouped according to their level of aggressiveness and fungi to their level of pathogenicity. Because the critical threshold of attack density has been determined for very few species only, bark beetle aggressiveness has been estimated according to the importance of damage on living trees, especially on healthy trees, mainly by using information from Furniss and Carolin (1977) in North America, and Chararas (1962) and Grégoire and Evans (2004) in Europe. References for fungal pathogenicity are indicated in Table I. Clearly, even when considering only Ophiostomatoid fungi associated with conifer bark beetle, there is no relation between beetle aggressiveness and fungus pathogenicity, a conclusion already proposed by Harrington (1993a; 1993b) and Paine et al. (1997). There are three main possible explanations to such an observation:

1. Fungi do not play any role in beetle establishment and the association is not a real one, except in some cases when fungi provide complementary nutrition to the larvae;
2. Fungal pathogenicity is not the right parameter to appreciate the benefit brought by the fungus to beetle establishment on conifers (independently of possible food advantage);
3. Early tree death due to fungi is not a prerequisite for beetle establishment.

We think that each of these explanations could be valid, depending on the attack strategy used by the beetle species. Both explanations 1 and 3 are valid for beetles using the strategy of avoiding tree defenses. In such a strategy, the tree is not killed and, in order to minimize the development of the hypersensitive reaction (see Sect. 2.1), the association with a fungus able to stimulate it, must be avoided (Lieutier, 1992; 2002). When beetles use the strategy of overcoming tree resistance, we think that both explanations 2 and 3 are correct and that fungi effectively do play a role in beetle establishment. In the following part of this paper, we will concentrate on this latter strategy and try to explain why explanations 2 and 3 are valid. We will then report on results related to the modalities of fungal impact on tree defenses, a point of importance to appreciate the real role of fungi.

3.3. Fungal pathogenicity is not the right parameter to appreciate the benefit brought by fungi to beetle establishment on trees

The crucial aspect in the beetle strategy to overcome host resistance is to exhaust tree defenses or at least to considerably lower them so that the tree cannot put up effective resistance to the aggressors and cannot impede beetle establishment and larval development. In this strategy, everything stimulating the tree's defense reactions accelerates the tree's energy expenditure and thus lowers the critical threshold of attack density (Lieutier, 1992; 2002). Introducing fungi able to strongly stimulate the tree defensive reactions, into the beetle galleries, would thus be of great help for beetle populations, since it would allow them to establish on trees at a lower population level than without fungi (Berryman, 1972; 1976; Christiansen et al., 1987; Franceschi et al., 2005; Lieutier, 2002; Paine et al., 1997; Raffa and Berryman, 1983a). Consequently for establishment, the beetle population doesn't need fungus to kill the tree but to help in overcoming tree resistance through rapidly stimulating host defenses (by causing extensive reactions) in each place where it can be inoculated (Lieutier, 2002; Paine et al., 1997). In this context, there is no reason for an a priori relationship between fungus ability to stimulate tree defense reactions when introduced into the tree by a beetle, and its

Table I. Relationships between various conifer beetle species and their Ophiostomatoid fungi, while grouping beetles according to their level of aggressiveness [Furniss and Carolin (1977) and Dymerski et al. (2001) for North America; Chararas (1962) and Grégoire and Evans (2004) for Europe] and fungi according to their level of pathogenicity. H = High, M = moderate, L = low aggressiveness/pathogenicity.

		H					M			L												
		C. polonica (3, 9, 11, 12, 42)	*C. laricicola* (14, 28)	*C. rufipenni* (35, 37, 39)	*L. terebrantis* (8, 16, 27)	*L. wingfieldii* (4, 17, 19, 20, 38, 39)	*G. clavigera* (10, 36, 44)	*O. minus* (1, 19, 20, 22, 26, 29, 31, 38, 40)	*O. bicolor* (12, 13, 33, 34)	*O. ips* (2, 6, 13, 20, 20, 23, 24)	*O. brunneo-ciliatum* (7, 12, 20)	*O. montium* (36, 44)	*O. longiclavatum* (10, 18)	*O. canum* (41)	*O. brevicomi* (43)	*L. abietinum* (30, 32, 37)	*O.penicillatum* (33, 34, 42)	*0. piceae* (14, 16, 22)	*O. pseudotsugae* (25, 30, 37)	*O. europhioides* (37)	*O. ainoae* (14, 33, 34, 42)	*A. symbioticum* (5)
H	*I. typographus*	X							X								X	X		X	X	
	D. ponderosae						X					X	X									
	D. frontalis							X														
	D. brevicomis							X							X							
	D. pseudotsugae			X												X			X	X		
	D. rufipennis			X												X						
	D.micans													X								
	S. ventralis																					X
M	*I. cembrae*		X						X		X											
	I. sexdentatus							X		X	X										X	
	I. acuminatus							X		X	X										X	
	P. chalcographus								X									X			X	
	O. erosus									X												
	I. pini									X												
L	*T. minor*													X								
	D. valens				X					X												
	D. terebrans				X																	
	T. piniperda					X		X														

References related to the level of fungal pathogenicity or the association with beetle species: 1 = Basham (1970); 2 = Ben Jamaa et al. (2007); 3 = Christiansen (1985a); 4 = Croisé et al. (1998a); 5 = Filip et al. (1989); 6 = Francke-Grosmann (1963); 7 = Guérard et al. (2000); 8 = Highley and Tattar (1985); 9 = Hornvedt et al. (1983); 10 = Kim et al. (2008); 11 = Kirisits (1998); 12 = Kirisits 1999); 13 = Kirisits (2004); 14 = Kirisits et al. (2000); 15 = Klepzig et al. (1995); 16 = Krokene and Solheim (1998); 17 = Långström et al. (1993); 18 = Lee et al. (2006); 19 = Lieutier et al. (1989b); 20 = Lieutier et al. (1990); 21 = Lieutier et al. (2005); 22 = Mathiesen (1950); 23 = Mathre (1964a); 24 = Mathre (1964b); 25 = Neal and Ross (1999); 26 = Owen et al. (1987); 27 = Rane and Tattar (1987); 28 = Redfern et al. (1987); 29 = Rennerfelt (1950); 30 = Ross and Solheim (1997); 31 = Siemaszko (1939); 32 = Six and Bentz (2003); 33 = Solheim (1992b); 34 = Solheim (1993b); 35= Solheim (1995); 36 = Solheim and Krokene (1998a); 37 = Solheim and Krokene (1998b); 38 = Solheim and Långström (1991); 39 = Solheim and Safranyik (1997); 40 = Solheim et al. (1993); 41 = Solheim et al. (2001); 42 = Viiri and Lieutier (2004); 43 = Whitney and Cobb (1972); 44 = Yamaoka et al. (1995).

ability to kill a tree, especially when the latter is determined through the critical threshold of artificial inoculation density.

In fact, mass inoculations allow a comparison of the pathogenicity levels of different fungi or the level of resistance of different trees to be made, but they certainly mimic bark beetle attacks very badly. With artificial mass inoculations, every wound is inoculated with the fungus, which is often far from being the case during a beetle mass attack, as the frequency of beetle contamination is rarely 100% and can even be extremely low in certain populations: see for example Kirisits (2001), Viiri and Lieutier (2004) and Sallé et al. (2005) for *I. typographus* with *C. polonica*, Bridges et al. (1985) for *D. frontalis* with *O. minus*, Lieutier et al. (1989b) and Solheim and Långström (1991) for *T. piniperda* with *L. wingfieldii*, Lee et al. (2006) for *D. ponderosae* with *O. montium* and *G. clavigera*. The number of spores introduced by a beetle in its gallery may also differ largely from that contained in an inoculated disc of sporulated culture (see below). Gallery excavation by beetles contributes to fungal spread inside the host phloem, which is not the case for artificial inoculations (Raffa and Klepzig, 1992). Moreover, it has been reported that the pattern of host colonization by fungi after artificial mass inoculations differs from that observed following beetle mass attack (Parmeter et al., 1992).

Since the important factor is the ability of the fungi to stimulate tree defenses, results of inoculation experiments aiming at appreciating the role of the fungus in beetle establishment must be expressed in terms of tree defense stimulation rather than in terms of killing trees. In such a way, mass inoculations can be useful to assess the stimulation ability of various fungi or to examine the mechanisms of defense exhaustion, when approaching the critical threshold as done for example in the case of *G. clavigera* on *P. contorta* (Raffa and Berryman, 1983b). In addition to mass inoculations, low density inoculations (usually less than 20 inoculations per tree) far below the critical threshold of inoculation density, are also very useful to evaluate fungus ability to stimulate tree defenses, especially because the tree stays alive and can better exhibit the intensity of its response to aggression. Such inoculations allow understanding basic mechanisms of tree defenses and the processes leading to their stimulation, prior the alterations due to high inoculation densities (see for example Brignolas et al., 1995; Franceschi et al., 2005; Kim et al., 2008; Lieutier et al., 1995, 1996). Moreover, different fungal species and strains can be compared on the same trees, thus limiting variations due to trees (Rice et al., 2007a). It has also been suggested that taking into account the speed of reaction development is of importance for the interpretation of the results from low density inoculations (Wallin and Raffa, 2001). The two approaches thus complement each other. However, interpretations of results from artificial inoculations must be done carefully. In all cases, adult trees, i.e. comparable to trees attacked by the beetles, must be used. Fungus stimulation capability must also be estimated after a delay comparable to that involved for the issue (success or failure) of beetle attacks to be determined, which is often within a few days (from 4–5 to 20) depending on beetle aggressiveness and weather conditions (Anderbrandt et al., 1988; Lieutier, 2002; Payne, 1980; Raffa and Berryman, 1983a). The physiological status of the tree must also be taken into account since it can condition the phloem response in various ways (Croisé and Lieutier, 1993; Croisé et al., 1998b; Sallé et al., 2008; see also references in Paine et al., 1997; and Lieutier, 2004). Modalities of fungal impact on tree defenses should also be considered when interpreting the data (see Sect. 3.6).

Direct comparisons between phloem reaction zone length after low density inoculations and fungus pathogenicity after mass inoculation (or sapwood invasion after beetle attacks) have shown that pathogenicity is not related to fungus ablity to stimulate tree defenses (Krokene and Solheim, 1997; 1998; 1999; Solheim, 1988; 1992a; 1992b). As an example, in Figure 1, Norway spruce trees were inoculated with four fungal species, either at a low density (see left part of the figure) or at a high density (see right part of the figure) (Krokene and Solheim, 1999). Among all assayed fungal species, only *C. polonica* was really pathogenic (as indicated by its ability to stain the sapwood after mass inoculations), whereas all species were able to stimulate the phloem reaction significantly after low density inoculations (as indicated by the extension of the phloem reaction zone). It thus clearly appeared that the pattern of fungal ability to stimulate the phloem reaction differed largely from the pattern of fungal pathogenicity. Another significant example is *Ambrosiella symbioticum*, a fungus associated with *S. ventralis*. It strongly stimulates *A. grandis* phloem reactions after low density inoculations (Raffa and Berryman, 1982a; Wong and Berryman, 1977) but is unable to penetrate the sapwood after mass inoculations (Filip et al., 1989). It should be noted however that, when comparing isolates from the same species, a correlation between reaction zone length after low inoculation density and fungal pathogenicity can be observed (Lieutier et al., 2004; Plattner et al., 2008; Sallé et al., 2005).

Moreover, the fungus invading the sapwood after a beetle attack is not necessarily the one that plays a major role in stimulating tree defenses, even after high inoculations densities. In Figure 2, five different blue stain species are compared regarding their ability to stimulate the phloem hypersensitive reaction of Norway spruce after high density inoculation (Solheim, 1988), and their ability to grow into the sapwood after a successful natural infestation by *I. typographus* (Solheim, 1993a). Whereas *O. penicillatum* induced more extensive phloem reaction zone than *C. polonica* and *O. bicolor*, it stayed largely behind these latter two species during sapwood invasion. The pattern of the two fungal behaviors thus completely differed.

The pattern of fungal growth also largely differs between the phloem and the sapwood. In Figure 3 for example, four blue stain species were compared for their ability to grow in the tissues of Douglas fir after low density inoculations (Solheim and Krokene, 1998b). *Ophiostoma pseudotsugae* and *O. europhioides* extended much more than *L. abietinum* and *C. rufipenni* in the phloem, whereas no difference between species was observed in the sapwood although the two latter species tended to grow deeper. Clearly and as already reported, various blue stain species mainly infest the phloem and show a slow spread in the sapwood, whereas the opposite is true for other species (Solheim, 1992b; Solheim and Krokene,

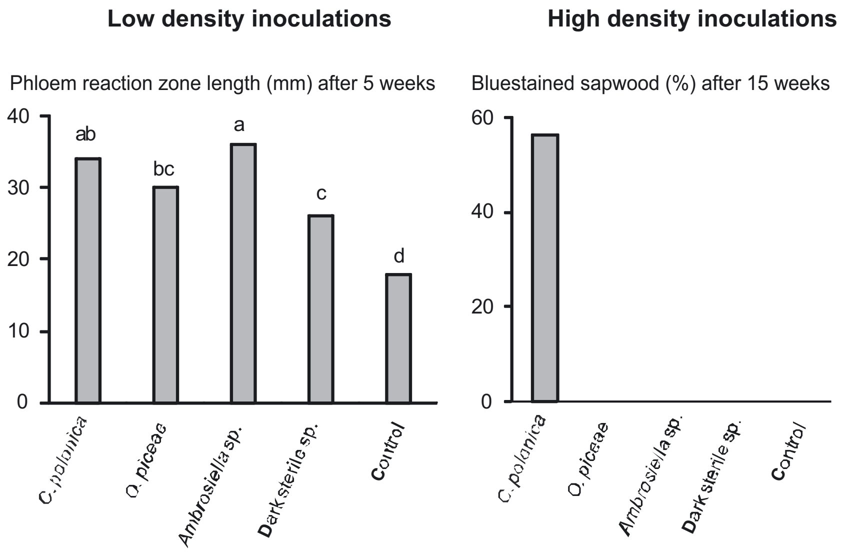

Figure 1. Direct comparisons between phloem reaction development after low density inoculations (three inoculations per fungal species in each tree) and fungus pathogenicity after mass inoculations (400 inoculations per m^2 of a given fungus per tree), in Norway spruce (*Picea abies*) inoculated with various blue stain fungal species (adapted from Krokene and Solheim, 1999). Values with the same letter did not differ significantly.

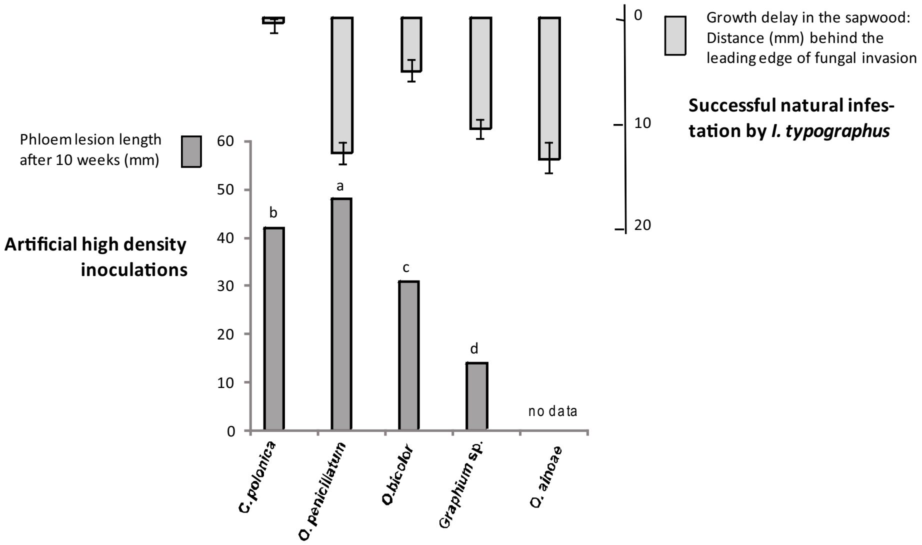

Figure 2. Extension of the phloem hypersensitive reaction zone after high density (475/m^2) inoculations of Norway spruce (*Picea abies*) with various fungal species, compared with fungal growth of the same species in the sapwood of Norway spruce after natural infestations by *I. typographus* (adapted from Solheim, 1988, 1993a). Values with the same letter did not differ significantly.

1998a,b; Uzunovic and Webber, 1998). Such different behaviors may be explained by referring to oxygen availability in tree tissues, the ability to invade sapwood being possibly related to high tolerance to oxygen deficiency (Solheim, 1991; 1992a; Solheim and Krokene, 1998a).

Figure 3 also shows that for all species, stimulation of tree defenses concerned both the phloem and the superficial sapwood. Between fungi comparisons related to reaction zone extension in the phloem show a tendency similar to that of fungus extension in the phloem. In the superficial sapwood however, *C. rufipenni* induced a more extended reaction zone than *O. pseudotsugae* and *O. europhioides*, while the reaction zone induced by *L. abietinum* was more extended than that of *O. pseudotsugae*. One can thus think that during a beetle attack, both fungal categories certainly interfere in the stimulation of tree defenses, those growing better in the phloem stimulating mainly phloem defenses, and those growing better in the sapwood stimulating mainly superficial sapwood defenses. Meanwhile, as it appears in Figure 3 related to Douglas fir, stimulation of tree defenses seems mainly a

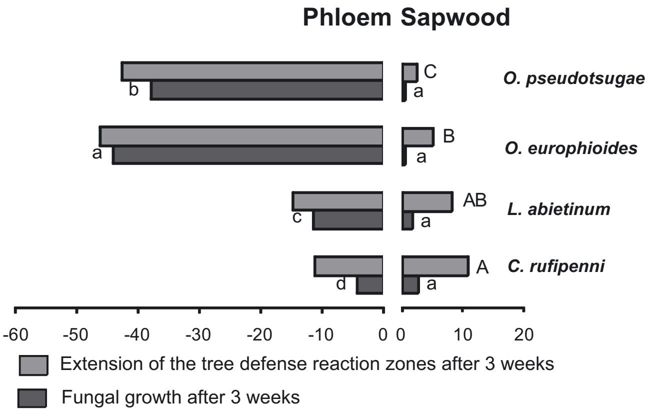

Figure 3. Fungal growth and extension of the tree defense reaction zones in the phloem and in the sapwood of Douglas fir (*Pseudotsuga menziesii*), three weeks after artificial low density inoculations with various fungal species (three inoculations per fungal species in each tree). Results of statistical tests were not available for phloem reaction zone extension (adapted from Solheim and Krokene, 1998b). In the same tissue, values with the same letter did not differ significantly.

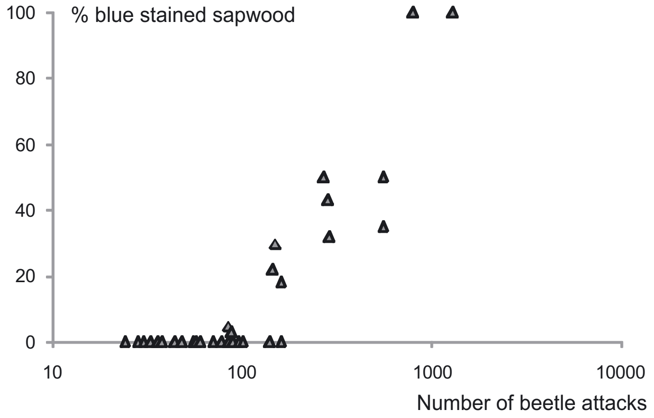

Figure 4. Development of the blue stained area in the sapwood of Norway spruce (*Picea abies*), with increasing numbers of *I. typographus* natural attacks (adapted from Christiansen, 1985b).

phloem phenomenon, especially when considering only the fungal species associated with the Douglas fir beetle (and thus excluding *C. rufipenni*). Evaluation of energy expenditure in building tree defenses is needed in each tissue to really compare the role of fungal species in exhaustion of tree defenses. In addition, a possible interference of other micro-organisms such as bacteria should also be taken into account (Cardoza et al., 2006). However, it is worth mentioning that stimulation of sapwood defenses concerns only the superficial part of this tissue. This is an additional reason for not using fungal pathogenicity and sapwood invasion to make conclusions about the role of fungi in beetle establishment.

3.4. Prior tree death due to fungi is not a prerequisite for beetle establishment

This statement is, in some ways, the consequence of the lack of relationship between fungus ability to stimulate tree reaction and fungus pathogenicity. As long as tree resistance is not overcome (below the critical threshold of attack density), it contains the aggressors. Fungi can extend into the sapwood only very little or not at all while beetles cannot establish in the phloem. As soon as tree defenses are exhausted (above the threshold), nothing impedes the development of the aggressors and, even if a low level of resistance may still persist, phloem and sapwood are invaded rapidly. As an example, Figure 4 shows that, during a natural attack by *I. typographus* on Norway spruce, no blue stain was observed in the sapwood until a certain number of beetle attacks was reached but this tissue was invaded rapidly above that threshold (Christiansen, 1985b). If, as generally thought, tree death effectively results from disruption of water transport in the sapwood, the trees were still alive when the threshold was reached. We thus think that beetle establishment begins after tree resistance is overcome, i.e. before tree is killed. Paine et al. (1997) have already concluded that trees are overcome well in advance of fungal growth in sapwood or changes in tree moisture status. By comparing the timing of increase in stem circumference with that of variations in sap flow velocity of loblolly pines naturally colonized by *D. frontalis*, Wullschleger et al. (2004) also reached the conclusion that disruption of water balance was not a prerequisite for beetle oviposition and larval development. In these conditions, sapwood (and phloem) invasion would simply be the unavoidable consequence of the considerable depletion of tree resistance. Considering that beetles are often associated with a complex group of fungi rather than with a single fungal species (see below, Sect. 4.1), at the moment when tree resistance is overcome, any present fungus, even with a low level of pathogenicity, depending on substrate characteristics and on its competitive ability, could invade the sapwood successfully and thus possibly be involved in tree death. Similarly, any insect present could also succeed in establishing in the phloem. Death would then result from all invaders acting together (Berryman, 1972; Lieutier, 2002; Paine et al., 1997), in addition to the sacrifice of tissues through the induced reactions (Lieutier, 2002), and would occur after the completion of the critical interactions between the tree and its aggressors (Franceschi et al., 2005; Lieutier, 2002; Paine et al., 1997).

The fastest growing species in the sapwood may have a dominant role in tree death, in agreement with the suggestion by Paine et al. (1997) that competition with other fungi could have been a driving force of pathogenicity. However, the presence of a fungus in the sapwood does not prove that it is responsible for tree death since sapwood at this moment can be colonized by any present pathogen. Several studies have concluded that fungi are not the primary factor of mortality in trees attacked by bark beetles (Nebeker et al., 1993; Parmeter et al., 1992; Stephen et al., 1993). Hobson et al. (1994) have even reported that during attacks by *D. brevicomis* on *Pinus ponderosa*, *Ophiostoma* species apparently invade the sapwood after the tree has died. In addition, there are several examples where trees were killed without fungus.

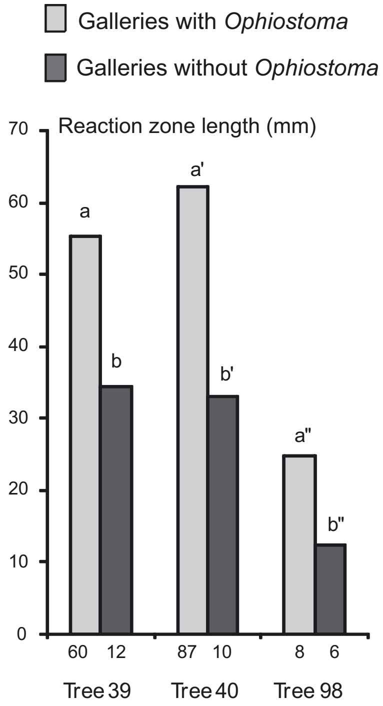

Figure 5. Phloem reaction zone length in three Scots pine (*Pinus sylvestris*) trees in front of beetle galleries, 6 to 9 weeks after *I. sexdentatus* attacks associated or not with an *Ophiostoma* species. The number of galleries is indicated under the histograms (adapted from Lieutier et al., 1995). Values with the same letter did not differ significantly.

That was reported in the case of *D. frontalis* (Bridges, 1983; Bridges et al., 1985; Hetrick, 1949). Similarly, pines have been killed by *D. ponderosae*, although *G. clavigera*, the only *D. ponderosae*-associated fungus able to kill trees after mass inoculation, was absent (Six and Klepzig, 2004).

3.5. Fungi do play a role in beetle establishment on trees

We think that the beetle strategy of being associated with moderately pathogenic fungi is really in operation in case of the strategy of exhausting tree defenses, and that it acts through the ability of the fungi to lower the critical threshold of beetle attack density by stimulating tree reactions to aggressions (Franceschi et al., 2005; Lieutier, 2002; Paine et al., 1997). This association is even a necessity for successful beetle attacks in trees with high resistance level. It has been suggested that nutrition is very likely an important benefit for bark beetles (Adams and Six, 2007; Bleiker and Six, 2007; Klepzig and Six, 2004; Six, 2003). We believe that stimulation of the tree defense reactions is also a real benefit brought by the fungi to the beetles. Following Paine et al. (1997) and Lieutier (2002), beetle establishment is suggested to proceed in two successive steps: (1) overcoming tree resistance (exhausting tree defenses by stimulation) in both the phloem and the superficial sapwood; (2) Invasion of tree tissues by beetles (in the phloem) and fungi (in the sapwood and phloem). Experiments with beetle attacks at low density have demonstrated that fungi are effectively able to stimulate tree reaction when introduced by a beetle into the tree. Figure 5 gives an example for *I. sexdentatus* on Scots pine, after beetle attraction to the trees with synthetic pheromones (Lieutier et al., 1995). Phloem reaction zones were significantly more extended in front of galleries with *Ophiostoma* than in front of those without *Ophiostoma*. The observation that *D. frontalis* attacks without fungi succeed at higher densities than attacks with fungi (Bridges et al., 1985; Whitney and Cobb, 1972) is also in agreement with the role of fungi in helping overcoming tree resistance.

3.6. Modalities of fungal impact on tree defenses

Several parameters can affect the role of the fungi during a beetle mass attack, which must be taken into account before concluding about the role that is or isn't played by a fungus in beetle establishment. These factors have already been mentioned as invalidating the use of artificial mass inoculations followed by measuring fungus pathogenicity to appreciate the benefit brought by the fungus to the beetle. They also condition the utilization of the results from artificial inoculations (at low or high densities) performed to appreciate beetle ability to stimulate tree defenses. They are the frequency of beetle contamination, the diversity of fungal species, the between-isolate variability in a fungal species, and the level of fungus load of a contaminated beetle.

– The frequency of beetle contamination by a fungus can vary largely between localities (even at a relatively short distance) as well as with time in a same locality, and such huge variations certainly affect the role of the fungus in beetle establishment.

In Europe, *C. polonica* has been reported as a common associate of *I. typographus* in several localities (Harding, 1989; Kirisits, 2001; Siemaszko, 1939; Solheim, 1986). It was however found less frequently and even very rarely in other places (Harding, 1989; Jankowiak, 2005; Kirisits, 2001; Mathiesen-Käärik, 1953; Sallé et al., 2005; Solheim, 1993b; Viiri, 1997; Viiri and Lieutier, 2004). Similar situations have been observed in North America with the associations between *D. rufipennis* and *C. rufipenni* (Six and Bentz, 2003; Six and Klepzig, 2004) or *L. abietinum* (Aukema et al., 2005), between *D. ponderosae* and *G. clavigera* or *O. montium* (Six and Bentz, 2007; Six and Klepzig, 2004), and between *D. frontalis* and *O. minus* (Bridges et al., 1985). The consequences, for beetle population dynamics, of huge variations in the frequency of fungal contamination have already been evoked through the effects on beetle nutrition and reproduction (Six and Bentz, 2007; Six and Klepzig, 2004). Certainly such variations interfere in beetle population dynamics also through effects on helping beetle establishment on trees.

– Additionally, the diversity of fungal species associated with a beetle population must also be considered. There are many examples of multiple associations in bark beetle species (Lee et al., 2006; Six, 2003; Six and Paine, 1999; as examples for North America, Kirisits; 2004, and references therein

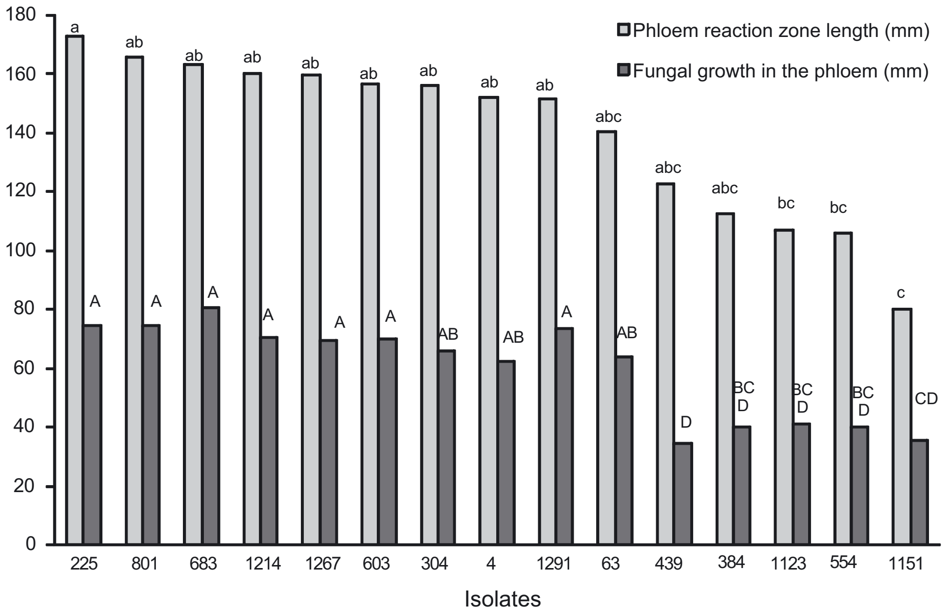

Figure 6. Phloem reaction zone length and fungal growth, 14 days after low density inoculations in Scots pine with 15 different isolates of *L. wingfieldii* from the Orléans forest (two inoculations per fungal isolate in each tree) (adapted from Lieutier et al., 2004). Values with a same letter did not differ significantly.

for Europe). In forests of southern Poland, the frequency of *I. typographus* beetles carrying *O. polonicum* with was 5.6%, while it was 54.8% for *O. penicillatum*, 30.8% for *O. bicolor*, 27% for *O. ainoae*, 23.3% for *O. piceaperdum* and 20.3% for *O. piceae* (Jankowiak, 2005). In these conditions, even if *O. polonicum* appears the most pathogenic and even if it is able to strongly stimulate tree responses after artificial inoculations, one may doubt its dominant role in beetle establishment (and even in sapwood invasion) in the natural conditions of these forests. Raffa and Smalley (1988) mentioned that "the relative abundance and proportions of various components of the fungal flora could have a strong effect on the colonization success". To appreciate the role of fungi in the establishment of a beetle population, it may thus be more important to consider the total percentage of beetles carrying blue stain fungi rather than the percentage of beetles associated with the fungus which is most efficient in stimulating tree defenses. The frequencies of the associations of *D. ponderosae* with *G. clavigera* and *O. montium* can vary largely, one or the other fungal species being the most prevalent depending on localities and time (Adams and Six, 2007; Bleiker and Six, 2009; Six and Bentz, 2007) probably in relation to differential performances of each species under different temperature or moisture conditions (Bleiker and Six, 2009; Rice et al., 2008; Six and Bentz, 2007). However, as the two fungal species play a similar role in beetle nutrition (Adams and Six, 2007), they are believed to complement each other, and the prevalence of one or the other is said to assure the insect a correct feeding substrate any time in all habitats of its geographic range (Bleiker and Six, 2009; Rice et al., 2008; Six and Bentz, 2007). Comparable complementarities could be suggested between fungal species involved in the stimulation of tree defenses, in this case and in the case of other beetle species. Variations of associations between *D. frontalis* and its symbiotic fungi, as well as between beetle-associated-mites and fungi, have also been reported to occur in relation to temperature (Hofstetter et al., 2007).

– The ability to stimulate tree defenses can also vary among isolates within the same fungal species. A comparison between 15 isolates of *L. wingfieldii* collected on the same date within the same forest revealed large variations in their ability to stimulate phloem tree defenses and to grow into the phloem of Scots pine, after artificial low density inoculations (Fig. 6) (Lieutier et al., 2004). The locality had no effect and, owing to the dispersal capabilities of the vector *T. piniperda*, it was concluded that all isolates were very likely to coexist throughout the forest. They thus certainly coexist also within the same beetle population. Similar results have been obtained among isolates from different localities, with *O. bicolor* and *O. piceaperdum* associated with *I. typographus* (Sallé et al., 2005) and with *G. clavigera* associated with *D. ponderosae* (Plattner et al., 2008; Rice et al., 2007a). In the latter association, variations among isolates in their adaptation to cold temperature have also been reported (Rice et al., 2008). Such phenomena could have evident consequences regarding the role of the associated fungus in stimulating tree defenses and consequently in beetle attack success.

– The level of fungus load carried by an individual beetle must also be considered to appreciate the real role of an associated fungus in stimulating tree defenses in the event of natural beetle attacks. Experiments with artificial low density inoculations have demonstrated that resin concentration in the phloem hypersensitive reaction zone that develops around a wound is positively related to the number of spores introduced, and that a minimum number of spores must be present to cause

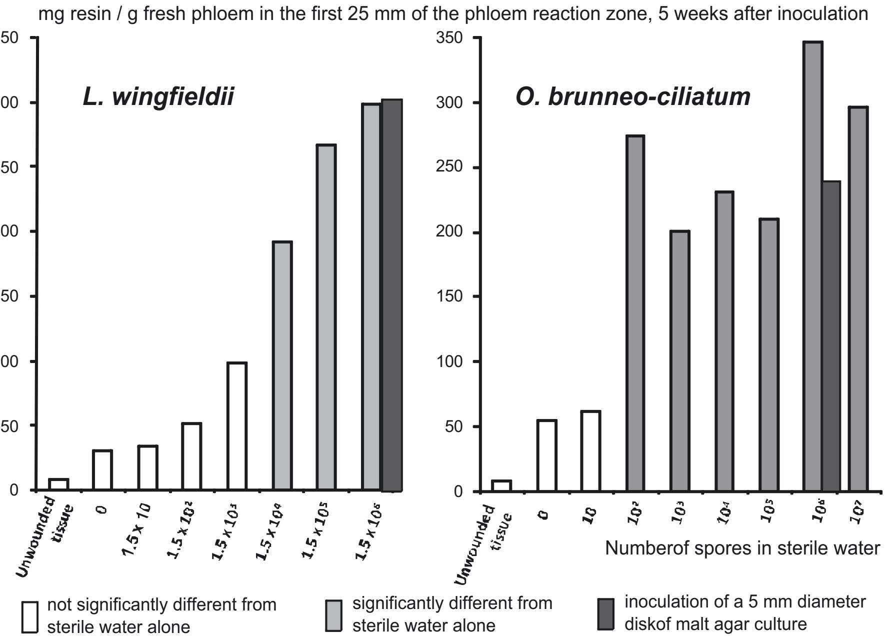

Figure 7. Resin concentration in the phloem hypersensitive reaction zone of Scots pine, 5 weeks after low density inoculations (16 to 18 inoculations per tree in total) with sterile water containing various numbers of fungal spores of *L. wingfieldii* and *O. brunneo-ciliatum*, and comparison with inoculations with 5 mm diameter disks of 3-week-old malt agar cultures (adapted from Lieutier et al., 1989a). Statistical tests were performed by comparing each resin concentration value to that obtained after inoculation with sterile water alone, and their results are indicated by the histogram color. For a tree response to be significantly different from that induced by sterile water, at least 10^4 spores were needed in the case of *L. wingfeldii*, and 10^2 in the case of *O. brunneo-ciliatum*.

a significant response (Lieutier et al., 1988; 1989a). For example (Fig. 7), this minimum number was higher than 10^4 spores for *L. wingfieldii*, a fungus vectored by *T. piniperda*, whereas it was 100 spores only for *O. brunneo-ciliatum*, vectored by *I. sexdentatus*. Certainly a contaminated *I. sexdentatus* beetle can introduce 100 spores of *O. brunneo-ciliatum* in its gallery when attacking a tree, but it is not certain that a contaminated *T. piniperda* beetle carries a sufficiently high number of *L. wingfieldii* spores to introduce at least 10^4 of them in its gallery. A 5 mm diameter disc of a 3-week-old sporulated agar culture of *L. wingfieldii* or *O. brunneo-ciliatum* (that is comparable to the discs commonly used in most experiments using artificial inoculations) contains at least 10^6 spores (Lieutier et al., 1989a) and induces a tree response comparable to that obtained with such a high number of spores (Fig. 7). This could explain why *L. wingfieldii*, although strongly stimulating tree reactions and having a high pathogenicity with artificial inoculations (Croisé et al., 1998a; Lieutier et al., 1989b; Solheim et al., 1993; Solheim and Långström, 1991), is not able to stimulate the phloem hypersensitive reaction during *T. piniperda* trunk attacks (Lieutier et al., 1989b; 1995) and thus not able to help this beetle in its establishment on the trunk. On the contrary, *O. brunneo-ciliatum* can play a real role during *I. sexdentatus* attacks on the same tree species (Lieutier, 1995; Lieutier et al., 1989a; 1989b; 1995). Using artificial inoculations of fungal culture to appreciate fungus ability to stimulate tree defenses may thus lead in some cases to overestimate the role of the fungus during natural beetle attacks.

4. WHY AND HOW BEETLES-FUNGI RELATIONSHIPS INTERFERE IN THE STRATEGY OF EXHAUSTING TREE DEFENSES

Here we discuss beetle – fungi relationships on a wider temporal scale, by proposing functional and evolutionary hypotheses for the role of Ophiotomatoid fungi in beetle establishment through stimulating tree defenses. We propose: (1) that any beetle species can develop an association with a fungus species belonging to the Ophiostomatoid flora of its host tree, and can take advantage of this association for its establishment; (2) that the selection pressure that has led fungi to develop their ability to stimulate tree defenses is the necessity of a considerably low level of tree resistance for fungus extension into the tree. Meanwhile, we replace the stimulating role of the Ophiostomatoid fungi in the general context of conifer-bark beetle-fungi relationships, leading us to present our views on the general functioning of these relationships and finally to hypothesize that beetle species are associated with fungal complexes, of which species assume various roles regarding their relationships with beetles and trees.

4.1. Origin of the associations and existence of fungal complexes

Beetle-associated fungal flora is generally similar between two conifer species belonging to the same genus, even attacked by beetle species from different genera. Conversely, there are often large differences between the beetle-associated fungal floras of conifers belonging to different genera, even attacked by closely related beetle species. As examples in Europe, the fungal flora associated with *I. typographus*, *I. duplicatus* and *I. amitinus* on spruce resembles that of *P. chalcographus* (also attacking spruce) much more than that associated with *I. sexdentatus* and *I. acuminatus* on pines, which on the contrary resembles that of *O. proximus* (also attacking pines) (Kirisits, 2004). This suggests that the host tree has more importance than the beetle in speciation of Ophiostomatoid fungi (Harrington, 1993b; Kirisits, 2004). Phylogenetic studies of the *Ceratocystis* species that develop on conifers have also concluded that adaptation of these fungi to trees is older than their adaptations to bark beetles (Harrington and Wingfield, 1998). In these conditions, it is logical to suppose that fungal ability to stimulate tree defenses is also adapted to the host tree.

We thus first hypothesize that any beetle species would be helped in its establishment on a given tree species by developing an association, even loosely, with a fungus species belonging to the Ophiostomatoid flora of that tree species. In addition, because several fungi often cohabit in a tree, we suppose that the association could be developed with various species in the same beetle population, each fungal species playing a comparable or complementary role, even they aren't as efficient. Such a comparable and complementary role has already been suggested for *G. clavigera* and *O. montium* in their feeding association with *D. ponderosae* (see Sect. 3.6). The beetle population would thus be associated with a fungal complex. For *I. typographus* as an example, a similar background of fungal species is generally found, *C. polonica*, *O. anoiae*, *O. bicolor*, *O. penicillatum* and *O. piceaperdum* being the most common and ecologically important species (Kirisits, 2004), but varying in proportion depending on locality and time (see above Sect. 3.6). In addition, competition certainly occurs between the different fungal species of a complex in a bark beetle habitat (Bleiker and Six, 2009; Klepzig et al., 2001a; 2001b). The efficiency of the beetle fungus associations in beetle population establishment would then depend on several components acting complementarily: the intrinsic ability of the beetle-associated fungi to stimulate tree defenses, the percentage of contaminated beetles in the attacking population, the level of individual inoculum, and the possible competitive interactions between fungal species. As each of these components can be affected by environmental factors and substrate variability, it is not surprising that the nature of the dominant beetle-associated fungal species and the efficiency of the insect-fungus associations vary considerably in space and time, especially for beetle species with large geographic distribution and high dispersal capabilities. Moreover, diversified environmental conditions favor a considerable mixing between beetle individuals and associated fungal flora, certainly resulting in limiting selective pressures, which in return would favor the coexistence of different fungal species in a same beetle population.

4.2. Why fungi stimulate tree defenses

According to the above hypothesis, the beetle-fungus associations can be weak and variable, which proves that certainly no coevolution has occurred between beetles and Ophiostomatoid fungi (Harrington, 1993a). Being basically adapted to trees, blue stain fungi have then adapted to transportation by beetles that live on these trees because they benefit from being easily disseminated and then introduced, through wounding, into a suitable host (Harrington, 1993a; 2005; Kirisits, 2004; Six, 2003). However, as said above, fungus extension in sapwood is allowed only if tree resistance is depleted or at least considerably lowered.

We thus hypothesize that the necessity of a considerably low level of tree resistance for fungus extension into the tree is the selection pressure that has led fungi to develop their intrinsic ability to stimulate tree defenses. In healthy vigorous trees attacked by very aggressive beetles, fungi must have developed a high ability to stimulate tree defenses. This selection could have been induced, directly or indirectly, through the ability of the fungus to grow into the phloem. Indeed, in a context of interspecific competition, growth speed is a crucial element for a fungus, since in both dead and living trees it conditions fungus access to resources like food or ability to produce fruiting bodies in the galleries of dispersing beetles, and fungal extension is positively related to phloem reaction zone length after low density inoculations (Ben Jamaa et al., 2007; Lieutier et al., 1989b; 2004; 2005; Wong and Berryman, 1977). That aggressive beetle species develop more frequently in living trees than moderately or low aggressive species would thus have lead, for the former, to more specific associations with fungi able to grow rapidly in living trees, and thus to strongly stimulate tree reactions. Moreover, in the context of an association with bark beetles for transportation, it must be noted that developing the ability to stimulate tree defenses to invade a tree is a better strategy for a fungus than developing its own pathogenicity, because this latter choice would have led to competition with the insect for the tree tissues (see below).

4.3. The necessity of controlling fungus extension

Being associated at a high frequency with a fungus that extends quickly in the tree is a risk for beetle progeny if fungus extension is not controlled after tree defenses are overcome (Harrington, 1993a; Lieutier, 2002; Six and Klepzig, 2004, among others). There are several examples indeed where phloem invaded by fungi is unsuitable for beetle larvae, such as in the case of *O. minus* for *D. frontalis* (Barras, 1970; Klepzig et al., 2001a; 2001b), *O. montium* for *D. ponderosae* (Six and Paine, 1998) or *O. ips* for *I. avulsus* (Yearian, 1966; in Klepzig and Six, 2004). These observations raised the question of how a fungus that is antagonist to the beetle larval development can be maintained in a beetle population at a high level of association, instead of being strongly counter-selected. Klepzig et al. (2001a) and Six and Klepzig (2004) proposed that an explanation could be the benefits brought by such fungi to the phoretic mites carried by the beetles. Certainly also, the benefit brought by the fungus in stimulating tree defense reactions is a valid reason.

For the beetle, equilibrium must be maintained between benefit from stimulating tree defenses and antagonism to larval development. *Dendroctonus frontalis* has achieved such an equilibrium by developing an association with a mycangial species of the genus *Entomocorticium*, antagonist to *O. minus* in addition to bringing nutrients to beetle larvae (Klepzig et al., 2001a; 2001b). Similar associations, eventually with other micro-organisms such as bacteria (Cardoza et al., 2006), certainly also exist in other bark beetle species. In the fungal complexes associated with bark beetles, there is often one relatively virulent blue stain species, while others are less virulent (Kirisits et al., 2000; Kirisits, 2004; Lee et al., 2006; Lieutier et al., 1989b; Six and Klepzig, 2004; Solheim, 1988). The role of each of them is ignored. Possibly antagonisms comparable to those described in the *D. frontalis* complex also exist, resulting in beetle brood protection and nutrition. Phloeomycetophagous species with sac mycangia should be concerned (Klepzig and Six, 2004; Six, 2003; Six and Klepzig, 2004), amongst which are *D. ponderosae* (Six and Paine, 1998; Whitney and Farris, 1970), *D. jeffreyi* (Six and Paine, 1997), *D. brevicomis* (Paine and Birch, 1983) or *I. acuminatus* (Francke-Grosmann, 1967). Beetles with pit mycangia may also be concerned especially if pits have glands, such as *I. sexdentatus* (Levieux et al., 1991). However, in this later species, no mycangial fungus has been identified and fungi-free insects develop the same way as fungi-contaminated beetles (Colineau and Lieutier, 1984). Klepzig and Six (2004) have suggested that, on poor substrates like dead trees or trees that die rapidly after attack, beetles need complementary food (mainly nitrogen and sterols) and could get it from associated fungi even if they are not mycangial. Possibly and similarly to the mycangial fungi, non mycangial fungi that serve as additional food may also serve as antagonists to blue stain fungi after tree resistance is overcome.

4.4. Multiple roles of associated fungal complexes, a final hypothesis

As a consequence of the discordance between fungal pathogenicity and beetle aggressiveness, of the tree killing process proposed above, and of our above comments and hypotheses on relationships between beetles and fungi, we finally hypothesize that a beetle species using the strategy of overcoming tree resistance is generally associated with a fungal complex, of which species could assume four roles regarding relations with beetles and trees: (1) to stimulate tree defenses in the phloem and superficial sapwood, (2) to grow into the sapwood after tree resistance is overcome, (3) to control phloem extension of the first other two categories, and (4) to bring nutrients to the beetle progeny, at least for the phloeomycetophagous beetles. Depending on beetle species and host trees, as well as environmental factors and beetle population levels, the nature and the relative abundance of the beetle associated fungal species can vary (see Sects. 3.6 and 6, references included), and thus the above roles could be played by different fungal species, or the same fungal species could be involved in several roles, which could have consequences for beetle population dynamics (Hofstetter et al., 2006).

As suggested by Paine et al. (1997), multiple and complex interactions among beetle-associated fungi and between fungi and beetles thus certainly exist. Their complexity is even increased, at least in some cases, by additional interactions with other partners such as nematodes, bacteria and mites (Cardoza et al., 2006; 2008; Klepzig et al., 2001a; 2001b). By interfering in beetle establishment and brood development in trees, these complex interactions must play an important role in the beetle population dynamics and outbreaks.

5. TYPES OF BEETLE-FUNGUS ASSOCIATIONS

There is a large diversity of types of bark beetle – Ophiostomatoid fungus associations, from absent or very simple to very elaborate. In the following section, we tentatively classify these associations by using the above considerations. Among beetle species able to develop on living trees, we are able to define three main groups while indicating the possible levels of beetle aggressiveness within them. For species using the strategy of overcoming tree resistance, we also tentatively distinguish fungal species mainly stimulating tree defenses from those rather specialized in invading the sapwood after tree defenses are exhausted. In these cases, we present known examples when fungus roles have been observed, or predictions when fungus roles are unknown.

5.1. Group 1 – Beetle species not using the strategy of exhausting tree defenses and without an effective association with Ophiostomatoid fungi

This group gathers beetle species from both ends of the aggressiveness scale.

– Group 1a is composed of highly aggressive beetles attacking living trees and succeeding in establishing and developing in their hosts without killing them, by using the strategy of avoiding tree defenses. They are not associated with fungi because they must avoid stimulating tree defenses. Typical examples are *D. micans* and *D. punctatus* on spruces. *D. micans* carries *O. canum* but at a very highly variable frequency (0.5 to 90 %) and the fungus does not play any role in the success of beetle establishment (Lieutier et al., 1992), hence corresponding to no real association.

– Group 1b gathers the very secondary beetle species that are able to attack living trees but with very low or without defense ability. They do not need to exhaust tree defenses and thus have not developed fungal associations for establishing in trees. Some secondary beetles, that are weakly or even frequently associated with more or less pathogenic black stain fungi, also belong to this group. They are however passive vectors, apparently unaffected by the presence of the fungus, and their associations are rather commensal (Klepzig and Six, 2004). For example, *D. terebrans* attacks only very weak pines, although it has already been found to be highly associated (up to 100% of the beetles) with *L. terebrantis*, a fungus which, in addition to its high pathogenicity (Harrington and Cobb, 1983), is able to induce violent tree reactions (Rane and Tattar, 1987; Wingfield, 1983). Likewise, occasionally, *Hylastes* and *Hylurgus* carry *L. serpens*, *L. procerum* or *L. wageneri* (Harrington, 1993a; Jacobs and Wingfield, 2001; Klepzig et al., 1991). *Hylurgops* are also vectors of *L. procerum*, and *D. valens* can carry *L. serpens* and even *L. terebrantis* (Klepzig et al., 1995; Jacobs and Wingfield, 2001). In all these situations however, the insect doesn't need fungus to be present in order to establish itself on trees and certainly only plays the role of a vector for the pathogen, making the association not a true one (Klepzig et al., 1991). The fact that fungus has no role to play in beetle establishment on trees, however, does not discard the possibility that associations with fungi have been developed to bring nutrients to the insect, at least at the larval stage (Eckhardt et al., 2004; Klepzig and Six, 2004).

5.2. Group 2 – Beetles species using the strategy of exhausting tree defenses but very loosely associated with fungi

Beetle species of this group attack living trees but only a very low proportion of individuals carry fungi and/or the fungi are unable to stimulate tree defenses when introduced into the tree by a beetle. The strategy of exhausting tree defenses is used but with little success because the fungal association is not effective enough to stimulate tree defenses. The best example seems to be *T. piniperda* on pines during the reproduction phase of its life cycle when it attacks trunks. It is associated with two blue stain fungi, *L. wingfieldii* rather pathogenic and *O. minus* moderately pathogenic, but the latter species has a very low and variable frequency, while the former is unable to stimulate tree defenses when introduced into the tree by a beetle (see above Sect. 3.6) (Lieutier et al., 1989a; 1989b, 1995). Probably *Cryphalus abietis* on fir (Kirschner, 1998 in Kirisits, 2004) and *Pityogenes quadridens* on Scots pine (Mathiesen-Käärik, 1953; in Kirisits, 2004) also belong to this group because of the low frequency of their associated fungi. In this group, stimulation of tree defenses is only due to beetle tunneling activity resulting, as observed during *T. piniperda* reproductive attacks, in a high critical threshold of attack density (Långström and Hellqvist, 1993; Långström et al., 1992) and a very moderate or low aggressiveness (Långström and Hellqvist, 1988; Lieutier et al., 1995).

5.3. Group 3 – Beetle species using the strategy of exhausting tree defenses and highly associated with blue stain fungi

Beetle species attack living trees, with a high percentage of individuals (sometimes up to 100%) carrying blue stain fungi. Within this group it is possible to distinguish beetle species according to their level of aggressiveness, while comparing it with the ability of their associated blue stain fungi to stimulate tree defenses (Tab. II). The problem is however that, in no situation, is the ability of the fungus to stimulate tree defenses after introduction into the host tree by a beetle known. We are thus using the fungus stimulating ability after artificial low density inoculations, and making predictions in situations where this ability is still unknown.

In Table II, considering species where data on fungus ability to stimulate tree defenses is available, the highly aggressive beetle species, *D. frontalis*, *D. ponderosae*, *D. jeffreyi*, *D. pseudotsugae*, *S. ventralis* and *I. typographus* (Furniss and Carolin, 1977; Grégoire and Evans, 2004) are all frequently associated with Ophiostomatoid fungi amongst which at least one species is able to highly stimulate tree phloem defenses (references in Tab. II). This is the same for *I. cembrae* in Scotland, its area of introduction (Redfern et al., 1987). inversely, *I. acuminatus*, *I. sexdentatus*, *O. erosus* and *I. pini*, known to be moderately or weakly aggressive (Chararas, 1962; Furniss and Carolin, 1977; Grégoire and Evans, 2004), are all associated with fungal species that stimulate the phloem response only moderately or weakly (references in Tab. II). There is thus a close correspondence between beetle species aggressiveness and the ability of their main associated fungal species to stimulate tree defenses.

The stimulating ability of the fungi associated with the other beetle species presented in Table II is not known but we can make the following predictions. Since *D. brevicomis* is known to be a very aggressive beetle for pines (Furniss and Carolin, 1977), *O. brevicomi* certainly stimulates pine defenses very strongly during attacks by *D. brevicomis*. Similarly, *D. rufipennis* is a highly aggressive beetle species on spruce (Dumerski et al., 2001) and is highly and frequently associated with *L. abietinum* (Aukema et al., 2005; Reynolds, 1992; Six and Bentz, 2003). We predict that *L. abietinum* strongly stimulates spruce defenses when introduced by *D. rufipennis* in its host. Conversely, the aggressiveness of *I. cembrae* for the European larch is usually only moderate in continental Europe (Grégoire and Evans, 2004; Redfern et al., 1987), its area of origin. Certainly, *C. laricicola* or another highly associated fungal species stimulates larch defenses only moderately in that area.

At this stage of the discussion, one may wonder if a relation exists between beetle aggressiveness and the ability of the fungal complex or other organisms to control blue stain extension in the phloem. Indeed, when a possibility of controlling fungus extension exists, it should allow beetles to tolerate fungal species which are particularly able to grow well in the phloem and thus be able to strongly stimulate tree reactions. However the lack of sufficient knowledge on the existence of antagonistic species does not allow the validity of this speculation to be checked.

6. SPECULATIVE CONCLUSION

Bark beetles and fungi maintain very complex and multiple interactions, making it difficult to propose a general model of beetle - fungus relationships. Bark beetles vary considerably in their dependence on fungal complexes for nutrition and establishment on trees, according to both their strategy of establishment and the physiological state of their usual host tree. For the species using the strategy of exhausting tree defenses, however, to be associated at a minimum frequency with fungi

Table II. Aggressiveness of bark beetles species highly associated with fungi, and the ability of their main associated fungal species to stimulate host tree defenses. H = high; M = moderate; L = low.

Observation/ prediction	Beetle species and host tree	Beetle aggressiveness	Main fungal species stimulating tree defenses after low density inoculations, and level of stimulation (H, M, L)	References / ability to stimulate tree defenses
Observation	*D. frontalis* (pines)	H	*O. minus* (H)	2, 3, 10, 15
	D. ponderosae (pines)	H	*G. clavigera* (H), *O. longiclavatum* (H), *O. montium* (M)	13, 14, 17
	D. jeffreyi (Jeffrey pine)	H	*G. clavigera* (H)	17
	D. pseudotsugae (Douglas fir)	H	*O. europhioides* (H), *O. pseudotsugae* (H)	18
	S. ventralis (Grant fir)	H	*A. symbioticum* (H)	19
	I. typographus (Norway spruce)	H	*O. penicillatum* (H), *C. polonica* (H) *Ambrosiella* sp. (H)	4, 5, 16,
	I. cembrae in UK (introduction area) (European larch)	H	*C. laricicola* (H)	12
Prediction	*D. brevicomis* (pines)	H	*O. brevicomi* (H)	
	D. rufipennis (spruces)	H	*L. abietinum* (H)	
Observation	*I. acuminatus* (pines)	M	*O. ips* (M), *O. brunneo-ciliatum* (M)	8
	I. sexdentatus (pines)	M	*O. ips* (M), *O. brunneo-ciliatum* (M)	6, 7
	O. erosus (pines)	M	*O.ips* (M)	1, 9
	I. pini (pines)	M	*O. ips* (M), *O. nigrocarpum* (L)	11
Prediction	*I. cembrae* in cont. Europe (area of origin) (European larch)	M	*C. laricicola* (M) or another species (M)	

References regarding fungus ability to stimulate tree defenses: 1 = Ben Jamaa et al. (2007); 2 = Cook et al. (1986); 3 = Cook and Hain (1987); 4 = Krokene and Solheim (1997); 5 = Krokene and Solheim (1999); 6 = Lieutier et al. (1989b; 7 = Lieutier et al. (1990); 8 = Lieutier et al. (1991b); 9 = Lieutier et al. (2005); 10 = Paine and Stephen (1987); 11 = Raffa and Smalley (1988); 12 = Redfern et al. (1987); 13 = Rice et al. (2007a); 14 = Rice et al. (2007b); 15= Ross et al. (1992); 16 = Solheim (1988); 17 = Solheim and Krokene (1998a); 18 = Solheim and Krokene (1998b); 19 = Wong and Berryman (1977).

able to naturally stimulate tree defenses is a necessity for an efficient attack. The association, even loosely, with Ophiostomatoid blue stain fungi allows that.

For the aggressive beetle species, one may speculate that no association existed at the origin, except in some cases for nutrition, which is currently the case for the saprophytic and very secondary beetles of group 1b. The conquest of living trees would have then diverged in two directions, one towards avoiding tree defenses without developing a fungal association (today group 1a), the other towards exploiting tree defenses, with the progressive help of fungi. In this latter situation, the association would have begun first as occasional, possibly comparable to the current situation for *T. piniperda* in group 2. Then, fungi would have been selected for their ability to grow in the phloem and thus to stimulate tree defenses (group 3). But this step would not allow selecting fungi that were particularly efficient in stimulation because such efficient fungi would grow too fast in the phloem after tree defenses had been exhausted. The final step would thus have been to develop novel associations with additional fungi (or other micro-organisms) able to control the phloem extension of the former after defense exhaustion, thus allowing the development of associations with fungi that were fast growing in the phloem and very efficient in stimulating tree defenses. As already mentioned, the most active sapwood invading species are most often not those that have helped the beetle in previously exhausting tree defenses. In such a context, most sapwood invading fungi might simply be "cheaters" which have taken advantage of the increasing efficiency of the relationships between the beetles and the fungi stimulating tree defenses. The rather high level of pathogenicity of some of them would result only from competition with other sapwood invaders. Such a scenario of building beetle-fungi associations based on primary association with fungal species specialized in stimulating tree defense, completed later with the arrival of pathogenic "cheaters", can be interestingly compared with the suggestion that weakly pathogenic fungi are "established associates" whereas pathogenic fungi are incidental late arriving "invaders" (Six, 2003; Six and Paine, 1999; Six and Klepzig, 2004).

Moving from group 1b to groups 2 and then 3 gives the appearance of moving from very simple relationships (nutritional only) to more and more elaborate relationships (nutritional plus stimulation plus eventual control of extension) between beetles and fungal complexes. However, these successive groups do not reflect the phylogeny of bark beetles, at least when referring to the *Dendroctonus* genus (Kelley and Farrell, 1998; Six and Klepzig, 2004). The above speculated process of building beetle-fungi associations would have thus evolved independently for the different beetle species of a same group. This is in agreement with the conclusion that no coevolutionary process has occurred between beetles and their associated fungi (Harrington, 1993a).

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