

# 9

## From Attack to Emergence: Interactions between Southern Pine Beetle, Mites, Microbes, and Trees

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### Abstract

Bark beetles are among the most ecologically and economically influential organisms in forest ecosystems worldwide. These important organisms are consistently associated in complex symbioses with fungi. Despite this, little is known of the net impacts of the fungi on their vectors, and mites are often completely overlooked. In this chapter, we will describe interactions involving the southern pine beetle (SPB), among the most economically damaging of North American forest insects. We examine SPB interactions with mites, fungi, and other microbes, following the natural temporal progression from beetle attack to offspring emergence from trees. Associations with fungi are universal within bark beetles. Many beetle species possess specialized structures, termed mycangia, for the transport of fungi. The SPB consistently carries three main fungi and numerous mites into the trees it attacks. One fungus, *Ophiostoma minus*, is carried phoretically on the SPB exoskeleton and by phoretic mites. The mycangium of each female SPB may contain a pure culture of either *Ceratocystiopsis ranaculosus* or *Entomocorticium* sp. A. The mycangial fungi are, by definition, transferred in a specific fashion. The SPB possesses two types of gland cells associated with the mycangium. The role of these cells and their products remains unknown. Preliminary studies have observed yeast-like fungal spores in the mycangium and several surrounding tubes that presumably carry secreted chemicals from gland cells (or bacteria) to the mycangium. The degree to which there is selective activity of the glandular chemical secretions remains to be seen. While *O. minus* may play some role in tree killing, none of these three fungi are highly virulent in their pine hosts. All three fungi grow within the phloem, sporulating heavily in beetle tunnels within which the SPB larvae graze. Though their ecological roles are complex and context-dependent, these three fungi can be divided into an antagonist (*O. minus*) and two mutualists (both mycangial fungi, though *Entomocorticium* sp. A appears to be of greater benefit to the beetles than *C. ranaculosus*). Naturally, all three of the fungi compete for access to uncolonized pine phloem. The results of these competitions can have significant impacts on their beetle and mite hosts, and ultimately on the population dynamics of this destructive pest.

## 9.1. INTRODUCTION

Bark beetles (Coleoptera: Scolytidae, altern. Curculionidae: Scolytinae) are among the most ecologically and economically influential organisms in forest ecosystems worldwide. These important organisms are consistently associated in complex symbioses (from casual commensalisms to obligate mutualisms) with fungi (Harrington 2005, Paine and others 1997) and mites (Kinn 1971, Lindquist 1969, Moser and Roton 1971). Despite this, little is known of the net impacts of the fungi on their vectors, and mites are often completely overlooked. In this chapter, we will describe interactions involving the southern pine beetle (*Dendroctonus frontalis* Zimmermann) (SPB), among the most economically damaging of North American forest insects (Price and others 1998). We will examine SPB interactions with its associated mites and fungi, following the natural temporal progression from beetle attack to offspring emergence from trees.

### 9.1.1. Phoretic Mites

Mites (Chelicerata: Acariformes) are commonly associated with bark beetles and fungi (Kinn 1971). Although mites are often believed to be passive inhabitants of a community, they can have strong interactions with nonmite species (Hofstetter and others 2006a), are important indicators of disturbance (Schelvis 1990, Steiner 1995), impact natural and agricultural systems (Dicke and Sabelis 1988, Hill and Stone 1985, Lindquist 1986), and are major components of biological diversity (Walter and Proctor 1999). Details of the biology and ecology of this important group of organisms are handled in chapter 11. We focus here on their roles and activities within the beetle-tree interaction.

Dispersal and migration pose major challenges for mites living in discontinuous, ephemeral habitats (e.g., bark beetle-infested trees). Because mites are very small and wingless, movement between resources that are patchy in space and time requires assistance. The use of one organism by another for transport or phoresy, is common among mites, small insects, pseudoscorpions, nematodes, and microbes associated with bark beetles. Mites are especially adept at phoresy and often have highly modified phoretic stages (phoretomorphs) and appendages (Kinn 1971, Moser and Cross 1975). Phoretic individuals often go through a sequence of behaviors or morphological changes that are quite different from nonphoretic individuals of the same

species. Many of these behaviors are analogous to those used by parasites to find their hosts (Athias-Binche and Morand 1993). In a sense, all phoresy can be considered an exploitation of the carrier, and therefore, parasitic. However, species interactions should be defined in terms of their ultimate effects on the fitness of the participants if they are to make ecological and evolutionary sense (Walter and Proctor 1999). Under most conditions, phoretic mites can be classified as commensal, in that they do not affect the carrier but the phoront benefits (Houck 1994). However, when mites are abundant they may interfere with carrier movement and reduce travel distances (Kinn 1971, Kinn and Witcosky 1978).

### 9.1.2. Phoretic Fungi

Associations with fungi are universal within bark beetles. Many beetle species possess specialized structures, termed mycangia, for the transport of fungi (Batra 1963, Klepzig and Six 2004, Levieux and others 1989, Paine and others 1997). Broadly defined, a mycangium is any structure that consistently transports fungi regardless of form (Beaver 1989, Farris and Funk 1965, Furniss and others 1987, Livingston and Berryman 1972). The mycangium may exist in a variety of forms, from simple pits to highly evolved integumental invaginations lined with glandularized cells (as in SPB).

Ophiostomatoid fungi are well adapted to dispersal on the exoskeletons of bark beetles and associated arthropods (Klepzig and Six 2004). Most of these fungi produce perithecia with necks that extrude sticky spores at heights where they are likely to be encountered by invertebrates. These spores are also shaped such that multiple contact points with the vector are likely, and easy removal is not. Asexual fruiting structures also produce mucilaginous masses of spores that readily adhere to insects (Malloch and Blackwell 1993). These adhesive coats of spores are easily dispersed in resin (though not in water) ensuring release only in the presence of an appropriate substrate (i.e., a new host tree) (Whitney and Blauel 1972).

## 9.2. THE SOUTHERN PINE BEETLE AND ITS SYMBIOTIC COMMUNITY

The SPB consistently carries three main fungi and numerous mites into the trees it attacks. One ophiostomatoid fungus, *Ophiostoma minus*, is

carried phoretically on the SPB exoskeleton and by phoretic mites (Bridges and Moser 1983, Rumbold 1931). The phoretic transport of this fungus by SPB and its arthropod associates, especially mites, is the only means of access *O. minus* has to new host tissue (Dowding 1969). The mycangium of each female SPB (the structures are not found in males; Barras and Perry 1972, Happ and others 1971) may contain a pure culture of either *Ceratocystiopsis ranaculosus* (Barras and Taylor 1973, Jacobs and Kirisits 2003, Zipfel and others 2006) or *Entomocorticium* sp. A (aka, SJB122; Barras and Perry 1972, Happ and others 1976), an amber-colored basidiomycete (Hsiau 1996). A small percentage of beetles may not carry fungi in their mycangium. Only mycangial fungi, if present, are found in live beetles.

While *O. minus* may play some role in tree killing (see below), none of these three fungi are highly virulent in their pine hosts. All three fungi grow within the phloem, sporulating heavily in beetle tunnels within which the SPB larvae graze. Though their ecological roles are complex and context-dependent (also reviewed in greater detail below), these three fungi can be divided into an antagonist (*O. minus*) and two mutualists [both mycangial fungi, though *Entomocorticium* sp. A appears to be of greater benefit to the beetles than *C. ranaculosus* (Bridges 1985, Coppedge and others 1995, Goldhammer and others 1990, Klepzig and Wilkens 1997)]. Naturally, all three of the fungi compete for access to uncolonized pine phloem (Klepzig and Wilkens 1997). The results of these competitions can have significant impacts on their beetle and mite hosts. Interestingly, *O. minus* and *C. ranaculosus* spores are also carried by *Tarsonemus* mites (Acarina: Tarsonemidae) that can occur in high densities on SPB (Bridges and Moser 1983, Hofstetter and others 2006b, Moser 1985, Moser and Bridges 1986). *Tarsonemus* release fungal spores throughout newly excavated beetle galleries (Lombardero and others 2000c). Feedback between mites and *O. minus* (as well as *C. ranaculosus*) can affect SPB population dynamics and influence forest dynamics (Hofstetter and others 2006a; Lombardero and others 2000c, 2003).

### 9.2.1. Beetles in Flight

#### *Mite Community*

The mite community of the SPB is reviewed elsewhere in this volume (see chapter 11). The phoretic members of this assemblage are faced with a choice among a number of

conspecific hosts, which may vary in quality as carriers. The best host is one that is predictable, available, and abundant. Southern pine beetles provide mites with the additional advantage of delivering their mite phoronts, such as *Tarsonemus krantzi*, to substrate suitable for their favored fungus (*O. minus*) (Hofstetter and others 2006a). Thus, *Tarsonemus* spp. occur frequently on SPB and have strong interactions with Ophiostomatoid fungi (Hofstetter and others 2006a, 2006b; Klepzig and others 2001a, 2001b; Lombardero and others 2000c, 2003) (Figure 9.1). Abundances of mite species vary with time of year, beetle density and emergence patterns, fungal abundance, and geographic location (Hofstetter and others 2006a, 2006b; Kinn 1971, 1982).

#### *Fungal Community*

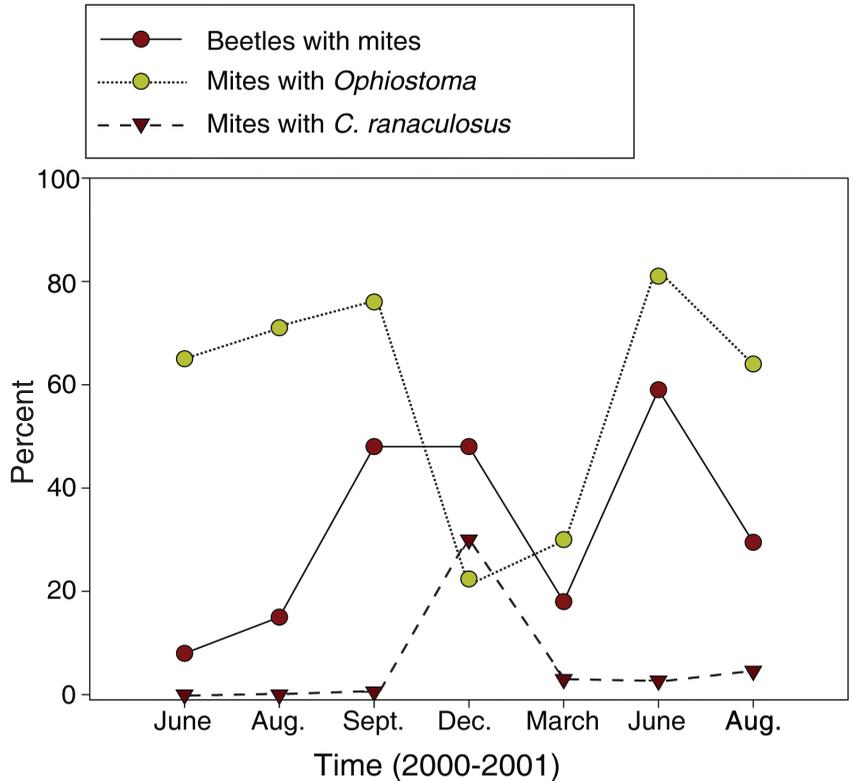
The relative abundance of each mycangial fungus within SPB populations varies with location and time of year (Harrington 2005, Hofstetter and others 2006b). Interestingly, 5-20 percent of females within a population have both mycangial fungi, one within each side their mycangium. A small percentage of SPB females within a population do not carry mycangial fungi (Hofstetter and others 2006b). Spores of *O. minus* (and *O. nigrocarpum*; Harrington 2005) are commonly found on the exoskeleton of SPB (Bridges and Moser 1983, Rumbold 1931). *O. minus* is the most abundant associate, but its abundance varies greatly among beetle populations and across regions (Harrington 2005).

### 9.2.2. Beetles on the Attack

#### *Beetle Interactions with Highly Defended Host Trees*

As beetles enter living/more or less healthy/well defended trees, they are met with an exudation of oleoresin (Hodges and others 1979). If sufficient oleoresin flows from each wound, and if that resin is viscous enough and crystallizes quickly enough, the SPB entomb or “pitch-out”. Although SPB attack can be successfully resisted (Trapp and Croteau 2001), it is generally accepted that no potential host tree is immune to attack at high SPB densities (Strom and others 2002). Resistance by individual trees does vary, and environmental attributes that affect oleoresin production can have substantial impacts on the success of beetle attack. So far, it has not been possible to experiment with SPB effects on host trees in the absence of fungi and mites. It is also very

**Figure 9.1**—Percentage of beetles with *Tarsonemus* mites and percentage of *Tarsonemus* that have  $\geq 1$  spore of *Ophiostoma minus* or *Ceratocystis ranaculosus*. Each time period represents beetles from five infestations in Bankhead National Forest, Alabama. (data collected by R.W. Hofstetter)



difficult to artificially infest healthy trees with SPB (Cook and Hain 1987a, 1987b). Most work on this insect-tree interaction has therefore been limited to artificial wounding and inoculation, without the direct inclusion of the insect itself. Fortunately, the fungal associates of SPB can be readily cultured on media and used as a practicable surrogate for SPB (Klepzig and Walkinshaw 2003).

### Fungal Interactions with Highly Defended Host Trees

#### *Ophiostoma minus*

While the exact role of *O. minus* at this stage of the SPB life cycle continues to be debated, the insect-tree interaction is affected by and affects the fungus immediately. For example, SPB that have been exposed to tree resin carry dramatically reduced populations of viable fungal propagules on their exoskeleton (Veysey and others 2003). As SPB enter trees, the spores they carry may become detached and begin to germinate. This inoculation of *O. minus* into phloem begins the colonization process. While *O. minus* is not a primary pathogen within pine trees, it may work with the beetles to hasten tree death (Paine and others 1997). Studies examining the virulence of *O. minus* have varied in methodologies and results. Most investigators accept increased lesion

size as an indication of greater growth within the host tissues. The relationship between growth *in situ* and degree of virulence is less clear. *Ophiostoma minus* is capable of growing some distance—and causing a relatively high degree of resinosis and necrosis—within well defended pine trees. In no case, however, have single point inoculations with *O. minus* been found to be capable of causing death of, or even external symptoms on, host trees. The spate of inoculation studies in the 1980s led to a change in thinking about the possible role of this fungus in the SPB life cycle. Contrary to initial investigations (Bramble and Holst 1940, Nelson 1934), *O. minus* was no longer seen as a virulent pathogen which killed the tree and allowed for development by its mutualistic insect vector, SPB. The observations of tree mortality attributed to SPB in the absence of *O. minus* (Bridges and others 1985, Hetrick 1949) added to this line of thinking.

A mass inoculation study, perhaps more closely reflecting the inoculation technique used by SPB, has cast a different light on the role of *O. minus* in the SPB attack process. Recognizing that previous work (Table 9.1) (Christiansen and others 1999; Cook and Hain 1986, 1987b; Guérard and others 2000; Horntvedt and others 1983; Krokene and Solheim 2001; Långström and others 2001; Lieutier 2002; Raffa and

Berryman 1983; Solheim and others 1993) in other systems had demonstrated the feasibility and utility of using mass inoculation to test host responses to beetles and vectored fungi, Klepzig and others (2005) tested whether mass inoculation with *O. minus* had lasting effects on resin defenses in loblolly pine. They quantified oleoresin production response to wounding alone, and wounding plus inoculation, relative to untreated controls. They also quantified a secondary defense of pines against *O. minus*, by measuring the extent to which this fungus colonized tree tissue. Finally, they tested the ability of loblolly pine to exhibit induced systemic resistance to fungi in response to mass inoculation, a phenomenon previously observed in Norway spruce (Krokene and others 1999), Scots pine (Krokene and others 2000), Monterey pine (Bonello and others 2001), and Austrian pine (Bonello and Blodgett 2003).

Trees mass inoculated with *O. minus* produced higher resin yields than control or wounded-only trees as soon as 15 days and as late as 105 days post-treatment. While fungal stimulation of resin flow was previously known (Hepting 1947, Popp and others 1991), it had never been studied in the context of a mass inoculation/simulated beetle attack context. Lieutier (2002) does caution against strong reliance on artificial inoculation studies in making conclusions about resistance to bark beetles. There are likely important differences between artificial inoculations and natural beetle attacks. For example, the percentage of SPB carrying *O. minus* varies significantly even within a population (Hofstetter and others 2006a, 2006b). However, in some cases there is close

correspondence between results from artificial inoculations and natural attacks (Guérard and others 2000, Långström and others 2001). Klepzig and others (2005) did conclude that beetles attacking previously attacked trees—within the zone of the previous attack—would face a more extensive resinous response from their host than would beetles attacking unattacked trees. However, this effect does not appear to extend beyond a single season. While recently published studies have indicated that mass wounding might decrease tree resistance to SPB and *O. minus* (Tisdale and others 2003a, 2003b), these studies did not incorporate simultaneous impacts of wounding and fungal inoculation, as typically occurs in the natural SPB infestation process.

Klepzig and others (2005) did not observe signs of systemic induced resistance in loblolly pine. Lesions from inoculations outside the mass wounding/inoculation site did not differ between treatments. Fungal success, as measured by the area of host tissue colonized by the invading fungus before it was stopped by the host defensive response, was unaffected by prior fungal inoculation experience by the host. These results are in agreement with Krokene and others (1999) who, working with Norway spruce, only noted this phenomenon in the immediate vicinity of mass inoculations.

In this study, mass inoculations with *O. minus* did not result in the death of a single tree at either site. However, SPB likely inoculate *O. minus* at much higher rates (up to 1,900 m<sup>2</sup>; Fargo and others 1978). While some bark beetle-associated fungi can kill trees (Krokene and Solheim 1998, Lieutier 2002, Solheim and

**Table 9.1—Summary of inoculation studies using SPB associated fungi (literature summary by K.D. Klepzig)**

Lesion size (mm)	Sampling time (days)	Wound (mm)	Inoculum type	Reference
42,68,62,69	7,14,21,28	13	Mycelia in broth	Cook and Hain 1985
100-150	14	13	Mycelia in broth	Cook and Hain 1986
103	14	13	Mycelia in broth	Cook and Hain 1987a
18,20,80,90	1,2,7,14	10	Mycelia in broth	Cook and Hain 1987b
74-97	14	13	Mycelia in broth	Cook and Hain 1988
65,49,85,87	21	3,6,12,24	Phloem disk	Paine and Stephen 1987b
111.5		48		
45	20	12	Phloem disk	Paine and Stephen 1987c
70-120	14	12	Phloem disk	Paine and others 1988
70,110,81	3,7,28	10	Cotton	Ross and others 1992
24	70	10	Malt extract agar	Nevill et al. 1995

others 1993), numerous inoculation studies have failed to demonstrate a similar role for *O. minus* (see Table above). Paine and others (1997) note that the long-held assumption that bark beetle-associated fungi kill trees is based primarily on the vector relationships, the association of staining with dead trees, and instances of artificial mass inoculation killing trees. However, Lieutier (2002) explains that this does not necessarily indicate involvement of phoretic ophiostomatoid fungi in tree death. Rather, the role of bark beetle associated fungi, e.g., *O. minus*, may be that of cofactors (Kopper and others 2004, Lieutier 2002)—biotic agents that are not pathogenic in and of themselves but do function in compromising host defenses (Beckage 1998).

In the SPB system, the primary role of *O. minus* may be to aid in exhausting tree defenses and allowing for beetle establishment. During the critical period that beetles first enter a tree and either succeed or fail to trigger aggregation, it appears that the host defensive response to fungal inoculation can dramatically reduce resin flow. The tree may be at this stage—and due to the fungus—more vulnerable to beetle infestation. Anything that contributes to depletion of the tree’s ability to synthesize secondary metabolites during beetle aggregation improves the probability of successful beetle mass attack (Lieutier 2002). Subsequently, the ultimate death of the tree likely occurs from a combination of bark beetle and fungal effects (Lieutier 2002, Paine and others 1997). Via this mechanism and/or detoxification of host chemistry, *O. minus* appears to aid SPB in overcoming its tree host.

#### Mycangial fungi interactions with highly defended host trees

The early pine-SPB fungus interaction literature painted a contradictory picture of the relative virulence of these fungi. Some focused on the small resinous lesions formed by mycangial fungi within trees and inferred an avoidance of host defense, or greater virulence (Paine and Stephen 1987b, Paine and others 1988). Others concluded that a more extensive host response indicated a greater degree of fungal virulence (Cook and others 1986, Cook and Hain 1985, 1988). Cook and Hain (1988) noted that mechanical wounds alone produced shorter lesions than mycangial fungi, which produced shorter lesions than *O. minus*, concluding that “The more virulent invader appeared to evoke a stronger response... the less energy a tree uses in

defending itself against less virulent attackers, the more energy it would have for future defensive responses.” In general, mycangial fungi are now seen as weak pathogens of healthy trees (Paine and others 1997).

#### Mite Interactions with Highly Defended Host Trees

Little is known about how bark beetle-associated mites interact with trees during the attack stage, though it has been hypothesized that the heavy resin flow serves to scrub beetles clean of phoretic mites, at least to some extent. By comparing the number of mites on beetles that attack a tree (before entering the tree) with the number of mites on those same beetles when they reemerge from the tree, Hofstetter and others (unpublished) found that a majority (~90 percent) of phoretic mites leave their beetle host or are killed between the time a beetle lands on the tree and after it has mated and laid eggs. The trigger or cue that results in mites dismounting from a host beetle varies with the mite species and tree condition. In general, mites appear to leave host beetles when the host is in the appropriate habitat for the mite (Kinn 1971) or when the beetle is stressed (e.g., captured by a predator) or dying (e.g., old age) (Hofstetter, personal observation). Mites of most species are capable of surviving more than 30 days without food, indicating that phoretic mites can survive during the phoretic stage and through the early stages of bark beetle attack on trees (Kinn 1971). This extended survival allows time for fungi, nematodes, and beetle larvae to establish in trees.

### 9.2.3. Beetles in the Tree

#### Beetle Interactions with Poorly/Undefended Host Trees

Southern pine beetles must attack living trees for their offspring to develop properly, and do not attack dead or cut material. The SPB attack process transforms highly defended hosts into poorly defended hosts, which are good substrates for SPB larval and mycangial fungus development. However, new attacks by SPB on hosts that are already poorly defended are generally unsuccessful. These poorly defended hosts are often occupied by secondary bark beetles and saprophytic fungi that may be competitive and antagonistic towards SPB and its associated fungi. By the time defensive compounds in an attacked tree have diminished in quantity, most SPB larvae in that tree have moved through several developmental stages,

and the mycangial fungi introduced by the mother beetle are well established in the surrounding phloem. Larvae may feed on a fungus that originates from an adjacent gallery of a conspecific or another arthropod. The fact that 20 percent of emerging SPB have two mycangial fungi suggests that this is not a rare occurrence (Hofstetter and others 2006b).

### *Fungus Interactions with Poorly/Undefended Host Trees*

Competitive interactions among SPB-associated fungi occur largely within trees that have succumbed to beetle attack. These poorly defended trees pose a different context to the interacting fungi and their insect vectors.

#### *Ophiostoma minus*

While the aggressiveness of *O. minus* within well defended trees may aid in stimulating and/or overcoming host defenses, this same aggression may make it a detriment to its beetle vector (Lieutier 2002). Highly pathogenic fungi that exhaust tree defenses and rapidly kill them are also very likely to invade the whole tree very rapidly, even before the beetle and its brood can become established. This makes these host tissues unsuitable for beetle development. This phenomenon has been well documented in the *O. minus*/SPB association in which the blue stain fungus is highly antagonistic to SPB mutualistic fungi (Klepzig and Wilkens 1997) and thus to larval development (Barras 1970). The moderately virulent habit of *O. minus* may be the best strategy for SPB. This allows SPB to successfully mass attack trees, but limits the extent to which the fungus can grow during beetle development (Lieutier 2002). By the time larvae begin developing within host tissues, *O. minus* becomes a competitor and antagonist of SPB (Barras 1970). The mechanism of this antagonism is likely due to, in large part, the interactions of SPB with its two other significant fungal associates. Areas fully colonized with *O. minus* are correlated with reduced developmental success in SPB—inhibited egg production, slower larval growth and development, even larval mortality (Barras 1970, Hofstetter and others 2006a). In addition, overall levels of *O. minus* within SPB infestations are negatively correlated with SPB population increase (Bridges 1985, Hofstetter and others 2006a, Lombardero and others 2000c).

### *Mycangial fungi*

Most SPB infestations (from 1 to more than 100 colonized trees) have both mycangial fungi present (Bridges 1983, Coppedge and others 1995, Hofstetter and others 2005). This indicates the importance of both of these species to SPB and/or their phoretic mites. *Ophiostoma minus*, *Entomocorticium* sp. A, and *C. ranaculosus* compete for the uncolonized pine phloem (Klepzig and Wilkens 1997) and engage in primary resource capture, followed by direct interaction, which can lead to defense, and/or secondary resource capture. Competitive wins by mycangial fungi will result in successful development and emergence of fit adults. Wins by *O. minus* will likely result in poorly fed, weakened larvae and few, if any, emerging as adults. The most appropriate time to evaluate the role of the mycangial fungi is post-mass attack. Once the tree's resistance is overcome and the eggs hatch, early instar larvae begin feeding, constructing fine, sinuous galleries in the phloem as they develop and move (Payne 1983). Eventually, the larvae enlarge their feeding area into obovate feeding chambers that become lined with either of the two mycangial fungi. It appears extremely likely that larval SPB get most of their nutrition from the fungal growth within their feeding chambers rather than directly from the phloem itself. The mycangial fungi may, in fact, provide their most substantial benefits to SPB by concentrating dietary nitrogen for larvae (Ayres and others 2000). Beetles carrying *Entomocorticium* sp. A within their mycangia are more fecund and heavier, and have higher lipid contents than those containing *C. ranaculosus*. In turn, beetles containing *C. ranaculosus* tend to be more fit than those whose mycangia contain no fungi (Bridges 1985, Coppedge and others 1995, Goldhammer and others 1990). These interactions, however, are mediated by a variety of abiotic factors.

Differences in fungal tolerance to various tree compounds, for example, can have significance for fungal colonization, growth rate, and fungal-fungal competition. High levels of secondary metabolites (e.g.,  $\alpha$ -pinene) may favor the growth of one mycangial fungus over another. Seasonal variation in secondary metabolites can also lead to dynamic changes in mycangial fungi throughout the year. Phloem chemistry affects the nature of interactions between fungi by altering the production, diffusion, or volatilization of fungal-produced compounds (Boddy 2000). Differences in compound

concentrations or lack of particular compounds and the abundance of *O. minus* within trees may influence the relative frequencies of mycangial fungi within beetle infestations. Because these fungi differ in their benefits to the beetle, allelochemical effects on competitive interactions can drastically affect beetle success. For example, *C. ranaculosus* is a better competitor with *O. minus* than *Entomocorticium* sp. A in the presence of  $\alpha$ - or  $\beta$ -pinene. High levels of uric acid (a component of SPB frass) in growth media reduces *C. ranaculosus* and *O. minus* growth, but increases the growth rate of *Entomocorticium* sp. A (Goldhammer and others 1989). Uric acid also reduces *O. minus*' ability to capture resources previously captured by *Entomocorticium* sp. A (Hofstetter and others 2005). Uric acid levels likely increase in larval chambers as beetles develop, and thus become more important for competing fungi during the late larval or pupal stage. Differences in the tolerance of the two mycangial fungi to phloem chemistry, temperature, moisture, interactions with *O. minus*, or other species-specific organisms (e.g., nematodes associated with one of the fungal species) may have selected for two fungal mutualistic associates of SPB.

Competitive interactions among bark beetle-associated fungi are also potentially influenced by water potential, which undergoes marked changes over the course of beetle colonization of tree hosts. Soon after SPB attack, the phloem tissue the beetles inhabit rapidly dehydrates (Wagner and others 1979, Webb and Franklin 1978). Subsequent changes in water relations strongly affect the growth and competition of fungi (Klepzig and others 2004). At especially low water potentials, fungal growth is reduced to the extent that *C. ranaculosus* can equally compete with *O. minus*. This demonstrates the effects of an abiotic factor on fungal interactions and helps explain the success of mycangial fungi in SPB infested hosts, despite the nearly overwhelming competitive ability exhibited by *O. minus* in most cases (Klepzig and Wilkens 1997, Klepzig and others 2004).

Temperature also strongly affects the growth rates of all three fungi (Klepzig and others 2001b). *Entomocorticium* sp. A grows near maximum levels at cooler temperatures, suggesting that seasonal changes can alter relative fungal abundances within this community. In a study of several sites in northern Alabama, Hofstetter and others (2006b) observed that *Entomocorticium* sp. A became more abundant in winter and spring but tended to be supplanted

by *C. ranaculosus* during the summer. Experimental manipulations of temperatures within infested logs were consistent with this seasonal pattern.

In summary, the variety of consistent SPB fungal associates may insulate SPB, to a degree, from the variety of abiotic conditions they face.

### *Mite Interactions with Poorly/Undefended Host Trees*

*Tarsonemus* mites are important in the propagation of *O. minus* between and within beetle-infested trees (Hofstetter and others 2006a, Lombardero and others 2003). Although ascospores of *O. minus* are abundant on the bodies of most mites within trees, only *Tarsonemus* spp. carry ascospores in the phoretic state (Moser 1985).

The feeding habits and interactions of mites underneath bark remain largely unknown. Food resources are readily available in undefended, infested host trees, and it is during this period that mites reproduce, feed, and propagate fungi throughout the inner bark. Mite populations are capable of staggering growth rates (Bruce and Wrensch 1990)—more than 300 fold in some cases (Lombardero and others 2000c) during this time.

Conditions within hosts can drastically worsen or improve for mites with time; phloem moisture changes, temperatures become more extreme, predator densities likely increase, microbial communities change, and pathogens may increase as the tree dies. Thus mite population growth rate and survival change as time progresses, depending upon the biology and trophic nature of the mite, insect, and microbial species present. For example, feeding by large woodborer larvae prior to beetle emergence may reduce fungal and mite levels under the bark. More specifically, *Tarsonemus* spp. and *O. minus* suffer direct losses from woodborer larval feeding and likely disrupt mite dispersal within bark (Hofstetter, unpublished).

### *Beetle Interactions with Fungi and Other Microbes in Poorly Defended Trees*

By the time trees have succumbed to SPB attack, *O. minus* acts as an antagonist of the developing larvae. This negative effect is indirect: 1. there is strong asymmetric competition between mycangial fungi and *O. minus* (Klepzig 1998); 2. SPB larvae require mycangial fungi (Barras 1973); and 3. beetles without fungal mutualists

are relatively less affected by phoretically vectored blue stain fungi (Klepzig and Six 2004, Yearian and others 1972). However, we cannot exclude direct effects from fungal compounds [(phenolics and isocoumarins (Hemingway and others 1977) or melanin (DeAngelis and others 1986)]. It is also possible that *O. minus* (or a colorless relative; e.g., Cartapip®) could be deployed as a biological control agent (Klepzig 1998).

Insects can harbor gut microbial communities that range from simple to complex (Cruden and Markovetz 1987, Handelsman and others 2005, Leadbetter and others 1999, Lilburn and others 2001). Little is known about gut symbionts of bark beetles. Previous studies have suggested a role in pheromone synthesis (Brand and others 1975, Conn and others 1984) and protection from gallery-invading fungi (Cardoza and others 2006). The importance of symbiotic fungi in the life cycles of bark beetles (Hofstetter and others 2006a, 2006b), the nutrient-poor substrate on which they feed, and the cellulolytic and nitrogen-fixing activities of some microbes associated with wood-boring insects (Bridges 1981, Delalibera and others 2006) suggest that gut symbionts could play important roles in the biology of bark beetles. Recently, Vasanthakumar and others (2006) found  $\alpha$ - and  $\gamma$ -Proteobacteria and Firmicutes in SPB larvae. Adult SPB guts contained only  $\gamma$ -Proteobacteria. They concluded that the presence of *Bacillus* sp. and *Leuconostoc* sp. in larval but not adult guts indicated a role in growth and development for these bacteria. *Enterobacter* spp., *Rahnella aquatilis*, *Klebsiella* spp., and *Pantoea* spp. were all commonly found in SPB larvae, and are known to fix nitrogen in other environments (Behar and others 2005), indicating a possible role for these bacteria, together with some fungal associates, in nitrogen concentration for larvae (Ayres and others 2000, Bridges 1981, Klepzig and Six 2004). These microbes might also detoxify conifer defensive compounds (monoterpenes, diterpene acids, phenolics) (Lewinsohn and others 1991, Martin and others 1999, Raffa and others 2005, Yu and Mohn 1999). Neither Vasanthakumar and others (2006) nor Delalibera and others (2006) found cellulolytic bacteria with SPB. This important role is likely performed by other microbial associates. Recent work has identified yet another bacterial partner of SPB (Scott and others 2008). A new species of actinomycete bacterium occurs within the mycangium and larval galleries of SPB. Via a newly characterized antibiotic, mycangimycin, the bacterium strongly inhibits

the fungal antagonist *O. minus*, but only weakly inhibits the mutualist, *Entomocorticium* sp. A. (Scott and others 2008).

### *Fungus Interactions with Fungi in Poorly/Undefined Host Trees*

The fungi associated with SPB compete with one another and other fungi for host substrate and access to beetles and mites. While these competitions have been quantitatively characterized (Klepzig and Wilkens 1997), the mechanisms behind them remain poorly understood. One possible explanation for the observed antibiotic effects may lay with the molecules responsible for the common name of some ophiostomatoid fungi—stain fungi. Melanins are dark biological macromolecules that can protect fungi against irradiation, enzymatic lysis, temperature extremes, and desiccation (Butler and Day 1998). These secondary metabolites can also be toxic (Henson and others 1999), can act as virulence factors, and may account for as much as 30 percent of the dry weight of a cell (Butler and Day 1998). The role of melanins in the ecology and pathology of bark beetle-associated stain fungi remains unknown. Klepzig (2006) found that *Entomocorticium* sp. A was significantly inhibited by added melanin, though *C. ranaculosus* was not.

### *Fungus-Mite Interactions in Poorly/Undefined Host Trees*

Mites and associated insects play a significant role in the dispersal of fungi within SPB-infested trees. Mite densities can be extremely high (> 100 individuals per cm<sup>2</sup> in phloem) in areas where food sources are plentiful. Food resource abundance and mite abundance are often interrelated, in that particular resources (e.g., a species of nematode or fungus) are closely associated with mite species (Lindquist 1986). For instance, there is strong evidence that *O. minus* abundance (number of distinct units) is strongly correlated with *Tarsonemus* abundance (Hofstetter and others 2006a, 2006b; Lombardero and others 2000c, 2003).

Mite-fungal interactions are dynamic and change as the tree decays or climatic conditions change. For example, in cool temperatures a larger proportion of *Tarsonemus* carry ascospores of *C. ranaculosus*. Presumably, hyperphoresy of *C. ranaculosus* on *Tarsonemus* tends to promote the extent of phloem that is colonized by *C. ranaculosus*, and therefore the proportion of SPB that later emerge carrying

*C. ranaculosus* (Hofstetter and others 2006b). Interestingly, changes in temperature and moisture might influence genetic variability in *C. ranaculosus* via effects on mite behavior. Temperature, or any other factor that influences the relative abundance of *C. ranaculosus* and *Entomocorticium* sp. A, would likely affect both the mite and beetle populations. Because *C. ranaculosus* represents an inferior nutritional resource for SPB (Bridges 1983; Coppedge and others 1995; Goldhammer and others 1990; Klepzig and others 2001a, 2001b) but a superior nutritional resource for *Tarsonemus* (Lombardero and others 2000c), seasonal changes in the ratio of mycangial fungi species could influence beetle and mite population dynamics in opposite directions. Field studies by Miller and Parresol (1992) and Bridges (1983) demonstrated increased reproduction in beetle populations when *Entomocorticium* sp. A was the dominant mycangial fungus. Likewise, Hofstetter and others (2006a, 2006b) recorded increased mite reproduction and decreased beetle reproduction during periods when *O. minus* and *C. ranaculosus* were particularly abundant. These results and interpretations predict that the abundance of *C. ranaculosus* relative to *Entomocorticium* sp. A would tend to be highest in the warmest climates where SPB occurs (for example, Florida and Mexico). Preliminary surveys of SPB mycangia in Mexico reveal that *Entomocorticium* sp. A is very uncommon (Hofstetter and others, unpublished).

## 9.2.4. Beetles Emerging

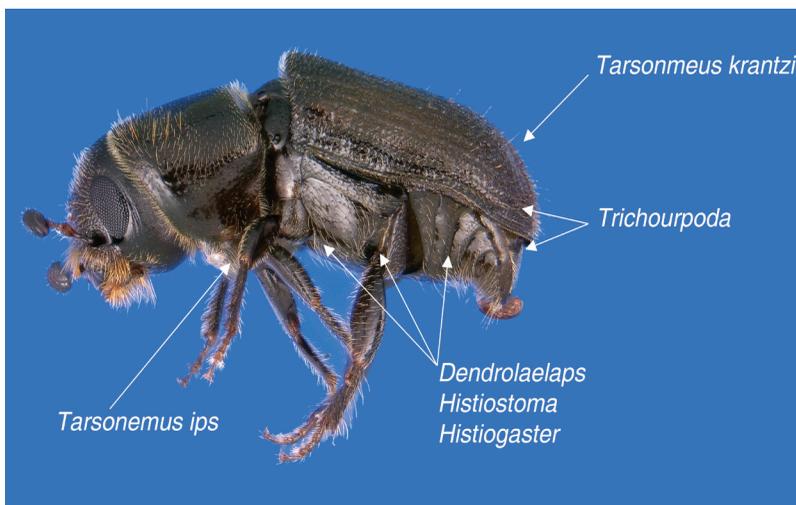
### *Acquisition of Mites during Emergence*

Typically only one life history stage is phoretic in a given species. Most phoretic mites of many different taxa share similar morphologies: dorsoventral flattening, oval or circular bodies, and flanges covering all or some appendages (Athias-Binche and Morand 1993, Binns 1982, O'Conner 1994). This convergent morphology may serve to reduce loss of moisture when on the host and to present a smooth dorsal surface, making it difficult for the host to remove mites by grooming or rubbing (Figure 11.2 in chapter 11).

Mites may be attracted, or inhibited from attaching, to a given insect carrier via kairomonal secretions or acoustic emissions from the insect, the insect's fungal component or activity level (e.g., tunneling), as well as the condition, sex, and age of the insect. Abiotic factors, such as low moisture and high temperature within bark, can stimulate mites to search for phoretic hosts (Kinn 1971). Mites exhibit some site specificity, reminiscent of niche partitioning, in attaching to SPB adults (Figure 9.2). Interestingly, most SPB exiting a tree have just a few or even no mites (Hofstetter and others 2006a, 2006b; Kinn 1971).

### *Acquisition and Transport of Fungi*

Many insects emerging from SPB-infested bark acquire *O. minus* while traveling through SPB galleries or stained phloem. Approximately 80 percent of SPB-associated species carry *O. minus*. Likewise, up to 80 percent of *Tarsonemus* may carry *O. minus* spores. No *O. minus* was observed on mite species phoretic on associated insects (Table 9.2). The mycangial fungi are, by definition, transferred in a much more specific fashion. Happ and others (1971) first described the mycangium of the SPB, identifying two types of gland cells associated with this structure. The role of these cells and their products remains unknown. In preliminary studies, Klepzig and others (unpublished) have observed yeast-like fungal spores in the mycangium and several surrounding tubes that presumably carry secreted chemicals from gland cells to the mycangium. The degree to which there is selective activity of the glandular chemical secretions remains to be seen.



**Figure 9.2**—General locations of phoretic mite species on SPB during flight. (figure by R.W. Hofstetter)

**Table 9.2 — The presence or absence of *O. minus* and mites on organisms captured in emergence traps on 36 SPB-infested *Pinus taeda* in Talladega National Forest, Alabama 2000 (Data collected by R.W. Hofstetter)**

Insect	N	Percent insects with <i>Ophiostoma minus</i>	Percent insects with <i>Tarsonemus</i>	Percent insects with mites (all species)*
<i>Crematogaster</i> sp.	1	100.0	0	0
<i>Aradus</i> sp.	4	0	0	0
<i>Aulonium</i> sp.	2	100.0	0	50.0
<i>Atanycolus comosifrons</i>	2	100.0	0	0
<i>Cossonus corticola</i>	27	74.1	0	16.0
<i>Corticus</i> sp.	1	0	0	0
<i>Crypturgus aleutaceus</i>	4	0	0	0
<i>Dendroctonus frontalis</i>	247	59.1	35.1	49.2
<i>D. valens</i>	1	100.0	0	0
<i>Gnathotricus materiarius</i>	26	30.8	0	21.0
<i>Platysoma</i> sp.	2	100.0	0	50.0
<i>Hylastes</i> sp.	2	100.0	0	50.0
<i>Ips avulsus</i>	1	100.0	100.0	100.0
<i>I. grandicolus</i>	1	100.0	0	0
<i>Leptacinus</i> sp.	1	100.0	0	0
<i>Lyctocoris</i> sp.	5	80.0	0	0
<i>Platysoma attenuata</i>	11	72.7	0	0
<i>P. parallelum</i>	22	72.7	0	46.0
<i>Plegaderus</i> sp.	1	100.0	0	0
<i>Roptroceris</i> sp.	1	100.0	0	0
<i>Silvanus bidentatus</i>	3	66.7	0	0
<i>Tenebroides collaris</i>	1	0	0	0
<i>Thanasimus dubius</i>	1	100.0	0	0
<i>Temnochila</i> sp.	2	0	0	50.0
<i>Tenebroides collaris</i>	2	50.0	0	50.0
<i>Tenebroides marginatus</i>	1	0	0	100.0
<i>Xyleborus</i> sp.	5	60.0	0	25.0

### 9.3. CONCLUSIONS AND EVOLUTIONARY CONSIDERATIONS

The SPB creates ephemeral habitats that are occupied by a large community of insects, mites, and fungi. These organisms interact with each other and the host tree in ways that modify the phloem resource on which they all depend. Some of the stronger interactions, such as those between the mycangial fungi, *O. minus*, SPB, and *Tarsonemus*, have been thoroughly covered in this chapter. Long-term evolutionary changes in these symbiotic associations may have resulted in specialized behaviors and dependencies. Closely related taxa that exhibit a variety of ecological relationships allow the testing of hypotheses about the direction of

evolution (e.g., phoresy leads to parasitism) and about forces behind such changes and associations. Mites and fungi associated with bark beetles provide numerous clear examples of switches in lifestyle that encompass parasitism, antagonism, commensalism, and mutualism. More studies are needed to evaluate how these interactions and others change over geographic space and evolutionary time, and how bark beetle communities influence beetle dynamics and beetle-tree interactions.

Factors that affect the evolution of obligate associations or promote the virulence of mites and microbes are important topics relating to disease dynamics and species preservation. How important are neutral symbionts (e.g., commensal mites) in promoting pathogens and

diseases? Do microbes transmitted vertically (mites or fungi transmitted from parent beetle to offspring) produce greater effects on host dynamics than those transmitted horizontally between unrelated beetles within the tree? How would global climate change affect the stability of organisms within a tight community or the dynamics of an outbreak species? How do cheaters (e.g., *C. ranaculosus*) join and persist within communities? These are some questions that can be addressed through studies of interactions between bark beetles and their associated organisms.