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Phoretic Symbionts of the Mountain Pine Beetle (*Dendroctonus ponderosae* Hopkins)

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During its life cycle, the tree-killing mountain pine beetle *Dendroctonus ponderosae* Hopkins interacts with phoretic organisms such as mites, nematodes, fungi, and bacteria. The types of associations these organisms establish with the mountain pine beetle (MPB) vary from mutualistic to antagonistic. The most studied of these interactions are those between beetle and fungi. The least studied are interactions with bacteria, but these have received increased attention recently. Nematodes remain little studied. We reviewed the significant literature pertaining to MPB phoronts. A number of potentially important interactions and contributions resulting from associations between MPB and its phoronts are discussed. A wealth of literature exists on this topic, yet many questions remain unanswered, and the effects of some phoronts on population levels remain unexplored.

Keywords: bacteria, bark beetles, fungi, mites, nematodes, symbiosis

The mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) is a natural disturbance agent in western North American coniferous forests, which uses various species of *Pinus* as hosts. Eruptive populations can cause extensive levels of tree mortality. When beetles arrive at a tree they carry a large array of ecto- and endosymbiotic organisms, which exhibit highly complex interactions that can contribute to the success or failure of population establishment in the new host. These include several species of mites (Reboletti 2008, Mori et al. 2011), external and internal nematodes (Reid 1958, Massey 1974), fungi and yeasts (Whitney 1982, Paine et al. 1997, Six 2003), and bacteria (Cardoza et al. 2009, Winder et al. 2010, Hulcr et al. 2011). This collection of organisms comprises an entire functioning community including fungivores, herbivores, detritivores, scavengers, parasites, and predators. The roles of some microorganisms, particularly certain fungi, are relatively well understood in the MPB community (Six and Paine 1998, Six 2003, Bentz and Six 2006). Some fungi and bacteria may facilitate digestion of host tissues, aid pheromone synthesis, or serve as a food source for beetles (Six 2003, Harrington 2005, Bentz and Six 2006). Bacterial and yeast symbionts may benefit beetle hosts by modifying the microbial community, particularly by inhibiting antagonistic fungi (Cardoza et al. 2006a). Entomopathogenic fungi, such as *Beauveria bassiana* (Bals.-Criv.) Vuill., can easily infect MPBs, especially during epidemics, playing a role in population dynamics (Hunt et al. 1984, Safranyik et al. 2001). Mites are commonly associated with bark beetles and have a suite of interactions (Cardoza et al. 2008, Hofstetter 2011). Many of these associates probably exert both positive and negative effects on beetles (Eckhardt et al. 2004, Klepzig and Six 2004, Kopper et al. 2004). For example, in the southern United States, *Tarsonemus ips* Lindquist carries *Ophiostoma minus* (Hedgc.) H. and P. Syd., which is antagonistic to southern pine beetle (SPB) (*Dendroctonus frontalis* Zimm.); however, in Chiapas, Mexico, the same mite is associated with *Ceratocystiopsis ranaculosa* T.J. Perry & J.R. Bridges (Moser and Macías-Sámano 2000) a known mutualist of SPB. Hence, the effects of phoretic mites may be context-dependent, or they can be considered conditional mutualists.

Species that attach to other organisms, called phoronts, are highly adapted for transport in or on other organisms and often have highly modified structures in their phoretic stage. In this form of symbiosis, the organisms often go through behavioral changes (such as cessation of feeding and host searching) or morphological changes that are quite different from those of nonphoretic individuals of the same species. Under most conditions, phoretic organisms can be

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classified as commensal, in that they do not cause any direct harm or benefit to the carrier but benefit by being transported to a new habitat (Houck 1994). When these phoronts are abundant, they may interfere with carrier movement, reduce travel distances, and be energetically costly (Kinn 1971, Kinn and Witcosky 1978). Phoronts may provide direct or indirect benefits or harm to their carrier or influence ecological interactions within host trees. Thus, the relationship of the phoront and its carrier may be mutualistic, neutral (e.g., commensal, benefiting the phoront), or antagonistic (predatory, parasitic, or toxic), resulting in a loss of fitness to the carrier.

Symbionts of the MPB

Mites

The Phoretic Mite Fauna

Trees colonized by bark beetles often become home to a large variety of other invertebrates. Mites (Acari: Acariformes) are common phoronts on bark beetles (Kinn 1971, Moser and Roton 1971), and their numbers on individual beetles can vary greatly from none to hundreds of mites on a single beetle (Hofstetter 2011). Mites of bark beetles are now known to have strong interactions with associated organisms, are major components of biological diversity, and can have an impact on bark beetle population dynamics and fungal interactions (e.g., Hofstetter et al. 2006a, 2006b).

An extensive body of literature exists on phoretic mites associated with several bark beetle species (reviewed by Hofstetter et al. 2013) such as SPB (Moser 1976, Kinn and Witcosky 1978, Hofstetter et al. 2007), spruce beetle (SP) (*Dendroctonus rufipennis* Kirby) (Cardoza et al. 2008), European spruce bark beetle (*Ips typographus* L.) (Takov et al. 2009), species of *Pityokteines* Fuchs (Pernek et al. 2008), and *Scolytus* Geoffroy (Moser et al. 2010). Most mites associated with bark beetles are in the order Sarcoptiformes (Kinn 1971, Moser and Roton 1971, Hofstetter et al. 2013). Tarsonemid mites in the order Trombidiformes include parasites of beetle eggs (Lindquist 1986) and fungivores that often have intricate relationships with fungi associated with beetles (Moser 1985, Bridges and Moser 1986, Moser et al. 1989a, 1989b, Cardoza et al. 2008). Mesostigmata mites, including many genera found in decaying fungi, are especially prominent as predators of nematodes and other mites and as phoronts on adult bark beetles (Kinn 1971, Moser and Roton 1971, Lindquist 1975, Lindquist and Wu 1991). Oribatid mites that often associate with bees and wasps are also common on bark beetles and may act as commensal organisms, mutualists, or predators (Kinn 1971). Phoretic mites may be specific on bark beetle species or found on multiple insect species, including predatory insects (Hofstetter et al. 2013).

There are published records of mites associated with MPB (Lindquist and Hunter 1965, Lindquist 1969, 1971, Moser and Roton 1971, Mori et al. 2011), including 13 phoretic mite species (Table 1). Studies in Alberta, Canada (Mori et al. 2011), and South Dakota (Reboletti 2008) showed that the percentage of adult beetles carrying phoretic mites varied by collection method but typically averaged 30–50% of beetles. The mean numbers of mites varied by site and time of year (Mori et al. 2011), ranging from 0.93 to 2.75 mites per beetle (Reboletti 2008). Reboletti (2008) recorded the location of several phoretic mite species on the beetle exoskeleton. *Proctolaelaps subcorticalis* Lindquist were found under the elytra, whereas *Tarsonemus endophloeus* Lindquist were found on the metathoracic wing origin or the sternum. Other mite species were found on various places on the beetle's exoskeleton (Reboletti 2008, Mori et al. 2011) (Figure 1). Despite the best efforts of past investigators, our understanding of MPB mite fauna remains incomplete, partly because of understudied areas of their geographic distribution.

Table 1. Feeding guild and abundance of phoretic mites found on adult *Dendroctonus ponderosae* in North America.

Mite symbiont	Feeding guild[a]	Frequency in *D. ponderosae*[b]
Histiogaster arborsignis Woodring	Mycetophagous	Infrequent
Macrocheles schaeferi Walter	Predacious	Rare
Nanacarus spp.	Omnivorous	Rare
Parawinterschmidtia (Khaustov) spp.	Unknown	Rare
Proctolaelaps hystricoides Lindquist & Hunter	Predacious	Common
Proctolaelaps subcorticalis Lindquist	Predacious	Frequent
Schweibea spp.	Unknown	Rare
Tarsonemus endophloeus Lindquist	Mycetophagous	Rare
Tarsonemus ips Lindquist	Mycetophagous	Frequent
Trichouropoda lamellose (Hirschmann)	Omnivorous	Infrequent
Trichouropoda spp.	Omnivorous	Rare
Winterschmidtia spp.	Unknown	Rare
Tydeidae (undetermined genus)	Unknown	Rare

[a]Omnivorous, feeds on a variety of organisms: fungi, bacteria, dead invertebrates, and others; mycetophagous, feeds on fungi, often transports and disperses reproductive structures of fungi; and predacious, feeds on living organisms such as nematodes, invertebrate eggs, or larvae.
[b]We categorize phoretic mite abundance on beetles as rare (<1% have the particular mite species), infrequent (1–5%), common (5–20%), and frequent (>20% have the species).

Mite Feeding Guilds and Diversity

The phoretic mite diversity associated with MPB seems moderate to low compared with that of phoretic mite assemblages found on other species of *Dendroctonus* Er. (Moser and Roton 1971, Cardoza et al. 2008, Hofstetter et al. 2013). For instance, only five species of phoretic mites were commonly associated with MPB in Alberta, Canada. Mori et al. (2011) suggested that this may be due to recently established relationships on the leading edge of the MPB outbreak spreading to novel range expansions in Alberta. However, sampling intensity and methodology may influence levels of species diversity reported in various studies. For example, in South Dakota, Reboletti (2008) catalogued 10 mite species on MPB when sampling was conducted over multiple years. As found for the SPB, additional differences in mite diversity could be associated with MPB population levels or environmental conditions that may affect species differentially, among other factors (Hofstetter et al. 2006b).

In terms of trophic effects and community interactions, several mite species are known to be predators of other invertebrates or early MPB developmental stages. *Macrocheles* Latr. and *Proctolaelaps* Berlese mites are known to feed on nematodes, other mites, and bark beetle eggs and early larvae (Lindquist and Hunter 1965, Moser and Roton 1971, Kinn 1983, Hofstetter et al. 2013). *Tarsonemus* Canestrini and Fanzago and *Histiogaster* (Griffiths) are known to be fungal feeders (Moser and Roton 1971, Moser 1985, O'Connor 1990, Cardoza et al. 2008, Moser et al. 2010, Hofstetter et al. 2013). The feeding habits of other mite genera found on MPB, such as *Parawinterschmidtia* (Khaustov), *Schweibea* Oudemans, *Trichouropoda* Berlese, and *Winterschmidtia* Oudemans, are unknown, but they may be omnivores (Hofstetter et al. 2013).

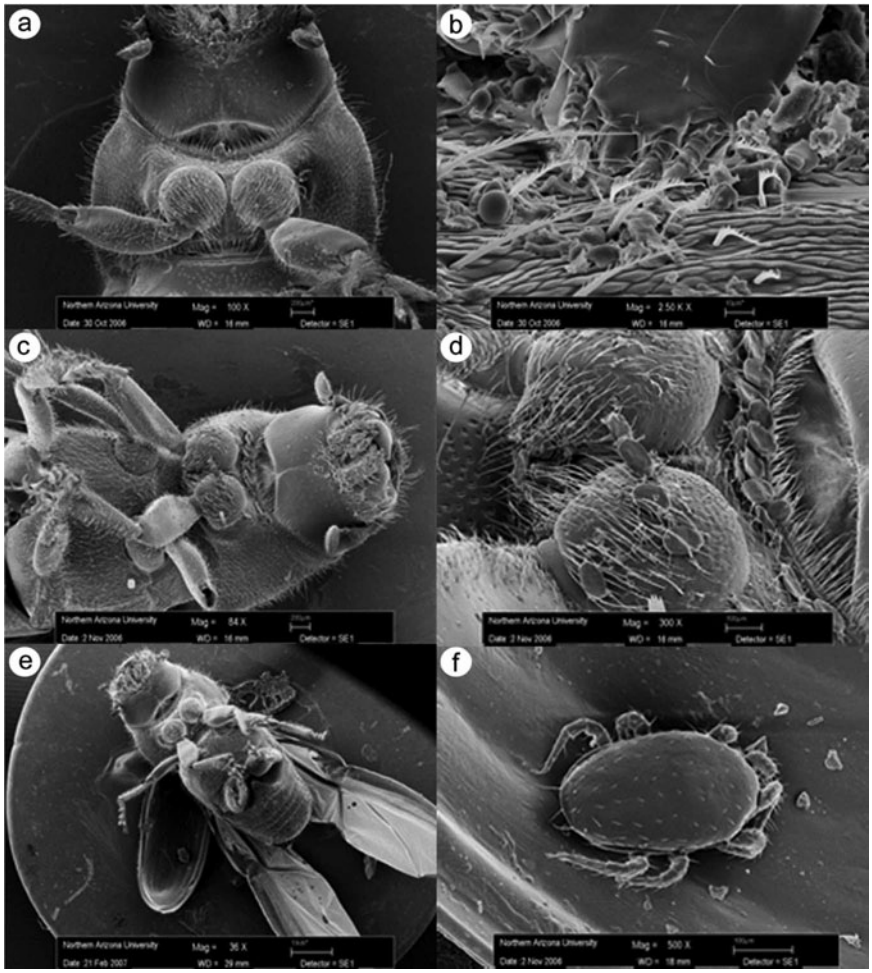

Figure 1. Scanning electron microscope images. (a) Phoretic *Tarsonemus* mites located near the first coxa of an adult MBP. (b) Closer look at the first two sets of legs of *Tarsonemus;* note that there is a spore located in the left-hand portion of the image. (c) Species of *Tarsonemus* located on the thorax of an adult MPB. (d) A closer image of (c). The phoretic mites appear in a necklace-like fashion on the beetle. (e) *Proctolaelaps* located in the elytra. (f) A closer image of the body surface of *Proctolaelaps*. (Images by R.W. Hofstetter and D.M. Reboletti.)

Seasonal changes and environmental conditions, among other factors, may influence the bark beetle-associated mite fauna (Hofstetter et al. 2006b). Like MPB, mites are subject to the effects of weather, predation, and disease. Mite abundance, from other bark beetle species studied, is known to fluctuate seasonally (e.g., Hofstetter et al. 2007), develop at rates mediated by temperature (e.g., Lombardero et al. 2000), and experience mortality from extreme temperatures (e.g., Evans et al. 2011) or diseases (Schabel 1982).

Interactions between Mites and MPB

Phoretic mites can have a direct impact on MPB by effects on beetle free movement or by predation on immature stages. Mites can directly reduce the flight velocity of individual SPBs (Moser 1976). The presence of clusters of mites at the tips of the elytra of Douglas-fir beetle (DFB) (*Dendroctonus pseudotsugae* Hopk.) reduced its wing-beat frequency (Atkins 1960). Although no specific studies have been conducted on MPBs, the findings of Moser (1976) and Atkins (1960) suggest that effects may potentially occur by decreasing dispersal and colonization by the beetle.

Mites can also be predators of eggs and larvae of species of *Dendroctonus* (Lindquist and Bedard 1961, Moser and Roton 1971, Kinn and Witcosky 1978, Lindquist 1986). Moser (1975) studied mite species found associated with brood of SPB and concluded that eight species could be useful as natural control agents in reducing field infestations. These included *Histiogaster arborsignis* Woodring, *Proctolaelaps dendroctoni* Lindquist & Hunter, *Macrocheles boudreauxi* Krantz, *Dendrolaelaps neodisetus* Hurlbutt, *Eugamasus lyriformis* Mc-Graw & Farrier, *Dendrolaelaps neocornutus* Hurlbutt, *Dendrolaelaps isodentatus* Hurlbutt, and *Proctolaelaps fiseri* Samsinak. Species of mites belonging to these genera are associated with MPB (Reboletti 2008, Mori et al. 2011), yet it is not known what affect they may have on its population dynamics.

Mites can indirectly affect MPB by altering the presence and abundance of antagonistic or mutualistic fungi, yeast, bacteria, nematodes, or other invertebrates. For instance, *Tarsonemus* spp. are known to influence the abundance of fungi in SPB-infested trees (Lombardero et al. 2003) and can potentially affect the population dynamics of beetle populations (Hofstetter et al. 2006a). Such interactions may exist in the MPB subcortical environment since mites have been observed in areas of blue-stain fungi such as *Ophiostoma montium* (Rumbold) Arx and *Grosmannia clavigera* (Rob.-Jeffr. and R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wing. in MPB-infested trees.

Figure 2. Four *Trichouropoda* spp. (tortoise mites) transported around the front coxae of a MPB that rests on a pine needle. (Photograph by Javier E. Mercado.)

Some of the most common phoretic mite species on MPB are known to carry fungal spores, suggesting that they could vector fungi between trees and within trees. Mori et al. (2011) observed fungal spores attached directly to the cuticle of *P. subcorticalis*, and Reboletti (2008) observed *P. subcorticalis* and *T. ips* carrying spores. In South Dakota, where both mites disseminated spores, Reboletti (2008) found that approximately 70% of the spores transmitted by the two mites were *O. montium*, whereas approximately 30% were *G. clavigera*. Mites in the genus *Histiogaster* Berlese are also known to carry fungal spores when associated with SB (Cardoza et al. 2008) and thus may vector and feed on the beetle's associated fungi as well. Similarly, a species in the genus *Trichouropoda*, a genus found on MPB in at least South Dakota (Reboletti 2008), Alberta (Knee et al. 2012), and Colorado (J.E. Mercado, USDA Forest Service, unpubl. observ., July 2013) (Figure 2), is one of the principal vectors of ophiostomatoids in *Protea* L. flowers in South Africa (Roets et al. 2011). It is possible that MPB-transported *Trichouropoda* mites introduce fungi that could alter the fungal composition in subcortical environments. *T. ips* could help augment the frequency of *O. montium*, the mycangial fungus that prefers warmer temperatures (Six and Bentz 2007), throughout the season in the subcortical niche. Mites thus have the potential to influence fungal communities and abundance within MPB-infested trees and potentially the frequency of mycangial fungi dispersed by MPBs.

Indirect negative effects can also occur between MPB and its phoretic mites. Mites have been shown to carry *B. bassiana* on their surfaces (Renker et al. 2005) and transmit it to the pales weevil (*Hylobius pales* [Herbst]) (Peirson 1921). Another fungal species, the green muscardine fungus (*Metarhizium anisopliae* [Metschn.] Sorokin), was effectively transmitted by a species of *Macrocheles* mite to the pales weevil, causing widespread mortality to the beetles (Schabel 1982). Thus, it is pertinent to examine the potential of mite symbionts in vectoring entomopathogens of MPB.

Changing densities of MPBs within trees can affect the abundance of mycetophagous mites by influencing the fungal species composition within the host trees. The overall phoretic mite community assemblage and abundance may increase with augmentation of MPB densities. As MPB density increases within the tree, total phoretic mite abundance on emerging beetles could increase. Mites that remain within habitats after MPBs have left probably have the greatest mortality or must find other phoretic hosts, such as clerid beetles, secondary bark beetles, or woodborers, to locate new habitats. Thus, mutualism may better explain the relationship between mites that both transport and feed on MPB beneficial fungi.

Table 2. Feeding guild and transport site of 13 species of ecto- and endosymbiotic nematodes described for *Dendroctonus ponderosae* in North America.

Nematode symbiont	Feeding guild	*D. ponderosae* transport site
Aphelenchoides tenuidens (Thorne 1935)	Ectoparasite	Digestive tract
Bursaphelenchus conurus (Steiner 1932) Goodey 1960	Mycetophagous	Under elytra
Bursaphelenchus talonus (Thorne 1935) Goodey 1960	Mycetophagous	Under elytra
Contortylenchus reversus (Thorne 1935) Rühm 1956	Endoparasite	Hemocoel
Cryptaphelenchus latus (Thorne 1935) Rühm 1956	Mycetophagous	Under elytra
Ektaphelenchus josephi (Massey 1974)	Mycetophagous	Under elytra
Ektaphelenchus obtusus (Massey 1956)	Mycetophagous	Elytra, hemocoel
Mikoletzkya inedia (Massey 1966)	Egg predator	Under elytra
Mikoletzkya pinicola (Thorne 1935) Baker 1962	Egg predator	Under elytra
Neoditylenchus pinophilus (Thorne 1935) Goodey 1963	Mycetophagous	Undetermined
Panagrolaimus dentatus (Thorne 1935) Rühm 1956	Unknown	Under elytra
Parasitaphelenchus acroposthion (Steiner 1932) Rühm 1956	Endoparasite	Hemocoel
Sphaerulariopsis hastatus (Khan 1957) Nickle 1963	Endoparasite	Hemocoel

Nematodes

The Symbiotic Nematode Fauna

Nematodes are one of the most diverse groups of invertebrates and include many functional groups (Bongers and Bongers 1997) that are common internal and external symbionts in many subcortical beetles (Massey 1974). However, the interactions between bark beetles, nematodes, and other associated organisms have received little attention. The nematode fauna of *Dendroctonus* species in North American has been described for the roundheaded pine beetle (*Dendroctonus adjunctus* Blandf.) (Massey 1966), DFB (Furniss 1967), SB (Cardoza et al. 2008), SPB (Massey 1956), and MPB (Steiner 1932, Thorne 1935, Reid 1958, Massey 1974). Steiner (1932) made the first contribution to the taxonomy of nematode fauna associated with MPB by describing three species collected from MPBs colonizing western white pine (*Pinus monticola* ex D. Don) from northeastern Washington. Subsequently, nine species were described by Thorne (1935) from beetles collected on lodgepole pine (*Pinus contorta* ex Loudon) from northeastern Utah and British Columbia, Canada (Reid 1958). Massey (1974) summarized the biology and taxonomy of species associated with North American bark beetles, adding one species from central New Mexico to that of the MPB. Currently, the associated nematode fauna of MPB includes 13 species that are known to establish ecto- and endosymbiotic relationships with the beetle (Table 2).

Nonparasitic nematodes are typically transported externally on the beetle, whereas parasitic species are usually transported internally. Phoretic and parasitic nematodes transported by bark beetles undergo an alternate third larval state known as a dauerlarvae, which is a resting or diapause stage (Poinar 1969). In MPB, dry clusters of dauerlarvae travel at the inner base of each elytron (Cardoza et al.

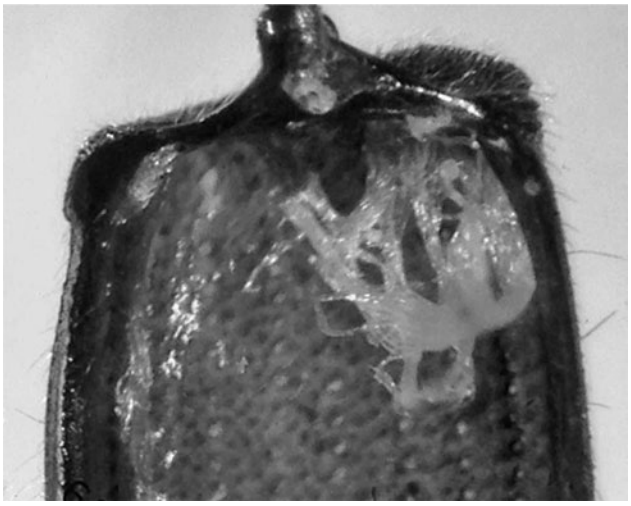

Figure 3. A mass of unidentified nematode dauerlarvae travels inside a MBP's elytral base. (Photograph by Javier E. Mercado.)

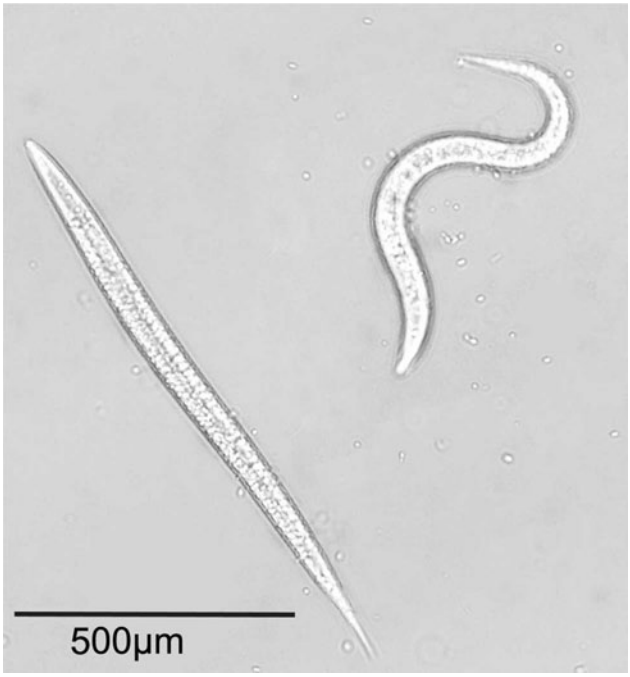

Figure 4. Unidentified nematode from inside the elytra of a MPB. Note the spores floating on the medium around the nematode. (Photograph by Javier E. Mercado.)

2006b) (Figure 3). Those in the genus *Ektaphelenchus* Fuchs (Figure 4) build a leathery cocoon in which up to 75 immature females have been found (Massey 1974) (Figure 5). *Ektaphelenchus obtusus* Massey has been found in pocket-like structures, termed "nematangia" in the hind wing of the SB, but in MPBs collected from Utah, nematangia were not found (Cardoza et al. 2006b).

Effects of Nematodes on MPB Populations

Nematodes can develop an array of symbiotic strategies with their transporting hosts. These strategies can be phoretic, parasitic, necromenic (completing development after natural death of host), or predatory (Massey 1974). Studies of parasitic nematode symbionts occurring in *Dendroctonus* species have shown that both null and negative effects can occur. In laboratory experiments in British

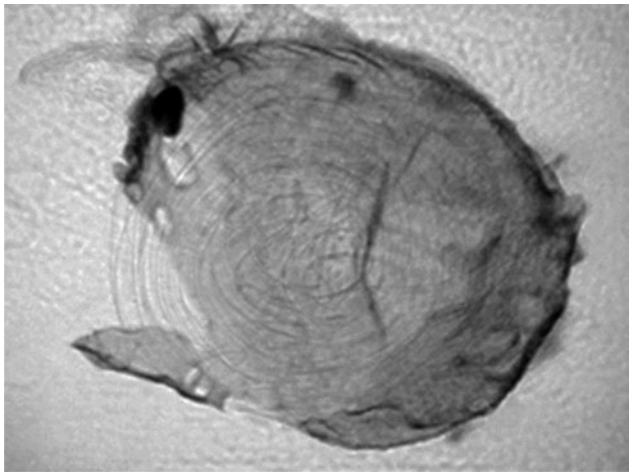

Figure 5. Cocoon-like structure (<1 mm in diameter) created by a phoretic nematode. The structure was removed from under the elytra of a SPB caught in a flight trap. Note that the nematode is still within the structure. (Photograph by Richard W. Hofstetter.)

Columbia, Canada, Atkins (1961) found in general a null effect of phoretic nematodes on DFB during its dispersal to new host trees. The results suggested that the overall flying range capacity of 90 DFBs was not different between beetles lacking nematodes or those with any combination of ecto- and endoparasitic nematodes; however, the first of several induced flights during a period of 8 hours was significantly shorter for beetles carrying nematodes. Physiologically, nematodes can negatively affect the reproductive success of their beetle hosts. Adult female DFB showed a reduction of 20% of total protein and size of their oocytes when harboring the endoparasitic nematode *Contortylenchus reversus* (Thorne) Rühm (Thong and Webster 1975). Southern pine beetles serving as hosts to *Contortylenchus brevicomi* (Massey) showed a 74% reduction in brood in contrast with that of uninfested individuals (MacGuidwin et al. 1980) and Massey (1956) indicated a reduction in egg production in SB in northern Colorado infested by nematodes in the genus *Sphaerulariopsis* Wachek. In British Columbia, females with the nematode *Sphaerulariopsis hastatus* (Khan) Nickle produced approximately 33% less brood than females lacking these. The infested individuals also exhibited lethargic behavior unlike that of those uninfested by the nematode (Reid 1958). Amman and Cole (1983) found that *Mikoletzkya pinicola* (Thorne) Baker was the principal cause of egg mortality through predation. In addition to reducing egg numbers, nematodes can directly affect the development of various insect stages. MacVean and Brewer (1981) indicated that *Steinernema carpocapsae* (Weiser) can infect all developmental stages of MPB, but only at very high concentrations per individual beetle. They found that early developmental stages were more susceptible, but inoculations of 3,000 nematodes were needed to kill 44 and 66% of larvae and pupae, respectively. The nematode *C. reversus* has a potentially high rate of transmission into MPB brood from parent MPBs. For instance, in Utah, females of *C. reversus* were reported to produce hundreds of eggs in both the larvae and adult hemocoel (Thorne 1935).

Nematodes may indirectly affect MPB through interactions of the associated microbe biota of both organisms. In SB and SPB, mycetophagous nematodes in the genera *Ektaphelenchus* and *Parasitorhabditis*, genera also found in MPB, associate with fungi as well as bacteria different from those normally associated with their beetle

carriers (Cardoza et al. 2006b, Carta et al. 2010). Fungal spores have been observed on nematodes from Colorado (J.E. Mercado, USDA Forest Service, unpubl. observ., July 2013.) (Figure 4). However, the interactions between MPB microbes and those of their phoretic nematodes have not been studied.

Little has been published on the direct effects of nematodes on bark beetles, and with the exception of Cardoza et al. (2008), no recent studies have been conducted with nematodes in bark beetles. The available literature indicates that nematodes can reduce the fitness of bark beetles, including MPB. Consequently, nematodes may contribute to maintenance of bark beetle populations at endemic levels. Moreover, the decline of an outbreak of the SB in Colorado during the late 1950s was attributed in part to nematode infections of a species in the genus *Contortylenchus* Rühm due to reduced female fecundity as quantified by McCambridge and Knight (1972). To better understand the population dynamics of MPB, it is important to examine the interaction of nematodes with other associated organisms. Nematode-vectored microorganisms could influence the microbial composition found in carrier beetles and, therefore, that of their subcortical niche.

Fungi

Fungal symbionts are common in the Scolytinae in which they can contribute to beetle nutrition in MPB (Six and Paine 1998, Bleiker and Six 2007) and SPB (Ayres et al. 2000) as well as to important physiological processes, such as metamorphosis and sexual maturation of beetles (Bentz and Six 2006). Fungal groups in the MPB system include filamentous Ascomycetes (blue-stain fungi), unicellular Ascomycetes (yeasts), and filamentous Basidiomycetes. Many Scolytinae have evolved mycangial harboring structures to transport fungi, suggesting that benefits are derived from these microorganisms (Batra 1967, Whitney and Farris 1970, Farrell et al. 2001). Symbiotic fungi can sometimes negatively affect the health of the beetle's host tree (Brasier 1991, Kolařík et al. 2011).

Symbiotic Fungi of the MPB

The most studied group of symbiotic fungal associates of bark beetles are the Ascomycota in the class Sordariomycetes (Linnakoski et al. 2012). This group of fungi is responsible for some of the most severe impacts to plant communities in the United States. The widespread mortality in chestnut (*Castanea dentata* [Marshall] Borkh.) caused by the fungus *Cryphonectria parasitica* (Murrill) M.E. Barr and the mortality caused by the fungi *Ophiostoma ulmi* (Buisman) Nannf. and *Ophiostoma novo-ulmi* Brasier in elms (*Ulmus* L. species) are two classic examples of fungal diseases vectored by bark beetles. Within the Sordariomycetes, all species found in MPB belong to the family Ophiostomataceae. Three sexual genera in this family are associated with MPB: *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr., *Grosmannia* Goid. (nonsexual form *Leptographium* Lagerberg & Melin), and *Ophiostoma* H. & P. Syd. (De Beer et al. 2013). Although the sexual form of *Leptographium longiclavatum* Lee, Kim, and Breuil has not been described, it is considered part of the *G. clavigera* species complex (De Beer and Wingfield 2013). It appears that this species does not reproduce sexually (Roe et al. 2011).

Almost 40 years after the first observation of blue-stain fungi symptoms on infected pines (Von Schrenk 1903), the first of the three mycangial fungi associated with MPB, *O. montium*, was described (Rumbold 1941). This species is morphologically similar to *Ophiostoma ips* (Rumbold) Nannf., a species with a much broader distribution that has been collected on rare occasions externally on

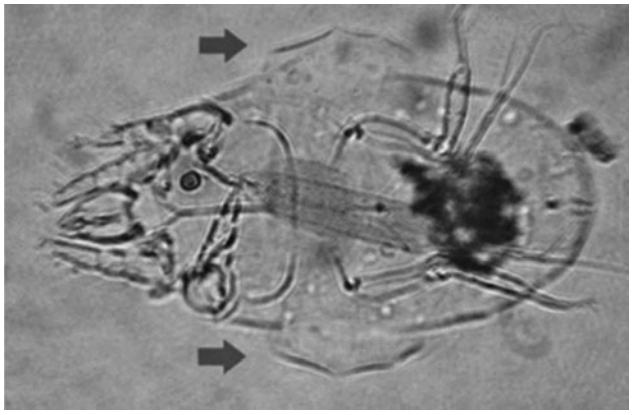

Figure 6. *Tarsonemus ips* with view of sporothecae (arrows). Fungal spores are carried and stored in each sporotheca. The mite was collected from a live MPB in the Black Hills National Park, South Dakota. (Image by D. Reboletti.)

MPBs (Six 2003). The second MPB mycangial fungus to be described was *G. clavigera*. Both *O. montium* and *G. clavigera* are found throughout the MPB's distribution, from Northern Baja California in Mexico (Mock et al. 2007) to areas where MPB has recently expanded into northwestern Alberta, Canada (Cullingham et al. 2012). As suggested by studies looking at numerous loci, *G. clavigera* may actually be a complex of two cryptic sibling species, each better equipped to inhabit either lodgepole or ponderosa pines (Alamouti et al. 2011). The most recently described mycangial blue-stain fungus was L. *longiclavatum*. Since its discovery in lodgepole pine from Canada (Lee et al. 2005), it has been found in the northern range of MPB across Canada where it also occurs in lodgepole × jack pine (*Pinus banksiana* Lambert) hybrids in areas of Alberta (Rice and Langor 2009). In the United States, this species has been found in AZ, CO, ID, MT, NV, OR, SD, UT, and WA (Dario Ojeda, University of British Columbia, pers. comm., Feb. 24, 2014). It is probable that because its optimal growing temperature is similar to that of *G. clavigera* (Lee et al. 2005) and because of its ability to grow in widely distributed lodgepole and ponderosa pines, *L. longiclavatum* may be as widely distributed as *G. clavigera*.

These fungi have evolved to be transported by bark beetles and their phoronts in several ways of varying complexity. Sexual and nonsexual spores of the Ophiostomatales are dispersed in sticky secretions that adhere to the exoskeleton of insects living in their subcortical niches (Malloch and Blackwell 1993). The asexual spores or conidia of *O. montium* and *G. clavigera*, two mutualistic ophiostomatoids, are commonly transported in the beetle's mycangia (Bleiker and Six 2009). Externally, phoretic fungi are transported by beetles in several ways. Their spores can attach to setae or to exoskeletal pits. The pits in the elytra are thought to work as simple mycangia and have been shown to carry *G. clavigera* and *O. montium* (Six 2003, Bleiker and Six 2009). In addition, fungi can be transported by phoretic mites (Moser 1985, Moser et al. 1989b, 2010, J.E. Mercado, USDA Forest Service, unpubl. observ., July 13, 2013) (Figure 6) and nematodes (Cardoza et al. 2008, Suh et al. 2013) (Figure 4). The proportion of fungal spores that adhere to beetles can differ between unemerged and emerged beetles (Six 2003). Six (2003) reported that MPBs contained higher spore loads of *O. montium* when still in their galleries compared with after emergence.

Other nonstaining Ascomycetes are also vectored by MPB. A

Table 3. Common fungal symbionts of *Dendroctonus ponderosae* in North America.

Fungal symbiont	Symbiotic relationship	*D. ponderosae* transport site (primary, secondary)
Ophiostomatales (blue-stain fungi)		
Ophiostoma montium (Rumbold) Arx	Mutualist	Mycangia, exoskeleton
Grosmannia clavigera (Rob.-Jeffr. and R.W. Davidson) Zipfel, Z.W. de Beer & M.J. Wing.	Obligate mutualist	Mycangia, exoskeleton
Leptographium longiclavatum S.W. Lee, J.J. Kim & C. Breuil	Mutualist	Mycangia, exoskeleton
Saccharomycetales (yeasts)		
Ogataea pini (Holst) Y. Yamada, M. Matsuda, K. Maeda & Mikata	Mutualist	Gut, exoskeleton
Kuraishia capsulata (Wick.) Y. Yamada, K. Maeda & Mikata	Mutualist	Gut, exoskeleton
Nakazawaea holstii (Wick.) Y. Yamada, K. Maeda & Mikata	Mutualist	Gut, exoskeleton
Yamadazyma scolyti (Phaff & Yoney.) Billon-Grand	Mutualist	Gut, exoskeleton
Russulales (filamentous yeast)		
Entomocorticium dendroctoni Whitney	Mutualist	Mycangia

Ophiostomatales follow the nomenclator in De Beer et al. (2013).

species similar to *Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhyay & W.B. Kendr. but genetically close to *Cop. ranaculosa* (Plattner et al. 2009) has been documented on MPBs in Colorado (Upadhyay 1981) and British Columbia, Canada (Robinson 1962, Kim et al. 2005, Lee et al. 2006a). Khadempour et al. (2012) found that in British Columbia, probably that same species (*Ceratocystiopsis* sp. 1 until formal description) was the only fungal species having a development cycle that positively correlates significantly with developing MPBs. *Cop. ranaculosa* is a nutritionally important mycangial mutualist of SPB (Klepzig et al. 2001), and similar relationships are possible in other *Dendroctonus* species.

Other fungi, including Basidiomycetes and yeasts, have been found in MPB mycangia. Whereas yeasts are frequently found in the mycangia, the associations with Basidiomycetes in the genus *Entomocorticium* H.S. Whitney, Bandoni & Oberw. (perhaps not a true symbiont but treated as such in this article) seem looser, because these have not been found in that specialized transporting structure (Lim et al. 2005, Lee et al. 2006a, Khadempour et al. 2012). The agaricomycete *Entomocorticium dendroctoni* Whitney and another undescribed species in that genus were found less frequently than *G. clavigera* but more abundantly than *L. longiclavatum* in parts of Canada (Lee et al. 2006a, Khadempour et al. 2012). Other species of *Entomocorticium* as well as species in the genus *Phlebiopsis* Jülich have been documented on MPBs from California, Colorado, and Canada (Hsiau and Harrington 2003), but it is not known whether these represent frequent phoretic associates.

Several yeasts that were originally placed in the genus *Pichia* Hansen (Table 3) and that are now placed in the genera *Ogataea* Yamada, Maeda, et Mikata; *Kuraishia* Yamada, Maeda, et Mikata; *Nakazawaea* Yamada, Maeda, et Mikata; and *Yamadazyma* Billon-Grand (Billon-Grand 1989, Yamada et al. 1994, 1995) have also been collected externally from MPB, although these are typically found in the insect's gut. A variety of Ascomycota and Basidiomycota including saprobes have been collected externally from MPB (Six 2003, Kim et al. 2005, Lim et al. 2005) but are not usually associated with the beetle and could represent opportunistic "hitchhikers."

Virulence of MPB Fungal Symbionts

Phytopathogenicity is the ability of an organism to cause disease to plants (Shaner et al. 1992). In some Ophiostomatales associated with bark beetles, phytopathogenicity is indicated by the staining of the wood. Wood stain diseases are not always a cause of death in mature trees, as is evidenced by *Ophiostoma piliferum* (Fr.) Syd. & P. Syd. which is used as biological control of related virulent fungal

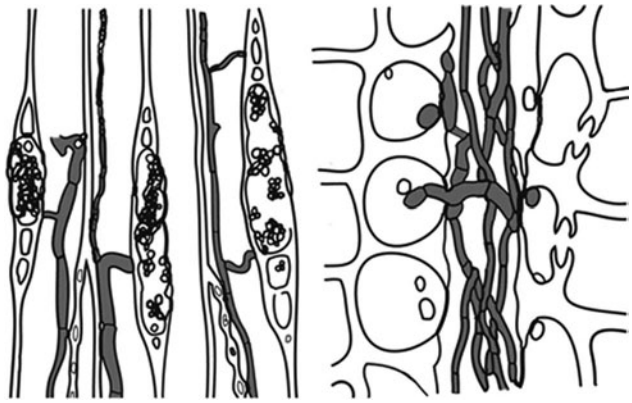

Figure 7. A tangential (left) and a radial (right) view of the blue-stain fungal hyphae (gray) growing on the tracheids and on the xylem's parenchyma rays as described from ponderosa pine in the Black Hills in the early 1800s. (Modified from Von Schrenk 1903.)

species (Dunn et al. 2002). However, on rare occasions symbiotic Ophiostomatales are virulent, contributing to or killing the trees in which they are vectored by beetles (Parker et al. 1941, Solheim and Safranyik 1997, Kolařík et al. 2011). For instance, the most destructive tree-killing bark beetle species in the northern hemisphere, the MPB and the European spruce bark beetle (*Ips typographus* L.), vector at least one tree-killing fungal associate (*G. clavigera* and *Ophiostoma polonicum* [Siemaszko] C. Moreau, respectively). This has been shown under artificial inoculations while trying to remove the beetles' tree killing effect (Christiansen and Solheim 1990, Solheim and Krokene 1998).

The virulence of the mycangial ophiostomatoids associated with MPB has been suspected for a long time. Von Schrenk (1903) first described the fungus development in sapwood. His depiction of the blue-stain fungus growing into the parenchyma ray cells and into the tracheids (Figure 7) may represent *G. clavigera* or *O. montium* and not *O. piliferum*, the species he considered as the pathogen affecting ponderosa pine in the Black Hills. MPB vectors three pathogenic (blue-stain) ophiostomatoids: *G. clavigera*, *O. montium*, and *L. longiclavatum* to their conifer hosts, each exhibiting different degrees of virulence. After being introduced into a tree, these fungi grow into the phloem, penetrating the xylem through the parenchyma rays and destroying them (Ballard et al. 1982, 1984). After this, hyphae extend radially, reaching the heartwood margin, where radial growth stops. The hypha grows through the half-bordered pits into adjacent tracheids (Von Schrenk 1903, Rumbold 1941)

where it displaces the tori (Ballard et al. 1982, 1984). Both *O. montium* and *G. clavigera* have been shown to reach the heartwood of lodgepole pines in Canada in a period of about 5 weeks; however, *G. clavigera* is usually the first to reach the heartwood (Solheim 1995) because of a greater tolerance of that species to the high moisture and less oxygenated properties of healthy sapwood (Solheim and Krokene 1998). Blue-stain fungi growing into pine sapwood is believed to occlude it with fungal material or resin (Tyree and Sperry 1989, Wullschleger et al. 2004). A symptom of sapwood occlusion may be the decline in transpiration usually observed within 10 days after infection (Yamaoka et al. 1990, Hubbard et al. 2013). In experiments in Colorado, Hubbard et al. (2013) observed that transpiration in infected trees declined to 40% of that observed in control trees 2 months after the infection.

A fascinating aspect of the MPB-fungi interactions is teasing out the contribution that these two organisms may have in causing tree death. The plurality of the research addressing this question has been driven by what Six and Wingfield (2011) elegantly called the "classic paradigm." As they describe it, the paradigm itself is composed of two hypotheses. The first hypothesis suggests that tree mortality is the result of fungal invasion into the xylem, disrupting water flow; alternatively, the second suggests that fungal invasion of the phloem results in the depletion of tree defenses, which allows a successful bark beetle colonization (Six and Wingfield 2011), resulting in girdling and tree death.

Several studies have examined the separate impact of fungal inoculation and girdling to trees: the physiological effect of the fungus (i.e., xylem blockage) and the "mechanical effect" of the beetle (i.e., girdling). The impacts of fungal inoculations are difficult to isolate because these usually require some amount of damage to the phloem. The virulence to pines caused by blue-stain ophiostomatoids found on MPB was tested during artificial inoculations (Mathre 1964, Strobel and Sugawara 1986, Yamaoka et al. 1995, Lee et al. 2006b, Solheim and Krokene 1998) with different amounts of phloem removal. Virulence of *O. montium* was tested in 10- to 15-year-old ponderosa pines by inoculating trees on top of a girdled 40-cm band (Mathre 1964). Tree mortality caused by *O. montium* infection occurred within 15–22 days. Although this method did not remove the effects of girdling, it caused a much faster tree death than otherwise would have been observed by girdling alone (see Mathre 1964). In other studies, the killing capacity of *O. montium* in lodgepole pine was shown to be significant by Strobel and Sugawara (1986), only when a considerably large portion of the phloem was removed, for which they recorded 88% mortality; but death occurred two seasons after treatment. However, in the same study, a method leaving most of the phloem intact only killed one of six infected trees. Yamaoka et al. (1990) measured sap flow under a healthy section of lodgepole nested between two girdled bands that were reattached after they were inoculated with *G. clavigera* and *O. montium*, among other fungi. Their results showed that *G. clavigera* reduced sap flow more significantly than the other fungi. Yamaoka et al. (1995) artificially inoculated *G. clavigera* and *O. montium* under phloem flaps, carefully leaving alternate portions of phloem intact. In these experiments, the capacity of *G. clavigera* to kill mature lodgepole pines was found to be greater than that with *O. montium*. The virulence of the mycangial fungus *L. longiclavatum* was inferred by the length of lesions caused during artificial inoculations to lodgepole pine (Lee et al. 2006b), jack pine, and to jack × lodgepole pine hybrids in Canada (Rice et al. 2007). Jack pine appeared to be more susceptible than lodgepole pine to the three mycangial MPB fungal associates (Rice et al. 2007). In addition to carrying the cold-tolerant *G. clavigera*, the novel host association of MPB with *L. longiclavatum* may have contributed to the beetle's expansion into new areas in Alberta, Canada and may increase its chance of dispersal through the boreal forest and into the eastern United States (Safranyik et al. 2010).

If we hypothetically remove the fungi from the beetle, the sole effect of MPB to tree death is the girdling damage of the phloem. However, even the complete girdling of a pine tree does not cause death in less than 1 year or "rapid death" (Craighead 1928, Hubbard et al. 2013). Girdled trees are capable of maintaining healthy physiological activity related to transpiration. For example, girdled lodgepole pines maintained transpiration rates well into the growing season after a beetle attack (Hubbard et al. 2013), and girdled ponderosa pine presented changes in xylem embolism and conductivity similar to those of control trees during the growing season (Domec and Pruyn 2008). The time it takes for a girdled conifer to die is highly variable, but often exceeds 1 year (Noel 1970, Wilson and Gartner 2012). However, pines completely and successfully colonized by the MPB and its associated blue-stain fungi typically die within 1 year after the attack. One of the first symptoms presented in trees colonized by MPB is a rapid reduction in transpiration (Yamaoka et al. 1990, Hubbard et al. 2013).

In summary, studies on the separate effects of fungal inoculation and girdling suggest that no single component causes rapid tree mortality but that perhaps there is a synergistic effect achieved by the combined impacts of both organisms. We may need to pursue new thinking and creative avenues of research to explain these interactions. For example, the loss of sapwood conductivity recorded by Yamaoka et al. (1990) and Hubbard et al. (2013) is a symptom of embolism in the sapwood (Tyree and Sperry 1989). A mechanism of embolism repair proposed by Salleo et al. (1996, 2004) involves the translocation of sugars in addition to water to embolized sapwood by healthy phloem (Salleo et al. 1996, 2004). The disruption of this mechanism, caused by damage to the phloem, could explain the rapid tree mortality caused by the combined effect of MPB and its blue-stain fungi.

Beneficial and Antagonistic Relationships between MPB and Its Fungal Symbionts

Many insects have evolved intimate relationships with fungi and derive benefits from species they culture and protect. The culture of fungi or fungiculture has evolved in three different groups of insects: ants, termites, and bark beetles (Mueller et al. 2005). Recently, behaviors such as mycocleptism, the stealing of fungi (Hulcr and Cognato 2010), and the use of certain bacteria as selective fungicides (Cardoza et al. 2006a, Scott et al. 2008) that protect the bark beetle's beneficial fungi have been documented.

The presence of protective mycangia, specialized for the transport of fungi in MPB, suggests a long-established mutualism. Fungi may benefit MPB by providing hospitable conditions in areas occupied by the beetle's developing brood. For instance, the ophiostomatoid *G. clavigera* may aid in the establishment of the beetle by exhausting tree defenses (Lieutier et al. 2009, but see Six and Wingfield 2011), and the fungi benefit from the beetles rapid vectoring through a recently attacked tree where little competition with other fungi occurs. Both blue-stain fungi and MPB have life strategies that mutually benefit their establishment. These have probably coevolved to develop in a tree before tree death. Killing a tree too rapidly (i.e., before MPB saturates the available phloem space) is

probably detrimental to both fungi and MPB as the two colonizers benefit from low competition during early attack stages until their successful establishment (Kim et al. 2005, Khadempour et al. 2012).

G. clavigera can also benefit MPB by detoxifying the terpenoids present in the defensive oleoresin of attacked trees (DiGuistini et al. 2011), creating a safer environment for its developing brood. The two primary mycangial fungi can also benefit the beetles by redistributing nutritional components, such as nitrogen, where concentrations are not adequately available on the tree's phloem (Bleiker and Six 2007). *G. clavigera* is better at concentrating nitrogen in the beetle's developing area than *O. montium* (Cook et al. 2010). In addition, these fungi may provide sterols that are required for the synthesis of pheromones by adult beetles (Bentz and Six 2006) and potentially serve as a maturation resource for the insect (Six 2003).

Dendroctonus individuals with a greater body mass (10.8 mg versus 10.0 mg) were found to have better reproductive fitness (Elkin and Reid 2005), a greater flying capacity (Williams and Robertson 2008), and a greater tolerance against host tree defenses (Reid and Purcell 2011) than smaller conspecifics. The fitness of MPB can be affected by the species of fungal associates consumed during its development. In laboratory experiments MPB brood that fed on stem sections inoculated with *G. clavigera* developed more rapidly and in greater numbers than those in stem sections inoculated with *O. montium*, and broods were not produced in the absence of any of these fungi (Six and Paine 1998). The symbiotic relationship between *O. montium* and MPB could be less specific than the one with *G. clavigera*, because it has been found to be less restricted to the mycangia (Six 2003). Mutualistic organisms benefit from the synchronization of their development (Boucher et al. 1982). Although the development of *O. montium* overlaps with all developmental stages of MPB, that of *G. clavigera* was found to be more common during the teneral stage (Khadempour et al. 2012). The synchronization of *G. clavigera* with the dispersal stage of MPB suggests a stronger affinity of that fungus with the beetle. Because *O. montium* has been documented from *Ips pini* (Say) (Lim et al. 2005), *Ips perturbatus* (Eich.) (Alamouti et al. 2007, Rudski 2011), and mites carried by MPB (Reboletti 2008, J.E. Mercado, USDA Forest Service, unpubl. observ., July 2013), this species does not rely exclusively on MPB for its establishment on new trees, although the beetle is perhaps its most important vector. Several findings suggest that *O. montium* is less important to MPB than *G. clavigera*. MPB can survive and reproduce successfully with *G. clavigera* alone (Six and Paine 1998), making the mutualistic relationship between MPB and *O. montium* not obligate but facultative. *O. montium* is considered to have established an association with MPB more recently than *G. clavigera* (Bleiker et al. 2009). In addition, *O. montium* may provide fewer nutritional benefits to MPB, making it less favorable to the beetle, as suggested by the smaller beetle brood size produced in association with this fungus versus that with *G. clavigera* (Six and Paine 1998, Bleiker and Six 2007). However, both species appear to benefit MPB, given their ability to grow during different environmental conditions (Six and Bentz 2007), providing a nutrition source in a changing environment (Six 2012), a scenario that may indicate adaptive characteristics of MPB-fungal associations.

Yeasts also make important contributions to the establishment and development of MPB on newly attacked trees. The species *Ogataea pini* (Holst) Y. Yamada, M. Matsuda, K. Maeda & Mikata can indirectly benefit MPB by promoting the growth of at least one of the mycangial fungi, *O. montium* (Rumbold 1941). The yeasts *Kuraishia capsulata* (Wick.) Y. Yamada, K. Maeda & Mikata and *O. pini* were found to benefit MPB both indirectly and directly by oxidizing tree terpenes that are harmful to both fungus and beetle (Hunt and Borden 1990). They may also contribute to the attack behavior of MPB by regulating the conversion of *cis-* and *trans-*verbenone into verbone (Hunt and Borden 1990), an anti-aggregation pheromone that fosters secession of attack, which may provide benefits by reduced competition. In addition to the benefits provided by the Ascomycota, Basidiomycetes are considered important nutritional mutualists of MPB (Whitney et al. 1987). For example, *Entomocorticium* species contribute to the nutrition and brood success of *Dendroctonus* species such as the SPB (Klepzig et al. 2001, Hofstetter et al. 2006a) and the western pine beetle (*D. brevicomis* LeConte) (Paine et al. 1997, Davis et al. 2011). Species in this genus enrich the beetles' developing substrate more efficiently than other associated fungi (Ayres et al. 2000). *E. dendroctoni* was found to increase the egg production of MPBs by 19% (Whitney et al. 1987). Species of *Entomocorticium* are known to positively interact with yeasts in other *Dendroctonus*-fungal systems. The yeast *O. pini* was shown to increase the growth of the beneficial *Entomocorticium* sp. B in the western pine beetle system (Davis et al. 2011), and similar interactions may occur in the MPB system.

Antagonistic relationships have also been identified between beetles and their microbial biota. Entomopathogenic fungi have been mentioned in the literature as being widely distributed along with MPB (Safranyik et al. 2001); however, this information has not been quantified. One pathogen from this group, *B. bassiana*, has been collected from oral secretions of beetles from Colorado and Utah (Cardoza et al. 2009). The pathogenicity of a sympatric wild strain of this entomopathogen was tested against MPB under laboratory conditions in British Columbia, Canada, and although the pathogen had a low germination rate on the beetle's body surface, it was effective in killing (Hunt et al. 1984). It is probable that entomopathogenic fungi reach MPB surfaces indirectly through phoretic mites (see Mites section); however, this phenomenon remains undetermined.

Indirect Multitrophic Effects of Fungal Symbionts of MPB Phoronts

Some phoretic mites and nematodes transported by MPBs are mycetophagous, carrying the fungus on which they feed to freshly attacked trees. Like their insect vectors, several phoretic mites have coevolved life histories with mutualistic fungi and transport them in specialized "flaplike" structures, called sporothecae (Figure 6) (Moser 1985). The fungi vectored by MPB's phoretic mites may affect MPB fitness. For example, in the SPB, *T. ips* transports *O. minus*, an antagonistic species that diminishes the reproductive success of SPB by outperforming the growth of its beneficial mycangial fungi and *Entomocorticium* sp. A (Klepzig et al. 2001, Lombardero et al. 2003). It will be important to examine whether *T. ips* vectors antagonistic fungi in the MPB system as well.

As in mites, the effects to trees and to the beetle of fungi associated with phoretic nematodes are unknown. Nematodes transported by other phytophagous beetles can vector fungi; the most notable example might be the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner and Buhrer] Nickle), transported by species of *Monochamus* Dejean. This nematode was found associated with *O. ips* and *O. minus* in parts of the United States (Wingfield 1987). The ectosymbiotic nematode genus *Ektaphelenchus* also has been found in association with ophiostomatoid fungi. Cardoza et al.

(2006b) cultured species of *Ophiostoma* different from those typically associated with the SB from the nematangia (a nematode harboring structure) of *E. obtusus*. The potential indirect effects of fungi that may be carried by species of *Bursaphelenchus* Fuchs and *Ektaphelenchus* on MPB are not known.

Bacteria

Other important microbial symbionts of MPB are bacteria, which are usually transported internally but can also be excreted in the beetle's frass or secreted orally (Cardoza et al. 2006a, 2009). Their roles are not well understood, but some species have been found to have fungicidal, nutritional, and antagonistic effects on the fungal fauna present in bark beetle systems (Cardoza et al. 2006a, Adams et al. 2008).

MPB Bacterial Associates

All insects are associated with bacteria that can either be transported phoretically outside the body or as primarily gut symbionts where they perform different functions. The study of bacteria in bark beetles has expanded recently, including that with MPB. The associations between bacteria and MPB's blue-stain fungi were first noticed by Rumbold (1941). A single actinobacterium in the genus *Microbacterium* Lehmann and Neumann was isolated from the gut of MPB specimens from Colorado and Utah, whereas more recently two species of *Streptomyces* Waksman & Henrici were recovered from the body surface of 14% of sampled MPBs (Hulcr et al. 2011). In addition, 12 species of bacteria were cultured from living MPB larvae in British Columbia (Winder et al. 2010). Recently, six bacteria in the genera *Serratia* Bizio; *Rahnella* Izard, Gavini, Trinel, and Leclerc; *Pseudomonas* Migula; and *Brevundimonas* Segers were documented from MPB in Alberta and British Columbia, Canada (Boone et al. 2013). Because bacteria are some of the most diverse groups of microorganisms on earth, the current knowledge probably represents only a small fraction of the total diversity and interactions associated with MPB.

Beneficial and Antagonistic Effects of Bacterial Symbionts

Complex interactions occur in the insect groups practicing fungiculture. For example, species of *Acromyrmex* Mayr leaf-cutting ants have evolved the capacity to transport bacterial species of *Streptomyces*. These ants exploit the antifungal properties of *Streptomyces* by using them as fungicides to control the growth of antagonistic fungi in their fungal gardens (Currie et al. 1999). As for the leaf-cutting ants, SPBs have been shown to use bacteria in their oral secretions to kill unwanted fungi (Cardoza et al. 2006a). The common soil bacterium, *Micrococcus luteus* (Schr.) Cohn inhibited the growth of a species of *Aspergillus* Micheli found invading the galleries of SBs in Alaska (Cardoza et al. 2006a).

Some species of bacteria have been found to inhibit the growth of mycangial symbionts of MPB. *Bacillus subtilis* (Ehrenberg) Cohn was found to inhibit the growth of *G. clavigera* and *O. montium*. This bacterium was collected from portions of the phloem that were neither attacked by MPB nor colonized by mycangial symbionts in Montana (Adams et al. 2008). Moreover, *B. subtilis* was found in oral secretions of MPB, suggesting it may be an associated bacterium (Cardoza et al. 2009). Whether the MPB avoids areas infected by this bacterium or whether there is a type symbiosis between MPB and this bacterium warrants further investigations. Several species of bacteria have been shown to cause different effects on the two most common MPB mycangial ophiostomatoids. In western Montana, a species of *Micrococcus* Cohn antagonized the growth of MPB's most beneficial blue-stain fungi, *G. clavigera*, whereas it benefited the growth of *O. montium* in bioassays in which the three organisms were grown together (Adams et al. 2008). Conversely, *Pseudomonas fluorescens* Migula and *Pectobacterium cypripedii* (Hori), recovered from the beetle's mouthparts and larvae in another Montana study, significantly increased the growth and spore formation of *G. clavigera* in the presence of α-pinene, whereas *P. cypripedii* significantly decreased the growth of *O. montium* (Adams et al. 2009). A situation in which the most affected blue-stain fungus was *O. montium* was observed in British Columbia in areas of significant larval mortality, where *Serratia liquefaciens* (Grimes and Hennerty) and *Serratia plymuthica* (Lehmann and Neumann), isolated from 16% of live cultured MPB larvae, decreased the growth of *O. montium* by about 70% and that of *G. clavigera* by 40% (Winder et al. 2010). Similar to fungi, bacteria may also help detoxify terpenoids in the MPB host tree, which may contribute to the successful establishment of the insects. Adams et al. (2013) and Boone et al. (2013) found that bacteria in the genera *Serratia*, *Rahnella*, and *Brevundimonas* can metabolize tree defensive terpenoids such as diterpene abietic acid, a terpenoid shown to inhibit the growth of *G. clavigera* and *O. montium* (Boone et al. 2013). These important findings indicate that the study of bacteria in MPB and their potential use by the beetle is of importance and deserves more attention.

Concluding Remarks

An array of symbiotic organisms are carried by and shares life histories with MPB. Our general knowledge is based on a limited number of places across the vast distribution of the beetle. Studies to date suggest that mites affect colonization and dispersal and can drive populations of related bark beetle species. The most common mites found in the MPB may be mutualistic because they vector blue-stain fungi species beneficial to the beetle, but the prevalence of these associations vary under different population and climate scenarios. Nematodes can affect flight capacity and reproductive potential and thus have an impact on MPB, but to become effective, these may require high numbers. Fungi and bacteria often synergize, modifying the otherwise inhospitable subcortical niche where beetles develop. After more than 100 years of learning about MPB, many questions on the association among these organisms and the MPB still remain. For example, although we have considerable knowledge about the association between *G. clavigera* and *O. montium*, it still needs further clarification. In addition, the growing study field of bacterial associates suggests specialized interactions between these and beetles; for instance, their use in fungiculture, in which some aid mutualistic fungi while simultaneously limiting the growth of antagonistic species.

Multitrophic interactions in the subcortical niche are rich and complex and many remain unexplored. Obtaining a broader understanding of the multitrophic interactions in different contexts may uncover a wealth of information concerning ecological factors that may drive population fluctuations of this important landscaping agent. The cryptic habitat that MPB and its microscopic symbionts use presents challenges to investigation. Slow-growing species that do not invade the sapwood such as *Ceratocystiopsis* sp. 1 may provide new information on the nutritional requirements of the MPB, yet the identity of this species remains unresolved. The mechanisms explaining the synergistic effects between the girdling caused by MPB and the sapwood invasion by blue-stain fungi demand research that satisfies the different theories about their impact on rapid

tree mortality. Among the most common blue-stain fungi, *G. clavigera* has been found to be more beneficial to the MPB than *O. montium*, but how its benefit compares with those of *L. longiclavatum* and *Ceratocystiopsis* sp. 1 remains to be explored. Khadempour et al. (2012) initiated this endeavor by connecting the developmental synchronicity of fungi with the beetle and the dietary significance for the insect.

The type, strength, and reliability of the symbiotic relationships can greatly influence MPB dynamics. Palmer et al. (2003) outlined three general factors that influence mutualism strength or specificity: the variability in the "quality" (in terms of benefits) of alternative partner species; the reliability/dependence of mutualist species; and the effectiveness of partner selections. Thus, partnership consistency is a key element of long-term mutualist associations, and mycangial fungi and MPB are a good example of it (Klepzig and Six 2004). It is thought that the relative strength and importance of most mutualisms vary temporally and spatially with respect to the extent that they confer reciprocal benefits (Bronstein 2001). This hypothesis implies that some level of context dependence is inherent in many mutualisms (Bronstein 1994), and, in fact, many symbiotic interactions in the MPB community range from mutualistic to commensal to antagonistic, given various sets of environmental conditions, resource quality, and the presence of particular species (Klepzig and Six 2004). We are beginning to see a picture in which MPB symbionts can be consistently found in association with the insect and filling the gaps of information will clarify the symbiotic characters of these organisms.

A missing piece of the puzzle is how interactions may be affected under a climate change scenario. The main blue-stain fungal species composition seems to be sensitive to temperature changes (Six and Bentz 2007), but whether this relates solely to the fungi growing requirements or to population variations of other phoretic symbionts that also vector the fungi still needs to be explored. Extreme climatic events and migration of host trees will undoubtedly have an effect on the distribution of climate-sensitive associates and dynamics of the interactions. Expanding our knowledge base about these effects will further increase our capacity to explore potential ways of using the actions of MPB-associated organisms as methods of biological control. We hope that the information presented will help students, managers, and scientists in formulating and addressing key ecological processes among these fascinating organisms.

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