Symbiosis, 37 (2004) 189–205
Balaban, Philadelphia/Rehovot

Review article.

Bark Beetle-Fungal Symbiosis: Context Dependency in Complex Associations

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Received October 1, 2003; Accepted December 20, 2003

Abstract

Recent thinking in symbiosis research has emphasized a holistic consideration of these complex interactions. Bark beetles and their associated microbes are one group which has previously not been addressed in this manner. We review the study of symbiotic interactions among bark beetles and microbes in light of this thinking. We describe the considerable progress already made, examine areas where further progress could be made, and propose new approaches to be used, in understanding these important organisms. We place emphasis on the manner in which context (e.g., abiotic and biotic factors) can influence these interactions and change their nature.

Keywords: *Ophiostoma*, Scolytidae, mites, mutualism, antagonism

1. Defining Symbiosis

The problem associated with the use of the term symbiosis has been framed this way, "the term symbiosis has been used with different meanings, and the

Presented at the 4th International Symbiosis Congress, August 17–23, 2003, Halifax, Canada

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question of its correct meaning and even the desirability of its use at all has been debated. The term, indeed, raises the question as to how far it is possible to distinguish a definitely beneficial association between the two or more organisms from certain states of parasitism on the one hand and from complex ecological interactions on the other" (Sapp, 1994). We navigate similar terrain in considering symbioses involving bark beetles. We recognize the flexibility provided by the classic definitions arrived at by Frank in 1877 ("... all the cases where two different species live on or in one another under a comprehensive concept which does not consider the role which the two individuals play but is based on their mere coexistence") and DeBary in 1888 ("the living together of differently *named* organisms"). We prefer the specificity of the definition coined by Zook (1998) who defines symbiosis as being "the acquisition and maintenance of one or more organisms by another that results in novel structures and (or) metabolism" (we add the "or" to indicate our belief that the existence of modified structures *or* metabolism is sufficient to qualify as symbiosis). However, De Bary described the most significant aspect of symbiosis as being its leading to morphological variations. He saw symbiosis as a major source of evolutionary novelty that could and should be investigated experimentally (Sapp, 1994). It is in this spirit that we approach our consideration of bark beetles and their associates within the context of symbiosis.

2. Bark Beetles as Symbiotic Systems

Bark beetles and their fungal associates represent a rich source of studies in the field of symbiosis. Reciprocal effects are commonly found between these insects and their microbial partners, and a diverse array of interactions, from antagonism to commensalism to mutualism may be found when the group is considered as a whole.

Bark beetles (Scolytidae, alt. Curculionidae: Scolytinae) range in life history from highly aggressive tree-killers to facultative colonizers of weak or recently killed trees to parasites of living trees to saprophagous exploiters of dead trees (Paine et al., 1997). As is common in many insect-microbial symbioses (Martin, 1987), bark beetles feed in nutritionally poor substrates including woody tissues, bark and phloem, fruits, and the pith of twigs (Wood, 1982). Most feed on phloem and cambial tissues of living or recently killed of trees (Wood, 1982). Associations with fungi are apparently universal within this group and many species possess specialized structures, termed mycangia (treated below), for the transport of fungi. The low levels of virulence of some of the fungal associates of these beetles may allow the fungi to use living or freshly killed plant tissues and consequently experience less interspecific

competition from saprophytes (Harrington, 1993).

Many bark beetles feed on fungi as well as plant tissues both as larvae and as adults. The degree of dependence of beetles on fungi likely ranges from obligate, to facultative, to opportunistic, to none. Unfortunately, for most species, virtually nothing is known regarding fungal associates, specificity, or degree of obligacy of association.

Although the association of true yeasts such as *Pichia* and *Hansenula* spp. with bark beetles has been well documented (Whitney, 1971), relatively little is known of their taxonomy or effects on the beetle host. True yeasts are associated with all developmental stages of the beetle (Grosmann, 1930; Leach et al., 1934; Shiffrine and Phaff, 1956; Lu et al., 1957), and are commonly carried in mycangia and in pits of the exoskeleton of adult beetles (as may be yeast-like stages of ophiostomatoid fungi) (Whitney and Farris, 1970; Whitney and Cobb, 1972; Lewinsohn et al., 1994; Furniss et al., 1990; 1995), and are limited to colonizing tree tissues relatively early in the colonization process (Bridges et al., 1984).

Although bacteria are well established as symbionts of other insects, little study has been devoted to their role in bark beetle systems. However, numerous species of bacteria have been recovered from bark beetle guts (Moore, 1972), including nitrogen fixing *Enterobacter* species (Bridges, 1981).

In this article, we review the symbiotic associations of bark beetles with filamentous fungi, consider the relationships from the myco- and entomo-centric views, and discuss the state of our knowledge of bark beetle-fungus associations in light of recent symbiosis theory.

3. Beetle Adaptations

Many bark beetles possess structures – mycangia (Batra, 1963) – that function in the transport of fungi and the maintenance of bark beetle-fungus symbioses. A mycangium (*sensu stricto*) is defined as an invagination of the integument lined with glands or secretory cells that is specialized for the acquisition and transport of fungi (Batra, 1963; Levieux et al., 1991). More loosely defined, the term mycangium has been applied to any structure that consistently transports fungi regardless of form or presence of secretory cells (Farris and Funk, 1965; Livingston and Berryman, 1972; Nakashima, 1975; Beaver, 1986; Furniss et al., 1987). This broader definition allows the inclusion of shallow pits and setae along with deeper pockets that act as fungal repositories but are not known to be associated with glands.

Given that there is a wide variety of structures including pits, punctures, setal brushes and highly-developed sac-like structures that function in a biologically similar manner, and that few of these structures have been

investigated for the presence of glands, it may be appropriate to consider any structure that consistently functions to transport specific fungi as a mycangium regardless of fine structure (Six, 2003). Thus, one of us (Six, 2003) has described mycangia on the basis of coarse structure (morphological form). The three morphology form classes are:

Pit mycangia – all fungal repositories formed by shallow depressions of the exoskeleton, with or without setae.

Sac mycangia – complex invaginations forming deep pockets, tubes or cavities in the exoskeleton.

Setal brush mycangia – dense brushes of setae that may or may not arise from depressions in the exoskeleton.

These coarse structure classes can be further divided by fine structure (presence or absence of glands) if and when such fine structure is investigated (Six, 2003).

The glands most commonly associated with bark beetle mycangia are type III glands which have direct ducts to the surface of the exoskeleton within the lumen (Farris and Funk, 1965; Schneider and Rudinsky, 1969; Barras and Perry, 1971; Levieux et al., 1991) although some mycangia may be associated with more than one gland type (Happ et al., 1971; Cassier et al., 1996). The secretions produced by mycangial glands probably protect and support the growth of fungal propagules, and may act selectively against non-symbiotic fungi (Schneider and Rudinsky, 1969; Happ et al., 1971; Barras and Perry, 1971; Barras and Perry, 1972; Paine and Birch, 1983). Though little is known of the secretions of bark beetle mycangial glands, secretions of glands of several closely related ambrosia beetles contain fatty acids, phospholipids, sterols, and amino acids, and influence the growth of ambrosial fungi while in the mycangium (Norris, 1979). Mycangia have arisen independently several times in the Scolytidae, underscoring the importance of fungi to this group of beetles.

4. Fungal Adaptations

Ophiostomatoid fungi are well adapted to dispersal by arthropods (Malloch and Blackwell, 1993). Most produce sexual fruiting bodies with necks that extrude the spores at heights where they are most likely to be encountered by insects and other arthropods. The spores of most of these fungi are sticky, and shaped in ways to allow for multiple contact points with the vector, ensuring that they are not easily removed in transit. The adhesive coats of the spores disperse in resin but not in water, ensuring that the sticky spores release from beetles only when an appropriate substrate (ie. the new host tree) is encountered (Whitney and Blauel, 1972). Bark beetle-associated fungi also produce a wide range of asexual fruiting structures that produce spores in slimy

masses that readily adhere to the insect cuticle (Tsuneda and Hiratsuka, 1984; Tsuneda, 1988; Malloch and Blackwell, 1993). These conidia are also often found in pit mycangia on the beetle exoskeleton.

Some nutritional mutualists of bark beetles may either have lost their capacity for sexual reproduction, or possess a dual life history, only producing sexual spores in a free living, wood colonizing stage (Hsiau and Harrington, 2003). This lack or rarity of sexual recombination in some fungal associates may be an adaptation to mutualism. If a fungus possesses a genotype that confers high fitness to both itself and to the host beetle, sexual reproduction may be disadvantageous by continually breaking up successful gene combinations (Wulff, 1985).

5. Effects of Symbiosis

Nutrition for bark beetles

As we mentioned previously, bark beetles feed on nutritionally poor substrate. Fungal symbionts likely aid in beetle nutrition in at least two ways: concentration of nitrogen, and provision of sterols. Ayres et al. (2000) have shown that the southern pine beetle, *Dendroctonus frontalis*, is aided in meeting its nitrogen nutritional needs by its mycangial fungal symbionts. The southern pine beetle's mycangial fungi were found to concentrate nitrogen better than an antagonistic non-mycangial associate, *Ophiostoma minus*, reducing the amount of phloem required for development. In addition, *O. minus* acts as a strong antagonist to *D. frontalis* larval development (discussed below). Furthermore, the amount of phloem consumed by this beetle was also found to be much less than that consumed by the eastern six-spined pine engraver (*Ips calligraphus*), a species of beetle not known to be associated with mutualistic fungi and not known to possess mycangia. Similarly, other non-mycangial bark beetles typically must feed more extensively in phloem to compensate for the low nutrient content of their diet in the absence of nutritionally beneficial fungi (Furniss and Carolin, 1977). In contrast, mycangial species typically produce short feeding galleries (terminating in fungal "feeding chambers") (Wood, 1982).

Another likely nutritional benefit bark beetles receive from fungi is the provisioning of a source of sterols to their host beetles. A dietary source of sterols is essential to provide elements of cellular structure, necessary precursors for hormone synthesis, and is critical for the production of viable eggs (Clayton, 1964; Svoboda et al., 1978). However, the sterol concentrations in most plant tissues, especially phloem, are typically low. Indicative of a fungal role in sterol chemistry are observations that fungus-free larvae must tunnel farther

(eat more), are smaller than normal, and do not pupate (Webb and Franklin, 1978; Strongman, 1982). Strengthening the case is the fact that ergosterol, a major (often only) sterol produced by many fungi, is one of the most suitable sterols for insect nutrition. Many plant-produced sterols are not usable by insects (Clayton, 1964).

While the literature is replete with examples of insect dependence upon sterols provided by fungal associates, including for other scolytids (Kok et al., 1970; Norris et al., 1969; Norris, 1972; Maurer et al., 1992; Morales-Ramos et al., 2000; Mondy and Corio-Corset, 2000), the evidence is scarcer for bark beetles. Nevertheless, several bark beetle species exhibit reduced levels of pupation or oviposition when their symbiotic fungi are absent (Strongman, 1982; Fox et al., 1993; Six and Paine, 1998). While the exact nutrients are not known, evidence strongly indicates that, at the least, fungus-derived compounds are crucial to at least some beetles in this group.

Transport of fungi

Bark beetle-associated fungi are completely dependent, or nearly so, upon their hosts for transport from tree to tree (Dowding, 1969). While bark beetles act as the primary vectors for these fungi, spores of these fungi may also sometimes be isolated from the bodies of other insects that develop in bark beetle-colonized trees such as predators, and saprophagous woodborers (D.L. Six, unpublished), however, the reliability of dissemination by these potential alternate vectors is unknown. The important role of beetle-associated mites as vectors should not be underestimated (Klepzig et al., 2001a, b).

Protection of fungi

Beetle-associated fungi gain direct physical protection from desiccation and UV light while in transit within mycangia. Likewise, the growth and competitiveness of mutualistic (usually mycangial) fungi may offer some protection of beetle brood from contact with antagonistic fungi. Competition amongst bark beetle-associated fungi is apparently a common phenomenon (Klepzig and Wilkens, 1997; Klepzig, 1998; K. Bleiker and D.L. Six, unpublished data, A.S. Adams and D.L. Six, unpublished data). The outcome of these competitions has a direct impact on which fungi bark beetle larvae contact and ingest, and hence on their development, survival, and dynamics. It also has a direct effect on determining which fungi will ultimately be carried by new adults leaving the natal tree, thus substantially affecting the dynamics of the fungi.

6. Additional Partners in Bark Beetle-Fungus Symbioses

Over 96 species of mites are associated with *D. frontalis* (Moser and Roton, 1971). At least 14 of these species are phoretic-transported on the external surface of the beetle, not feeding or reproducing during transport (Lindquist, 1969; Moser and Roton, 1971; Smiley and Moser, 1974). *Tarsonemus ips* Lindquist, *Tarsonemus krantzii* Smiley and Moser, and *Tarsonemus fusarii* Cooreman (Moser and Roton, 1971; Smiley and Moser, 1974; Moser, 1976; Bridges and Moser, 1983; Moser and Bridges, 1986) are phoretic on *D. frontalis*, and not directly deleterious to their beetle host. However, the mites do appear to indirectly impact the *D. frontalis*-fungus-tree interaction (Lombardero et al., 2000; Klepzig et al., 2000; Lombardero et al., 2003). All three mites possess specialized, flap-like structures of the integument (sporothecae) which frequently contain *O. minus* and *C. ranaculosus* ascospores (Bridges and Moser, 1983; Moser, 1985; Moser et al., 1995).

7. Examples of Bark Beetle Symbioses

In the following sections, we provide examples of some interesting symbiotic interactions among bark beetles and fungi. We begin with those which appear to be the most primitive and conclude with those which are likely the most advanced. Coincidentally, perhaps, the least advanced and the most advanced systems are those about which we know the least, and most, respectively.

Hylastes/Leptographium

Beetles in the genus *Hylastes* are non-aggressive, generally secondary (not primary colonizers of healthy tree hosts) insects. These root-feeding bark beetles typically attack unhealthy, declining, wounded, even recently dead pines (Wood, 1982; Drooz, 1985; Klepzig et al., 1991), and have been associated with decline diseases in pines (Klepzig et al., 1991; Eckhardt, 2003). Many species within this genus possess pit-non-glandular mycangia which transport saprogenic staining fungi (*sensu* Klepzig et al., 1996), primarily in the genera *Ophiostoma* and *Leptographium* (Harrington, 1988; Jacobs and Wingfield, 2001). Their interactions with fungi are mostly considered to be commensal. The fungi obtain transport, while the beetles appear to be mostly unaffected by the presence of their fungal symbionts.

However, very little is actually known about the interactions among these organisms, and even less about specific effects of the fungi on their beetle vectors. Less still is known about the mites associated with these beetles and

their roles, if any, in facilitating or interfering with the beetle-fungus interactions.

Recent work with *H. salebrosus* and *H. tenuis*, however, hints at a possible mutualistic relationship between these secondary beetles and their saprogenic fungi. In laboratory studies, beetles which were covered with *Leptographium* spores and then reared in aseptic pine logs produced substantially more brood than did non-inoculated beetles (Eckhardt, 2003). The positive effects of the fungi are apparently not related to their ability to kill trees.

Ips species (engravers)/Ophiostoma, Leptographium and Entomocorticium

Interactions between this group of secondary bark beetles and fungi are variable. Most pine engravers (*Ips* spp.) carry one or more *Ophiostoma* and/or *Leptographium* species. Larvae feed on these mycelial fungi as early instars and teneral adults, and probably on yeasts throughout development. In many cases, mycelium, yeast cells, and conidia are ingested; however, *I. avulsus* and *I. calligraphus* have been observed to seek out and ingest entire *O. ips* perithecia during maturation feeding as young adults (Yearian et al., 1972). These beetles are considered to be non-mycangial by some and potentially possessing of pit mycangia by others (Furniss, 1995). These beetles appear to be less specific in their associations and are likely to be less dependent upon their fungal associates than beetles with complex (sac) mycangia. For example, fungus-free *I. calligraphus* and *I. grandicollis*, are more fecund than beetles reared with fungi (Yearian et al., 1972). However, while fungus-free *I. paraconfusus* also can develop successfully to adulthood, they exhibit reduced size of brood and developmental rates compared to beetles developing with fungi (Fox et al., 1993).

Mites can serve as co-vectors in *Ips* spp.-fungus symbiotic systems (Malloch and Blackwell, 1993). Up to 85% of individual mites associated with *Ips typographus*, for example, carry ophiostomatoid fungi (Moser et al., 1989) and can serve as major vectors of these fungi (Levieux et al., 1989). Mites are also especially important vectors in the *Ips avulsus*-fungus symbiosis. The small southern pine engraver, *I. avulsus*, carries a bluestain fungus – *Ophiostoma ips* – which renders pine phloem unsuitable for *I. avulsus* (Yearian, 1966); few larvae survive within areas fully colonized by *O. ips*. Apparently this fungus competes for host tissue (phloem) with an *Entomocorticium* sp., the nutritional mutualist of *I. avulsus* larvae (Yearian, 1966; Yearian et al., 1972). This basidiomycete fungus is also transported by adult Ips avulsus, though apparently not in a mycangium. Wild populations of *I. avulsus* (with their full fungal complement) are significantly more fecund and successful than are fungus-free or *O. ips*-infested populations (Yearian et al., 1972), though it is possible to successfully

rear *I. avulsus* minus their fungal mutualist.

Ips avulsus is also associated with a number of mite species, at least 13 of which are phoretic (Moser and Roton, 1971). The most significant mite associate of *I. avulsus* is *Elattoma bennetti*. This mite feeds on fungus within the *Entomocorticium* sp. lined galleries of *I. avulsus* (Klepzig et al., 2001b). As the females feed they become physogastric (massively swollen) from the developing larvae within their abdomens. Still within the adult female abdomen, these mites mate with their siblings. Eventually, after prolonged feeding and swelling, the adult female ruptures to give birth to reproductively mature adult mites. These mites are then carried phoretically to the next tree (and next source of fungal food) by emerging *I. avulsus*. The degree to which the mite affects the *I. avulsus-Entomocorticium* sp. relationship is unclear. At the very least, the life cycles of all three organisms are tightly interwoven, even interdependent.

8. Conditional Outcomes and Context Dependency

Bronstein (1994b) observes that, although cooperation and mutual benefit between organisms are reasonably common in nature, we know much more about antagonistic interactions (e.g., predation, competition, etc.). We still, relatively speaking, understand little about how and why organisms benefit each other. In an effort to shift research on mutualism away from mainly description and story-telling, and towards a rigorous, evolutionarily based, and question-oriented approach, Bronstein posed several questions regarding mutualisms in nature (1994a, and b). Which we briefly consider below.

1. Are mutualisms delicately balanced antagonisms? There are, as reviewed above, elements of antagonism in the southern pine beetle system, for example. More information is needed, however on the net effects of these organisms on one another.

2. How common are mutualisms involving asymmetrical dependence; when and where do they occur? It would seem that, in many cases, fungi are much more dependent upon bark beetles than vice-versa. However, again, we have much to learn about the degree to which beetles rely on fungi for nutritional and other benefits.

3. Where are we most likely to see a tight match between mutualist traits, and what processes can produce it? We would argue that this is most likely to be found in highly co-evolved systems, within predictable substrates. Such is the case, of course, with most of the beetle-fungus symbioses we have already described. A close association, within a reliable set of nutritional and environmental conditions should facilitate an increasing degree of reliance of two or more organisms upon one another.

4. Is there positive feedback in mutualist population sizes? In the case of the southern pine beetle, higher fitness of female beetles is closely associated with greater abundance of their most beneficial fungal mutualist (Goldhammer et al., 1990; Coppedge et al., 1995). This may be true in other bark beetle systems as well.

5. How much do individual species invest in the different forms of mutualism in which they are involved? Clearly the southern pine beetle has made a significant investment in the maintenance of its mutualism with *E.* sp. *A*. The highly adapted mycangial structures found in this beetle (Happ et al., 1971, 1976) are evidence of evolutionarily costly contributions to this symbiosis. The primary fungal mutualist may have lost its ability to sexually reproduce (Hsiau and Harrington, 2003) which indicates both a level of investment on the part of the fungus and the selectively stable environment provided for the fungus by its beetle host.

6. How are within-species and between-species cooperative interactions similar and different? Within species, many bark beetle species cooperate – via mass attack – to overcome host resistance (Raffa et al., 1993). Similarly, interactions with mutualistic fungi may aid beetles in overcoming host resistance. The most obviously mutualistic interactions species in bark beetle-fungus symbioses occur between species. As discussed above, beetles and fungi provide multiple, direct benefits to one another.

7. How common are cheaters in mutualistic interactions; when and where do they occur? We have little information as to the frequency of cheaters in bark beetle-fungus mutualisms. It is possible, however, that "invader" fungi were initially cheaters within beetle fungal mutualisms.

8. When are the costs and benefits within mutualisms context dependent?

One question that we find especially intriguing, given the multiple associates of bark beetles and the substantial changes to their environment that occur over time during their development in the host tree is one of context dependency of outcomes. We address this question for the remainder of the paper.

Conditional outcomes and context dependency

Like Bronstein, Callaway and Walker (1997) consider the manner in which context may determine the classification of a symbiotic relationship. While the natural reality is that symbiotic interactions occur within a complex, dynamic milieu of abiotic and biotic conditions (including time, space and resources), most systems are studied at one point in their interaction. A relationship between two interacting organisms may – early on – be mutualistic (when resources and space are not limiting, for example). Later, these same two

organisms may become competitors (for space, nutrients, light, etc.) and be understandably classified as antagonists. The context within which the interaction is considered is, therefore, key to describing the nature of the relationship. As described above, the southern pine beetle may initially interact with *O. minus* as a mutualist, providing transport to a new host in response to aid from the fungus in killing that same host tree. Later, however, the relationship is seen to change. Southern pine beetle larvae, dependent upon growth of mutualistic fungi directly and indirectly compete for host tissue (a biotic resource) with *O. minus*. The organisms have remained the same, but the context, and hence the relationship, has changed.

Implicit in this theoretical framework is that biotic and abiotic factors may influence, and even alter the nature of the interactions among closely associated organisms (Callaway and Walker, 1997). However, investigators studying, for example, the response of host trees to bark beetle associated fungi have typically inoculated unattacked, healthy, relatively moisture rich, trees (e.g., Cook and Hain, 1985; Paine and Stephen, 1987; Ross et al., 1992; Nevill et al., 1995). Our own investigations into competitive interactions have utilized relatively dry pine bolts or moisture rich artificial media (Klepzig and Wilkens, 1997; Klepzig, 1998). However, under natural conditions, soon after beetle attack, the phloem tissue the beetles inhabit dehydrates, rapidly losing moisture. Subsequent changes in water potential may occur which seem very likely to affect the ability of fungi to grow and compete with one another. Previous work has demonstrated the importance of water potential in determining the colonization success of tree pathogens (Hong and Michailides, 1999; Whiting and Rizzo, 1999). In our study, fungi grown together in competition experiments exhibited reductions in growth (Klepzig et al., 2004). However, at –5 to –10 MPa (a range of water potentials similar to that measured in loblolly pines within a southern pine beetle infestation), *C. ranaculosus*, the moderately mutualistic associate, was nearly equal in competitive ability to the antagonistic *O. minus*. Thus lowered water potentials may alter competitive interactions in ways that favor bark beetle success.

Although yearly population dynamics of *D. frontalis* are not strongly correlated with climatic patterns (Turchin et al., 1991), small changes in temperature can indirectly affect beetle populations by altering demographic processes within the community. For instance, temperature can enhance or reduce *O. minus* growth relative to mycangial fungi (Klepzig et al., 2001a) and alter *Tarsonemus* mite reproduction and mobility (Lombardero et al., 2000; 2003). In addition, positive feedback between *O. minus* and *Tarsonemus* mites can be disrupted by differential responses to temperature (Hofstetter, unpublished). Over-wintering temperatures may strongly impact annual populations of beetles, mites and fungi (reviewed in Klepzig et al., 2001a).

Finally, just as interactions among multiple symbionts must be considered when characterizing bark beetle-fungus symbioses, it is also important to consider the influence of organisms external to the symbioses. Recent studies have shown that the dynamics of mutualists may at times be significantly impacted, and sometimes even largely determined, by the dynamics of species external to the mutualism (Agrawal and Fordyce, 2000; Morales, 2000; Bacher and Friedli, 2002). For example, Bacher and Friedli (2002) found that the dynamics of weevil and rust mutualists were largely determined by the dynamics of their host plant. With bark beetles, host defensive chemistry can affect both the ability of beetles to successfully attack trees and produce brood, and the ability of their associated fungi to grow (and hence interact with their beetle hosts). Although much research has emphasized the inhibitory effects of host allelochemicals, on beetle-associated fungi, recent research hints at stimulatory effects of some secondary compounds on fungal growth (Hofstetter, unpublished). This opens up the possibility that some bark beetle-associated fungi may benefit from defensive reactions of trees giving them an edge against other, co-occurring bark beetle associated fungi. Again, the context – healthy trees with stronger defenses vs. stressed trees with weaker defenses (Klepzig et al., 1996) – within which these interactions occur, may differentially affect the interacting organisms. Ultimately, to gain a clear understanding of the net impacts of fungi on their symbiotic fungal partners, we must understand how interactions among symbionts and hosts change in response to exogenous factors, as well as how they are affected by endogenous relationships.

REFERENCES

Agrawal, A.A. and J.A. Fordyce. 2000. Induced direct defense in a lycaenid-ant association: the regulation of a resource in a mutualism. *Proceedings of the Royal Society of London B* **267**: 1857–1861.

Ayres, M.P., Wilkens, R.T., Ruel, J.J., and Vallery, E. 2000. Fungal relationships and the nitrogen budget of phloem-feeding bark beetles (Coleoptera:Scolytidae). *Ecology* **81**: 2198–2210.

Bacher, S. and Friedli, J. 2002. Dynamics of a mutualism in a multi-species context. *Proceedings of the Royal Society of London B* **269**: 1517–1522.

Barras, S.J. and Perry, T. 1971. Gland cells and fungi associated with prothoracic mycangium of *Dendroctonus adjunctus* (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **64**: 123–126.

Barras S.J., and Perry, T.J. 1972. Fungal symbionts in the prothoracic mycangium of *Dendroctonus frontalis*. *Zeitschrift für angewandte Entomologie* **71**: 95–104.

Batra, L.R. 1963. Ecology of ambrosia fungi and their dissemination by beetles. *Transactions of the Kansas Academy of Science* **66**: 213–236.

Beaver, R.A. 1986. The taxonomy, mycangia, and biology of *Hypothenemus curtipennis* (Schedl), the first known cryphaline ambrosia beetle (Coleoptera: Scolytidae). *Entomologica Scandinavica* **17**: 131–135.

Bridges, J.R. 1981. Nitrogen-fixing bacteria associated with bark beetles. *Microbial Ecology* **7**: 131–137.

Bridges, J.R. and Moser, J.C. 1983. Role of two phoretic mites in transmission of bluestain fungus, *Ceratocystis minor*. *Ecological Entomology* **8**: 9–12.

Bridges, J.R., Marler, J.E., and McSparrin, B.H. 1984. A quantitative study of the yeasts associated with laboratory-reared *Dendroctonus frontalis* Zimm. (Coleopt., Scolytidae). *Zeitschrift für angewandte Entomologie* **97**: 261–267.

Bronstein, J.L. 1994a. Our current understanding of mutualism. *Quarterly Review of Biology* **69**: 31–51.

Bronstein, J.L. 1994b. Conditional outcomes in mutualistic interactions. *Trends in Ecology and Evolution* **9**: 214–217.

Callaway, R.M. and Walker, L.R. 1997. Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* **78**: 1958–1965.

Cassier, P., Levieux, J., Morelet, M., and Rougon, D. 1996. The mycangia of *Platypus cylindrus* Fab. and *P. oxyurus* Dufour (Coleoptera: Platypodidae) structure and associated fungi. *Journal of Insect Physiology* **42**: 171–179.

Clayton, R.B. 1964. The utilization of sterols by insects. *Journal of Lipid Research* **5**: 3–19.

Cook, S.P. and Hain, F.P. 1985. Quantitative examination of the hypersensitive response of loblolly pine, *Pinus taeda* L., inoculated with two fungal associates of the southern pine beetle, *Dendroctonus frontalis* Zimmerman. *Environmental Entomology* **14**: 396–400.

Coppedge, B.R., Stephen, F.M., and Felton, G.W. 1995. Variation in female southern pine beetle size and lipid content in relation to fungal associates. *Canadian Entomologist* **127**: 145–154.

Dowding, P. 1969. The dispersal and survival of spores of fungi causing bluestain in pine. *Transactions of the British Mycological Society* **52**: 125–137.

Drooz, A.T. 1985. *Insects of Eastern Forests*. USDA, Forest Service. Miscellaneous Publication No. 1426, Washington, DC. 608 p.

Eckhardt, L.G. 2003. Biology and ecology of *Leptographium* species and their vectors as components of loblolly pine decline. Ph.D. Dissertation, Louisiana State University.

Farris, S.H. and Funk, A. 1965. Repositories of symbiotic fungus in the ambrosia beetle, *Platypus wilsoni* Swaine (Coleoptera: Platypodidae). *Canadian Entomologist* **97**: 527–536.

Fox, J.W., Wood, D.L., and Akers, R.P. 1993. Survival and development of *Ips paraconfusus* Lanier (Coleoptera: Scolytidae) reared axenically and with tree-pathogenic fungi vectored by cohabiting *Dendroctonus* species. *Canadian Entomologist* **125**: 1157–1167.

Furniss, M.M. 1995. Biology of *Dendroctonus punctatus* (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **88**: 173–182.

Furniss, R.L. and Carolin, V.M. 1977. *Western Forest Insects*. USDA, Forest Service. Miscellaneous Publication No. 1339, Washington, DC. 653 pp.

Furniss, M.M., Woo, Y., Deyrup, M.A., and Atkinson, T.H. 1987. Prothoracic mycangium on pine-infesting *Pityoborus* spp. (Coleoptera: Scolytidae). *Annals of the Entomological Society of America* **80**: 692–696.

Furniss, M.M., Solheim, H., and Christiansen, E. 1990. Transmission of blue-stain fungi by *Ips typographus* (Coleoptera: Scolytidae) in Norway spruce. *Annals of the Entomological Society of America* **83**: 712–716.

Furniss, M.M., Harvey, A.E., and Solheim, H. 1995. Transmission of *Ophiostoma ips* (Ophiostomatales: Ophiostomataceae) by *Ips pini* (Coleoptera: Scolytidae) in ponderosa pine in Idaho. *Annals of the Entomological Society of America* **88**: 653–660.

Goldhammer, D.S., Stephen, F.M., and Paine, T.D. 1990. The effect of the fungi *Ceratocystis minor* (Hedgcock) Hunt, *Ceratocystis minor* (Hedgcock) Hunt var. *barrasii* Taylor, and SJB 122 on reproduction of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). *Canadian Entomologist* **122**: 407–418.

Grosmann, H. 1930. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. *Zeitschrift für Parasitenkunde* **36**: 56–102.

Happ, G.M., Happ, C.M., and Barras, S.J. 1971. Fine structure of the prothoracic mycangium, a chamber for the culture of symbiotic fungi, in the southern pine beetle, *Dendroctonus frontalis. Tissue and Cell* **3**: 295–308.

Happ, G.M., Happ, C.M., and Barras, S.J. 1976. Bark beetle-fungal symbiosis II: Fine structure of a basidiomycetous ectosymbiont of the southern pine beetle. *Canadian Journal of Botany* **54**: 1049–1062.

Harrington, T.C. 1988. *Leptographium* species, their distributions, hosts and insect vectors. In: *Leptographium Root Diseases on Conifers.* Harrington, T.C. and Cobb, F.W., Jr, eds. APS Press, St. Paul, MN, USA, pp. 1–40.

Harrington, T.C. 1993. Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. In: *Ceratocytis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity.* Wingfield, M.J., Seifert, K.A., and Webber, J.F., eds. APS Press, St. Paul, MN, USA. pp. 161–172.

Hong, C. and Michailides, T.J. 1999. Mycelial growth, sporulation, and survival of *Monilinia fructicola* in relation to osmotic potential and temperature. *Mycologia* **91**: 871–876.

Hsiau, P.T. and Harrington, T.C. 2003. Phylogenetics and adaptations of basidiomycetous fungi fed upon by bark beetles (Coleoptera: Scolytidae). *Symbiosis* **34**: 111–131.

Jacobs, K. and Wingfield, M.J. 2001. *Leptographium* species: Tree pathogens, insect associates, and agents of blue-stain. APS Press, St. Paul, MN USA. 207 p.

Klepzig, K.D. 1998. Competition between a biological control fungus, *Ophiostoma piliferum*, and symbionts of the southern pine beetle. *Mycologia* **90**: 69–75.

Klepzig, K.D. and Wilkens, R.T. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. *Applied and Environmental Microbiology* **63**: 621–627.

Klepzig, K.D., Raffa, K.F., and Smalley, E.B. 1991. Association of an insect- fungal complex with red pine decline in Wisconsin. *Forest Science* **37**: 1119–1139.

Klepzig, K.D., Smalley, E.B., and Raffa, K.F. 1996. Interactions of ecologically similar saprogenic fungi with healthy and abiotically stressed conifers. *Forest Ecology and Management* **86**: 163–169.

Klepzig, K.D., Moser, J.C., Lombardero, M.J., Ayres, M.P., Hofstetter, R.W., and Walkinshaw, C.J. 2001a. Mutualism and antagonism: Ecological interactions among bark beetles, mites and fungi. In: *Biotic Interactions in Plant-Pathogen Associations.* Jeger, M.J. and Spence, N.J., eds. CAB International, Wallingford, Oxon, UK. pp. 237–267.

Klepzig, K.D., Moser, J.C., Lombardero, M.J., Hofstetter, R.W., and Ayres, M.P. 2001b. Symbiosis and competition: complex interactions among beetles, fungi and mites. *Symbiosis* **30**: 83–96.

Klepzig, K.D., Flores-Otero, J., Hofstetter, R.W., and Ayres, M.P. 2004. Effects of available water on growth and competition of southern pine beetle associated fungi. *Mycological Research* **108**: in press.

Kok, L.T., Norris, D.M., and Chu, H.M. 1970. Sterol metabolism as a basis for mutualistic symbiosis. *Nature* **225**: 661–662.

Leach, J.G., Orr, L.W., and Christiansen, C. 1934. Interrelations of bark beetles and blue-staining fungi in felled Norway pine timber. *Journal of Agricultural Research* **49**: 315–341.

Levieux, J., Cassier, P., Guillaumin, D., and Roques, A. 1991. Structures implicated in the transportation of pathogenic fungi by the European bark beetle, *Ips sexdentatus* Boerner: Ultrastructure of a mycangium. *Canadian Entomologist* **123**: 245–254.

Levieux, J., Lieutier, F. Moser, J.C., and Perry, T.J. 1989. Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. *Journal of Applied Entomology* **108**: 1–11.

Lewinsohn, D., Lewinsohn, E., Bertagnolli, C.L., and Partridge, A.D. 1994. Blue-stain fungi and their transport structures on the Douglas-fir beetle. *Canadian Journal of Forest Research* **24**: 2275–2283.

Lindquist, E. 1969. New species of *Tarsonemus* (Acarina:Tarsonemidae) associated with bark beetles. *Canadian Entomologist* **101**: 1291–1314.

Livingston, R.L. and Berryman, A.A. 1972. Fungus transport structures in the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae). *Canadian Entomologist* **104**: 1793–1800.

Lombardero, M.J., Klepzig, K.D., Moser, J.C., and Ayres, M.P. 2000. Biology, demography, and community interactions of *Tarsonemus* (Acarina: Tarsonemidae) mites phoretic on *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Agricultural and Forest Entomology* **2**: 193–202.

Lombardero, M.J., Ayres, M.P. Hofstetter, R.W. Moser, J.C., and Klepzig, K.D. 2003. Strong indirect interactions of *Tarsonemus* mites (Acarina, Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Oikos* **102**: 243–252.

Lu, K.C., Allen, D.G., and Bollen, W.B. 1957. Association of yeasts with Douglas–fir beetle. *Forest Science* **3**: 336–343.

Malloch, D. and Blackwell, M. 1993. Dispersal biology of the Ophiostomatid fungi. In: *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. Wingfield, M.J., Seifert, K.A., and Webber, J.F., eds. APS Press, St. Paul, MN, USA. pp. 195–206.

Martin, M.M. 1987. *Invertebrate Microbial Interactions: Ingested Fungal Enzymes in Arthropod Biology*. Cornell University Press, Ithaca, NY, USA. 148 p.

Maurer, P., Debieu, D., Malosse, C., Leroux, P., and Riba, G. 1992. Sterols and symbiosis in the leaf-cutting ant *Acromyrmex octospinosus* (Reich) (Hymenoptera, Formicidae: Attini). *Archives of Insect Biochemistry and Physiology* **20**: 13–21.

Mondy, N. and Corio-Costet, M.F. 2000. The response of the grape berry moth (*Lobesia botrana)* to a dietary phytopathogenic fungus (*Botrytis cinerea*): The significance of fungus sterols. *Journal of Insect Physiology* **46**: 1557–1564.

Moore, G.E. 1972. Microflora from the alimentary tract of healthy southern pine beetles, *Dendroctonus frontalis* (Scolytidae), and their possible relationship to pathogenicity. *Journal of Invertebrate Pathology* **19**: 72–75.

Morales, M.A. 2000. Mechanisms and density dependence of benefit in an ant-membracid mutualism. *Ecology* **81**: 482–489.

Morales-Ramos, J.A., Rojas, M.G., Sittertz-Bhatkar, H., and Saldana, G. 2000. Symbiotic relationship between *Hypothenemus hampei* (Coleoptera: Scolytidae) and *Fusarium solani* (Moniliales: Tuberculariaceae). *Annals of the Entomological Society of America* **93**: 541–547.

Moser, J.C. 1976. Surveying mites (Acarina) phoretic on the southern pine beetle (Coleoptera: Scolytidae) with sticky traps. *Canadian Entomologist* **108**: 809–813.

Moser, J.C. 1985. Use of sporothecae by phoretic *Tarsonemus* mites to transport ascospores of coniferous bluestain fungi. *Transactions of the British Mycological Society* **84**: 750–753.

Moser, J.C. and Roton, L.M. 1971. Mites associated with southern pine bark beetles in Allen Parish, Lousiana. *Canadian Entomologist* **103**: 1775–1798.

Moser, J.C. and Bridges, J.R. 1986. *Tarsonemus* mites phoretic on the southern pine beetle: attachment sites and numbers of bluestain ascospores carried. *Proceedings of the Entomological Society of Washington* **88**: 297–299.

Moser, J.C., Perry, T.J., and Solheim, H. 1989. Ascospores hyperphoretic on mites associated with *Ips typographus*. *Mycological Research* **93**: 513–517.

Moser, J.C., Perry, T.J. Bridges, J.R., and Yin, H.-F. 1995. Ascospore dispersal of *Ceratocystiopsis ranaculosus*, a mycangial fungus of the southern pine beetle. *Mycologia* **87**: 84–86.

Nakashima, T. 1975. Several types of mycetangia found on Platypodid ambrosia beetles (Col. Platypodidae). *Insecta Matsumura* **7**: 1–69.

Nevill, R.J., Kelley, W.D., Hess, N.J., and Perry, T.J. 1995. Pathogenicity to loblolly pines of fungi recovered from trees attacked by southern pine beetles. *Southern Journal of Applied Forestry* **19**: 78–83.

Norris, D.M. 1972. Dependence of fertility and progeny development of Xyleborus ferrugineus upon chemicals from its symbiotes. In: *Insect and Mite Nutrition: Significance and Implications in Pest Management*. Rodriguez, J.G., ed. Amsterdam, Netherlands, North Holland. pp. 299–310.

Norris, D.M. 1979. The mutualistic fungi of *Xyleborus* beetles. In: *Insect-Fungus Symbiosis*. Batra, L.R., ed. Allanheld, Osmun and Co., Montclair, NJ, USA. pp. 53–63.

Norris, D.M., Baker, J.M., and Chu, H.M. 1969. Symbiontic interrelationships between microbes and ambrosia beetles III: Ergosterol as the source of sterol to the insect. *Annals of the Entomological Society of America* **62**: 413–414.

Paine, T.D. and Birch, M.C. 1983. Acquisition and maintenance of mycangial fungi by *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae). *Environmental Entomology* **12**: 1384–1386.

Paine, T.D. and Stephen, F.M. 1987. Response of loblolly pine to different inoculum doses of *Ceratocystis minor* a bluestain fungus associated with *Dendroctonus frontalis*. *Canadian Journal of Botany* **65**: 2093–2095.

Paine, T.D., Raffa, K.F., and Harrington, T.C. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology* **42**: 179–206.

Raffa, K.F., Phillips, T.W., and Salom, S.M. 1993. Strategies and mechanisms of host colonization by bark beetles. In: *Beetle-Pathogen Interactions in Conifer Forests*. Schowalter, T.D, and Filip, G.M., eds. Academic Press, San Diego, CA, USA. pp. 102–128.

Ross, D.W., Fenn, P., and Stephen, F.M. 1992. Growth of southern pine beetle associated fungi in relation to the induced wound response in loblolly pine. *Canadian Journal of Forest Research* **22**: 1851–1859.

Sapp, J. *Evolution by Association: A History of Symbiosis*. Oxford University Press, Oxford, UK. 255 p.

Schneider, I.A. and Rudinsky, J.A. 1969. Mycetangial glands and their seasonal changes in *Gnathotrichus retusus* and *G. sulcatus*. *Annals of the Entomological Society of America* **62**: 39–43.

Shiffrine, M. and Phaff, H.J. 1956. The association of yeasts with certain bark beetles. *Mycologia* **48**: 41–55.

Six, D.L. 2003. Bark beetle-fungus symbioses. In: *Insect Symbioses.* Bourtzis, K. and Miller, T. eds. CRC press, Boca Raton, FL, USA. pp. 97–114.

Six, D.L. and Paine, T.D. 1998. Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Environmental Entomology* **27**: 1393–1401.

Smiley, R.T. and Moser, J.C. 1974. New Tarsonemids associated with bark beetles (Acarina:Tarsonemidae). *Annals of the Entomological Society of America* **67**: 713–715.

Strongman, D.B. 1982. The relationship of some associated fungi with cold-hardiness of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. MS Thesis. University of Victoria, BC, Canada.

Svoboda, J.A., Thompson, M.J., Robbins, W.E., and J.N. Kaplanis. 1978. Insect sterol metabolism. *Lipids* **13**: 742–753.

Tsuneda, A. 1988. Pleomorphism in beetle-gallery conidial fungi and protoplast reversion. *Proceedings of the Japan Academy* **64 Ser. B**: 135–138.

Tsuneda, A. and Hiratsuka, Y. 1984. Sympodial and annelidic conidiation in *Ceratocystis clavigera. Canadian Journal of Botany* **62**: 2618–2624.

Turchin, P., Lorio, P.L., Jr., Taylor, A.D., and Billings, R.F. 1991. Why do populations of southern pine beetles (Coleoptera: Scolytidae) fluctuate? *Environmental Entomology* **20**: 401–409.

Webb, J.W. and Franklin, R.T. 1978. Influence of phloem moisture on brood development of the southern pine beetle (Coleoptera: Scolytidae). *Environmental Entomology* **7**: 405–410.

Whiting, E.C. and Rizzo, D.M. 1999. Effect of water potential on radial colony growth of *Armillaria mellea* and *A. gallica* isolates in culture. *Mycologia* **91**: 627–635.

Whitney, H.S. 1971. Association of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) with blue-stain fungi and yeasts during brood development in lodgepole pine. *Canadian Entomologist* **103**: 1495–1503.

Whitney, H.S. and Farris, S.H. 1970. Maxillary mycangium in the mountain pine beetle. *Science* **167**: 54–55.

Whitney, H.S. and Blauel, R.A. 1972. Ascospore dispersion in *Ceratocystis* spp. and *Europhium clavigerum* in conifer resin. *Mycologia* **64**: 410–414.

Whitney, H.S. and Cobb, F.W. Jr. 1972. Non-staining fungi associated with the bark beetle *Dendroctonus brevicomis* (Coleoptera: Scolytidae) on *Pinus ponderosa. Canadian Journal of Botany* **50**: 1943–1945.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Naturalist Memoirs* **6**: 1–1359.

Wulff, J.L. 1985. Clonal organisms and the evolution of mutualism. In: *Population Biology and Evolution of Clonal Organisms.* Jackson, J.B.C., Buss, L.W., and Cook, R.E., eds. Yale University Press, New Haven, CT, USA. pp. 437–466.

Yearian, W.C. 1966. Relations of the blue stain fungus, *Ceratocystis ips* (Rumbold) C. Moreau, to *Ips* bark beetles (Coleoptera:Scolytidae) occurring in Florida. Ph.D. Dissertation. University of Florida. 81 pp.

Yearian, W.C., Gouger, R.J., and Wilkinson, R.C. 1972. Effects of the blue stain fungus, *Ceratocystis ips,* on development of Ips bark beetles in pine bolts. *Annals of the Entomological Society of America* **65**: 481–487.

Zook, D.P. 1998. A new symbiosis language. *Symbiosis News* **1**: 1–3.