



Temperature-dependent effects on mutualistic, antagonistic, and commensalistic interactions among insects, fungi and mites

R. W. Hofstetter^{1*}, T. D. Dempsey², K. D. Klepzig³ and M. P. Ayres²

¹ School of Forestry, Northern Arizona University, Flagstaff AZ 86011 USA

² Department of Biological Sciences, Dartmouth College, Hanover NH 03755 USA

³ Southern Research Station, USDA Forest Service, Pineville LA 71360 USA

* Corresponding author. Mailing address: School of Forestry, Northern Arizona University, Flagstaff, AZ 86011-5018. Phone: (928) 523-6452. Fax: (928) 523-1080. Email: Rich.Hofstetter@nau.edu

Keywords: Antagonism, Bark beetle, Climate change, Commensalism, *Dendroctonus*, Fungi, Mites, Mutualism, *Ophiostoma*.

Abstract. The relative abundance and nature of associations between symbiotic species can be affected by abiotic conditions with consequences for population dynamics. We investigated the effects of temperature on the community of mites and fungi associated with the southern pine beetle, *Dendroctonus frontalis*, an important pest of pine forests in the southern United States. First, we determined whether the growth rates of mutualistic and antagonistic fungi associated with *D. frontalis* differed in their responses to temperature. Second, we tested the effects of temperature on the abundance of, and interactions among, fungi, mites and beetles within *D. frontalis*-infested trees. Fungi differed in their growth responses to temperature, resulting in changes in fungal-beetle associations. Mite species associated with *D. frontalis* also differed in their responses to temperature, resulting in different mite communities associated with bark beetle progeny. The effects of temperature on beetle reproduction could not be assessed because of high wood borer density, but inter-relations among surviving beetles, mites and fungi were altered by temperature. Results support the hypothesis that temperature can produce direct and indirect effects on the web of mutualistic and antagonistic relationships within the community of *D. frontalis* and their symbiotic mites and fungi.

Introduction

Global climate change is expected to have drastic effects on populations through direct impacts on demographics and through disruption of community interactions (Stireman et al. 2005). Few studies have addressed the potential impact of changing annual and seasonal temperatures on the interactions and associations of symbiotic species within communities (Post et al. 1999, Ness and Bressmer 2005). Cascading effects from climate change are increasingly likely in communities where multiple symbiotic associations occur, and differential responses of symbiotic species to climate change can disrupt vital associations (Walther et al. 2002). We experimentally test the effects of temperature on the abundance and interaction among multiple mutualistic, commensalistic, and antagonistic associations with a keystone species, the southern pine beetle (*Dendroctonus frontalis* Zimmermann).

Bark beetles (Curculionidae: Scolytinae) have obligate and facultative associations with many microbial and invertebrate species. For instance, symbiotic fungi are introduced by aggressive bark beetles to help kill host trees (Paine et al. 1997) or used as sustenance for developing beetle larvae (Webb and Franklin 1978, Ayres et al. 2000). Alternatively, microbes introduced by beetles into host trees have antagonistic effects on beetle fitness (Baras 1970, Lombardero et al. 2000b). Bark beetles are also phoretic hosts to many mite species which further introduce microbes or alter existing relationships between bark beetles and fungi (Klepzig et al. 2001b). Recent studies of beetle communities reveal that mites, bark beetles and associated fungi form continuous or complex chains of species connections (Lombardero et al. 2000b, Hofstetter et al. 2005a). Non-linear relationships between linked species with intricate feedback structures and dependencies within bark beetle community contribute to the complex dynamics exhibited by many bark beetle species (Six and Paine 1998, Lombardero et al. 2003, Hofstetter

et al. 2005a, 2006). The inter-relations between bark beetles and symbionts (e.g., microbes and mites) may at times be significantly impacted by factors external to the interaction such as seasonal temperatures or changes in precipitation (Klepzig and Six 2004) that can drastically affect the population dynamics and the composition of species within a community.

Variation in abiotic conditions may affect a community by altering the relative abundance of species and/or the strength of species interactions (Callaway and Walker 1997). Hofstetter et al. (2006) observed strong seasonal changes in the relative abundances of mites and fungi associated with *D. frontalis*, and Lombardero et al. (2000a, 2003) showed marked differences between *D. frontalis* and mites in their developmental sensitivity to temperature. Here, we quantified the effects of temperature on abundances and interactions between *D. frontalis* and its major fungal and mite associates. Specifically, we focused on the response of beetle-mutualistic fungi: *Ceratocystiopsis ranaculosus* Perry and Bridges, *Entomocorticium* sp. *A*; a beetle-antagonistic fungus: *Ophiostoma minus* (Hedgc.); and three genera of mites: *Tarsonemus*, *Dendrolaelaps*, and *Trichouropoda* to various temperatures, and assessed how potential differences in temperature-responses might affect community interactions. We experimentally manipulated the temperatures experienced by intact communities of *D. frontalis* and their symbionts within naturally infested trees. We hypothesized that fungal growth rates and abundances, as well as mite abundance within *D. frontalis*-infested trees are differentially affected by various temperatures. Autecological responses to temperature predict that increasing temperatures will lead to (1) higher mite loads on beetles (due to increased mite reproduction and population abundance; Lombardero et al. 2000b); (2) differing ratios of the mutualistic fungi: *C. ranaculosus* and *E. sp. A* (due to expected differences in individualistic temperature responses; Klepzig et al. 2001a); and (3) increased abundance of the antagonistic fungus *O. minus* (due to expected increased *O. minus* and mite growth rates with increasing temperature). We determined whether autecological responses to temperature match the observed abundances of species exposed to various temperatures.

Study system

Dendroctonus frontalis undergoes extreme fluctuations in abundance (Turchin et al. 1991) that result in extensive mortality of host trees and produce broad, economically significant, patterns of forest disturbance (Pye et al. 2006). Interspecific interactions have been well documented between the southern pine beetle, three

fungi, and several phoretic mites (Moser et al. 1995, Klepzig et al. 2001b, Lombardero et al. 2000b, 2003, Hofstetter et al. 2005a, 2006; also see Fig. 4). Two of these fungi, *Ceratocystiopsis ranaculosus* (J.R. Bridges and T.J. Perry) Hausner (= *Ophiostoma ranaculosum*, Jacobs and Kirisits 2003) and *Entomocorticium* sp. *A* (Hsiao and Harrington 1997), are actively transported in specialized structures within the beetle (mycangia) and introduced into new host trees during beetle entry (Francke-Grosmann 1967, Barras and Perry 1972, Happ et al. 1975, 1976). In addition to depending upon beetles for dispersal, these fungi provide vital nutrition for beetle larvae (Barras and Perry 1972, Barras 1973, Goldhammer et al. 1990). A third fungal species, *Ophiostoma minus* (Hedgc.) H. & P. Sydow is passively transported on the beetle's exoskeleton and significantly reduces beetle reproductive success (Barras 1970, Franklin 1970, Hofstetter et al. 2005a). The relationship between *D. frontalis* and these fungi is further complicated by phoretic *Tarsonemus* mites which, though not directly affecting the beetles, actively transport, disperse and feed upon *O. minus* and *C. ranaculosus* (Plate 1) (Moser et al. 1974, Kinn and Witcosky 1978, Bridges and Moser 1983, Moser 1985, Bridges and Moser 1986, Moser and Bridges 1986, Stephen et al. 1993, Lombardero et al. 2000b). Other mite genera, such as *Dendrolaelaps* and *Trichouropoda*, are prominent but their interactions with fungi and beetles are not well known.

Materials and methods

Temperature-fungal growth rates on artificial media

Community-level temperature effects on fungi were first determined by growth experiments on media. Ten strains each of *E. sp. A* and *C. ranaculosus* were isolated from the mycangia of twenty female *D. frontalis*, and ten strains of *O. minus* were isolated from the surface of ten male and female *D. frontalis* collected during the summer of 1999 in Bankhead National Forest, AL. Representative isolates of these fungi were deposited in the culture collection of the Forestry and Agriculture Biotechnology Institute (FABI) at the University of Pretoria, Pretoria, South Africa (culture numbers CMW15445, CMW15412 and CMW15903 for *O. minus*, *C. ranaculosus*, and *E. sp. A*, respectively). Fungi were maintained and periodically transferred on 2.5% malt extract agar (MEA) (Fisher Scientific Inc.) plates and stored at 25°C in the dark. Fungal growth was measured in 15 cm long glass vials (2 cm diameter) containing 2 ml of MEA. Vials were placed horizontally so that the agar filled the entire length of the vial to a thickness of approximately 0.5 cm. We aseptically added a disk of colonized MEA (0.5 cm plug) from one

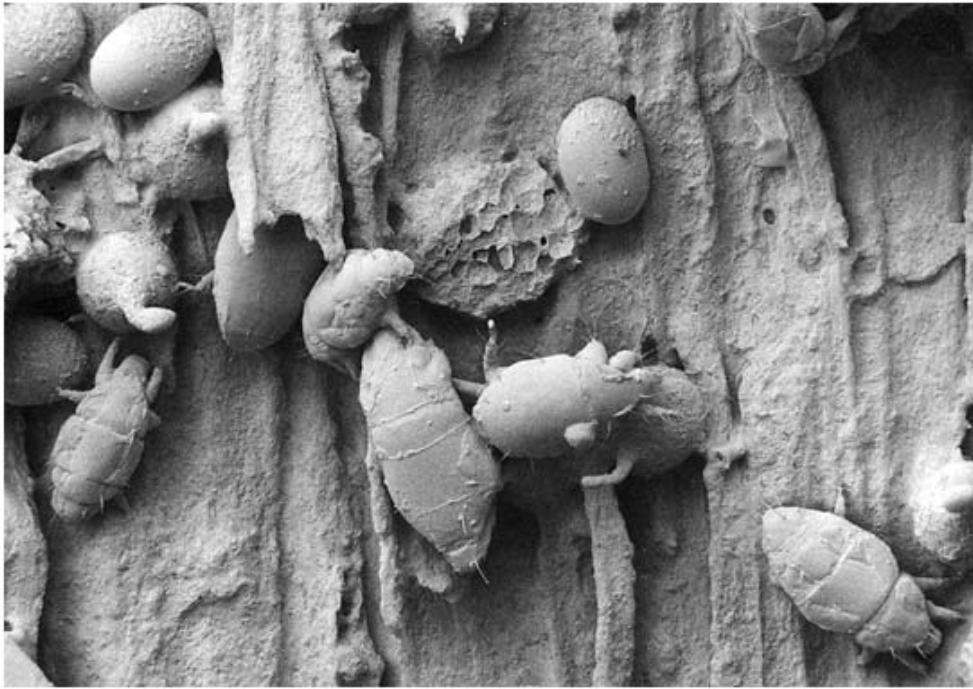


Plate 1. Scanning Electron Microscopy image of *Tarsonemus* mites on *Ophiostoma minus*. *Tarsonemus* eggs and immature are feeding on mycelia and ascospores from perithecia of *O. minus*. Photo credit: R. Ochoa, E. Erbe and K. Klepzig.

of the fungi to the mouth of a vial and resealed the vial. We placed five replicates of each fungus into dark growth chambers at a constant temperature of either 8, 15, 22, 28, or 32°C, as in a previous study with mites (Lombardero et al. 2003). Linear growth rate was measured each day until the fungus reached the end of the vial. The relationship between temperature and acceleration of fungal growth (Q_{10}) was estimated using the vant'Hoff equation (Fry 1947, as cited Andrewartha and Birch 1954):

$$Q_{10} = (Y_1 / Y_2)^{10 / (T_1 - T_2)}$$

Y_i = growth of fungi at temperature T_i ; T_i = absolute temperature in °C.

Test of temperature effects on species abundances and interactions in trees

To test the effects of temperature on within-bark communities, we placed *D. frontalis*-infested *Pinus taeda* L. (loblolly pine) billets under three different temperature regimes. Infested trees were located within an active infestation (USDA Forest Service Infestation #1184) in the Bankhead National Forest, Alabama, USA. At the time of sampling, this infestation consisted of approximately 20 dead trees, 45 trees currently infested with brood and 10 trees under early stages of beetle colonization.

On 17 June 2001, five trees under beetle colonization were chosen (approximately 30 years old, 30 to 40 cm di-

ameter, located within 10 meters of one another). Using Lindgren traps, we captured 50 attacking beetles ("Parent beetles") near these five trees. We placed each captured beetle into a sterile 1-ml centrifuge vial (Fisher Scientific Inc.) and stored the vials at 5 °C. On 25 June 2001 (8-10 days after *D. frontalis* initially colonized the trees) we felled the trees and cut 3 billets (55 cm long) from the bole of each tree between 3 and 5 m high. On 27 June 2001, we randomly assigned a billet from each tree to be placed into one of three temperature regimes (cool 16-23°C, warm 23-25°C, hot 25-31°C). Each billet was stored vertically within a sealed metal rearing container over a glass-collecting jar. Moist towels were kept in the collecting jar to prevent desiccation of exiting beetles. The rearing chambers were kept on a 10L:14D cycle, with temperature ramped from the coolest temperature at 01:00 and the warmest temperature at 13:00. The average temperature in the cool, warm and hot temperature regimes was 20, 24 and 28°C, respectively. Humidity was held between 50-80%.

All emerging offspring were collected. However, high damage from wood borer larvae (Buprestidae and Cerambycidae) prevented total emergence estimates for each treatment. Offspring beetles collected between 29th and 31st of July 2001 were used to assess the effects of temperature treatments on the abundance of fungi and mites associated with *D. frontalis*. We harvested live beetles from the collecting jars using sterile forceps, and immediately placed each beetle into a sterile 1 ml centrifuge

vial (stored at 5°C). Later, phoretic mites were removed from all beetles and the prothorax was removed from female beetles (Hofstetter et al. 2005a). All male beetles, and the remaining parts of female beetles, were placed on MEA amended with cycloheximide (100ug/500ml MEA; ICN biomedical, Inc., Aurora, Ohio) to determine the incidence of *O. minus* on the beetle exoskeleton. All phoretic mites were identified and counted, and placed on glass slides to evaluate the presence of spores of *O. minus* and *C. ranaculosus* (Moser 1985, Moser and Bridges 1986, Moser et al. 1995). The mycangium from the thorax of female beetles were broke open and the yeast-like growth forms were identified under a compound microscope as either *E. sp. A* or *C. ranaculosus* (Bridges 1983).

Two 6 dm² bark samples (20 cm × 30 cm) were removed on 3 August 2001 from each billet and the percent *O. minus*, beetle attacks / dm², woodborer damage, and mite densities were measured. The abundance of *O. minus* within the trees was recorded by tracing the areas of xylem containing blue stain and *O. minus* perithecia onto 8 × 11 inch mylar acetate sheets (methods similar to Bridges and Moser 1983). Beetle entry density was measured by scraping the outer bark and recording the number of resinous entry holes. Woodborer damage was measured by tracing borer galleries within the phloem onto mylar acetate sheets. Mite density in each bark sample was estimated by counting mites in three 1-cm² plots within blue stain areas and three randomly selected 1-cm² plots within non-blue stain areas. We had intended to compare per capita reproduction of beetles across treatments, but this was not possible because the extensive wood-borer damage prevented measurement of successful pupal chambers within bark.

Analyses. The effect of temperature on fungal growth rates on growth media was analyzed using a two-way ANOVA with temperature and fungal species as fixed effects (JMP 3.2.1, SAS Institute Inc. 1997). To improve normality and homogenize variances among treatments, growth values were square-root transformed. Differences between fungal species across temperature were evaluated using Student t-tests. The effect of temperature on bluestain quantity in the phloem and xylem, woodborer damage, and phoretic mite abundance was evaluated with an ANOVA with TREE as a random factor. The fraction of beetles with *O. minus*, each mycangial fungus, and phoretic mites was analyzed using a nominal logistic model (Wald X^2) that included temperature as a fixed effect. The effect of tree and height of removed bolt on attack density were each analyzed with ANOVA that included tree and height as fixed effects. Correlations among mite and fungal abundances (means for each bil-

Table 1. Q_{10} estimates for fungi associated with *D. frontalis* (when grown on malt-extract agar). Q_{10} 's represent the factor by which a metabolic process increases with a 10 °C increase in temperature.

Q_{10}	<i>Entomocorticium</i>	<i>C. ranaculosus</i>	<i>O. minus</i>
8-15°C	34.80	7.51	5.01
15-22°C	2.69	3.68	1.85
22-28°C	1.08	1.50	1.47
28-32°C	NA	NA	NA

let) were computed using the Pearson product-moment correlation coefficient.

Results

Temperature-fungal growth rates on media

For all three fungi, growth rates on media increased with temperature from 8 to 28 °C, and then decreased from 28 to 32°C (Fig. 1). However, there appeared to be differences among species in the details of their temperature responses (Table 1, Fig. 1). Growth rate of *E. sp. A* increased sharply from 8 to 22 °C, and then remained nearly constant from 22 to 28 °C. In comparison, growth rates of both *C. ranaculosus* and *O. minus* increased less sharply from 8 to 15 °C and increased more between 22 and 28 °C. Maximum growth rates were high for *O. minus*, intermediate for *C. ranaculosus*, and low for *E. sp. A*: mean ± SE = 5.0 ± 0.3, 1.6 ± 0.2, and 0.31 ± 0.07 mm / d, respectively. Growth rates of the fungi were significantly different between species across all temperatures (growth rates were square-root transformed: $F_{2,12} = 5.31$, $P < 0.01$). Given that *E. sp. A* obtained maximum growth rates at both 22 °C and 28 °C while *O. minus* and *C. ranaculosus* had maximum growth rates at 28 °C, we expected *E. sp. A* to perform relatively better with trees at 22 °C ('cool temperatures') than the other two fungi.

Test of temperature effects on species abundances and interactions in trees

Fungi and mites associated with parent beetles. Attacking beetles carried on average 4.74 ± 0.84 (SE) *Tarsonemus* and 1.94 ± 0.54 (SE) non-*Tarsonemus* mites. Ninety-one percent of the *Tarsonemus* were *T. krantzii* Smiley and Moser and 9% were *T. ips* Lindquist. *Ophiostoma minus* was present on the exoskeleton of 38% of the beetles, and the ratio of *E. sp. A* relative to *C. ranaculosus* in female mycangia was 54.5% to 45.5% (Fig. 2 – Parent beetles). Density of attacking beetles differed significantly by tree ($F_{4,29} = 6.16$, $P = 0.002$) but not by height ($F_{2,29} = 2.81$, $P = 0.09$).

Effects of temperature on mites. Temperature had strong effects on the abundance of mites but patterns differed among taxa (Fig. 3; Mite × Temperature interaction: $F =$

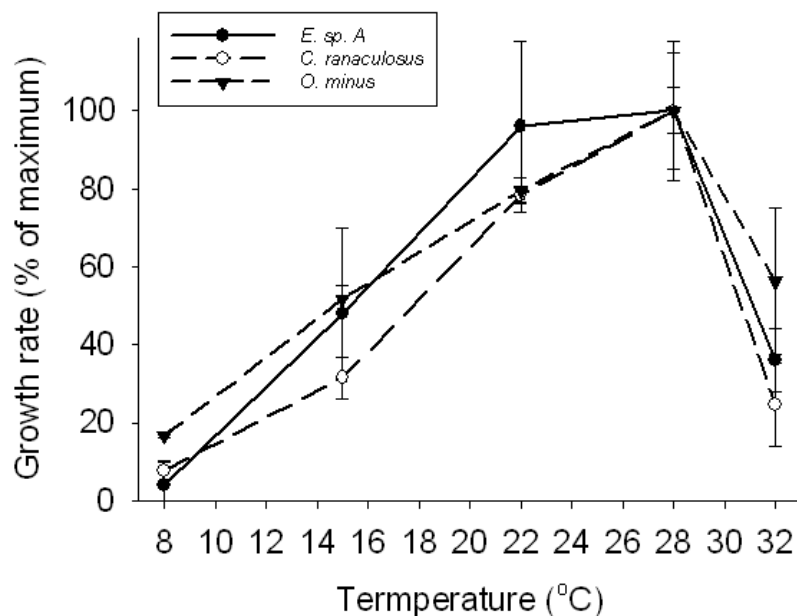


Figure 1. Linear growth of each fungus (as a percentage of their maximum) on malt extract agar at different temperatures. Five replicates per treatment. Bars represent the standard deviation.

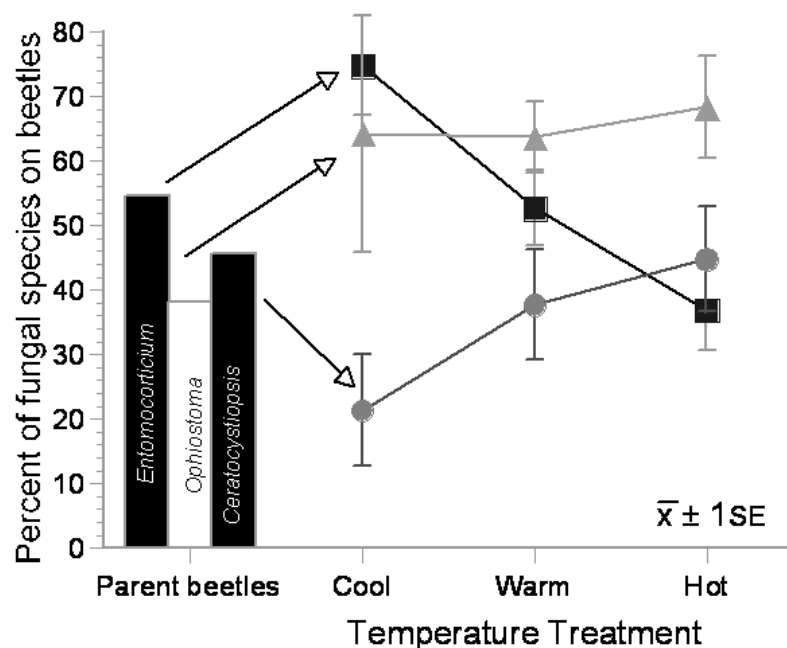


Figure 2. Fungal composition on adult *D. frontalis*. *Entomocorticium* sp. A and *Ceratocystiopsis ranaculosus* occurred as a yeast-like growth form within the prothoracic mycangia of beetles, and *Ophiostoma minus* spores occurred on the beetle exoskeleton. The bars represent adult beetles (parent beetles) captured during tree colonization. The ‘Cool, Warm and Hot’ represent emerging offspring beetles from each temperature treatment. Each point represents beetles from 5 tree bolts. Abundances of *Ceratocystiopsis* and *Entomocorticium* significantly changed with temperature ($X^2 > 14.5$, $P < 0.001$) while *Ophiostoma* abundances did not significantly change with temperature ($X^2 = 0.0$, $P = 0.99$). The Fungi X Temperature interaction was significant: ANOVA $F = 2.90$, $P = 0.035$.

5.24, $P < 0.01$). Mite abundances were not significantly different across trees ($X^2 < 2.0$, $P > 0.20$). *Tarsonemus krantzii* were more abundant on beetle offspring than *T. ips*, comprising 70%, 78% and 77% of the phoretic *Tarsonemus* in the “cool”, “warm” and “hot” treatments, respectively. *Tarsonemus* (mites / dm²) within bark did not significantly differ between the cool and warm treatments ($F_{1,18} = 1.35$; $P = 0.20$), but in the hot treatment there were no living *Tarsonemus* during bark sampling. *Trichouropoda* and *Dendrolaelaps* comprised 90% of the non-

Tarsonemus mites found in the bark and on *D. frontalis*. *Histiostoma*, *Histiogaster* and *Proctolaelaps* spp. comprised most of the remaining phoretic mites, and there were no apparent temperature effects on the abundance of this group (1.1 ± 0.6 , 2.1 ± 0.3 , 1.2 ± 0.4 mites per beetle (mean \pm standard error) in the cool, warm and hot treatments, respectively).

Effects of temperature on beetle-mutualistic fungi. Temperature affected the relative abundance of the two mutualistic fungi in female beetles that developed successfully

Table 2. Correlations among fungi, mites and beetles associated with five trees (3 bolts per tree). *Tars.* = *Tarsonemus* mite adults, BS = bluestain coverage within bark, SPB = southern pine beetle adults, borer = damage from woodborer larvae (Cerambycidae, Buprestidae), *E. sp. A.* = *Entomocorticium sp. A.* *C. r.* = *Ceratocystiopsis ranaculosus*. Correlations with absolute values > 0.52 were significant at $P < 0.05$.

	<i>Tars.</i> /beetle	<i>Dendro.</i> /beetle	<i>Trich.</i> / beetle	%borer /dm ²	% BS (xylem)	%SPB <i>O. minus</i>	%SPB <i>E. sp. A</i>
<i>Dendrolaelaps</i> / beetle	0.31						
<i>Trichouropoda</i> / beetle	-0.50	-0.46					
% borer/dm ²	-0.58	-0.61	0.54				
% bluestain (in billets)	0.02	0.01	0.36	-0.26			
% SPB w/ <i>O. minus</i>	0.21	0.22	0.25	-0.49	0.09		
% SPB w/ <i>E. sp. A.</i>	-0.61	0.59	-0.71	-0.50	0.30	-0.20	
% SPB w/ <i>E.sp.A.&C. r.</i>	-0.18	-0.14	0.54	-0.46	0.03	-0.03	-0.12

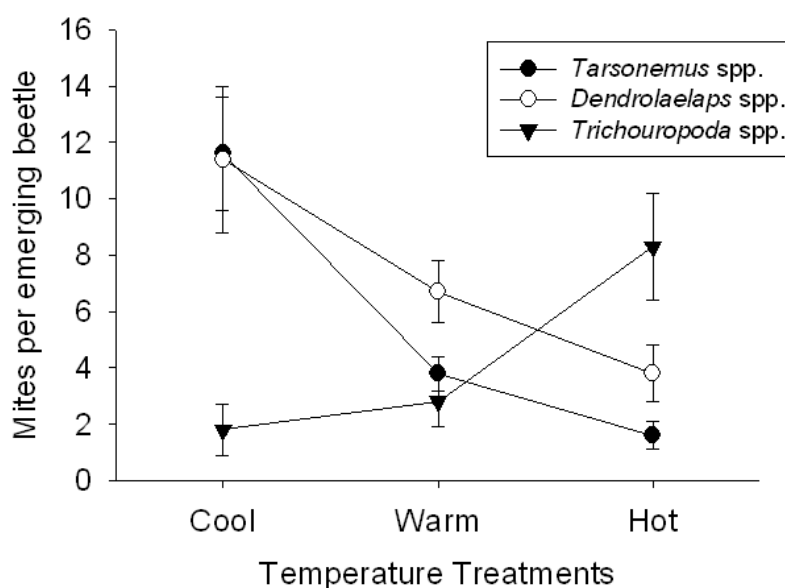


Figure 3. Phoretic mites on *D. frontalis* emerging from infested logs exposed to three temperature regimes (Cool = 16-23°C; Warm = 23-25°C; Hot = 26-31°C). Each point represents mean number of mites per beetle from 5 tree bolts. Error bars represent standard errors. All mite species significantly changed with temperature ($X^2 > 10.0$, $P < 0.01$).

and exited the logs (Fig. 2). Ratios of the mycangial fungi from emerging females in the “cool” treatment did not significantly differ from the ratios on parent beetles ($X^2 = 3.42$, $P = 0.06$). However, in the warm and hot treatments, offspring beetles differed significantly from parental beetles in carrying less *E. sp. A* relative to *C. ranaculosus* ($X^2 = 9.54$ and 28.45 , $df = 1$, $P < 0.002$). *Ceratocystiopsis ranaculosus* spores are also sometimes carried in the sporothecae of phoretic *Tarsonemus* (Moser 1985), but in this study that occurred only rarely and only in *Tarsonemus* from the cool treatment.

Effects of temperature on the beetle-antagonistic fungus. The percent of xylem containing *O. minus* (measured by area stained blue) increased slightly with temperature ($X^2 = 6.57$, $P < 0.01$) as predicted based on growth rates on media. Abundance of *O. minus* was also significantly different across trees ($X^2 = 18.05$, $P < 0.01$). Seventy-eight

percent of the phoretic *Tarsonemus* carried at least one *O. minus* ascospore. The percentages of offspring beetles and phoretic *Tarsonemus* carrying *O. minus* spores were unrelated to temperature ($X^2 < 0.10$, $P > 0.98$), but the number of *Tarsonemus* per beetle declined with temperature (Fig. 3). Besides *Tarsonemus*, no other mite species were observed carrying ascospores of *O. minus*.

Effects of temperature on woodborer activity. The percent of phloem consumed by woodborers increased significantly with temperature from 56 ± 5 to 70 ± 4 to 77 ± 3 (means \pm SE for cool, warm, and hot treatments, respectively; $X^2 = 5.59$, $P = 0.02$). Woodborer activity did not differ between trees ($X^2 = 1.84$, $P = 0.80$).

Discussion

Temperature affected species abundances in ways that were not predicted based on autecological studies alone.

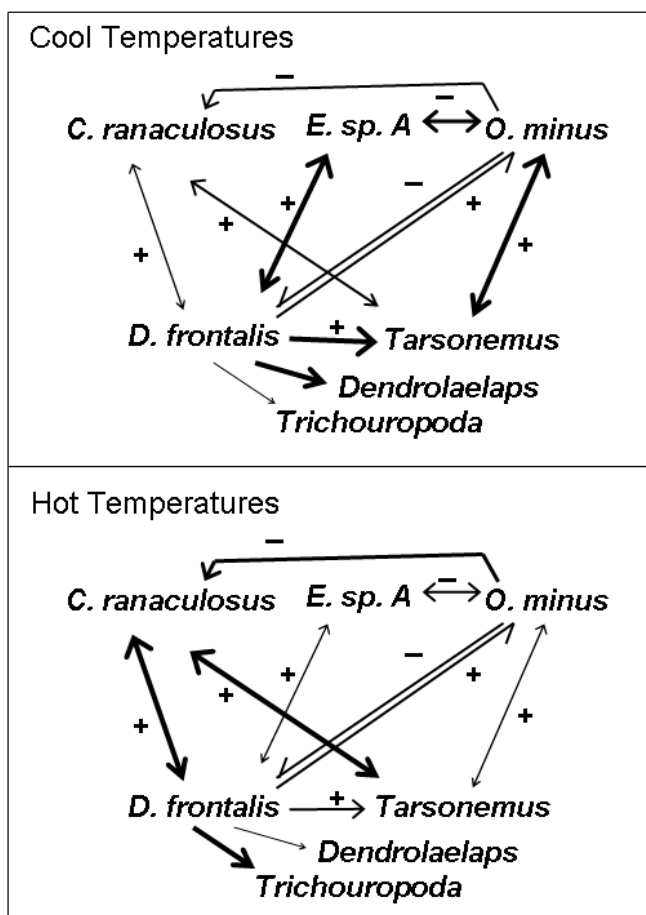


Figure 4. Diagram of observed interactions among mites (*Tarsonemus*, *Dendrolaelaps*, *Trichouropoda* spp.), bark beetles (*Dendroctonus frontalis*), beetle-mutualistic fungi (*Entomocorticium* sp. A, *Ceratocystiopsis ranaculosus*), and beetle-antagonistic fungus (*Ophiostoma minus*). Arrow thickness emphasizes intensity of interaction due to increased abundances of species at cool or hot temperatures.

Differential responses in behavior, reproduction, and movement of species to temperature appeared to change the nature of interactions and composition of species within this community (Fig. 4). These results have consequences for understanding interactions between biotic and abiotic factors and the complex relationships between fungi, mites, and an economically important bark beetle species.

Growth rates of all three fungi within this community were strongly affected by temperature but differed slightly in the details of their temperature responses. The beetle-mutualistic fungus *E. sp. A* exhibited nearly maximum growth at cooler temperatures on growth media than the other mutualistic fungus, *C. ranaculosus*, and the antagonistic fungus, *O. minus* in both the infested logs and on growth media. The pattern exhibited by the beetle-mutualistic fungi in response to temperature matches observed seasonal variation in fungal abundances in natural beetle populations. Hofstetter et al. (2006) surveyed multiple isolated beetle populations in northern Alabama and

observed that *E. sp. A* became more abundant in winter and spring but tended to be supplanted by *C. ranaculosus* during the summer. The beetle-antagonistic fungus, *O. minus*, in experimental logs increased with temperature, matching previous field surveys of beetle infestations that show increases in *O. minus* as temperatures climb from early spring to late summer (Hofstetter et al. 2006).

Contrary to predictions based on seasonal patterns of *O. minus* abundance, temperature had no effect on the proportion of emerging *D. frontalis* adults carrying *O. minus* on their sporothecae. Furthermore, abundance of phoretic *Tarsonemus* per emerging beetle actually declined with increasing temperature. Thus, even though the ‘warm’ and ‘hot’ treatments maximized *O. minus* growth within infested trees, the percentage of *O. minus* colonization within trees subsequently attacked by beetle progeny would decrease due to reduced transport and propagation by phoretic *Tarsonemus* (Lombardero et al. 2003). These results suggest that *O. minus* and *Tarsonemus* abundance should be relatively low in hot climates and

thus, these organisms should have little effect on bark beetle dynamics within tropical climates or in regions with projected increases in temperature. With expected increases in annual temperature due to climate change, fewer negative effects from *O. minus* and mites are expected to occur. However, this may be offset or at least mediated by increased abundance of *C. ranaculosus*. Environmental impacts on the growth and proliferation of *O. minus* deserves further study because this fungus has a strong negative effect on *D. frontalis* reproduction (Barras 1970, Lombardero et al. 2003, Hofstetter et al. 2005a). Temperature appears to influence *O. minus* both directly through growth rate and indirectly via interactions with other organisms. Factors, other than temperature, such as phloem phytochemistry (Hofstetter et al. 2005b) and phloem water potential (Klepzig et al. 2004) can also impact fungal growth rate and fungal-fungal competition.

Because *C. ranaculosus* represents an inferior nutritional resource for *D. frontalis* (Bridges 1983, Goldammer et al. 1990, Coppedge et al. 1995, Klepzig et al. 2001a, b) but a superior nutritional resource for *Tarsonemus* (Lombardero et al. 2000b), seasonal changes in the ratio of the beetle-mutualistic fungi could influence beetle and mite population dynamics in opposite directions. Field studies by Miller and Parresol (1992) and Bridges (1983) demonstrated increased reproduction in beetle populations when *E. sp. A* was the dominant mycangial fungus. Likewise, Hofstetter et al. (2006) recorded increased mite reproduction and decreased beetle reproduction during periods when *O. minus* and *C. ranaculosus* were particularly abundant within bark. Our results and interpretations predict that the abundance of *C. ranaculosus* relative to *E. sp. A* would tend to be highest in the warmest climates where *D. frontalis* occurs (e.g., Florida and Mexico). Supporting this prediction, preliminary surveys of *D. frontalis* mycangia in Mexico reveal that *E. sp. A* is very uncommon (Hofstetter et al., unpublished).

Though *Tarsonemus* population growth is maximized when the mites feed on *C. ranaculosus* and *O. minus* (Lombardero et al. 2000b, Klepzig et al. 2001b), we found that *Tarsonemus* abundance on beetles peaked at cooler temperatures when *E. sp. A* was plentiful. This result, contrary to the predicted pattern, could indicate that seasonal patterns in *Tarsonemus* are more strongly influenced by beetle survival or synchrony with beetle development rate (impacted by temperature) than by fungal composition within the bark. For instance, better survival of beetle larvae results in more hosts for mites to attach to for transport to another tree. Alternatively, the rate at which perithecia are produced by fungi at particular temperatures, and its effect on mite development, could be the

critical factors in determining mite abundance (Klepzig and Wilkens 1997).

Other than *Tarsonemus*, the biology of mites and their impact on species within bark beetle communities are poorly understood. *Dendrolaelaps* spp. are believed to be nematode predators while the *Trichouropoda* spp. are believed to be generalist feeders (Kinn 1971). The abundances of *Tarsonemus* and *Dendrolaelaps* were inversely correlated with temperature, woodborer activity, and *C. ranaculosus* but positively correlated with *E. sp. A*. Alternatively, *Trichouropoda* were inversely correlated with *Tarsonemus* and *Dendrolaelaps* abundances. We believe that these mites do not interact with one another, but that their relative abundances were differentially impacted by temperature effects on mortality and natality, and indirectly via effects on food resources.

The effects of temperature on large wood borers (Buprestids and Cerambycids) are also not well known. We found that as temperature increased, wood borer larvae consumed relatively more phloem, along with fungi, mites, and beetle larvae that were within it. Because woodborer larvae can consume much phloem, their activity impacts the rest of the community. There would be value in studies that explicitly address the role of woodborers in this community, especially because their activity depends upon temperature.

The community of fungi and mites associated with the keystone species, *D. frontalis* transforms as average temperatures change. For instance, both the ratio of the two beetle-mutualistic fungi transferred to beetle progeny as well as the relative abundances of mite species are temperature-dependent. The observed variability in species responses suggests that, though flexibility exists within this community, ultimately species loss is possible. Such variability in species responses suggest that there is flexibility within this community but species loss will likely occur. Increases in average temperature will likely lead to a reduction in community richness and a predominance of a few species. However, increased variability in mean temperatures might counter this trend and promote symbiont diversity and community complexity.

Acknowledgements. We thank R. Arnold, J. Fryar, J. Moan, J. Moser, E. Vallery, and T. Ylloja for their assistance in the field and laboratory. Thanks to the researchers and staff at the USDA Forest Service, Pineville LA and Bankhead National Forest, AL. We also appreciate the constructive comments of J. Moser and manuscript referees. Research was supported by the USDA Forest Service Southern Research Station, NRI CGPs #9835302, #2001-35302-09921, and NSF DEB-0316522.

References

- Andrewartha, H.G. and L.C. Birch. 1954. *The Distribution and Abundance of Animals*. The University of Chicago Press, Chicago, Illinois.
- Ayres, M.P., R.T. Wilkens, J.J. Ruel and E. Vallery. 2000. Fungal relationships and the nitrogen budget of phloem-feeding bark beetles (Coleoptera: Scolytidae). *Ecology* 81:2198-2210.
- Barras, S.J. 1970. Antagonism between *Dendroctonus frontalis* and the fungus *Ceratocystis minor*. *Ann. Entomol. Soc. Am.* 63:1187-1190.
- Barras, S.J. 1973. Reduction of progeny and development in the southern pine beetle following removal of symbiotic fungi. *Can. Ent.* 105:1295-1299.
- Barras, S.J. and T.J. Perry. 1972. Fungal symbionts in the prothoracic mycangium of *Dendroctonus frontalis*. *Z. ang. Entomol.* 71:95-104.
- Bridges, J.R. 1983. Mycangial fungi of *Dendroctonus frontalis* (Coleoptera: Scolytidae) and their relationship to beetle population trends. *Env. Ent.* 12:858-861.
- Bridges, J.R. and J.C. Moser. 1983. Role of two phoretic mites in transmission of bluestain fungus, *Ceratocystis minor*. *Ecol. Ent.* 8:9-12.
- Bridges, J.R. and J.C. Moser. 1986. Relationship of phoretic mites (Acari: Tarsonemidae) to the bluestaining fungus, *Ceratocystis minor*, in trees infested by southern pine beetle (Coleoptera: Scolytidae). *Env. Ent.* 15:9-12.
- Callaway, R.M. and L.R. Walker. 1997. Competition and facilitation: A synthetic approach to interactions in plant communities. *Ecology* 78:1958-1965.
- Coppedge, B.R., F.M. Stephen and G.W. Felton. 1995. Variation in female southern pine beetle size and lipid content in relation to fungal associates. *Can. Ent.* 127:145-154.
- Francke-Grosman, H. 1967. Ectosymbiosis in wood-inhabiting insects. In: S. M. Henry (ed.), *Symbiosis*, Volume II. Academic Press, New York. pp. 141-438.
- Franklin, R.T. 1970. Observations on the blue stain-southern pine beetle relationship. *J. Georgia Entomol. Soc.* 5:53-57.
- Goldhammer, D.S., F.M. Stephen and T.D. Paine. 1990. The effect of the fungi *Ceratocystis minor* Hunt, *Ceratocystis minor* Hunt var. *Barrasii* Taylor, and SJB 122 on reproduction of the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae). *Can. Ent.* 122:407-418.
- Happ, G.M., C.M. Happ and S.J. Barras. 1975. 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., C.M. Happ and S.J. Barras. 1976. Bark beetle-fungal symbiosis. II. Fine structure of a basidiomycetous ectosymbiont of the southern pine beetle. *Can. J. Bot.* 54:1049-1062.
- Hofstetter, R.W., J.T. Cronin, K.D. Klepzig, J.C. Moser and M.P. Ayres. 2005a. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. *Oecologia* 147: 679-691.
- Hofstetter, R.W., J.B. Mahfouz, K.D. Klepzig and M.P. Ayres. 2005b. Effects of tree phytochemistry on the interactions between endophloedic fungi associated with the southern pine beetle. *J. Chem. Ecol.* 31:551-572.
- Hofstetter, R.W., J.C. Moser, K.D. Klepzig and M.P. Ayres. 2006. Seasonal dynamics of mites and fungi and their effects on the southern pine beetle. *Env. Ent.* 35:22-30.
- Hsiau, P.T.W. and T.C. Harrington. 1997. *Ceratocystiopsis brevicomi* sp. nov., a mycangial fungus of *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *Mycologia* 89:661-669.
- Jacobs, K. and T. Kirisits. 2003. *Ophiostoma kryptum* sp. nov. from *Larix decidua* and *Picea abies* in Europe, similar to *O. minus*. *Mycol. Res.* 107:1231-1242.
- Kinn, D.N. 1971. The life cycle and behavior of *Cercoleipus coelonotus* (Acarina: Mesostigmata): Including a survey of phoretic mite associations of California Scolytidae. *University of California Publications in Entomology*, Volume 65. Univ. of California Press, London, England.
- Kinn, D.N. and J.J. Witcosky. 1978. Variation in southern pine beetle attack height associated with phoretic uropodid mites. *Can. Ent.* 110:249-252.
- Klepzig, K.D., J. Flores-Otero, R.W. Hofstetter and M.P. Ayres. 2004. Effects of available water on growth and competition of southern pine beetle associated fungi. *Mycol. Res.* 108:183-188.
- Klepzig, K.D., J.C. Moser, M.J. Lombardero, M.P. Ayres, R.W. Hofstetter and C.J. Walkinshaw. 2001a. Mutualism and antagonism: ecological interactions among bark beetles, mites and fungi, In M. J. Jeger and N. J. Spence (eds.), *Biotic Interactions in Plant-Pathogen Associations*. CABI Publishing, New York. pp. 237-268.
- Klepzig, K.D., J.C. Moser, M.J. Lombardero, R.W. Hofstetter and M.P. Ayres. 2001b. Symbiosis and competition: Complex interactions among beetles, fungi, and mites. *Symbiosis* 30:83-96.
- Klepzig, K.D. and R.T. Wilkens. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. *Appl. Environ. Microbiol.* 63:621-627.
- Klepzig, K.D. and D.L. Six. 2004. Bark beetle fungal symbiosis: Context dependency in complex interactions. *Symbiosis* 37:189-206.
- Lombardero, M., M.P. Ayres, B.D. Ayres, and J.D. Reeve. 2000a. Cold tolerance of four species of bark beetle (Coleoptera: Scolytidae) in North America. *Env. Entomol.* 29:421-432.
- Lombardero, M.J., K.D. Klepzig, J.C. Moser and M.P. Ayres. 2000b. Biology, demography and community interactions of *Tarsonemus* (Acarina: Tarsonemidae) mites phoretic on *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Agric. For. Entomol.* 2:1-10.
- Lombardero, M.J., M.P. Ayres, R.W. Hofstetter, J.C. Moser and K.D. Klepzig. 2003. Strong indirect interactions of *Tarsonemus* mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). *Oikos* 102:243-252.
- Miller, M.C. and B.R. Parresol. 1992. Winter increase of *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae) – a precursor for outbreaks. *J. Appl. Entomol.* 114:520-529.
- Moser, J.C. 1985. Use of sporothecia by phoretic *Tarsonemus* mites to transport ascospores of coniferous bluestain fungi. *Trans. Brit. Mycol. Soc.* 84:750-753.
- Moser, J.C. and J.R. Bridges. 1986. *Tarsonemus* mites phoretic on the southern pine beetle: attachment sites and number of bluestain ascospores carried. *Proc. Entomol. Soc. Wash.* 88:297-299.
- Moser, J.C., T.J. Perry, J.R. Bridges and H-F Yin. 1995. Ascospore dispersal of *Ceratocystiopsis ranaculosus*, a mycangial fungus of the southern pine beetle. *Mycologia* 87:84-86.
- Moser, J.C., R.C. Wilkinson and E.W. Clark. 1974. Mites associated with *Dendroctonus frontalis* Zimmerman (Scolytidae: Coleoptera) in Central America and Mexico. *Turrialba* 24:379-381.

- Ness, J.H. and K. Bressler. 2005. Abiotic influences on the behavior of rodents, ants, and plants affect an ant-seed mutualism. *Ecoscience* 12: 76-81.
- Paine, T.D., K.F. Raffa and T.C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Ann. Rev. Entomol.* 42:179-206.
- Post, E., R.O. Peterson, N.C. Stenseth and B.E. McLaren. 1999. Ecosystem consequences of wolf behavioural response to climate. *Nature* 401: 905-907.
- Pye, J.M., T.S. Price, S.R. Clarke and R.J. Huggett, Jr. 2006. *A History of Southern Pine Beetle Outbreaks in the Southeastern United States through 2004*. Georgia Forestry Commission, Macon, Georgia.
- SAS Institute. 1997. JMP 3.2.1. SAS Institute Inc., Cary, NC.
- Six, D.L. and T.D. Paine. 1998. Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Env. Ent.* 27:1393-1401.
- Stephen, F.M., C.W. Berisford, D.L. Dahlstein, P. Penn and J.C. Moser. 1993. Invertebrate and microbial associates. In: T. D. Schowalter and G.M. Filip (eds.), *Beetle-Pathogen Interactions in Conifer Forests*. Academic Press, San Diego, CA. pp. 129-147.
- Stireman III, J.O., L.A. Dyer, D.H. Janzen, M.S. Singer, J.T. Lill, R.J. Marquis, R.E. Ricklefs, G.L. Gentry, W. Hallwachs, P.D. Coley, J.A. Barone, H.F. Greeney, H. Connahs, P. Barbosa, H.C. Morais and I.R. Diniz. 2005. Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proceedings of the National Academy of Sciences* 102(48): 17384-17387.
- Turchin, P., P.L. Lorio, A.D. Taylor and R. Billings. 1991. Why do populations of southern pine beetles (Coleoptera: Scolytidae) fluctuate? *Env. Ent.* 20:401-409.
- Walther, G-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T.J.C. Beebee, J-M. Fromentin, O. Hoegh-Guldberg and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* 416: 389-395.
- Webb, J.W. and R.T. Franklin. 1978. Influence of phloem moisture on brood development of the southern pine beetles (Coleoptera: Scolytidae). *Env. Ent.* 7:405-410.

Received November 11, 2006
 Revised February 12, 2007
 Accepted February 23, 2007