Full Length Research Paper

Interactions between multiple fungi isolated from two bark beetles, *Dendroctonus brevicomis* and *Dendroctonus frontalis* (Coleoptera: Curculionidae)

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Accepted 29 June, 2010

Antagonism between the fungal symbionts of bark beetles may represent a biologically significant interaction when multiple beetle species co-occur in a host tree. Since high density bark beetle populations rapidly and dramatically shift forest characteristics, patterns of competition between the obligate fungal associates of sympatric bark beetle species may have broad ecological effects. Primary and competitive resource acquisition between allopatric and sympatric isolates of mutualist fungi associated with the bark beetles *Dendroctonus frontalis* and *Dendroctonus brevicomis* were investigated. Growth assays at multiple temperatures suggest that primary resource acquisition by fungi growing in the absence of competitors varies regionally, and that optimal growth rate is likely to correspond to average summertime maximum temperatures. In competition assays, interactions were asymmetric between fungi isolated from sympatric beetle populations and fungi isolated from allopatric beetle populations: sympatric isolates out-competed allopatric isolates. However, competition between fungi from beetle populations in sympaty was found to be equal. These studies are the first to investigate interactions between the mycangial fungi of multiple *Dendroctonus* species, and the results suggest that competition is likely to occur when the mycangial fungi of multiple beetle species occur together.

Key words: Allopatric, competition, coexistence, mutualism, mutualist, mycangial fungi, sympatic.

INTRODUCTION

In many ecosystems, symbiotic associations are ubiquitous (Bronstein, 1994). For species that co-evolve with symbionts, interaction with symbionts is strongly correlated with population performance (e.g. fig/fig wasp, yucca/ yucca moth, ants/myrmecophytes, and beetle/fungus mutualisms; Bronstein, 1992; Huth and Pellmyr, 1997; Klepzig et al., 2001; Palmer et al., 2003); however, little research has investigated competitive symmetry among species with multiple symbionts (Palmer et al., 2003). *Dendroctonus* beetles (Coleoptera: Curculionidae) represent a useful system for studying interactions between symbiotic species (Six and Klepzig, 2004). *Dendroctonus* beetles associate with an extensive community of microorganisms, including mites, nematodes, fungi, yeasts, and bacteria (Whitney, 1982; Klepzig et al., 2001; Kenis et al., 2004; Kirisits, 2004; Scott et al., 2008). The composition and abundance of these microbial communities considerably impact beetle population dynamics (Bridges, 1983; Paine et al., 1997; Hofstetter et al., 2006), and previous studies have cited a need for investigating interactions among the microbial associates of *Dendroctonus* beetles (Klepzig and Wilkins, 1997; Harrington, 2005).

*Dendroctonus* species construct tunnels in the vascular tissue of host conifers in order to lay eggs (Wood, 1982). During excavation, tunnels are inoculated with fungal symbionts that grow throughout host tissues and deve-
Beetle larvae feed upon them (Harrington, 2005). In most cases Dendroctonus species are allopatric or colonize different tree species (Wood, 1982; Lieutier et al., 2004), so the opportunity for antagonism among fungal symbionts are avoided during larval development (Schlyter and Anderbrandt, 1993). However, in the ponderosa pine (Pinus ponderosa var brachyptera) forests of Southwestern North America, Dendroctonus brevicornis LeConte and Dendroctonus frontalis Zimmerman have been reported to co-colonize tissues of ponderosa pine (Pinus ponderosa) and with no apparent negative impacts on the fitness or fecundity of either beetle species (Davis and Hofstetter, 2009).

For D. brevicornis and D. frontalis, fungal symbionts in the genera Ceratocystispias and Entomocorticium confer important benefits to developing larvae (Klepzig and Wilkens, 1997; Hsiau and Harrington, 1997). For example, the presence of these filamentous fungi in feeding chambers is correlated with adult beetle size, fecundity, and nitrogen content (Bridges, 1983; Coppejse et al., 1995; Ayres et al., 2000). Also, beetles have evolved granular structures (termed mycangia) that are used for transporting these fungi between host trees (Barras and Perry, 1971; Hsiau and Harrington, 1997; Yuceer et al., 2010). Due to the prevalence of association and interdependence of mycangial fungi and multiple Dendroctonus species, the beetle-fungal relationship is often considered a mutualism (Six, 2003). However, the sign (+, −) of interaction between multiple fungal symbionts of sympatric Dendroctonus species is unknown. But, interactions between fungal species that co-inhabit a niche have been shown to be antagonistic in many natural systems (Klepzig and Wilkens, 1997; Yuen et al., 1999; Murphy and Mitchell, 2001; Klepzig, 2006; Boddy, 2007; Licyayo et al., 2007). Uncolonized pine vascular tissue is a limiting resource for mycangial fungi; this should create an interface for antagonism between fungal mutualists when multiple beetle species co-colonize a host. Among sympatric Dendroctonus species that co-inhabit host tissue, competition between fungal symbionts of beetles may represent an interaction that limits beetle fitness or population growth.

Here, the authors report on patterns of primary and competitive resource acquisition by mycangial fungi of sympatric and allopatric Dendroctonus beetles. Throughout this report, “primary resource acquisition” is defined as the rate at which fungal isolates acquired resource area in the absence of competitors. “Competitive resource acquisition” is defined as the average proportion of trials in which individual fungal isolates acquired resource space that was occupied by another fungal isolate. The authors experimentally investigated the effects of temperature and biotic interactions on growth patterns of 33 isolates of mutualistic mycangial fungi associated with D. brevicornis and D. frontalis, from both sympatric and allopatric beetle populations. We ask two questions: (1) Does primary resource acquisition by mycangial isolates vary by fungal species, beetle species, or beetle populations? (2) Does the symmetry of competitive interactions by mycangial isolates vary with sympatry or allopatry of beetle populations?

**MATERIALS AND METHODS**

**Beetle collection and acquisition of fungal isolates**

Bark beetles used for fungal isolation were collected from three locations: Coconino National Forest in Arizona, U.S.A., Homochitto Ranger District National Forest in Mississippi, U.S.A., and Plumas National Forest in California, U.S.A. (Figure 1). In Arizona, populations of D. frontalis and D. brevicornis occur in sympathy and co-colonize ponderosa pine (Pinus ponderosa var brachyptera). In Mississippi only D. frontalis occurs and colonizes multiple pine species. In California only D. brevicornis occurs and colonizes P. ponderosa var benthamiana. There is moderate climatic variability between the three forests in terms of both annual mean precipitation and maximum temperature. On the Coconino National Forest annual precipitation averages 44.7 cm per year and mean summer maximum temperature is 25°C (Hereford, 2007), on the Homochitto National Forest annual precipitation averages 162.5 cm per year and mean summer maximum temperature is 30°C (Southern Regional Climate Center), and on the Plumas National Forest annual precipitation averages 38.8 cm per year and mean summer maximum temperature is 29.6°C (Western Regional Climate Center).

To obtain mycangial fungi, live beetles were trapped in the field using Lindgren funnel traps baited with pine beetle pheromones containing frontal, exo-brevicomin, and ω-pinene (Synergy Semi II, Corp, Lot No. WPP10416). Beetles were placed individually into clear, size 0 gelatin capsules (Torpac, Lot No. 1100049271), and stored in the lab in dark environmental chambers at 5°C until used for the isolation of fungi. All insects specimens used for microbial isolations mentioned in this study were collected between May 16, 2007 and July 18, 2007. Healthy female beetles were dissected and the thorax removed. Each thorax was surface sterilized using HgCl2 and de-ionized water described by (Kopp et al., 2004) and then split dorsoventrally and placed in 2% malt extract media (Malt extract – MP Biomedicals LLC, Lot No. 6753; Agar – BioServ, Lot No. 1740.01). The pH of the media was 4.7 ± 0.2 according to manufacturer specifications. Malt extract media (2%) and 95 × 15 mm Petri dishes (Fisherbrand) were used for all isolations and assays. Dishes containing isolates were sealed using Parafilm and incubated in dark environmental chambers at 15°C until used in assays.

**Fungal identification**

Fungal colonies were determined to be one of two fungal genera, Entomocorticium and Ceratocystis, and were positively identified based on microscopic observations of hyphal morphology and degree of melanization (Klepzig et al., 2004). Twenty strains of mutualistic mycangial fungi from D. brevicornis and D. frontalis in sympathy (9 D. frontalis and 11 D. brevicornis), and thirteen fungal strains from allopatric beetle populations (6 from D. frontalis in Mississippi and 7 from D. brevicornis in California) were isolated. Fungi from D. brevicornis and D. frontalis are Ceratocystispias brevicorni and Entomocorticium sp. B, and Ceratocystis ranaculosus (J.R. Bridges and T.J. Perry) Hausner and Entomocorticium sp. A, respectively (Hsiau and Harrington, 1997).

The identity of fungal strains were confirmed by sequencing of internal transcribed spacer (ITS) regions 1 and 2 between the ribosomal RNA genes 18S and 28S. DNA was extracted using a Qiagen DNeasy plant kit (Valencia, CA) with the modified protocol.
Figure 1. The locations of beetle populations where fungal isolates were collected. *D. brevicomis* and *D. frontalis* occur in sympatry in southern Arizona (Cochise National Forest), *D. brevicomis* in allopatry in California (Plumas National Forest), and *D. frontalis* in allopatry in Mississippi (Homochitto National Forest).

for yeasts, which includes a sorbitol buffer and lyticase enzyme to digest cell walls. The author used primers 5.8SF (5′-CGCTGCAGTTCTCATCG-3′) and 5.8SR (5′-TCGATGAGAACGCAAACG-3′) from White et al. (1990) and paired them with newly developed primers ITS-13S (5′-CTTSAACGAGGAATNCTAGTA-3′) and ITS-28S (CATWCCCAACWACYCGACTC) for ITS regions 1 and 2, respectively (Cindy Liu et al. unpublished manuscript).

The authors used the following parameters for a touchdown PCR: hot start 95°C for 4 min; then 20 cycles at 95°C for 30 s, 60°C for 1 min decreasing 0.5°C each subsequent cycle, 72°C for 1 min; 12 cycles at 95°C for 30 s, 45°C for 30 s, 72°C for 30 s; finishing with 72°C for 7 min. PCR reagents were used in the following final concentrations: Invitrogen PCR buffer 1x (Carlsbad, CA), primers 0.2 μM each, MgCl₂ 2.5 mM, dNTPs 0.8 μM, and Invitrogen Platinum taq polymerase 1.4 U. PCR amplons were cleaned up using ExoSAP-IT (USB, Cleveland, OH), cycle sequenced with ABI PRISM BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA), and run on an ABI 3130 × 1 Genetic Analyzer. Sequences for both reads were edited and compiled in Sequencher 4.9 (Gene Codes, Ann Arbor, MI) and BLASTed against all GenBank accessions. Identifications were based on the highest identity value (complete match) and read length. Representative voucher specimens of fungi were preserved in 80% glycerol/20% malt extract broth (MEB; Difco; Lot No. 7306921) and placed in storage freezers held at −80°C in the Microbial Genetics and Genomics Center in Flagstaff, Arizona, U.S.A.

Primary resource acquisition by mycangial fungi

Radial growth is a primary mode of resource acquisition for filamentous fungi, and primary resource acquisition was defined as the rate at which fungal colonies occupied media area in the absence of competitors. All fungal strains were incubated in dark environmental chambers at six temperatures of 5, 10, 15, 20, 25 and 28°C. Fungi were transferred from original isolates to sterile 2% malt extract agar by extracting a 1 × 1 mm section of growth media from hyphal tips of isolations during the linear growth phase using a flame-sterilized spatula. Hyphal growth was traced every 48 h beginning at day zero (initial transfer of colony) for 15 d. The growth rate for each fungal colony at each temperature was determined by dividing the distance between tracings by the number of days of growth. This study was replicated twice for each strain and growth rate was quantified in mm growth/day to 1 × 10⁻¹ mm.

Competitive resource acquisition by mycangial fungi

Competitive interaction between organisms is a secondary means of resource acquisition when limited resources are occupied by competitors (Tilman, 1982). The authors divided competitive resource acquisition into two parts: (1) Resource acquisition/capture: the mean frequency with which a fungal isolate colonized media resources occupied by a competing fungal isolate, and (2) Resource defense: the mean frequency with which a fungal strain resisted colonization by a competing fungal colony. Competitive interactions between mycangial fungi were tested using a pairwise approach. All fungi were paired in Petri dishes by placing 1 × 1 mm media sections containing fungal hyphae at opposing ends of the dish, and fungal strains were transferred from original isolate colonies as described above. Each isolate was also tested against itself. This full factorial design (33 × 33) was replicated twice (n = 2178 assays).

Variation in the growth patterns of these fungi on 2% malt extract
Table 1. Fungal growth rates (mm/day⁻¹) at multiple temperatures and passive / competitive resource acquisition patterns by fungal species and beetle host isolated from sympatric beetle populations. ANOVA results also shown. Bold values indicate significance differences in means at α = 0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>D. brevicomis Mean ± SE</th>
<th>D. frontalis Mean ± SE</th>
<th>F df P</th>
<th>Ceratocystis Mean ± SE</th>
<th>Entomocorticium Mean ± SE</th>
<th>F df P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>0.091 ± 0.033</td>
<td>0.040 ± 0.029</td>
<td>1.312  1.16 0.268</td>
<td>0.074 ± 0.027</td>
<td>0.057 ± 0.036</td>
<td>0.141  1.16 0.711</td>
</tr>
<tr>
<td>10°C</td>
<td>0.425 ± 0.117</td>
<td>0.368 ± 0.103</td>
<td>0.133  1.16 0.720</td>
<td>0.446 ± 0.094</td>
<td>0.347 ± 0.127</td>
<td>0.372  1.16 0.550</td>
</tr>
<tr>
<td>15°C</td>
<td>0.704 ± 0.150</td>
<td>0.665 ± 0.131</td>
<td>0.040  1.16 0.843</td>
<td>0.592 ± 0.121</td>
<td>0.777 ± 0.162</td>
<td>0.808  1.16 0.381</td>
</tr>
<tr>
<td>20°C</td>
<td>1.086 ± 0.254</td>
<td>1.361 ± 0.222</td>
<td>0.660  1.16 0.428</td>
<td>1.016 ± 0.205</td>
<td>1.432 ± 0.276</td>
<td>1.410  1.16 0.252</td>
</tr>
<tr>
<td>25°C</td>
<td>1.518 ± 0.299</td>
<td>1.999 ± 0.282</td>
<td>1.452  1.16 0.245</td>
<td>1.584 ± 0.242</td>
<td>1.933 ± 0.325</td>
<td>0.715  1.16 0.409</td>
</tr>
<tr>
<td>28°C</td>
<td>1.250 ± 0.228</td>
<td>0.95 ± 0.152</td>
<td>1.193  1.16 0.306</td>
<td>1.320 ± 0.216 a</td>
<td>0.880 ± 0.125 b</td>
<td>3.100  1.16 0.116</td>
</tr>
<tr>
<td>Resource defense</td>
<td>0.488 ± 0.047</td>
<td>0.497 ± 0.041</td>
<td>0.016  1.16 0.900</td>
<td>0.594 ± 0.038 a</td>
<td>0.391 ± 0.051 b</td>
<td>9.707  1.16 0.006</td>
</tr>
<tr>
<td>Resource capture</td>
<td>0.386 ± 0.076</td>
<td>0.425 ± 0.067</td>
<td>0.141  1.16 0.712</td>
<td>0.445 ± 0.061</td>
<td>0.366 ± 0.183</td>
<td>0.571  1.16 0.460</td>
</tr>
</tbody>
</table>

*Letters indicate differences in means (Tukey's HSD test) by row.

In comparisons between isolates across beetle populations (n = 33 fungal strains [20 sympatric/13 allopatric]), a one-way ANOVA was performed to analyze the fixed effect of sympathy or allopatry on response variables of radial growth rate, mean resource acquisition and mean resource defense. For this analysis sympatric isolates were considered as a single (Arizonan) population. The effects of temperature on fungal growth were analyzed as a fixed effect nested by location and differences between means were tested using contrasts. Statistical significance was established at α = 0.05 for statistical tests and ANOVA models for growth and competition assays were analyzed using F-tests to establish the significance of effects. Where differences in mean growth and competitive responses were detected in ANOVA models, directionality was established using Tukey's HSD Test.

RESULTS

Primary resource acquisition by mycangial fungi

Sympatric fungi: Growth rates of fungi from Arizona did not vary by beetle species, fungal species, or beetle species x fungal species interaction at 5, 10°C, 15, 20, 25 or 28°C (Table 1, Figure 2). Thus, fungi from sympatric beetle populations behaved statistically identically in terms of growth rates across temperatures by both fungal species and beetle species. However, fungal growth rates did consistently increase as temperature increased then declined once ambient temperature surpassed 28°C. Entomocorticium species exhibited greater variability in growth rates across temperatures than Ceratocystis species.

Sympatric and allopatric fungi: Growth rates did not vary among the three populations at 5 or 10°C (Table 2, Figure 2). Primary resource acquisition from the sympatric fungi was significantly higher than the rate of primary resource acquisition by fungi from D. brevicomis in California at 15 and 20°C, and fungi from D. frontalis in Mississippi were intermediate. There was no difference in primary resource acquisition by population at 25°C, but at 28°C primary resource acquisition by sympatric isolates...
Table 2. Fungal growth rates (mm/day\(^{-1}\)) at multiple temperatures and passive / competitive resource acquisition patterns by fungal species and beetle host isolated from sympatric and allopatric beetle populations. ANOVA results also shown. Bold values indicate significance differences in means at α = 0.05.

<table>
<thead>
<tr>
<th>Population</th>
<th>Sympatric (D. brevicomis and D. frontalis)</th>
<th>Allopatric (D. brevicomis)</th>
<th>Allopatric (D. frontalis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>5°C</td>
<td>0.064 ± 0.034</td>
<td>0.034 ± 0.054</td>
<td>0.187 ± 0.060</td>
</tr>
<tr>
<td>10°C</td>
<td>0.408 ± 0.065</td>
<td>0.237 ± 0.079</td>
<td>0.356 ± 0.086</td>
</tr>
<tr>
<td>15°C</td>
<td>0.799 ± 0.084 a</td>
<td>0.428 ± 0.100 b</td>
<td>0.588 ± 0.108 ab</td>
</tr>
<tr>
<td>20°C</td>
<td>1.341 ± 0.180 a</td>
<td>0.577 ± 0.216 b</td>
<td>1.136 ± 0.233 ab</td>
</tr>
<tr>
<td>25°C</td>
<td>2.016 ± 0.174 a</td>
<td>0.841 ± 0.259 b</td>
<td>1.810 ± 0.280 a</td>
</tr>
<tr>
<td>28°C</td>
<td>1.100 ± 0.201 b</td>
<td>2.362 ± 0.241 a</td>
<td>2.987 ± 0.260 a</td>
</tr>
<tr>
<td>Resource defense</td>
<td>0.800 ± 0.031 a</td>
<td>0.707 ± 0.038 b</td>
<td>0.616 ± 0.041 b</td>
</tr>
<tr>
<td>Resource capture</td>
<td>0.496 ± 0.078 a</td>
<td>0.185 ± 0.101 b</td>
<td>0.210 ± 0.109 b</td>
</tr>
</tbody>
</table>

*Letters indicate differences in means (Tukey's HSD test) by row.

was significantly lower than for allopatric isolates (Figure 2). Growth rates consistently increased with temperature for all fungal isolates until 25°C, where sympatric isolates showed a substantial decline but allopatric fungi achieved optimal growth.

Competitive resource acquisition by mycangial fungi

Sympatric fungi: Patterns of competitive resource acquisition varied significantly with fungal species but were not variable with beetle species (Table 1). Specifically, Ceratocystis species had higher mean frequencies of resource defense than Entomocorticium species (Table 1). Thus, neither beetle species was associated with a consistently more competitive fungal symbiont. Sympatric and allopatric fungi: Competitive resource acquisition by fungal isolates varied significantly with sympatry and allopatry of source beetle populations (Table 2). Isolates from the sympatric beetle populations exhibited significantly higher mean frequencies of competitive resource acquisition and resource defense of growth media than fungi isolated from either allopatric beetle population (Table 2). Fungi from opposing allopatric populations were not significantly different from each other.

DISCUSSION

Primary resource acquisition

The radial growth rates of fungi were found to vary by population at multiple temperatures (Table 2). This is in agreement with the findings of Six and Bentz (2007), which showed that ambient temperature was a mediator of fungal abundances, and that this variation was related to both site and seasonality in a Dendroctonus ponderosae system. Here, they show that the growth rates of mycangial fungi vary across beetle populations, which in the current study are separated by large geographic regions. However, growth rates did not vary among fungal isolates for fungal species or beetle species within sympatric populations. The studies did not sample fungi from multiple sites within sympatric populations, so they might have detected greater variation in growth rates by assessing multiple sites within each region.

In contrast to the present study, Hofstetter et al. (2007) showed that Entomocorticium sp. A, exhibited optimal growth at a lower ambient temperature than C. ranaculosus in a D. frontalis system in Mississippi. In the present study, no differences in response to temperature were detected for growth by fungal species from sympatric beetle populations in Arizona or an allopatric beetle population in California (Figure 2). However, the authors did support their findings that Entomocorticium sp. A, and C. ranaculosus had different optimal growth rates in a Mississippi population of D. frontalis (Figure 2). One explanation for this pattern is overall variability in daily temperature; daily temperature range is greater in Arizona and California than in Mississippi.
Figure 2. Growth rates of Ceratocystis spp. and Entomocorticium spp. isolated from sympatric bark beetle populations in Arizona, an allopatric population of D. brevicomis in California, and an allopatric population of D. frontalis in Mississippi. Symaptic populations were pooled in this figure since there were no significant differences between radial growth rates of sympatric fungi. Error bars represent one standard error.

(Hereford, 2007; Southern Regional Climate Center, 2008; Western Regional Climate Center, 2008). Thus, optimal growth rate and species abundances of mycangial fungi may be influenced by daily temperature range, in addition to regional and seasonally mediated temperature differences. The rates of primary resource acquisition by sympatric fungi corresponded to the average maximum summer temperature in the region. In northern Arizona, climate records show that maximum summer (June-September) temperatures average 25°C (Hereford, 2007), which is the range where the greatest average growth rates were observed for sympatric fungi (Figure 2). In the experiments, an increase in ambient temperature of only 3°C above the average summer maximum correlated with a dramatic decrease in growth rates of the sympatric fungi. In contrast, mycangial isolates from allopatric populations showed no apparent decrease in growth rates as ambient temperatures.
increased, and isolates from both populations grew optimally at 28 °C. Unfortunately, no inferences can be made about primary resource acquisition by fungi beyond ambient temperatures of 28 °C. However, previous work shows that the growth rates of mycanial fungi (both Entomocorticium and Ceratocystisipris) isolated from D. frontalis in Mississippi declines once ambient temperatures exceed 28 °C (Hofstetter et al., 2007). If it is true that fungal growth rates correspond to regionally defined average maximum temperatures during summer months, then they would predict that isolates from allopatric D. brevicomis will grow optimally at 29.6 °C, and isolates from allopatric D. frontalis will grow optimally at 30 °C. Future studies with these organisms could benefit from assaying growth rates of mycanial fungi from both within and between beetle populations across a broader temperature range and at smaller intervals, since isolates appear to be highly sensitive to relatively small incremental variations in temperature.

Competitive resource acquisition

Fungi isolated from the sympatric beetle populations exhibited significantly greater frequencies of competitive acquisition and defense of media in a resource-limited environment. In sympatric beetle populations, Ceratocystisipris species defended media resources from colonization by opposing isolates with significantly higher frequency than Entomocorticium species (Table 1). Data regarding competitive resource acquisition and resource defense by allopatric fungi were strongly asymmetric: in almost, no case (< 5%) was a fungal isolate from an allopatric beetle population able to colonize media occupied by a fungal isolate from a sympatric beetle population. Thus, competitive resource acquisition by allopatric isolates occurred almost exclusively in pairings with other allopatric isolates. The opposite was not true for sympatric fungi, which were able to competitively acquire media resources colonized by isolates from all beetle populations. However, all competition assays were performed at 25 °C, where sympatric fungi exhibited their optimal growth. Thus, it is possible that the observed differences in competitive performance between sympatric and allopatric isolates were due to localized adaptations to temperature or the seasonality of collection (Six and Bentz, 2007). However, optimal growth rate of mycanial fungi from D. frontalis collected in Mississippi and Alabama is reported between 25 - 28 °C (Klepzig et al., 2001; Hofstetter et al., 2007). The authors suggest that future studies related to competition between multiple mycanial species focus on testing interactions across a broader range of temperatures. In nature, many factors may contribute to the outcomes of competitive interactions between multiple fungi. For example, Licyayo et al. (2007) found that ammonia concentrations and pH had strong effects on interspecific interactions between fungal species. Similarly, melanin and other pigments have been shown to strongly mediate competition between fungal species (Yuen et al., 1999; Klepzig, 2006). In a D. frontalis system, water availability was determined to play an important role in fungal competition (Klepzig et al., 2004). The studies only account for differences in competitive ability among fungi between regions of sympatry and allopatry, and by beetle species and fungal species within sympatric populations. However, the present study represents a first assessment of combative interactions between the mutualists of two or more bark beetle species, and suggests that fungi are likely to adapt to a competitive environment when multiple mycohagous beetle species inhabit a single plant host. Future studies of competitive interactions could benefit by testing interspecific fungal interactions across a gradient of host plant Dendroctonus beetles are frequently exposed to a terpenoid-saturated environment during the colonization of host tissues, and exposure to terpenoid compounds strongly impacts the growth performance of beetle – associated fungi (Paine and Hanlon, 1994; Hofstetter et al., 2005).

In conclusion, the mycanial fungi associated with D. brevicomis and D. frontalis were variable with respect to primary resource acquisition and competitive interactions. Growth rates of fungi did not vary by beetle species or fungal species when beetle populations were sympatric, however fungal growth rates did show substantial variation across regions. In sympatric populations, Ceratocystisipris species were more likely to resist colonization by a competitor than Entomocorticium species. Mycanial isolates from sympatric beetle populations were more likely to competitively acquire resources. Interactions between sympatric isolates and allopatric isolates were asymmetric: sympatric isolates were better competitors and frequently colonized media resources inhabited by an allopatric isolate. Interestingly, competition also appeared to be symmetric among allopatric isolates.

These data reported here support the hypothesis that interactions between mycanial fungi of multiple Dendroctonus species are antagonistic ( –, –), since fungi that occurred in sympathy were stronger competitors. Furthermore, these findings may be extendable to other systems where multiple insects with fungal associates colonize the same plant host and insect-fungal associations have been increasingly recognized as a central theme in arthropod ecology (Blackwell and Vega, 2005). The importance of these interactions for beetle larval performance and fungal-beetle relationships are still unknown and further experiments are needed to determine how competition between multiple fungal associates of Dendroctonus species affect beetle fitness.

ACKNOWLEDGEMENTS

The author thanked Amanda Garcia, Sherri Smith, and
Danny Cluck for capturing and sending bark beetles to their lab. The author appreciated the efforts of the USDA Forest Service, Southern Research Station laboratory technicians and the USDA Forest Service, Rocky Mountain Research Station for providing us with laboratory space. The author thanked Brandy Francis for assistance with sequencing efforts, Cindy Liu for access to unpublished fungal sequencing primers, and Laine Smith for laboratory assistance. The author also thanked one anonymous reviewer for comments that improved the quality of the manuscript. The author acknowledged the USDA Forest Service funding sources: Southern Research Station Cooperative Agreement 06-CA-11330129-046 and Rocky Mountain Research Station Joint-Venture Agreement 05-PA-11221615-104.

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