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Abstract Feedback from community interactions involving mutualisms are a rarely explored mechanism for generating complex population dynamics. We examined the effects of two linked mutualisms on the population dynamics of a beetle that exhibits outbreak dynamics. One mutualism involves an obligate association between the bark beetle, *Dendroctonus frontalis* and two mycangial fungi. The second mutualism involves *Tarsonemus* mites that are phoretic on *D. frontalis* (''commensal''), and a blue-staining fungus, *Ophiostoma minus*. The presence of *O. minus* reduces beetle larval survival (''antagonistic'') by outcompeting beetle-mutualistic fungi within trees yet supports mite populations by acting as a nutritional mutualist. These linked interactions potentially create an interaction system with the form of an endogenous negative feedback loop. We address four hypotheses: (1) Direct negative feedback: Beetles directly increase the abundance of *O. minus*, which reduces per capita reproduction of beetles. (2) Indirect negative feedback: Beetles indirectly increase mite abundance, which increases *O. minus*, which decreases beetle reproduction. (3) The effect of *O. minus* on beetles depends on mites, but mite abundance is independent of beetle abundance. (4) The effect of *O. minus* on beetles is independent of beetle and mite abundance. High *Tarsonemus* and *O. minus* abundances were strongly correlated with the decline and eventual local extinction of beetle populations. Manipulation experiments revealed strong negative effects of *O. minus* on beetles, but falsified the hypothesis that horizontal transmission of *O. minus* generates negative feedback. Surveys of beetle populations revealed that reproductive rates of *Tarsonemus*, *O. minus*, and beetles covaried in a manner consistent with strong indirect interactions between organisms. Co-occurrence of mutualisms embedded within a community may have stabilizing effects if both mutualisms limit each other. However, delays and/or non-linearities in the interaction systems may result in large population fluctuations.

Keywords Feedback · Fungi · Mites · Mutualism · Population dynamics

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Introduction

Positive interactions among species (mutualisms and commensalisms) have been generally neglected by ecologists relative to negative interactions, such as competition and predation (Crawley 1990; Dickman 1992; Kearns et al. 1998; Menge 2000; Richardson et al. 2000; Waser et al. 2000). Even though positive interactions are common, we know little about their role in population dynamics (Addicott 1986; Holland and DeAngelis 2001; Holbrook and Schmitt 2004). Most studies of mutualisms have emphasized pairwise interactions (Herre et al. 1999) even though most mutualisms are embedded within multi-species systems, which can produce a diversity of indirect effects

(Wilson 1986; Wootton 1993; Jones et al. 1998; Stanton 2003). The potential for strong indirect effects may be especially high when the interaction system includes mutualisms, because strong positive feedback from +/+ interactions should tend to propagate, or even amplify, population fluctuations that arise for any reason elsewhere in the associated community. Thus, systems that include mutualisms may be more likely to exhibit high amplitude fluctuations from exogenous effects (Dean 1983).

Pairwise interactions among species may generate reciprocal dynamics that yield endogenous demographic feedback (density-dependence, Berryman 2002). Depending on the sign (+/−) and speed of feedback, populations may exhibit simple or complex dynamics (May 1976a; Turchin and Taylor 1992). Endogenous feedback could also be generated by multi-species interaction systems that form a feedback loop (Levins and Shultz 1996). If the interaction system produces density-dependent feedback with delays or nonlinearities, it would tend to generate endogenous fluctuations in populations (Lima and Jaksic 1999). Here we evaluate the effects of an interaction system that includes mutualisms, commensalisms, and antagonisms on the population dynamics of a notable outbreak bark beetle of North American pine forests.

The southern pine beetle, *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytinae), undergoes extreme fluctuations in abundance (Turchin et al. 1991) that result in extensive mortality of their host trees and produce broad, economically significant, patterns of forest disturbance (Price et al. 1997). Part of the temporal variance in beetle abundance is attributable to delayed density-dependence from specialist natural enemies, especially *Thanasimus dubius* (Coleoptera: Cleridae; Turchin et al. 1999; Cronin et al. 2000). However, *D. frontalis* also has strong interactions with symbiotic fungi that have been hypothesized to influence beetle population dynamics (Klepzig et al. 2001; Lombardero et al. 2000, 2003). For instance, the blue-staining fungus (*Ophiostoma minus*), commonly found with *D. frontalis*, competes within the phloem with other fungi that have an obligate mutualism with the beetles (Klepzig and Wilkens 1997; Klepzig et al. 2004). *O. minus* is introduced into phloem tissue both by adult beetles directly and by their associated mites (*Tarsonemus* spp., phoretic on beetles; Bridges and Moser 1983; Moser 1985; Moser and Bridges 1986). This suggests the possibility of an endogenous negative feedback loop (Fig. 1: H1, H2) in which the abundance of beetles affects *O. minus*, which affects per capita reproduction of beetles. This could be via direct effects of beetles on *O. minus* (Hypothesis 1) or indirect effects mediated by mites (Hypothesis 2). Alternatively, the demographic effects of *O. minus* on beetles could vary independently of beetle abundance (Fig. 1: H3, H4). In this case, the effect of *O. minus* on beetles might still depend on mite abundance, but mite abundances would be independent of beetle abundance (Hypothesis 3), resulting in no negative feedback. Finally, the effect of *O. minus* on beetles might be independent of either beetle or mite abundance (Hypothesis 4).

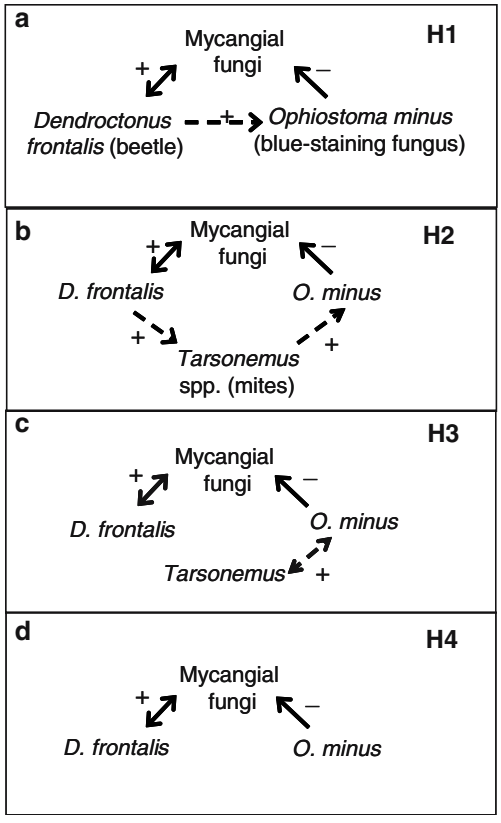

Fig. 1 Models of proposed hypotheses. *Solid arrows* indicate known effects of one species on the abundance of the other species, and *dashed arrows* indicate interactions tested in this study. The mycangial fungi and beetles (*D. frontalis*) are obligate mutualists. Any factor that affects the mycangial fungi will influence *D. frontalis*. **a** *H1*, Direct negative feedback: *D. frontalis* directly increase the abundance of *O. minus*, which reduces per capita reproduction of the *D. frontalis*. **b** *H2*, Indirect negative feedback: beetles indirectly increase the abundance of *O. minus* by increasing the abundance of mites (*Tarsonemus*), generating a subsequent loss of beetle per capita reproduction. **c** *H3*, No negative feedback, but the effect of *O. minus* on *D. frontalis* depends on *Tarsonemus* abundance, and *Tarsonemus* abundance is independent of *D. frontalis* abundance. **d** *H4*, No negative feedback: the effect of *O. minus* on *D. frontalis* is independent of beetle or mite abundances

Although the effects of *O. minus* on beetles occurs indirectly via interactions with their mycangial fungi (Goldhammer et al. 1990; Klepzig and Wilkens 1997), for the purposes of this study we have lumped beetles and their mycangial fungus together. Detailed studies of potential differential interactions between *O. minus* and mycangial fungi in beetle infestations are published elsewhere (Hofstetter et al. 2005a, b). Here, we tested for general effects of *O. minus* on beetle reproduction by manipulating the abundance of *O. minus* on beetle exoskeletons and measuring beetle reproduction. We evaluated one possible pathway for endogenous feedback (H1) by including a factorial manipulation of *O. minus* inoculation levels and *D. frontalis* attack

density. H1 would be supported if increasing density of parent beetles exacerbates the effect of *O. minus* on beetle reproduction (because of horizontal transmission of *O. minus* among beetle offspring within the phloem). In our experiment, this would be revealed as a statistical interaction between the density of beetles and the frequency of beetles with *O. minus* spores; i.e., if the effect of *O. minus* inoculation on per capita beetle reproduction is greatest when attack densities are high. We evaluated the role of mites (H2–H4) through an experimental manipulation of phoretic mites on beetles. We were unable to experimentally manipulate both beetle density and phoretic mite density, and thus could not experimentally test H2. Instead, we surveyed multiple beetle populations to test for predicted correlations among *O. minus* abundance, mite abundance, and beetle reproduction. The survey was conducted with a hierarchical sampling design, which allowed us to evaluate the spatial scale of variation in the abundance of *O. minus*, mites, and beetles. If the abundance of *O. minus* and mites is strongly influenced by climate rather than by beetle density (a likely mechanism under H3 and H4) then variation should be high at a coarse spatial scale.

Materials and methods

Study system

Dendroctonus frontalis is a native bark beetle that inhabits pine forests in Central and North America. *D. frontalis* uses pheromones to promote mass attacks on individual trees which must be killed to permit beetle larval development (Thatcher et al. 1980). Larvae feed upon phloem tissue and mutualistic fungi (*Entomocorticium* sp. *A* or *Ophiostoma ranaculosum* formerly *Ceratocystiopsis ranaculosus;* Jacobs and Kirisits 2003) introduced by the parent beetle (Barras 1973; Bridges 1983; Ayres et al. 2000). Female beetles cultivate these fungi within specialized glandular invaginations of the prothorax (mycangia; Happ et al. 1976; Hsiau 1996). Larvae fare poorly in the absence of the mycangial fungi (Barras 1973) and the fungi only occur in association with *D. frontalis*, so this is a strong obligate mutualism. *D. frontalis* have 3–6 generations per year depending upon temperatures (Thatcher et al. 1980; Ungerer et al. 1999).

During epidemics, populations of *D. frontalis* occur as multiple local aggregations that are scattered through a forest landscape. These infestations are generally comprised of neighboring trees, each containing beetles in one or more stages of development (Coulson 1980). Infestations are initiated by dispersing beetles in the spring and may enlarge through the rest of the year to included hundreds to thousands of trees containing thousands to millions of beetles. The following spring, beetles disperse and initiate a new set of infestations.

A large complex of insect predators and competitors develop concurrently in the host tree (reviewed in Thatcher et al. 1980). Commensal mites (Acarina: Tarsonemidae) are also commonly associated with *D. frontalis* and depend on the beetles for transportation (phoresy) between trees (Bridges and Moser 1983; Moser 1985; Moser and Bridges 1986). *Tarsonemus* mites feed on *O. minus* (Lombardero et al. 2000), which is one of the so called ''bluestain fungi'' that are common associates of bark beetles (Solheim 1986; Perry 1991; Seifert 1993; Jacobs and Wingfield 2001). *Tarsonemus* spp. carry spores of *O. minus* to newly attacked trees (Leach et al. 1934; Moser et al. 1995) and propagate *O. minus* within the oviposition galleries of *D. frontalis* (Lombardero et al. 2003). *O. minus* spores can also be transported directly on the exoskeleton of *D. frontalis* (Barras and Perry 1975). Within the tree, *O. minus* grows quickly (cm per day), forming vertical elliptical patches within the phloem (where it produces a black/blue stain). Beetle larvae that develop within or near patches of *O. minus* produce uncharacteristically long tunnels and often die (Barras 1970; Goldhammer et al. 1989; Lombardero et al. 2003). The negative effect of *O. minus* on *D. frontalis* is apparently an indirect result of asymmetric competition between *O. minus* and mycangial fungi within the phloem (Klepzig 1998).

Experimental manipulation of *O. minus* on beetles

To test for interacting effects of beetle density and *O. minus* inoculation levels on beetle reproduction (Hypothesis 1), we placed replicate field cages on trees and stocked each with low, medium, or high densities of attacking adults that carried low, medium or high levels of *O. minus* inoculum. Densities corresponded to approximately 6.6, 13.3, and 19.9 beetles/dm^2, encompassing the range of densities found in nature (Fargo et al. 1979).

We manipulated the prevalence of *O. minus* by altering the fraction of *D. frontalis* that carried the fungus. Adult beetles were surface-sterilized (collected from nature) by pouring White's solution over individuals held in a screen funnel for 20 s (Barras 1972). This process also removed phoretic mites, except under the elytra. One half of the beetles were forced to walk for 30 s on agar plates containing sporulating *O. minus* fungi. The remaining beetles were placed on sterile plates for 30 s to control for handling procedure.

We placed screen cages on 23 healthy loblolly pine (*Pinus taeda*, DBH of 20–28 cm) at the edge of a beetle infestation in the Kisatchie National Forest, Louisiana. On each tree, we attached two 0.5-m long cages centered at 3 and 5 m from the base of the tree, such that 1.5 m separated the upper from the lower cage along the bole of the tree. The distance between cages was sufficient to preclude the spread of beetle galleries or fungi between them (J.T. Cronin, unpublished data). We sealed the edges of the cage to the bark of the tree with caulk. We

baited the trees with frontalin and turpentine to attract *D. frontalis* from the surrounding area. Once the tree was under attack, we added *D. frontalis* (0, 50, or 100% infected with *O. minus*) to the cages (500, 1,000, or 1,500 beetles per cage, 1:1 sex ratio). We assigned treatments at random to a cage and established five replicates for each of the nine treatment combinations.

After the attack phase was complete and *D. frontalis* offspring were late larvae or pupae, we cut down each tree, placed the caged sections in rearing containers, and collected and counted the *D. frontalis* offspring that subsequently emerged. After all beetles emerged, we measured the percentage of bark undersurface (phloem) that was stained blue by *O. minus*. We tested for effects of initial beetle density and *O. minus* infection on per capita reproductive success of *D. frontalis* (adult offspring produced/parental adult) with a full-factorial ANOVA. Beetle density and *O. minus* infection rate were treated as fixed factors and tree (bearing two cages each) was treated as a randomized block effect in the model. We used the same model to test for effects on the proportion of phloem area with *O. minus*. To correct for heteroscedasticity, we ln-transformed progeny/adult and arcsine-square root transformed percent *O. minus*/phloem area.

Experimental manipulation of *Tarsonemus* mites on beetles

To test for indirect effects of phoretic mites on beetle reproduction (via increases in area colonized by *O. minus*) (Hypotheses 2, 3), we conducted a factorial manipulation of phoretic mites and *O. minus* on adult beetles. We screened sections on four *P. taeda* at the leading edge of an infestation in the Talladega N.F., Alabama, in July 2000. We covered the bole of the tree from 3 to 5 m in height with a fine woven dark brown cotton cloth, and sealed the edges of the cloth to the tree with caulk and black duct tape. We initiated *D. frontalis* attacks simultaneously on the four trees using frontalure and deployed three Lindgren funnel traps (Phero Tech, Inc.) to collect flying *D. frontalis*. Beetles captured in the traps were placed individually into sterile, 1-ml centrifuge vial-caps and stored on ice. We removed all phoretic mites from beetles (averaging 0.62 mites/beetle) and stored them on a sporulating culture of *O. minus*. We placed individual beetles into a clear gelatin capsule and randomly assigned each to one of four treatment groups: Control (mites removed and no *O. minus* added), Mite (one mite added), *O. minus* (beetles walked on *O. minus*-agar plate) and *O. minus* + Mite (beetles walked on *O. minus*-agar plate and one mite added). Mite treatments involved the addition of one female *Tarsonemus* mite (reared on *O. minus*) per beetle. For the *O. minus* treatments, we placed each beetle on a sporulating culture of *O. minus* for 30 s immediately before placing it into the tree. We did not surface-sterilize beetles with White's solution prior to infection. Isolates of *O. minus* on beetles revealed that 100% of beetles from the *O. minus* treatment and 49% of the control beetles carried *O. minus*.

Once each tree was under attack, we removed the brown cloth that had prevented beetles from attacking that section of the tree and divided the exposed bark into four 5×5 dm treatment areas. Each of the four treatments (Control, Mite, *O. minus*, *O. minus* + Mites) was randomly assigned to one of four treatment areas in each tree. Within each treatment area, we drilled 100 evenly spaced holes to the phloem (5 mm diameter). We placed one female beetle into each hole with the small end of a gelatin capsule sealing each exit and replaced the brown cloth. After 24 h, we introduced two males (wild beetles with mites removed) into each hole, and replaced the brown cloth to prevent additional *D. frontalis* attacks and prevent the introduction of competitors and predators into the treated areas. We removed the cloth after 5 weeks (1 week prior to expected emergence of offspring). During emergence, we removed a section of bark (2×3 dm) in the center of each treatment area and measured the percent of phloem area with bluestain indicative of *O. minus*, numbers of beetle attacks (each representing one pair of parental adults), number of pupal chambers (indicating successful larval development), and total length of oviposition gallery (correlated with eggs laid; Hain 1980). We estimated *Tarsonemus* densities in the bark by immediately counting mites in three 1-cm^2 areas inside and outside bluestain patches. We estimated brood survival by counting pupal chambers relative to eggs laid (estimated as 1.6 cm oviposition gallery; Foltz et al. 1976). We used an ANOVA model that included treatment and tree to test for effects of *O. minus* infection and phoretic *Tarsonemus* on per capita reproduction of *D. frontalis* (adult offspring/parental adult), *O. minus* abundance in phloem (arcsine-square root transformation), and mite population size (log transformed).

Natural variation and covariation in fungi, mites and *D. frontalis*

To evaluate the role of *Tarsonemus* and *O. minus* in natural *D. frontalis* populations, we tested for correlations in species abundance predicted by each hypothesis (Fig. 1). We studied beetle infestations in three National Forests in the southeastern US: the Homochitto N.F. in western Mississippi (760 km^2 containing loblolly pine, *Pinus taeda,* and shortleaf pine, *P. echinata*), Bankhead N.F. in the southern Appalachians of northern Alabama (720 km^2 containing loblolly pine and Virginia pine, *P. virginiana*) and Oakmulgee Ranger District of Talladega N.F. in south-central Alabama (620 km^2 containing loblolly pine and longleaf pine, *P. palustris*). We restricted our studies to *P. taeda*, which is the most common host of *D. frontalis* in each forest.

Within the three National Forests, we selected five discrete *D. frontalis* infestations (each involving 10–100

host trees) for surveys of *O. minus* abundance, beetle reproductive success, and *Tarsonemus* densities. We selected infestations approximately at random from the population of active infestations that had been detected during regular aerial surveys of each forest by U.S.D.A., Forest Service Forest Health Protection personnel. We excluded some infestations from the pool of potential study sites because they were targeted for imminent control treatments (suppression logging). We conducted surveys during June and July of 2000 and repeated with different infestations in 2001 (except for Homochitto N.F., which lacked beetle infestations during 2001). We monitored infestation status by periodically returning and recording tree colonization patterns and number of trees killed by *D. frontalis* at each site. Infestations were observed until October of each year to determine if beetle populations continued to grow, decline or go locally extinct (i.e., no beetles on or in trees for several months).

We removed two bark samples (each 21×28 cm) between 3 and 4 m from the base of five infested trees (containing pupae and callow adult beetles) in each infestation. We chose sample trees at random from the population of trees that contained beetle pupae. For each sample, we measured the percent area with bluestain (*O. minus*), number of beetle attacks (each representing a pair of colonizing adults), total length of oviposition gallery, and number of emergence holes or pupal chambers (indicating offspring that completed development). Areas damaged by wood borers (Cerambycidae and Buprestidae) could not be measured in the same way and were excluded from analysis. We sampled a total of 125 trees: 5 trees×5 infestations×5 forest-years. We evaluated *O. minus* abundance, gallery density, attack density, ln(progeny/beetle), and *Tarsonemus* per bark area using a nested ANOVA model (JMP 5.0.1, SAS Institute Inc. 2003) that included forest-year, infestation (within forest-year), and tree (within infestation and forest-year) as random effects. We used a linear regression model to characterize the effect of *O. minus* and attack density (and interaction between *O. minus* and attack density) on progeny/beetle, gallery production/beetle, larval survival (pupae/egg), and mite density. To correct for heteroscedasticity, we transformed percent area of phloem colonized by *O. minus* (arcsine-square root) and mite abundance [$\log(x+1)$]. We computed correlations among traits (means for each infestation) using a Pearson product-moment correlation coefficient, with a Bonferroni correction to control Type I errors for the full correlation matrix.

To determine phoretic *Tarsonemus* abundance and fungal composition within *D. frontalis* mycangia and on *D. frontalis* exoskeletons, we captured flying adult *D. frontalis* using Lindgren funnel traps placed against trees that were under attack. We monitored three traps every 15 min until a minimum of 50 *D. frontalis* were captured (usually 1–3 h) within each infestation. We placed captured beetles immediately into a sterile 1 ml centrifuge-cap vial and stored them on ice. We dissected the thorax of female beetles and mounted each mycangium on a microscope slide to identify the fungi present (Bridges 1983). After phoretic *Tarsonemus* were removed, we placed male *D. frontalis* and the head and abdomen of female *D. frontalis* onto selective media to determine the incidence of *O. minus* on the *D. frontalis* exoskeleton. We collected a total of 938 *D. frontalis*. We weighed a subset of intact female and male beetles to test for correlations between beetle mass and the presence of *O. minus* spores. We analyzed the incidence of *O. minus* on phoretic *Tarsonemus* or *D. frontalis* using a χ^2 test that included forest-year and infestation (within forest-year) as independent variables. For parametric statistics, we transformed *Tarsonemus* abundance as natural log of the square root of *Tarsonemus* plus one.

Results

Negative affects of *O. minus* on beetle per capita reproduction

In field manipulations of beetle density and phoretic *O. minus*, per capita reproduction of beetles declined with increased presence of *O. minus* in phloem (Figs. 2, 3). Negative effects of *O. minus* on per capita reproduction of beetles were also observed when bluestain area in phloem exceeded 23% ($Y = 0.89 - 0.038X$ %bluestain; $r^2 = 0.40$). In natural infestations, the parental replacement rate was less than 0 (progeny < adults) when phloem area colonized by *O. minus* among trees exceeded 56% (Fig. 4). Additionally, four of five infestations monitored crashed (local extinction) before September when average area colonized by *O. minus* exceeded 40% compared to only one of 20 infestations when *O. minus* coverage was below 40% (Table 1).

The presence of *O. minus* on beetles' exoskeletons was unrelated to beetle mass (paired *t*-test: $t_{41} = 0.66$, $P = 0.51$) or to whether individual females were carrying *E.* sp. *A* vs. *O. ranaculosum* within their mycangium (49 vs. 53%, respectively). Male and female beetles were equally likely to carry *O. minus* spores on their exoskeleton (49 vs. 51%; $\chi^2 = 0.37$, $P = 0.39$).

Test of direct negative feedback between beetles and *O. minus*

Although per capita reproduction of *D. frontalis* declined with increased presence of *O. minus* in phloem (experimental manipulation: $F_{1,32} = 22.7$, $P < 0.01$; field surveys: $r = -0.42$, $P < 0.01$, $n = 125$) and density of adult beetles (experimental manipulation: $F_{2,25} = 54.6$, $P < 0.01$; field surveys: $r = -0.45$, $P < 0.01$), increased density of adult beetles did not lead to higher abundance of *O. minus* in the phloem (manipulation study: $F_{2,25} = 0.79$, $P = 0.47$; $F_{4,25} = 0.47$, $P = 0.76$ for interac-

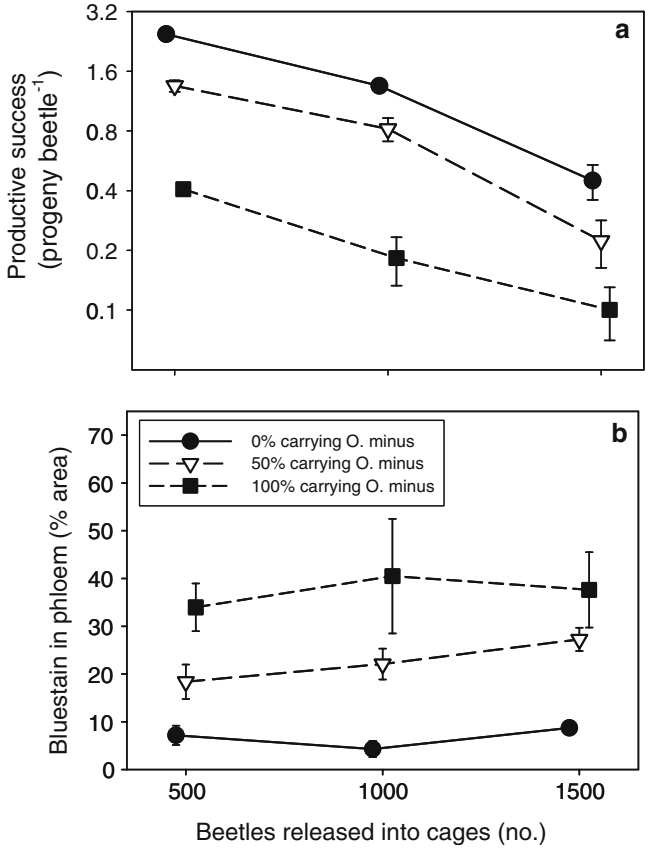

Fig. 2 The effect of *D. frontalis* beetle density and percentage of beetles carrying *O. minus* [0 (*filled circle*), 50 (*unfilled inverted triangle*), or 100 (*filled box*)] on **a** adult offspring per beetle and **b** the percent coverage of *O. minus* (bluestain) in phloem in the field-cage experiment. Means ± SE. Note log scale in **a**

tion of beetle density *x*% infected) (field surveys: $F_{1,208} = 0.95$, $P = 0.94$, Fig. 2b). In the manipulation study, the effects of density and proportion of adult beetles with *O. minus* were additive; i.e., the interaction term was small and nonsignificant ($F_{4,25} = 0.11$, $P = 0.98$), and there was no effect of tree (block effect) on per capita reproduction ($F_{1,25} = 0.03$, $P = 0.87$). Thus, the feedback loop (H1) in which the abundance of beetles influences per capita reproduction of beetles via changes in *O. minus* is not supported. Increased proportion of colonizing *D. frontalis* carrying *O. minus* spores, independent of adult density, resulted in an increase in phloem colonized by *O. minus* (indicated by bluestain; $F_{2,25} = 31.8$, $P < 0.01$) and a decline in beetle per capita reproduction ($F_{2,25} = 33.5$, $P < 0.01$, Fig. 2a). However, surveys of natural infestations revealed that the frequency of beetles carrying *O. minus* and bluestain within infestations was unrelated (Fig. 5b). The percentage of beetles carrying *O. minus* spores directly on their exoskeleton ranged from 15 to 93% across infestations and varied significantly among the three national forests and infestations (Appendix B, Table 2). The frequency of *O. minus* on beetles was unrelated to any measured attribute of infestations, including the abundance of mites in phloem, the abundance of phoretic mites, or the percentage of female adults carrying *E.* sp. *A* vs. the *O. ranaculosum* in their mycangium (Table 2). In addition, local beetle population size (estimated as number of infested trees) was unrelated to fungal abundance, mite abundance or beetle reproduction within infestations (Table 3, evidence against H1 and H2). The relative frequency of the two species of mycangial fungi within infestations was not related to percent *O. minus* in phloem or *Tarsonemus* densities (Table 3).

Test of indirect negative feedback via phoretic mites and *O. minus*

Because we did not experimentally manipulate mite densities and beetle densities together, we cannot directly test and conclude the indirect pathway from

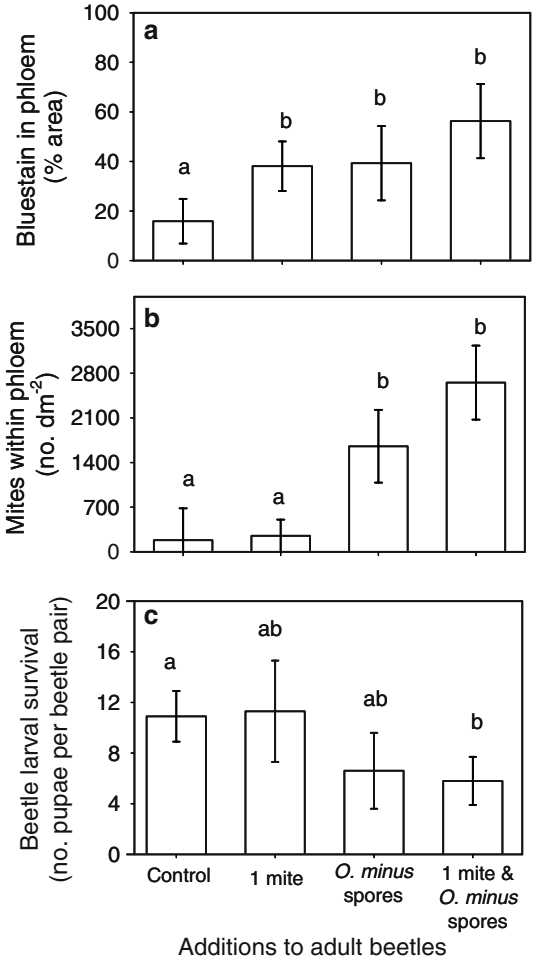

Fig. 3 Results from experimental additions of *O. minus* and *Tarsonemus* on *D. frontalis*. *Bars* show change (±SE) relative to control beetles in **a** extent of bluestain (*O. minus*) within phloem, **b** abundance of mites within phloem, and **c** larval survival. *Bars* with different *letters* indicate significant difference ($P < 0.05$) between means

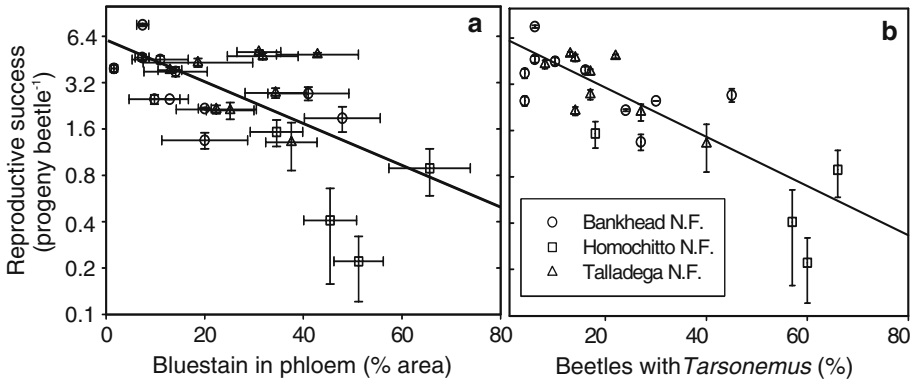

Fig. 4 Relationships between per capita reproductive success of *D. frontalis* and **a** percent bluestain (*O. minus*) in phloem ($y = 1.68 - 0.03x$, $P < 0.01$, $r^2 = 0.37$) and **b** percent of beetles carrying phoretic mites (*Tarsonemus;* $y = 1.83 - 0.037x$, $P < 0.01$, $r^2 = 0.54$). Each *symbol* represents one infestation within the Bankhead (*circle*), Homochitto (*square box*), or Talladega (*triangle*) National Forests (±SE based on five trees/infestation). Note the log scale used

beetles to *O. minus* via mites (H2). However, our experimental additions of phoretic *Tarsonemus* mites to colonizing beetles resulted in an increase in *O. minus* within phloem (support for H3 and partial support of H2, Fig. 3, Appendix A). Surveys of natural infestations also revealed a strong correlation between phoretic *Tarsonemus* abundance and *O. minus* abundance within infested trees (Fig. 5a). Beetle reproduction was negatively related to phoretic *Tarsonemus* abundance (Fig. 4b) among infestations within forests (support for H3, partial support for H2), and in general, beetle per capita reproduction within infestations was less than 0 when >50% of adult beetles carried ≥1 *Tarsonemus* (Fig. 4b). Field surveys of natural beetle infestations revealed that average beetle attack densities within tree were positively correlated with *Tarsonemus* and *O. minus* in phloem ($r = 0.50$–0.56), suggesting the possibility of density-dependence but these were not quite significant with Bonferroni corrections ($0.05 < P < 0.10$; Table 3). Dissections of field collected female beetles revealed that beetles carrying *O. ranaculosum* versus *Entomocortium* sp. *A* as their mycangial fungus were more likely to carry *Tarsonemus* (40% vs. 29%; $\chi^2 = 11.6$, $P = 0.008$).

Table 1 Relationship between abundance of *O. minus* (% phloem with bluestain) and *D. frontalis* population persistence: number of infestations that persisted (continuing attacks of live trees) or crashed (local extinction of beetles) before September of each year

Percent phloem area with bluestain	Infestations that persisted	Infestations that crashed
0–10	4	0
11–20	7	0
21–30	4	0
31–40	4	1
41–50	1	2
>50	0	2

Mean levels of *O. minus* per infestation were sampled in June

Test of feedback between *O. minus* and mites, independent of beetle abundance

Tarsonemus and *O. minus* (within trees) were strongly correlated within natural infestations (Table 3, Fig. 5a) and experimentally manipulated trees (Fig. 3, support for H3; evidence against H1, H4). For instance, average *Tarsonemus* densities in the phloem increased significantly following additions of *O. minus* and *O. minus* + *Tarsonemus* on attacking beetles (Fig. 3b; Student's t, $P < 0.05$). Across all natural infestations surveyed, 28% of flying beetles carried *Tarsonemus* and 69% percent of the phoretic *Tarsonemus* carried *O. minus* ascospores, averaging 18 ascospores per mite. Both *O. minus* and *Tarsonemus* were present in all 24 infestations surveyed and were positively correlated (Table. 2, 3). Also given that the abundance of *O. minus* and *Tarsonemus* were highly variable among and across forests (Appendix B, Table 1), there is little evidence that *O. minus* abundance functions independent of *Tarsonemus* (evidence against H4).

Discussion

Demographic effects of *O. minus* and *Tarsonemus* on *D. frontalis*

Dendroctonus frontalis undergoes extreme fluctuations in abundance (Turchin et al. 1991). Results here indicate that variation in abundance of symbiotic associates is a meaningful driver of variation in beetle population dynamics. In our study, the abundances of *O. minus* and *Tarsonemus* explained 37–54% of the variation in beetle reproduction among infestations (Fig. 4). Across 20–25 natural infestations, *O. minus* ranged from 2 to 65% of bark and *Tarsonemus* ranged from 0.1 to 7.1 per beetle. The apparent demographic consequences for *D. frontalis* are equal or greater than

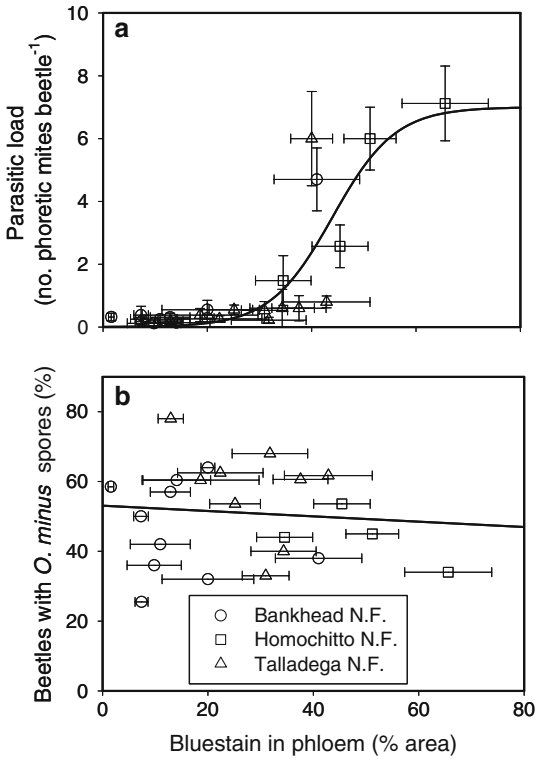

Fig. 5 Relationships between *O. minus* in phloem and **a** phoretic mites/beetle ($y = 7/e^{-(x-44/6)})x$, $P < 0.01$, $r^2 = 0.74$) and **b** percent of adult *D. frontalis* (phoretic mites removed) with *O. minus* spores on their exoskeleton ($y = 53-0.048x$, $P < 0.68$, $r^2 = 0.00$). Each *symbol* represents one infestation within the Bankhead (*circle*), Homochitto (*square box*), or Talladega (*triangle*) National Forests (±SE based on five trees per infestation)

other known sources of mortality. For example, an increase from 8 to 49% in average extent of *O. minus* in phloem corresponds to an 85% decline in progeny per beetle (Fig. 4a). In comparison, high densities of predators reduce survival of *D. frontalis* by 60%, and reduce the ratio of increase (N_t:N_{t-1}) by 50–70% (Reeve 1997; Turchin et al. 1999; Reeve and Turchin 2002).

The negative effect of *O. minus* on beetles is believed to be indirect, as indicated in Fig. 1, because we know that (1) there is strong asymmetric competition between mycangial fungi and *O. minus* (Klepzig 1998); (2) *D. frontalis* larvae require mycangial fungi (Barras 1973); and (3) non-mycomutualistic beetles seem to be less affected by phoretically vectored bluestain fungi (Yearian et al. 1972; Klepzig and Six 2004). However, we cannot exclude the possibility of allelopathic effects from phenolics and isocoumarins elicited by the tree in response to *O. minus*, or by melanin produced by *O. minus* (Hemingway et al. 1977; DeAngelis et al. 1986).

Regardless of the mechanism, recognition of demographic effects of *O. minus* may have value for management of pestilence from *D. frontalis*. For example, monitoring the abundance of *Tarsonemus* or *O. minus* within infestations may be a tool for predicting beetle population trends (Table 1). It is even possible that *O. minus* could be deployed as a biological control agent for *D. frontalis*. Abundances of *O. minus* and *Tarsonemus* can be enhanced within natural infestations though the use of baited open–exit Lindgren traps (R.W. Hofstetter et al., unpublished), but there remain technical challenges to operational use.

Variation among infestations in *O. minus* abundance appears to be driven much more strongly by the association between *O. minus* and phoretic mites ($r = 0.74$–0.80; Table 3) than by the association between *O. minus* and *D. frontalis* ($r = -0.06$). Furthermore, even with large experimental additions of *O. minus* spores to beetles, *O. minus* in the absence of *Tarsonemus* did not reach levels observed in natural infestations (Fig. 4). This survey, in combination with results from experimental manipulation of *Tarsonemus* on beetles (Table 3, Fig. 3, and Lombardero et al. 2003), suggest that *Tarsonemus* are a key factor for *O. minus* abundance and may be necessary for *O. minus* to reach levels high enough (>45% of phloem) to curtail beetle population

Table 2 Mean attributes of *D. frontalis* infestations in each National Forest and year (sampled between May and July)

	Homochitto 2000	Talladega 2000	Talladega 2001	Bankhead 2000	Bankhead 2001
% bluestain in phloem[a]	49 ± 6a	26 ± 5b	34 ± 3b	8 ± 2c	30 ± 6b
D. frontalis attacks/dm^2	7.3 ± 0.5a	5.0 ± 0.3b	5.3 ± 0.1b	5.6 ± 0.4bc	6.7 ± 0.3ac
ln(pupae/adult *D. frontalis*)	−0.52 ± 0.42a	1.34 ± 0.15bc	0.91 ± 0.28b	1.44 ± 0.15c	0.66 ± 0.18b
Cm *D. frontalis* gallery/dm^2	85 ± 7	77 ± 4	87 ± 5	84 ± 3	89 ± 5
Tarsonemus/dm^2[a]	241 ± 31a	281 ± 54a	189 ± 40ab	240 ± 38a	71 ± 19b
Tarsonemus/*D. frontalis*[a]	3.7 ± 1.7a	0.3 ± 0.1b	1.8 ± 1.1c	0.2 ± 0.1b	1.2 ± 0.8c
% *D. frontalis* w/*Tarsonemus*[a]	45 ± 9a	15 ± 2b	29 ± 5a	8 ± 2b	27 ± 5a
% *D. frontalis* w/*O. minus*[b]	42 ± 4a	66 ± 3b	55 ± 5ab	45 ± 3a	48 ± 3a
% *D. frontalis* w/*Ent.* fungus	50 ± 26	51 ± 9	46 ± 8	60 ± 6	60 ± 17
% *D. frontalis* w/*Ent.* and *O.r.*	17 ± 11ab	10 ± 4a	12 ± 5ab	21 ± 3b	13 ± 5ab

Standard errors based on $N = 5$ infestations/forest-year. Different letters indicate significant difference ($P < 0.05$) among forest-years. Bluestain indicated the presence of *Ophiostoma minus*, which is mutualistic with mites (*Tarsonemus* spp). *Entomocorticium* sp. *A.* (*Ent.*) and *Ophiostoma ranaculosum* (*O.r.*) are mutualistic fungi associated with *D. frontalis*

[a]Statistically analyzed with transformed data: log(sqrt(*Tarsonemus*/*D. frontalis*) + 1); arcsine sqrt(% *O. minus*); log(*Tarsonemus* + 1), sqrt(% *D. frontalis* with *Tarsonemus*)

[b]Only included propagules directly on beetle; excludes spores carried by phoretic mites

Table 3 Correlations among *D. frontalis* infestations ($n = 20$–25) in three National Forests

	% blue-stain	beetles/dm^2	ln(pupae/beetle)	gallery/attack	gallery/dm^2	mites/dm^2	mites/beetle	% beetles *E.* sp A	% beetles *O. minus*	% beetles w/mites
D. frontalis attacks/dm^2	0.56									
Ln(pupae/*D. frontalis*)	−0.73**	−0.83**								
Cm *D. frontalis* gallery/*D. frontalis* attack	−0.19	−0.77**	0.68*							
cm *D. frontalis* gallery/dm^2	0.32	0.25	0.06	0.26						
Tarsonemus/dm^2	0.58	−0.06	0.01	0.04	0.06					
Tarsonemus/*D. frontalis*	0.80**	0.52	−0.68*	−0.15	0.55	−0.01				
% *D. frontalis* with *Entomocorticium.* sp A	−0.06	−0.04	0.19	0.00	0.01	0.04	−0.15			
% *D. frontalis* with *O. minus*	−0.06	−0.27	0.06	0.14	−0.37	0.03	−0.13	−0.24		
% *D. frontalis* with *Tarsonemus*	0.74**	0.50	−0.82**	−0.26	0.33	−0.16	0.75**	−0.29	−0.06	
D. frontalis population size (no. infested trees)	−0.20	−0.18	0.34	0.37	0.22	0.06	−0.31	−0.19	0.10	−0.18

*$P < 0.05$; **$P < 0.01$ (with Bonferroni correction)

growth (Fig. 4). Apparently, *Tarsonemus* propagate *O. minus* both by transporting ascospores into newly attacked trees (69% of phoretic mites carried an average of 18 ascospores per mite) and by dispersing existing *O. minus* within the phloem of attacked trees (Lombardero et al. 2003). *Tarsonemus* presumably propagates *O. minus* because it feeds on it (Lombardero et al. 2000). Because *Tarsonemus* feeds on *O. minus*, it is logical that the addition of *O. minus* leads to an increase in mites (Fig. 3), and that infestations with high *O. minus* abundances have high mites/dm^2 within phloem (Table 3). In any case, *Tarsonemus*, *O. minus,* and beetle reproduction covary in a manner consistent with a system of strong direct and indirect interactions (i.e., mites to *O. minus* to mycangial fungi to beetles; support of H3; Table 3).

Negative feedback or exogenous dynamics?

Do the demographic effects of *O. minus* on beetles tend to increase with increasing abundance of beetles (H1, H2) or is *O. minus* best regarded as an exogenous force in beetle population dynamics (H3, H4)? Because interactions between *O. minus* inoculation per beetle and beetle attack densities were not evident (Fig. 2), our studies falsified the hypothesis (H1) that horizontal transmission of *O. minus* produces negative demographic feedback. Furthermore, beetle infestation size was unrelated to *O. minus* or *Tarsonemus* mite abundance (Table 3, lack of support for H1, H2). Our studies give the greatest support to H3, that the effect of *O. minus* on *D. frontalis* depends on *Tarsonemus* abundance, but *Tarsonemus* abundance is independent of *D. frontalis* population size. There is a suggestion of higher *O. minus* and *Tarsonemus* abundance within trees that had high beetle attack densities, but tree-specific attack densities were unrelated to infestation size (Table 3), so this seems unlikely to produce density-dependence at the population scale (Berryman 2002). The hypotheses of density-dependence feedback requires that changes in beetle populations from low to high somehow generate increases in the effects of *O. minus* on per capita reproduction of beetles. Positive correlations between mites, *O. minus,* and beetle densities within trees (Table 3) give support for frequency-dependent feedback via mites, *O. minus,* and beetles. Because beetle densities within trees are a function of beetle attack rate, attack behavior, tree susceptibility, and not necessarily population size (Coulson 1980), endogenous feedback may instead occur in relation to the rate of growth of the infested area (related to beetle density within trees) rather than total beetle abundance.

A key question is whether increasing abundance of beetles, leads to increases in the abundance of phoretic mites per beetle. We have strong evidence that increases in *Tarsonemus* lead to increases in *O. minus* (Fig. 3) and beetle decline (Fig. 4; Hypothesis 3), however the connections between beetle abundances and phoretic mite abundance remain to be tested. Factorial manipulations of phoretic mites and beetle densities could be used to test whether populations of phoretic mites grow more with high beetle densities. Another possible mechanism for endogenous feedback follows from the new observation reported here that *Tarsonemus* are more common on beetles that harbor *O. ranaculosum* instead of *E.* sp. *A* as their mycangial fungus. This could be because *O. ranaculosum* but not *E.* sp. *A* is nutritionally suitable for *Tarsonemus* (Lombardero et al. 2000). The vast majority of *Tarsonemus* within infested trees are found feeding in patches of *O. minus*, but *O. ranaculosum* might provide critical nutrition for mites during the days immediately following beetle attack when *O. minus* is not well established (Lombardero et al. 2000). The proportion of female beetles harboring *O. ranaculosum* versus *E.* sp. *A* has been reported to vary among populations (Bridges 1983), however seasonal surveys of fungi among *D. frontalis* infestations reveal no correlation between *O. minus* and either mycangial fungus (Hofstetter et al. 2005a). If beetle abundance tends to affect the proportion of mycangial fungi within a population, it is very plausible that this would generate a

pathway of endogenous feedback (change in mycangial species leads to change in mite abundance which leads to change in *O. minus* abundance and change in beetle population growth). Beetle populations that predominantly carry *O. ranaculosum* (rather than *E.* sp. *A*) may be particularly affected by increases in *O. minus* because larvae feeding on *O. ranaculosum* are believed to be more susceptible to antagonistic effects of *O. minus* (Goldhammer et al. 1990), however we found no correlation between the relative abundance of each mycangial fungus and *O. minus* (Tables 2, 3).

During epidemic outbreaks of beetles, such as were studied here, *Tarsonemus* and *O. minus* appear to exert their effects on beetles independently of beetle density (H3). High variation and co-variation in *O. minus* and *Tarsonemus* among forests suggests that climatic effects or some other coarse-grained environmental feature have important effects on the abundance of mites and fungi. Climatic patterns have been proposed to affect beetle populations through a variety of mechanisms (Craighead 1925; Kalkstein 1976; Ungerer et al. 1999; but see Turchin et al. 1991). Lombardero et al. (2003) suggested that differences between *Tarsonemus* and *D. frontalis* in their development rate as a function of temperature might make the community of mites, fungi, and beetles responsive to climatic variation. This is a plausible but untested hypothesis for the broad spatial autocorrelation between the abundance of mites and *O. minus* (Table 1).

Complex interactions within the *D. frontalis* community

The relationship between *O. minus* and *D. frontalis* is not easily classified within the normal framework of species interactions. *O. minus* is an obligate symbiont of bark beetles, chiefly *D. frontalis* in the southeastern US. *O. minus* requires *D. frontalis* for transport from recently killed trees to freshly attacked trees. Persistence of *O. minus* requires that this cycle be repeated every beetle generation (3–6 times per year). Also, *O. minus* appears to be generally incapable of killing trees by itself (Eckhardt et al. 2004; Wullschlager et al. 2004) and so depends upon mass attack by the beetles for breaking down tree defenses. Thus, *O. minus* depends upon *D. frontalis*, but is also a strong antagonist (Figs. 2, 3, 4). This is a $+/-$ relationship but differs from normal host/parasite or predator/prey systems because the negative effects are from competition (for phloem) and the positive effects are from transport and aid in overwhelming tree defenses. Symbiotic associations between bark beetles and Ophiostomatoid fungi are common, but most cases are regarded as $+/0$ relations, where the beetle experiences minimal effects, or $+/+$ relations where the beetles gain nutritional benefits or assistance in killing the host tree (Berryman 1972; Clark and Richmond 1977; Strobel and Lanier 1981; Owen et al. 1987; Krokene and Solheim 1998; Six and Paine 1998; Kopper et al. 2004). Why *O. minus* is a strong antagonist of the beetles on which it depends is somewhat puzzling. One possibility is that this is a transient, evolutionarily unstable, association. Both species are presumed to have been present in the southeastern US throughout the Holocene at least, so it is not transient on the scale of decades to centuries, but *O. minus* is rare in the putatively ancestral populations of *D. frontalis* in Mexico (Moser and Macias-Samano 2000). So it may be a transient condition on the scale of millennia. Also, there may be a parallel with vector-borne parasites. Biotic vectors are hypothesized to contribute to the evolutionary maintenance of virulence in parasites (Ewald 1995). If so, other examples of antagonistic effects of fungi on their insect vectors may be found in systems where phoretic mites are important intermediates in transport of fungi.

Our surveys of natural infestations reinforce earlier suggestions that there is a strong mutualism between *O. minus* and *Tarsonemus* (Moser 1985; Lombardero et al. 2000). The persistence of mutualisms is a long-standing puzzle in community ecology because simple models of $+/+$ interactions are intrinsically unstable (May 1982). One potentially general resolution is that the persistence of mutualisms in nature depends upon broader community interactions (Ringel et al. 1996). Our system suggests a special case of how that could occur. The community includes two pairs of mutualists (beetles–mycangial fungi and mites–*O. minus*), and the patterns of co-occurrence between the mutualists are so strong that each pair could each be thought of as one ecological module. Thus, the system represented in Fig. 1 could be abstracted to two modules (beetles–mycangial fungi and mites–*O. minus*) with a $-/-$ or $+/-$ relationship (depending on the relative strength of benefits from beetles vs. competition from mycangial fungi). Stability of $-/-$ or $+/-$ interactions is relatively easy to explain compared to mutualisms (May 1976b). Thus multiple mutualisms embedded within a community could be stable if the mutualisms limit each other. However, if there are nonlinearities or delays in the feedback system between mutualist pairs, the community could have a tendency for large population fluctuations, such as we see in *D. frontalis*.

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Table 4 Results from nested ANOVAs (and one χ^2) of measurements within 24 *D. frontalis* infestations

Source	DF	% *Ominus* in bark		ln(pupae/beetle)		Attacks/dm^2		cm gallery/dm^2		*Tars.*/dm^2 bark		*Tars.*/beetle[a]		% Beetles w/ *O. minus*	
		F	%	*F*	%	*F*	%	*F*	%	*F*	%	*F*	%	χ^2	*P*
Year-Forest	4	19.4**	41	10.9**	37	5.2*	11	6.4*	12	2.1*	10	7.7**	18	27.2	< 0.01
Infestation{Yr-For}	19	1.3	4	1.8*	10	1.0	1	1.0	1	3.4**	30	5.6**	9	46.2	< 0.01
Tree{Infest., Yr-For}	68–122	2.8**	27	3.3**	29	1.7**	24	1.9**	29	2.0**	21				
Residuals	80–137		28		24		64		58		39		73		
MS Error			0.027		0.378		3.69		327		0.218		0.034		

F-statistics test the null hypothesis of no variance among sample units at that hierarchical level; % Percent of total variance attributable to that hierarchical level
* $P < 0.05$; ** $P < 0.01$
[a]Residuals (error) DF = 912. Transformations: acrsine(sqrt(% *O. minus* in bark)), log(mites/dm + 1), sqrt(log(*Tars.* Mites/beetle + 1))

Appendix B

Table 4

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