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NITROGEN BUDGETS OF PHLOEM-FEEDING BARK BEETLES WITH AND WITHOUT SYMBIOTIC FUNGI

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Abstract. The nitrogen content of plant tissue is low relative to that of herbivores; as a consequence, dietary N can limit the growth and reproduction of herbivores and select for attributes that increase N acquisition. Bark beetles face a particularly severe challenge because the phloem that they consume is very low in nitrogen and phosphorus relative to their requirements. We quantified variation in the phloem concentrations of N and P in the host tree, *Pinus taeda*, and evaluated the following hypotheses regarding the role of symbiotic fungi in nutrient budgets of the herbivore *Dendroctonus frontalis*: *D. frontalis* experience variation in phloem nutrient concentrations across several spatial scales (H_1); mycangial fungi enhance the diet of *D. frontalis* larvae by contributing to the acquisition of N and P (H_2); *Ophiostoma minus*, an apparently antagonistic fungal symbiont, hinders *D. frontalis* larvae because it does not enhance nutrient concentrations of the phloem as much as mycangial fungi do (H_3); and larvae of bark beetle species that lack mycangial fungi must consume more phloem to accomplish the same growth as larvae of *D. frontalis* (H_4). In addition, we developed a general model for the N budgets of herbivorous insects that identifies the possible combinations of dietary and physiological parameters that can allow developmental success on low-nutrient diets.

Spatial variation in phloem N was mostly at the level of trees within sites (a scale of meters) while P mostly varied among sites (a scale of kilometers). Trees with higher N content produced larger *D. frontalis* adults. Prior to infestation by beetles, phloem nutrient concentrations were very uniform within trees and very low relative to that of the bark beetles (N and P concentrations of *D. frontalis* adults were 28 and 8 times greater, respectively). During infestation, phloem nutrient concentrations increased overall and became highly variable within trees. Nitrogen concentrations increased from 0.40 ± 0.01% (mean ± 1 SE) in uninfested phloem to 0.86 ± 0.03% in the phloem surrounding successfully developing *D. frontalis* larvae, which are typically associated with one or two species of mutualistic mycangial fungi. Nitrogen concentrations were intermediate in other microhabitats within infested trees, including regions with no adult colonization, with failed larval development, or colonized by the antagonistic bluestain fungus *O. minus*. We parameterized a general nutrient-budget model for *D. frontalis* and a sympatric non-mycangial bark beetle, *Ips grandicollis*, which indicated that (1) mycangial fungi provide their benefits by concentrating dietary N for larvae; (2) *O. minus* may exert its antagonistic effects on *D. frontalis* larvae by failing to concentrate dietary N as much as mycangial fungi do; (3) non-mycangial bark beetles meet their N budgets through high consumption of unaltered, low-N phloem; and (4) larvae should easily meet their P requirements with any combination of consumption rate and development time that allows them to meet their N requirements. Alternative strategies for N acquisition may have general consequences for the population dynamics and community interactions of bark beetles.

Key words: *bark beetles and dietary N;* Dendroctonus frontalis; *fungi, bluestain and mycangial; herbivory;* Ips grandicollis; *mutualism; mycangial fungi and N acquisition; nitrogen acquisition by herbivores;* Ophiostoma minus; *phosphorus; symbiotic fungi.*

INTRODUCTION

The nitrogen content of plant tissue is very low relative to that of herbivores. As a consequence, dietary nitrogen can limit the growth and reproduction of herbivores and select for attributes that increase nitrogen acquisition (Mattson 1980). Herbivores can potentially solve the problem of low dietary nitrogen by (1) increasing nitrogen-use efficiency, (2) increasing consumption rate, (3) protracting the duration of the feeding life stage, (4) adjusting life histories to exploit seasonal pulses in plant nutrient content, (5) feeding in tissue microsites of relatively high nitrogen content,

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and/or (6) manipulating the diet to increase nitrogen content (Forcella 1981, Tabashnik 1982, Robbins 1983, Ayres and MacLean 1987, Clancy et al. 1988, Clancy 1992, Slansky and Wheeler 1992, Slansky 1993, White 1993, Yang and Joern 1994, Trier and Mattson 1997). Phosphorus also occurs in lower concentrations in plants than in herbivores and is also a potentially limiting nutrient for herbivores (Ayres and MacLean 1987, Popp et al. 1989, Skinner and Cohen 1994). Bark beetles (Coleoptera: Scolytidae) face a particularly severe challenge because of the very low nutritional value of phloem (Scriber and Slansky 1981, Slansky and Scriber 1985). For example, nitrogen content of the phloem in healthy loblolly pine is ~0.38% (Hodges and Lorio 1969) compared to 1–5% in plant foliage (Mattson 1980). Because insects are typically 6–10% nitrogen themselves, growing bark beetles must therefore concentrate dietary nitrogen by 16–26 fold.

Some bark beetles, including *Ips grandicollis* (Eichhoff), appear to meet their nutritional needs with high consumption rates. Other bark beetles, including the southern pine beetle (*Dendroctonus frontalis* Zimmerman), appear to have relatively low consumption rates, but are thought to benefit from associations with fungi and other microorganisms that improve the nutritional quality of ingested phloem (Henry 1962, Becker 1971, Barras and Perry 1975, Martin 1979). Phloem nitrogen in one loblolly pine increased to 131% of control levels following the introduction of *D. frontalis* and associated microorganisms (Hodges et al. 1968). Adult female *D. frontalis* have elaborately modified structures (mycangium) that house and nurture two species of mutualistic fungi: *Ceratocystiopsis ranaculosus* Perry and Bridges and *Entomocorticium* sp. A (formerly SJB 122) (Barras and Perry 1972, Hsiau 1996). As the adults construct oviposition galleries, the fungal hyphae are exuded from the mycangium and begin to grow within the phloem tissue as *D. frontalis* progeny are hatching. Larvae subsequently feed upon the phloem–fungal complex. *D. frontalis* adults that lacked mycangial fungi laid half as many eggs as control beetles (Goldhammer et al. 1990) and *D. frontalis* larvae feeding in the absence of mycangial fungi had reduced survival and increased development time (Barras 1973, estimated potential population growth rate, r, to be 0.026 vs. 0.057 beetles·beetle^{-1}·d^{-1} in no-fungi and control populations, respectively).

The nutritional challenge for bark beetles can be compounded by intraspecific and interspecific competition for phloem. Interspecific competitors include other beetles (chiefly Scolytidae, Buprestidae, and Cerambycidae) and phoretic microorganisms. *D. frontalis* is not only associated with two species of apparently beneficial mycangial fungi but also with numerous other microorganisms such as the bluestain fungus (*Ophiostoma minus* H. & P. Sydow). Some bluestain fungi are thought to be mutualistic with some bark beetle species (Lewinsohn et al. 1994, Paine et al. 1997, Ross and Solheim 1997), but accumulating evidence suggests that *O. minus* is an antagonist of *D. frontalis*. There are strong negative correlations between the abundance of *O. minus* and the reproductive success of *D. frontalis* across infestation sites within a region, across trees within infestations, and across phloem patches within individual trees (Bridges 1983, Bridges 1985, Bridges et al. 1985, M. P. Ayres, *personal observations*). Experimental additions of *O. minus* in the laboratory reduce the growth performance of *D. frontalis* larvae (Barras 1970, Bridges and Perry 1985). The mechanisms underlying antagonistic effects of *O. minus* are not clear. It is possible that *O. minus* impedes beetle growth because it does not elevate nutrients as much as mycangial fungi do. Field and laboratory studies suggest that *O. minus* strongly outcompetes the mutualistic mycangial fungi (Bridges and Perry 1985, Klepzig and Wilkens 1997), indicating that the presence of *O. minus* may preclude the establishment of mycangial fungi in larval feeding chambers. It is also possible that secondary metabolites produced by *O. minus* (e.g., isocoumarins, Hemingway et al. 1977) have allelopathic effects on mycangial fungi or *D. frontalis* larvae.

In this study we evaluated the following hypotheses: (H_1) *D. frontalis* experience variation in phloem nutrient concentrations across many scales (within the bole of individual trees, among trees within infestations, and among infestation sites); (H_2) mycangial fungi enhance the diet of *D. frontalis* by contributing to their intake of nitrogen and phosphorus; (H_3) *O. minus* negatively impacts *D. frontalis* larval development because it does not enhance the nutrient concentrations of the phloem to the same degree as the mycangial fungi do; (H_4) larvae of *I. grandicollis,* which lack mycangial fungi, must consume more phloem to attain the same mass as *D. frontalis* larvae. In addition, we develop a general model for the nitrogen budgets of herbivorous insects that identifies the possible combinations of dietary and physiological parameters that can allow developmental success on low-nutrient diets.

Methods

Phloem nitrogen and phosphorus

Phloem samples were collected from five natural infestations of *Dendroctonus frontalis* within the Kisatchie National Forest of Louisiana and the Indian Mounds wilderness area of Texas (USA). Infestation sites were separated by 10–200 km. Within each infestation site, phloem samples were collected from five infested trees and five uninfested trees (all loblolly pine, *Pinus taeda* L., 15–30 cm in diameter at breast height). The infested trees that we sampled were all at the same stage of attack (most *D. frontalis* were late in the final larval stadium). The uninfested trees that we sampled within each site were within 40 m of the infested trees but were just outside the perimeter of the

infestation at the time of sampling. Within a site, infested and uninfested trees appeared to be comparable in all respects except for the presence or absence of *D. frontalis*. Samples from each infested tree were drawn from each of four mutually exclusive phloem classes (Fig. 1): (1) within 7 mm of adult galleries with successful brood (= good brood); (2) within 7 mm of adult galleries with unsuccessful brood (= failed brood); (3) separated from adult and larval galleries by >10 mm (= no brood); and (4) covered with fruiting bodies (perithecia) of *O. minus* (= bluestain). Phloem samples from the good brood, failed brood, and sometimes from the bluestain classes included the tissue that *D. frontalis* larvae were consuming. Good brood was defined by the presence of well-developed, oval, feeding galleries, while failed brood was defined by narrow, meandering feeding galleries that typically indicate larval mortality. We never observed successful larval development in regions with *O. minus* perithecia. Two replicate tissue samples from each phloem class in each tree were drawn from regions of phloem separated by 40–150 cm. Similarly, two replicate samples were drawn from each of the unattacked trees, but in this case there was only one phloem class (unattacked healthy phloem). Thus, each infestation site yielded a possible total of 50 phloem samples (40 from attacked trees and 10 from unattacked trees). In a few trees, not all possible phloem classes were represented (3 of 25 trees lacked *O. minus* samples and 5 of 25 trees lacked "no brood" samples). In 10 other trees, we could only obtain one sample from one of the phloem classes. In total, we analyzed 224 phloem samples.

Phloem samples were freeze-dried, ground, then analyzed for total nitrogen and phosphorus with a standard micro-Kjeldahl procedure (sulfuric acid digestion followed by analysis with a Technicon Auto-Analyzer [Pulse Instrumentation, Ltd., Saskatoon, Saskatchewan, Canada]). We also analyzed *D. frontalis* larvae (feeding final instars) and adults (~2 d after eclosion) that had been feeding upon the phloem. Plant tissue of known nitrogen concentration (National Institute of Standards and Technology, United States Department of Commerce) was included with each digestion lot to ensure quality control. Because results showed a surprising increase in the nitrogen concentration of adults relative to larvae, we made additional measurements on larvae, pupae, and adults removed from their feeding chambers (larvae) or pupation chambers (pupae and callow adults). Subsets of the callow adults were allowed to mature prior to measurements for 2 or 4 d within humidified petri dishes at ~24°C with no food (adults do not normally feed between emergence from one tree and attack of another tree).

Beetle success as a function of phloem nutrients

We evaluated patterns of variation in the size of adult beetles emerging from different trees and different infestation sites. *D. frontalis* adults from each of the attacked trees were collected as they emerged. A subsample of these beetles (8–10 of each sex from each tree) were measured for total length using an ocular micrometer (n_{total} = 5 infestation sites × 5 trees × 2 sexes × 8–10 beetles = 446 total adults sampled).

Statistics

We used an ANOVA to test for variation in phloem N and P content among infestation sites, trees nested within infestation sites, and (in the case of attacked trees) phloem classes within trees. Infestation sites and trees were treated as random effects, while phloem classes were treated as a fixed effect. We estimated the variance attributable to random effects (Sokal and Rohlf 1981). Infested and uninfested trees were analyzed separately because the phloem classes only applied to infested trees. A similar ANOVA model was used to evaluate variation in adult beetle size attributable to infestations, trees nested within infestations, and beetle sex (sex treated as a fixed effect). Correlation analyses tested for associations across trees between the average size of emerging beetles and the nitrogen and phosphorus content of phloem from the tree in which each developed; these analyses used nitrogen and phosphorus concentrations from the "good brood" phloem class because by definition most emerging beetles fed on phloem of this class.

Consumption and nitrogen-use efficiency

We estimated the consumption of phloem by *D. frontalis* larvae by measuring the size of successful larval galleries within five trees at each of two natural infestations within unthinned loblolly pine stands in the Homochitto National Forest of Mississippi (n_{total} = 100). We traced the galleries on clear mylar and calculated the area of phloem consumed using a video capture system. Some simpler measurements (with calipers) of *D. frontalis* gallery size in infestations within Lousiana, East Texas, and Alabama (USA) verified that there was no conspicuous variation in gallery size among forests. Virtually every successful *D. frontalis* gallery is in the range of 3 × 5 mm to 4 × 7 mm (see Fig. 1). We also measured the larval galleries of *Ips grandicollis* (3–13 individual galleries from each of four loblolly pine logs encompassing a range of phloem thicknesses). *I. grandicollis* galleries are long (40–130 mm) and narrow (<2 mm), not spherical like those of *D. frontalis*. So we measured initial and final gallery widths with digital calipers, measured the lengths with a digital planimeter after tracing the galleries on clear mylar, and estimated gallery size as the area of a trapezoid of these dimensions. For both species the mass of consumed phloem was calculated by multiplying the gallery area by phloem mass per unit area. Our primary estimate of phloem specific mass was based on two 1.25-cm^2 phloem samples collected from each *I. grandicollis* log during early larval development (logs: 29.8 ± 7.4 mg/cm^2 [mean ± 1 SD]). Because phloem specific

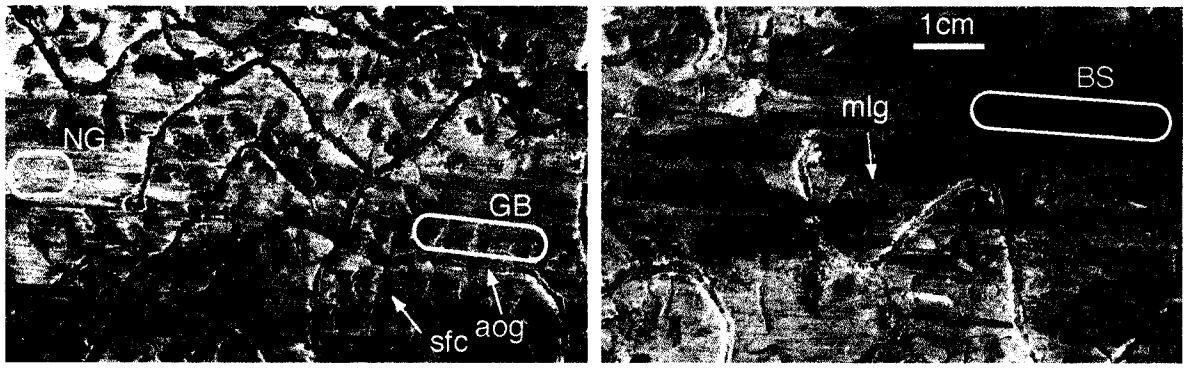

FIG. 1. Two patches of inner bark from *Pinus taeda* infested with *Dendroctonus frontalis*. *D. frontalis* had high reproductive success in the patch at left and low success in the patch at right. Darkly stained areas in the the patch at right are perithecia of the putatively antagonistic symbiotic fungi, *Ophiostoma minus*. Adult oviposition gallery (aog), successful larval feeding chambers (sfc), and meandering larval galleries (mlg; characteristic of dying larvae) are indicated. Ellipses show patches representative of the subsamples on which we measured the N and P concentrations of phloem: GB = good brood, NG = no gallery, and BS = bluestain. The remaining class, failed brood, was sampled from microhabitats with meandering larval galleries but no bluestain. At the time of our phloem sampling, successful feeding chambers still contained feeding late-instar larvae. In these photos, animals are pupating just out of sight in the outer bark below successful feeding chambers.

mass can vary depending upon tree physiological status (Wilkens et al. 1997), and because we could not measure phloem mass in the same individual trees where we measured *D. frontalis* galleries, we performed sensitivity analyses to evaluate the effects of phloem mass on estimates of *D. frontalis* nitrogen budgets. For this, we used phloem measurements from a survey of 11 loblolly pine stands that were chosen to span the range of growth conditions in our study area (12 trees/stand, 8 measurements/tree; M. P. Ayres, *unpublished data*).

We estimated nitrogen-use efficiency (NUE, percentage N consumed that is converted to insect tissue) for each beetle species using measurements of adult mass (M_{adult}), neonate mass ($M_{neonate}$), nitrogen concentration of adults (N_{adult}), total consumption (TC) and dietary nitrogen concentration (N_{diet}):

$$\text{NUE} = \frac{(M_{adult} - M_{neonate}) \times N_{adult}}{\text{TC} \times N_{diet}} \times 100. \quad (1)$$

For *I. grandicollis*, estimates of $M_{neonate}$ came from drying and weighing 4–7 eggs from each of 3–4 oviposition galleries on each of two logs, and estimates of M_{adult} and N_{adult} came from measurements of 3–10 adults from each of four logs and two multi-bolt emergence traps. For *D. frontalis*, estimates of $M_{neonate}$ came from 5–10 eggs from each of three oviposition galleries in naturally infested trees, and estimates of M_{adult} came from measurements of 446 adults that emerged from the bark samples collected for phloem nutrient analyses (lengths were converted to dry mass as M_{dry} = (0.76 × length) − 1.45; Clarke et al. 1979). Eq. 1 assumes that the percentage nitrogen in eggs is similar to that of adults; this assumption has little consequence because the mass of eggs is so small that they contain very little N regardless of the concentration.

Modeling insect nitrogen budgets

Eq. 1 can be rearranged to show that the minimum total consumption required for insect development (TC_{min}) is a function of adult mass (M_{adult}), neonate mass ($M_{neonate}$), adult nitrogen concentration (N_{adult}), nitrogen-use efficiency (NUE) and dietary N concentration (N_{diet}). Given a specified larval development time (T_{dev}), TC_{min} can be used to calculate the minimum relative consumption rate (RCR, in milligrams of biomass consumed per milligram of larva per day) as a function of minium total consumption (Eq. 2), which itself is a function of dietary nitrogen given NUE (Eq. 1). Eq. 2 follows Gordon (1968) where M_{exp} = mean exponential mass = $(M_{adult} - M_{neonate})/\ln(M_{adult}/M_{neonate})$. Alternatively, if RCR is specified, minimum development time ($T_{dev,\,min}$) can be calculated as a function of minimum total consumption (Eq. 3) or dietary nitrogen given NUE (Eq. 1).

$$\text{RCR}_{min} = \frac{\text{TC}_{min}}{M_{exp} \cdot T_{dev}} \quad (2)$$

$$T_{dev,min} = \frac{\text{TC}_{min}}{\left(\dfrac{\text{RCR}}{\ln\left(\dfrac{M_{adult}}{M_{neonate}}\right) \times M_{neonate}}\right) - \left(\dfrac{\text{RCR}}{\ln\left(\dfrac{M_{adult}}{M_{neonate}}\right) \times M_{adult}}\right)}. \quad (3)$$

Calculations used mean values of adult mass, neonate mass, and adult N concentration reported in this study. Larval development time for *D. frontalis* larvae (egg hatch to pupation) ranges from ~17 to 40 d at 25°C (Barras 1973, Gagne et al. 1982, Wagner et al. 1984) . Relative consumption rate and nitrogen-use efficiency have not been measured for *D. frontalis* larvae,

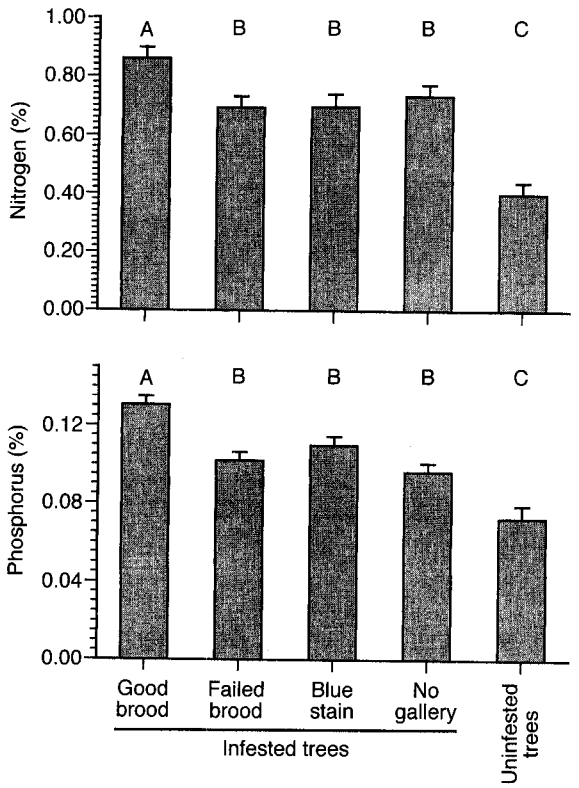

FIG. 2. Concentrations of nitrogen and phosphorus in four classes of phloem within infested trees and within the phloem of healthy uninfested trees. Data are means and 1 SE and are based on samples of five infested trees and five uninfested trees at each of five infestations within the Kisatchie National Forest in Lousiana, USA. Phloem classes (see *Methods: Phloem nitrogen and phosphorus*) with the same uppercase letter are not significantly different at $P < 0.05$ (Student-Newman-Keuls post-hoc multiple comparisons).

but, based on measured values for other insects (and theoretical limits of 0 to 1 for efficiencies), probably fall within the range of 1.5–3.5 $mg \cdot mg^{-1} \cdot d^{-1}$ for RCR (at 25°C) and 30–90% for NUE (Scriber and Slansky 1981, Slansky and Scriber 1985).

Nitrogen concentration of fungi associated with D. frontalis

Replicated pure cultures of *Entomocorticium, Ceratocystiopsis ranaculosus,* and *Ophiostoma minus* were grown in a malt-extract medium (15 g dehydrated malt extract/L distilled water), in a shaking water bath (150 rpm), for 14 d at 25°C. The resulting fungal tissue was isolated by filtration (0.2-μm pore size), lyophilized, and analyzed for nitrogen content.

RESULTS

Nitrogen and phosphorus concentrations of phloem

The N concentration of phloem around successfully developing *Dendroctonus frontalis* larvae was more than twice as high as that of the phloem in uninfested trees (0.86 ± 0.03% vs. 0.40 ± 0.01% [mean ± 1 SE], respectively, $P < 0.0001$; Fig. 2). The N concentration of phloem within infested trees that was associated with failed *D. frontalis* brood, bluestain, or no *D. frontalis* gallery was significantly higher than that of uninfested trees but significantly lower than that found in regions of good brood development (0.69–0.73%; Fig. 2, Phloem class in Table 1). Phosphorus concentrations in phloem averaged 6–7 fold less than N concentrations; the pattern across phloem classes matched that for nitrogen (Fig. 2, Table 1). Because *D. frontalis* attack virtually every pine tree along the advancing front of an outbreak (Thatcher et al. 1980), differences in N and P concentrations between infested and uninfested trees were almost certainly due to the introduction of microbes rather than to selection by the beetles for trees that were already high in phloem nutrients. Indeed, many of the trees included in our sample of uninfested phloem were subsequently infested by *D. frontalis.*

Within phloem classes, N concentrations varied considerably among trees within sites (Tables 1 and 2: Tree within infestation site). Among 18 infested trees, the N concentration in phloem with successful brood ranged from 0.52 to 1.08%. Variance among trees accounted for 76% of the total random variance in N

TABLE 1. Results from ANOVAs of phloem nitrogen and phosphorus concentration, for four phloem classes within loblolly pines infested with late-instar *Dendroctonus frontalis* (see Fig. 2).

Source of variation	df	Nitrogen (%)		Phosphorus (%)	
		MS × 10^4	F	MS × 10^6	F
Phloem class	3	2029	9.64**	7250	11.88***
Infestation site	4	627	0.82	11 092	20.46***
Phloem class × Infestation site	21	210	1.76	610	1.61
Tree (Infestation site)	13	763	16.44***	542	5.38***
Phloem class × Tree (Infestation site)	39	120	2.58***	379	3.76***
Error	63	46		101	

Notes: The F-test denominator for Phloem class was $MS_{\text{Phloem class} \times \text{Infestation site}}$; for Infestation site, it was $MS_{\text{Tree (Infestation site)}}$; and for Phloem class × Infestation site, it was $MS_{\text{Phloem class} \times \text{Tree (Infestation site)}}$. Others were tested over MS_{error}. (Table 2 shows corresponding analysis of uninfested trees.)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Results from ANOVAs of phloem nitrogen and phosphorus concentration in healthy uninfested loblolly pines adjacent to those infested with *Dendroctonus frontalis* (see Fig. 2).

Source of variation	df	Nitrogen (%)		Phosphorus (%)	
		MS × 10^4	F	MS × 10^6	F
Infestation site	4	83	1.61	2211	20.22***
Tree (Infestation site)	20	52	7.58***	109	2.51*
Error	25	7		66	

Note: The F-test denominator for Infestation was $MS_{Tree(Infestation)}$, and for Tree, it was MS_{error}.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

concentration in infested trees and 60% in uninfested trees. The variance in N concentration was dramatically higher among infested trees than uninfested trees ($\hat{\sigma}$ among trees = 0.196 vs. 0.047% N; $F_{13,20} = 17.36$, $P < 0.001$). The pattern of N concentrations among phloem classes within infested trees remained quite constant; although the phloem class × tree interaction was significant, it accounted for only 8% of the total random variance. There was no significant variation in phloem nitrogen attributable to infestation site (Tables 1 and 2).

In contrast to nitrogen, phloem concentrations of phosphorus varied markedly among infestation sites but relatively little (although still detectable) among trees within infestations (Tables 1 and 2). Variance among infestation sites accounted for 44% of the total random variance in P concentrations in infested trees and 74% in uninfested trees ($\hat{\sigma}$ among infestations = 0.0149 and 0.0203% phosphorus, respectively). Variance among trees within infestation sites accounted for 11% and 15% of the total random variance in P concentrations among infested and uninfested trees, respectively (Tables 1 and 2). As with N concentrations, the variance in P concentrations among infested trees was significantly greater than among uninfested trees ($\hat{\sigma}$ = 0.0153 vs. 0.0057% phosphorus; $F_{13,20} = 7.20$, $P < 0.001$). The pattern of P concentrations among phloem classes within infested trees remained quite constant across infestation sites and trees within infestations (phloem class × infestation site interaction was nonsignificant and phloem class × tree interaction, although statistically significant, accounted for only 16% of the total random variance). The variance among replicate phloem samples within trees (i.e., the error term in Tables 1 and 2) was also low, accounting for only 9–19% of the total random variance in N and P concentrations in infested and uninfested trees.

Concentrations of N and P in phloem seemed to vary independently of each other across uninfested trees, but were positively correlated across infested trees (Fig. 3). Among infested trees, there was a significant positive correlation between phloem nitrogen and phosphorus for all phloem classes except those without any *D. frontalis* gallery (r = 0.58, 0.58, and 0.62 for good brood, poor brood, and bluestain, respectively; r = 0.25 for no gallery).

Beetle size and phloem nutrition

Beetle size varied among trees within infestations ($F_{18,400} = 6.77$, $P < 0.0001$), and infested trees with higher N concentrations tended to produce larger beetles of both sexes (Fig. 4). Given the relationship between female adult size and fecundity (Clarke et al. 1979), the range in beetle size among trees translates into an 18% difference in fecundity (152 vs. 180 eggs/female at 0.5% vs. 1.1% N, respectively). Beetle size did not vary between sexes ($F_{1,4} = 5.39$, $P = 0.081$) or among infestation sites ($F_{4,18} = 2.49$, $P = 0.080$). Phosphorus concentration was weakly related to male size ($r^2 = 0.25$, $P = 0.035$) and unrelated to female size ($r^2 = 0.15$, $P = 0.12$). Multiple regressions that included both N and P did not provide a better fit than models that included only N.

N and P concentrations of bark beetles

The nitrogen content of *Dendroctonus frontalis* adults averaged 11.53 ± 0.13% (mean ± 1 SE; n = 45 individuals), which is 13.4-fold higher than the concentration of phloem in regions of successful brood. The phosphorus content of *D. frontalis* adults averaged 0.749 ± 0.058% (n = 17 individuals), which is ~5.8-

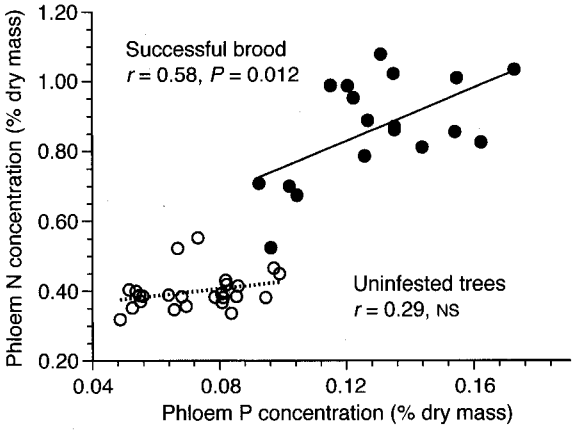

FIG. 3. Correlation between concentrations of nitrogen and phosphorus in the phloem of loblolly pine. Solid circles represent phloem samples from within infested trees that were associated with successful development of southern pine beetle brood; open circles represent healthy uninfested trees. Each symbol represents one tree.

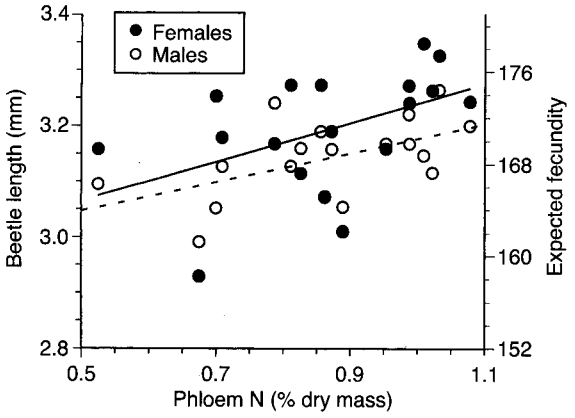

FIG. 4. Relationships between phloem nitrogen and the average size of emerging beetles in 18 naturally infested loblolly pines. For females, length = 2.89 + 0.35 × N (P = 0.048, r^2 = 0.22); for males, length = 2.92 + 0.26 × N (P = 0.020, r^2 = 0.29). The right-hand axis shows the expected fecundity (number of eggs per female) for southern pine beetle adults as a function of size (eggs = 140 × length − 277; after Clarke et al. [1979]).

fold higher than the concentration of phloem in regions of successful brood. Males and females did not differ in their concentrations of N or P ($P > 0.19$). *D. frontalis* larvae had significantly lower concentrations of N than adults (8.13 ± 0.35% N [mean ± 1 SE], $t_{59} = 3.40$, $P < 0.0001$), but similar concentrations of P (0.672 ± 0.026% P, $t_{19} = 0.62$, $P = 0.54$). The increase in N concentration from larvae to adults was due to decreases in total dry biomass (with no change in total N content) during developmental processes following adult eclosion (Fig. 5). Pupae, callow adults, and mature adults (2–4 d after eclosion) all contained an average of ~105 μg of N, but the total biomass decreased from an average of 1.3 mg in pupae and callow adults to 0.96 mg two days after eclosion (N concentration: 7.8 ± 0.2% N [mean ± 1 SE] for pupae, 8.2 ± 0.3% N for callow adults, 11.7 ± 0.4% N for adult at 2 d, and 13.4 ± 0.3% N for adults at 4 d). This developmental change (also reported by Barras and Hodges [1974]) does not affect the nitrogen-budget model so long as M_{adult} and N_{adult} in Eq. 1 were measured on the same developmental stage (the numerator of Eq. 1 equals ~105 μg N in any case). Our N budgets were based on 2-d-old adults, which were estimated to be 11.49 ± 0.13% N in one sample (biomass: 0.93 ± 0.05 mg) and 11.67 ± 0.46% N in another sample (biomass: 0.95 ± 0.07 mg). These measurements indicate that the outer bark through which prepupae and adults bore before emergence is nutritionally inert. The N concentration of 2-d-old adult *Ips. grandicollis* was 8.61 ± 0.27% N, significantly less than that of *D. frontalis* (t_{72} = 10.81, $P < 0.0001$ for comparison between species).

Nitrogen concentration of fungi

Nitrogen concentrations of fungi were 8.7–11.6 fold higher than that of uncolonized phloem and the N concentration of *Entomocorticium* was significantly higher than that of the other two species: 4.62 ± 0.07% N, 3.86 ± 0.23% N, and 3.55 ± 0.07% N (mean ± 1 SE) for *Entomocorticium, Ceratocystiopsis ranaculosus,* and *Ophiostoma minus,* respectively ($F_{2,9}$ = 21.95, P = 0.0006).

N and P budgets

Although *D. frontalis* adults were larger than *I. grandicollis* adults, *D. frontalis* larvae appeared to acquire their mass by consuming only 21% as much phloem as *I. grandicollis* (5.7 vs. 27.4 mg; Table 3). Analysis of nitrogen budgets indicated that *I. grandicollis* could meet their physiological demands with a realistic nitrogen-use efficiency of 46% (Table 3). The requisite consumption rate for *I. grandicollis* to complete development in 25–35 d is 3.5–4.0 mg biomass·(mg larva)$^{-1}$·d^{-1} (Fig. 6). This consumption rate is in the upper range of those reported for insects (Scriber and Slansky 1981, Slansky and Scriber 1985) and this development time is somewhat longer than the 20–30 d that appeared to be required for our colony, but in general it seems easy to reconcile the N budgets of *I. grandicollis* with an empirically and physiologically reasonable parameterization of Eqs. 1–3.

In contrast, the nitrogen budgets of *D. frontalis* could not be immediately reconciled (note impossible nitrogen-use efficiency in Table 3; see also analyses of Mishra et al. [1985] for a cerambycid beetle). *D. frontalis* larvae appeared to accumulate over twice as much nitrogen as was present in the phloem that they consumed from their feeding chamber (NUE = 216%, Table 3). Because a nitrogen-use efficiency >100% is impossible, one or more of the estimates used in our initial parameterization of Eq. 1 must be incorrect. If NUE is 80%, *D. frontalis* N budgets could be explained if the actual dietary nitrogen content is 1.5–2.0% (vs. estimate of 0.86%) and the actual total consumption is 8.8–6.6 mg (vs. estimate of 5.7 mg). This scenario is

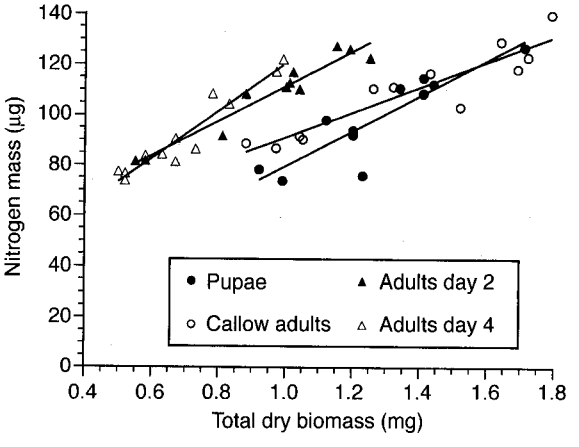

FIG. 5. Nitrogen content of *Dendroctonus frontalis* at different developmental stages following the cessation of larval feeding. Total dry mass decreased following adult eclosion, but total N content remained quite constant at 80–120 μg.

TABLE 3. Estimated consumption and nitrogen-use efficiency (and values used in the estimates) for larvae of *Dendroctonus frontalis* and *Ips grandicollis*.

Variable	*D. frontalis*			*I. grandicollis*		
	Mean	1 SE	*n*	Mean	1 SE	*n*
Egg mass (mg)	0.015	0.001	10	0.018	0.001	37
Adult mass (mg)	0.93	0.05	446	0.78	0.09	35
Adult N content (%)	11.56	0.13	45	8.61	0.27	29
Adult N content (μg)	107			67		
Area of feeding gallery (mm^2)†	19.1	1.1	10	92.8	13.5	4
Specific mass of phloem (mg/cm^2)†	29.8			29.8	3.9	4
Mass of consumed phloem (mg)†	5.7			27.4	5.0	4
Relative consumption (mg/mg)‡	6.2			35.9		
Nitrogen in consumed phloem (%)†	0.86	0.03	25	0.52	0.03	26
Nitrogen in consumed phloem (μg)	49			142		
Nitrogen-use efficiency, NUE (%)§	216			46		

† *n* = number of trees (based on 5–10 replicate measurements per tree).
‡ Consumed phloem mass/(adult mass − egg mass).
§ Calculated from Eq. 1. Note that the estimated NUE for *I. grandicollis* is reasonable, but that of *D. frontalis* is impossibly high.

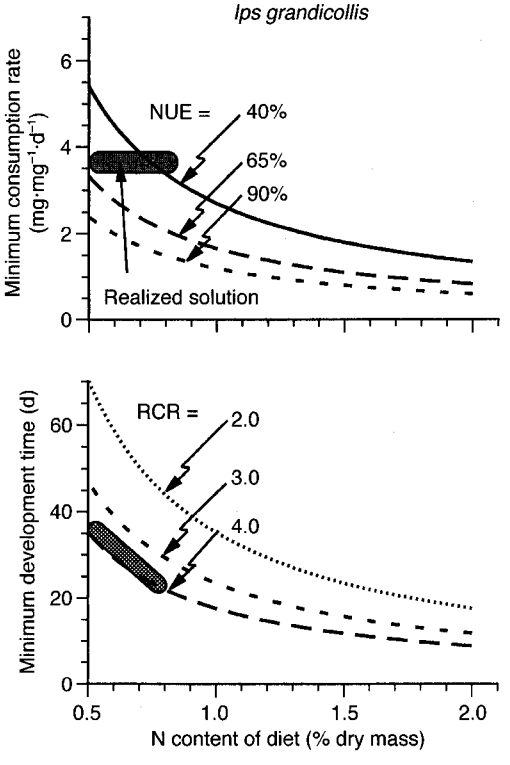

FIG. 6. Theoretical combinations of nitrogen-use efficiency (NUE), relative consumption rate (RCR), and development time that could allow successful development of *Ips grandicollis* larvae on diets with a range of nitrogen concentrations. Minimum consumption rates (top panel) were calculated from Eq. 2 assuming a larval development time of 30 d. Minimum development times (bottom panel) were calculated from Eq. 3 assuming NUE of 40%. Shaded ellipses indicate the realized parameter space for successful *I. grandicollis* larvae.

consistent with the hypothesis that *D. frontalis* feed extensively on fungal hyphae that grow within the feeding chamber and that import biomass and N into the feeding chamber. Any realistic budgets also require higher NUE for *D. frontalis* than the 46% estimated for *I. grandicollis*. The N budgets for *D. frontalis* cannot be reconciled by any realistic adjustments of phloem specific mass. Even if phloem mass was 45 mg/cm^2, which is 50% higher than our best estimate, and 15% higher than the maximum from a survey of 11 stands, the calculated nitrogen-use efficiency is still 143%. We could not identify any plausible N budgets for *D. frontalis* that do not require higher dietary nitrogen content and higher biomass consumption than the estimates in Table 3.

What are the consequences of variation in dietary nitrogen for bark beetle larvae? If consumption rate and NUE are held to some constant upper limit (as expected if larvae are routinely consuming and assimilating at their physiological limits; Ayres and MacLean 1987), then larvae would be forced to compensate for reduced dietary N with increased larval development time (Figs. 6 and 7, lower panels). For example, if the dietary nitrogen of *I. grandicollis* is 0.8% N in one tree vs. 0.5% N in another tree, then the minimum development time increases from 23.8 to 38.1 d (with NUE = 46% and RCR = 3.7 $mg \cdot mg^{-1} \cdot d^{-1}$; Fig. 6, lower panel). If the dietary nitrogen of *D. frontalis* larvae decreases from 1.5% to 0.5% (e.g., due to the absence of mycangial fungi) the minimum development time increases from 19.9 to 59.6 d (with NUE = 80% and RCR = 2.0 $mg \cdot mg^{-1} \cdot d^{-1}$; Fig. 7, lower panel).

Similar calculations with phosphorus indicate that larvae should easily meet their P requirements with any combination of consumption rate and development time that allows them to meet their N requirements (because the factor by which insects must concentrate N is 2.3 times greater than the factor by which they must concentrate P). Phosphorus requirements of *D.*

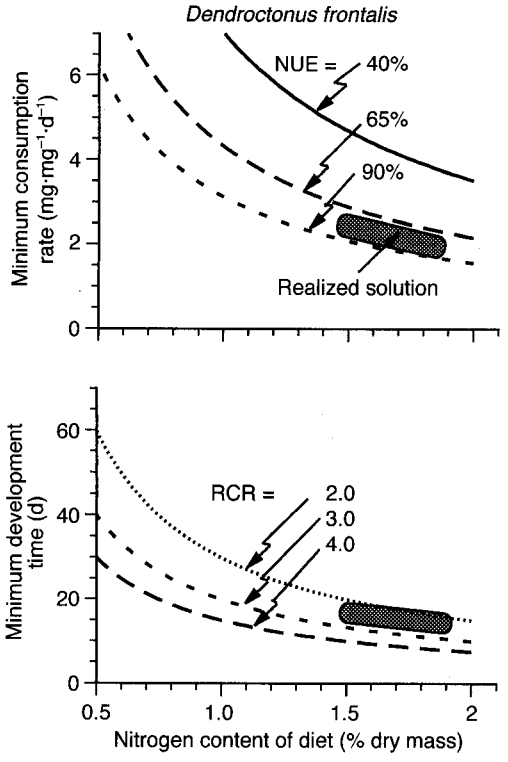

FIG. 7. Theoretical combinations of nitrogen-use efficiency (NUE), relative consumption rate (RCR), and development time that could allow successful development of *Dendroctonus frontalis* larvae on diets with a range of N concentrations. Minimum consumption rates (upper panel) were calculated from Eq. 2 assuming a larval development time of 17 d. Minimum development times (bottom panel) were calculated from Eq. 3 assuming NUE of 80%. Shaded ellipses indicate the realized parameter space for successful *D. frontalis* larvae.

frontalis could theoretically be met even from the 5.7 mg of phloem that is consumed out of their feeding chamber (phosphorus-use efficiency calculated from Eq. 1 = 92%). If *I. grandicollis* have the same P concentration as *D. frontalis,* their P budgets could be met with a phosphorus-use efficiency of only 21%.

DISCUSSION

H_1: Dendroctonus frontalis *experience variation in phloem nutrient concentrations across many scales*

D. frontalis experience significant variation in the nitrogen and phosphorus content of loblolly pine phloem (Fig. 2, Tables 1 and 2). However, the spatial scale of variance in these two dietary elements differed. For N, variance among trees within sites dominated the random sources of variation (a scale of meters), while for P, variance among infestation sites dominated the random variation (a scale of kilometers). There was great additional variation in both N and P content across phloem classes within infested trees (scale of centimeters). Our data provide strong correlative evidence for the importance of dietary nitrogen to *D. frontalis* larvae. Regions of phloem where larvae survived to pupate were those with the highest N content (Fig. 2) and trees that produced the largest adults were those with the highest N content (Fig. 4). For both bark beetle species, analysis of N budgets provides additional evidence that dietary N is likely to be limiting for larval development.

H_2: *Mycangial fungi enhance the diet of* D. frontalis *by increasing the concentrations of N and P*

Elevation of dietary nitrogen provides a mechanism to explain the beneficial effects of mycangial fungi for *D. frontalis.* Phloem with the highest N content (Fig. 2) was in regions of infested trees with small oval larval feeding chambers that are characteristically associated with thriving colonies of mycangial fungi (Barras and Perry 1972). We propose that the hyphae of mycangial fungi extract N from the phloem surrounding larval feeding chambers and concentrate it within the hyphae and conidia that grow into the feeding chamber (Barras and Perry 1972). When grown in culture, N concentrations in the hyphae of *Entomocorticium* are 4.6%, which is higher than the other fungal associates of *D. frontalis,* and very high relative to the 2.8% N that has been measured in soil-dwelling fungal species (also grown in culture, Laursen 1975). High N content may be an adaptation of *Entomocorticium* that is favored because of its benefits for *D. frontalis,* on which it depends. The high N content of *Entomocorticium* compared to the other mycangial associate, *Ceratocystiopsis ranaculosus,* may explain the higher rates of population growth, and higher lipid content, of *D. frontalis* populations with a high abundance of *Entomocorticium* relative to *C. ranaculosus* (Bridges 1983, Goldhammer et al. 1990, Coppedge et al. 1995). Also, transport of amino acids and proteins from the phloem to the feeding chamber could be facilitated by the multinucleate cellular morphology of *Entomocorticium* (Happ et al. 1975). This hypothesis to explain the elevation in phloem N predicts a depletion of N in the phloem tissue outside feeding chambers, especially when the mycangial fungi within the chamber is *Entomocorticium* rather than *C. ranaculosus.* It further predicts that the midguts of *D. frontalis* larvae contain significant amounts of fungal tissue; specifically, 2–3 mg of fungal tissue (at 4–3% N, respectively) added to 5.7 mg of phloem tissue at 0.86% N can reconcile the nitrogen budgets of *D. frontalis* larvae (see Fig. 7: ellipse of realized solutions). Additional tests of this hypothesis would benefit from protocols that isolate and identify the fungi occupying microsites within and around individual feeding galleries.

Alternative mechanisms to explain the elevation of N in colonized phloem include: (1) a relative increase due to depletion of carbon by microbial respiration, (2) a relative increase due to carbohydrate export from the phloem to support secondary metabolism in resin ducts during beetle attack, and (3) an increase due to activity

of N-fixing bacteria. Alternative 1 must be true to some extent (Martin 1979) because there is considerable CO_2 evolution from microbially infested phloem (M. P. Ayres, *unpublished data*), but neither it, nor alternative 2, can easily explain the differences in N content between phloem infested with mycangial fungi and *Ophiostoma minus*. Alternative 3 has been suggested (Peklo and Satava 1949, Peklo 1968), but discredited because of the very low abundance and metabolic activity of nitrogen-fixing bacteria associated with *D. frontalis* (Bridges 1981). Assuming that actual dietary N is 1.5–2.0%, a nitrogen budget model (Eq. 3) with nitrogen-use efficiency (NUE) of 80% and relative consumption rate (RCR) of 2.0 mg biomass·(mg larvae)$^{-1}$·d^{-1} accurately predicts that the minimum development time for *D. frontalis* larvae in the presence of mycangial fungi would be ~18 d vs. >40 d in the absence of mycangial fungi (Fig. 7; Barras 1973, Wagner et al. 1984).

H_3: Ophiostoma minus *negatively impacts the larval development of* D. frontalis

At the scale of our sampling (Fig. 1), phloem nitrogen concentrations were significantly lower in patches colonized by *O. minus* than in patches presumably dominated by mycangial fungi (Fig. 2). This difference in N content (0.86% vs. 0.70% N, respectively) would be enough to increase minimum development time of larvae from 34.7 d to 42.6 d (with RCR of 2.0 mg·mg^{-1}·d^{-1} and NUE of 80%; Fig. 7). There are no apparent concentrations of *O. minus* tissue analogous to the feeding chambers of *D. frontalis*. Instead, *O. minus* appears to be very evenly dispersed within patches of ~1 dm^2 or more. *D. frontalis* larvae feeding in phloem infested with *O. minus* create meandering tunnels (and almost always die) rather than the small feeding chamber typical of successful larvae (Fig. 1). Thus the larvae in phloem infested with *O. minus* are feeding at a similar scale to our sampling and the dietary nitrogen of these larvae is probably close to the 0.70% that we measured. If, as we hypothesize, the dietary N in bluestain vs. mycangial feeding sites is 0.70 vs. 1.5–2.0%, then the nutritional benefit of the mycangial fungi vs. *O. minus* is to more than halve larval development time from 42.6 d to ~20 d (with RCR of 2.0 mg·mg^{-1}·d^{-1} and NUE of 80%; Fig. 7). We cannot reject the hypothesis that *O. minus* further impedes *D. frontalis* reproduction by allelopathic effects of isocoumarins (Hemingway et al. 1977) or other secondary metabolites. However, Yearian et al. (1972) found no negative effects of *O. minus* on larvae of *Ips avulsus, I. calligraphus* or *I. grandicollis*. In any case, *O. minus* appears to be an antagonist of *D. frontalis*, and the antagonism is strengthened by strong competitive inhibition of mycangial fungi by *O. minus* (Klepzig and Wilkens 1997). Consequently, any factors that favor colonization of phloem by *O. minus* will have a negative impact on the potential reproduction of *D. frontalis*. Factors that might affect *O. minus* abundance and growth include phloem chemistry, temperature, relative humidity, and the abundance of *Tarsonemus* mites that are phoretic on *D. frontalis* adults and act as vectors of *O. minus* spores (Bridges and Moser 1986, Bridges 1987, Cook and Hain 1987, Lieutier and Yart 1989, Paine et al. 1997).

H_4: Ips grandicollis, *which lack mycangial fungi, must consume more phloem to attain the same mass as* D. frontalis

D. frontalis and *I. grandicollis* employ different strategies to meet their nitrogen budgets. *I. grandicollis* consumes large quantities of low-N phloem, while *D. frontalis* modifies the diet by introducing mutualistic fungi and consumes much less of a relatively high-N diet. We hypothesize that these two species represent alternative nutritional strategies of bark beetles. The Scolytidae includes ~6000 species worldwide (1430 species in the Nearctic). Based on the morphological diversity of specialized anatomical structures that house and nurture fungal associates, close mutualistic relationships between scolytids and fungi have evolved at least 6 times (Wood 1982). Genetic studies of the fungal associates also indicate multiple evolutionary origins (Cassar and Blackwell 1996). It seems unlikely that nutritional strategies intermediate to that of *D. frontalis* and *I. grandicollis* would be viable because the high consumption rates necessary without mycangial fungi are incompatible with the small feeding chambers needed to maintain physical associations with slow-growing mycangial fungi (Klepzig and Wilkens 1997). In this case, evolutionary transitions between strategies should be relatively rapid. Estimates of relative consumption provide one easily obtained measure for comparing the nutritional strategies of species; *I. grandicollis* larvae consume ~35.9 mg/mg compared to ~6.2 mg/mg for *D. frontalis* (Table 3). We predict that similar measurements of additional species will reveal a bimodal frequency distribution with the two peaks corresponding to alternative strategies. Sterilization studies allow a more direct, but technically challenging, means to test for effects of microbial associates on bark beetle development and reproduction (Barras 1973, Fox et al. 1992, Colineau and Lieutier 1994, Six and Paine 1998).

Population and community consequences of alternative nutritional strategies

The suite of traits that are correlated with mycangial vs. non-mycangial strategies may have general consequences for population dynamics and community interactions. For example, we predict that nonmycangial species (e.g., *I. grandicollis*) will generally be more sensitive than mycangial species (e.g., *D. frontalis*) to variation among trees in phloem nitrogen (because a unit change in dietary N has the greatest impact on larval nutrition at low dietary N; Figs. 6 and 7). Thus,

one effect of mycangial associations may be a damping of ecological effects for the consumer of variation in host plants (Auerbach and Strong 1981).

While populations of mycangial beetle species might be less sensitive than their non-mycangial counterparts to exogenous forces, they may be more influenced by complex endogenous dynamics because they exist within a community matrix that involves numerous strong interactions and therefore a greater potential for delayed density dependence (Berryman 1979, Turchin 1991, Hanski and Henttonen 1996). In fact, *D. frontalis* populations tend to cycle with a period of ~8 years and an amplitude of ~50×, but the cause of the cyclicity is unknown (Turchin et al. 1991, Reeve et al. 1995). We hypothesize that these cycles are produced by delayed density dependence resulting from the positive effect of growing beetle populations on the transmission of *O. minus,* which has an inverse effect on beetle population growth because *O. minus* outcompetes the mycangial fungi on which beetles depend to meet their nitrogen budgets. Similar community interactions may be common in mycangial species. At least it seems that a disproportionate number of the most economically important bark beetle species are mycangial: e.g., *D. ponderosae, D. jeffreyi, D. brevicomis, D. adjunctatus, Ips acuminatus, I. sexdentatus,* and *Scolytis ventralis* (Francke-Grosmann 1963, Barras and Perry 1971, Livingston and Berryman 1972, Paine and Birch 1983, Berryman 1986, Liebhold et al. 1986, Berryman and Ferrell 1988, Raffa 1988, Lévieux et al. 1991, Six and Paine 1996). In contrast, non-mycangial beetles exist within a weaker matrix of community interactions and should be less likely to experience endogenous feedback from the rest of the community (Hochberg and Holt 1990, Wilson et al. 1996).

Mycangial species should generally have higher resource-use efficiency (which translates into lower relative consumption, Table 3). Bark beetles frequently experience strong intra- and inter-specific competition (Raffa and Berryman 1983, Miller 1984, Flamm et al. 1987, Rankin and Borden 1991, Zhang et al. 1992, Schlyter and Anderbrandt 1993, Gara et al. 1995, Reeve et al. 1998). When phloem resources are limiting, and other factors are equal, mycangial species should be able to sustain larger populations than non-mycangial species and be favored in interspecific competition (Tilman 1982). Also because of their higher resource-use efficiency, populations of mycangial species may be less likely to experience intense intraspecific competition (larger numbers of beetles could successfully complete development in the same tree bole), and therefore be less likely to experience instantaneous density dependence and stable population dynamics. Studies that characterize the N acquisition strategies of additional bark beetle species will allow tests for the population and community consequences of alternative nutritional strategies.

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