# Interactions between the fungal symbiont of *Sirex noctilio* (Hymenoptera: Siricidae) and two bark beetle-vectored fungi

**Kathleen Ryan, Jean-Marc Moncalvo, Peter de Groot, Sandy M. Smith**

**Abstract**—The woodwasp *Sirex noctilio* F. is invading North American forests, where it will interact with a large guild of pine-inhabiting beetles and their associated fungi. The woodwasp's obligate fungal symbiont, *Amylostereum areolatum* (Fries) Boidin (Stereaceae), plays an essential role in the wasp's larval development but is expected to be a poor competitor in the presence of fungi vectored by co-occurring insects. We examined the outcomes of competitive interactions between *A. areolatum* and two fungal species vectored by bark beetles, *Leptographium wingfieldii* Morelet (Ophiostomataceae) and *Ophiostoma minus* (Hedgcock) H. and P. Sydow (Ophiostomataceae), and the effect of temperature and substrate on these interactions. Beetle-associated fungi were usually able to capture more uncolonized resource than *A. areolatum* regardless of substrate or temperature. *Amylostereum areolatum* was able to colonize relatively more space in some cases but could not gain substrate already colonized by the ophiostomatoid competitor. These findings suggest that competitive interactions between beetle-vectored fungal species and *A. areolatum* could influence the reproductive fitness and distribution of *S. noctilio* within individual trees and also across a wide geographic area.

**Résumé**—Le sirex européen du pin, *Sirex noctilio* F., est en train d'envahir les forêts d'Amérique du Nord où il va entrer en contact avec une importante guilde de coléoptères vivant sur les pins et avec leurs champignons associés. Le champignon symbiotique obligé du sirex, *Amylostereum areolatum* (Fries) Boidin (Stereaceae), joue un rôle essentiel dans le développement larvaire de la guêpe, mais risque d'être un mauvais compétiteur en présence des champignons portés par les insectes en cohabitation. Nous examinons les résultats d'interactions de compétition entre *A. areolatum* et deux champignons transmis par les scolytes de l'écorce, *Leptographium wingfieldii* Morelet (Ophiostomataceae) et *Ophiostoma minus* (Hedgcock) H. et P. Sydow (Ophiostomataceae), ainsi que les effets de la température et du substrat sur ces interactions. Les champignons associés aux scolytes sont généralement capables d'envahir une plus grande partie de la ressource non colonisée qu'*A. areolatum*, quels que soient la température et le substrat. Dans certains cas, *A. areolatum* peut occuper plus d'espace, mais il ne réussit pas à conquérir les substrats déjà colonisés par le compétiteur ophiostomatoïdé. Ces résultats indiquent que les interactions de compétition entre les espèces de champignons véhiculées par les scolytes et *A. areolatum* pourraient affecter la fitness reproductive et la répartition de *S. noctilio*, aussi bien au niveau des arbres individuels que sur de grandes aires géographiques.

[Traduit par la Rédaction]



## Introduction

A Eurasian woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae), was first discovered in North America in 2005 (Hoebeke *et al.* 2005; de Groot *et al.* 2006). This woodwasp has an important symbiotic relationship with a basidiomycete fungus, *Amylostereum areolatum* (Fries) Boidin (Stereaceae), (Talbot 1977), which may influence whether or not it becomes a significant

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pest in North America. Although it is a secondary pest in its native Eurasian range, where it has been introduced in the Southern Hemisphere, this woodwasp is of considerable economic concern (*e.g.*, Bedding 1993).

*Sirex noctilio* favours suppressed, physiologically stressed, or damaged pine trees, *Pinus* L. (Pinaceae) (Spradbery and Kirk 1978; Neumann and Minko 1981). During oviposition, a female woodwasp inoculates a tree with the fungus and a phytotoxic mucus that together impair the physiological and defensive responses of the tree and eventually cause its death (Coutts and Dolezal 1969; Fong and Crowden 1973). The fungal symbiont is also integral to the woodwasp's development. Egg eclosion is delayed when conditions in the tree impede fungal growth (Madden 1981), and the fungus provides an essential nutritional resource for at least the early larval instars (Madden and Coutts 1979). Larvae may starve if *A. areolatum* growth is inhibited (Coutts 1965; Coutts and Dolezal 1965; King 1966), but when conditions for fungal growth are optimal, larger adults are produced and their reproductive potential is greater (Madden 1981). Because the reproduction and development of *S. noctilio* are highly dependant upon its fungal associate, inhibition of the symbiont's growth could have considerable consequences for the population dynamics of this woodwasp.

In North America, potentially significant interactions may occur between *S. noctilio* and a large guild of bark and wood-boring beetles and their fungal associates. In Canada, the known distribution of *S. noctilio* in 2010 included all of southern Ontario and a few scattered locations in Quebec close to the border between Ontario and the United States of America (Canadian Food Inspection Agency 2010). There, it is reproductively active from early July to mid-September (Long *et al.* 2009) and may deposit eggs throughout the tree stem (Morgan and Stewart 1966; Neumann *et al.* 1982). An introduced bark beetle, *Tomicus piniperda* (L.) (Coleoptera: Curculionidae), is a common insect that uses similar host trees and may be associated with *S. noctilio* in this range in Canada (Morgan *et al.* 2004). *Tomicus piniperda* is reproductively active primarily in the early spring, although a second brood can be produced during the summer (Ryall and Smith 2000). *Leptographium wingfieldii* Morelet and *Ophiostoma minus* (Hedgcock) H. and P. Sydow (Ophiostomataceae) are common "blue-stain" fungal associates of *T. piniperda* in its native and Canadian ranges (Jacobs *et al.* 2004; Kirisits 2004; Hausner *et al.* 2005; Ben Jamaa *et al.* 2007). These fungal species are relatively virulent pathogens that can grow quickly in pine wood (Uzunović and Webber 1998; Solheim *et al.* 2001). They do not have an exclusive association with *T. piniperda* and are vectored by a number of other beetle species (*e.g.*, Jacobs *et al.* 2004; Kirisits 2004) that may also co-occur with *S. noctilio* in trees.

Based on field observations, it has been postulated that *A. areolatum* may be a weak competitor against blue-stain fungi and that this will subsequently impede the development and survival of *S. noctilio* larvae (Hanson 1939; Morgan and Stewart 1966; Titze and Stahl 1970). Most of these suggestions, however, are anecdotal although King (1966) noted that some common nonophiostomatoid fungi are strongly antagonistic to *A. areolatum*. The success of a fungus in colonizing a substrate depends on its competitive ability to capture the substrate either primarily, when the fungus rapidly exploits and gains control over the uncolonized resource, or secondarily, when one fungal species is able to gain access to or colonize an area already occupied by another (Rayner and Webber 1984).

The purpose of our study was to assess the outcome of *in vitro* competition between the woodwasp fungal symbiont *A. areolatum* and selected bark beetle-vectored ophiostomatoid fungi. Our objectives were to evaluate (*i*) the outcomes of primary capture of uncolonized substrate between *A. areolatum* and *L. wingfieldii* and *O. minus*; (*ii*) how temperature modifies the outcomes of these interactions; (*iii*) the ability of *A. areolatum* to establish in already occupied substrates (secondary resource capture); and (*iv*) the effect of substrate on the outcomes of competition experiments.

## Materials and methods

### Fungal isolates

Both strains of *A. areolatum* known to occur in Canada were used in these experiments.

**Table 1.** Provenance information for the strains of *Amylostereum areolatum*, *Leptographium wingfieldii*, and *Ophiostoma minus* used in all experiments.

| | Code | Collection No.* | Host | Location | Collector |
|---|---|---|---|---|---|
| *A. areolatum* | Aareol-1 | SSM 075 7011 | *Pinus sylvestris* | Sauble Beach, Ont. | C. Davis |
| | Aareol-2 | SSM 075 7013 | *Pinus sylvestris* | Eden Mills, Ont. | C. Davis |
| *L. wingfieldii* | Lwin-1 | SSM 025 7010 | *Pinus sylvestris* | Listowel, Ont. | C. Davis |
| | Lwin-2 | SSM 025 7011 | *Pinus sylvestris* | Barrie, Ont. | C. Davis |
| | Lwin-3 | SSM 025 7012 | *Tomicus piniperda* | Listowel, Ont. | C. Davis |
| *O. minus* | Omin-1 | SSM 075 7007 | *Pinus sylvestris* | Bracebridge, Ont. | C. Davis |
| | Omin-2 | WIN(M) 861 | *Pinus sylvestris* | Toronto, Ont. | L. Baumel |
| | Omin-3 | WIN(M) 1275 | *Pinus sylvestris* | Barrie, Ont. | C. Davis |

*SSM, Great Lakes Forestry Centre Culture Collection; WIN(M), University of Manitoba Culture Collection.



Three strains each of the *T. piniperda* fungal associates *L. wingfieldii* and *O. minus* were used as potential competitors. Multiple strains were used because they may have different growth rates (*e.g.*, Lieutier *et al.* 2004), which will modify the results of competition. All strains were obtained from existing culture collections (Table 1), inoculated onto sterile *Pinus sylvestris* L. woodchips, and then re-isolated to mitigate the effects of storage in culture. The strains were then grown on potato dextrose agar (PDA) for use in the following experiments.

### Interactions between *A. areolatum* and ophiostomatoid species on artificial media

In paired comparisons, each strain of *A. areolatum* was tested against each strain of the ophiostomatoid species. For each of the pairs, a 5 mm diameter PDA plug colonized by each fungus was inoculated onto 2.4% PDA on opposite sides of a 9 cm diameter Petri plate. The *A. areolatum* plug was inoculated 4 days prior to the contender to address the growth lag of this species (*A. areolatum* growth did not begin until 4 days after inoculation).

Five replicates of each pair combination were inoculated, and each strain was also inoculated on its own as a control. All plates were sealed with Parafilm and stored in the dark at 25 °C (±1 °C) in a growth chamber. The outer extent of each of the resultant fungal colonies was traced on the bottom of the plate every second day for 2 weeks, starting from day 2 after ophiostomatoid species inoculation. Fungal hyphae of each species are morphologically distinct, so, colony boundaries could be determined visually (Klepzig 1998) and confirmed under a dissecting scope. The sequential and final boundaries of the colony for each species were traced onto paper and then scanned and measured with Scion Image software (Scion Corporation, Frederick, Maryland).

### Interactions on wood substrate and effect of temperature

The interaction experiment was repeated on autoclaved (121 °C × 45 min submerged in distilled water) *P. sylvestris* wood chips (approximate dimensions 3 cm × 4 cm × 2 mm) over a range of temperatures. This was done because substrate type and temperature can affect fungal growth rate, and can do so differently for each species (*e.g.*, Uzunović and Webber 1998; Rice *et al.* 2007). In addition, the results of outcomes have rarely been compared between substrates.

Ten replicates of each of treatment combination and corresponding control chips were inoculated in a similar manner to the previous experiment: fungal strains were inoculated at opposite edges of the upper flat surface of each wood chip. Wood chips were suspended on glass stir rods bent into triangles, which were set in Petri plates on filter paper moistened with sterile water. Plates were stored in the dark in growth chambers at 10, 15, 20, 25, and 30 °C (±1 °C).

In a preliminary trial, the colony boundaries of each of the solo-growing strains seen under magnification were tested by taking wood samples from immediately outside of the outer colony limit but no fungi grew from these test

isolations, thus confirming the boundary. Hyphae of each contending species were distinctive in appearance, so colony boundaries were determined visually for this experiment. At 7 days, half of the replicates of each pair of strains were measured, colony boundary edges were marked, and each chip was photographed. The surface area colonized by each strain was measured from the photograph with Scion Image software. At 14 days, fungal colony boundaries on each of the remaining chips were similarly marked and measured. The set of interactions tests conducted at 25 °C were compared with those conducted on PDA to determine substrate-related differences in interaction outcomes.

### Ability to grow on precolonized resource

This experiment used the same species–strain combinations as in the previous experiments. Surface-sterilized *P. sylvestris* wood chips were inoculated with each of the strains and stored, as previously described, until approximately 75% of each woodchip was colonized. Half of the chips of each strain were then autoclaved. Competing strains were inoculated on top of colonized areas of autoclaved and live fungal colonies. There were five replicates of each pair of strains on each of the two substrate treatments (autoclaved and nonautoclaved) and five control replicates of a single strain growing on uncolonized wood chips. At 2 weeks postinoculation, the growth, or lack of growth, of the added second strain was recorded.

### Data analysis

To test for evidence of *A. areolatum* growth inhibition in the presence of contender fungi, the sequential area of growth on PDA of each *A. areolatum* strain in the presence of each ophiostomatoid species (three strains and control) was analyzed by fixed-factor repeated-measures ANOVA. Colony area of each strain at 25 °C at 7 days on wood chips was compared using ANOVA. To test differences in primary resource capture between strains, the colony area of each contending species at 14 days was compared using pooled variance *t* tests. The effect of temperature, ophiostomatoid species, and the interaction of the two on resource capture at 14 days was analyzed by ANOVA for each *A. areolatum* strain. Data were tested for normality and variance by inspecting graphs of the residuals from regression. The surface area of the woodchip colonized by each fungus was $\log_n$-transformed, met test assumptions after transformation, and analyzed in this form. For all analyses, the $\alpha$ value was set at 0.05. Analyses were conducted with SYSTAT (Systat Software Inc. 2007) (ANOVA) or calculated in Microsoft Excel (2003) (*t* test).

## Results

### Interactions on artificial media

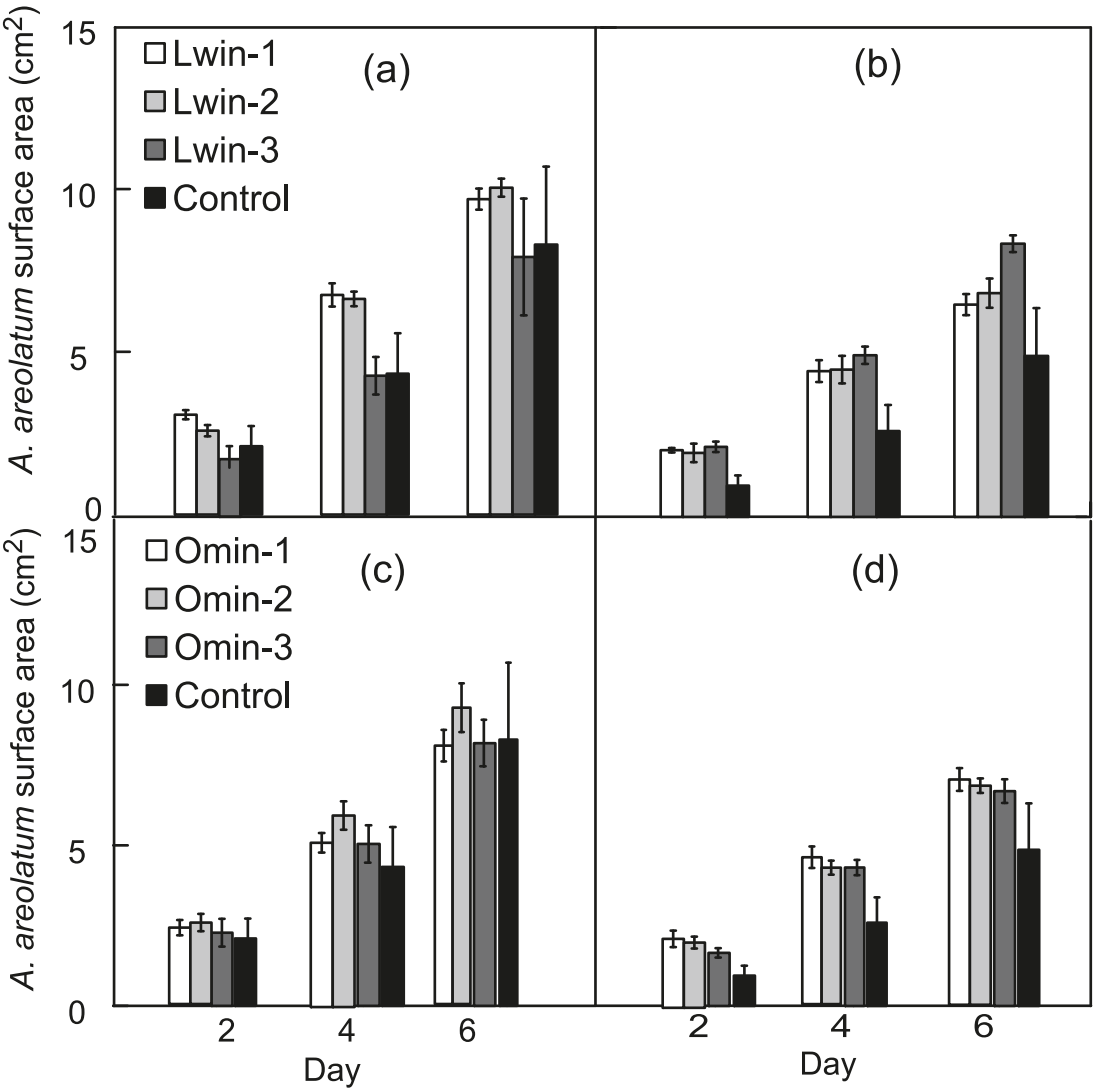
Some strains of the ophiostomatoid fungi grew quickly, resulting in fully occupied Petri plates within 6–8 days, so the repeated-measures analysis was restricted to the first three measurements (days 2, 4, and 6) to allow comparisons between all species and strains of fungi. There was a significant effect of the *L. wingfieldii* strains on the growth of both *A. areolatum* strains (Aareol-1: $F_{3,16} = 4.78$, $P = 0.02$; Aareol-2: $F_{3,16} = 7.91$, $P = 0.002$) over the first 6 days of growth; *A. areolatum* colonized more surface area in the presence of some *L. wingfieldii* strains than when growing alone (Figs. 1*a*, 1*b*). There was a similar effect of *O. minus* on the growth of one of the *A. areolatum* strains but not the other (Aareol-1: $F_{3,16} = 1.97$, $P = 0.16$; Aareol-2: $F_{3,16} = 5.59$, $P = 0.008$) (Figs. 1*c*, 1*d*).

When the plates were entirely colonized, each strain of *O. minus* and *L. wingfieldii* captured substantially more space than did each *A. areolatum* strain (compare columns 4 and 5 in Table 2). At 2 weeks, *A. areolatum* strains growing without competitors colonized significantly more area than the same strains growing with *L. wingfieldii* or *O. minus* (Table 2).

### Interactions on wood substrate at 25 °C

At 25 °C the colonized area of both *A. areolatum* strains at 7 days was significantly affected by the presence of *L. wingfieldii* (Aareol-1: $F_{3,16} = 17.3$, $P < 0.001$; Aareol-2: $F_{3,16} = 85.5$, $P < 0.001$). Both strains of *A. areolatum* colonized significantly less area in the presence of each of the *L. wingfieldii* strains than they did

**Fig. 1.** Growth of two *Amylostereum areolatum* strains in the presence of *Leptographium wingfieldii* (*a*, strain Aareol-1; *b*, strain Aareol-2) and *Ophiostoma minus* (*c*, strain Aareol-1; *d*, strain Aareol-2) compared with solo-growing control ($n = 5$ for each treatment pairing and control). Growth is presented as *A. areolatum* surface area (mean ± SE) of potato dextrose agar colonized at 2–6 days from the start of the experiment.






growing alone (Figs. 2*a*, 2*b*). *Amylostereum areolatum* area at 7 days was also significantly affected by the presence of *O. minus* (Aareol-1: $F_{3,16} = 5.2$, $P = 0.01$; Aareol-2: $F_{3,16} = 3.44$, $P = 0.04$); however, results varied between *O. minus* strains (Figs. 2*a*, 2*b*).

At 2 weeks, all strains of both ophiostomatoid species colonized substantially more space than did the *A. areolatum* strains, and the solo-growing *A. areolatum* colonized substantially more space than did colonies growing in the presence of competing species (Tables 2, 3). Inhibition zones were frequently present on chips inoculated with *A. areolatum* and *L. wingfieldii*, resulting in less than 100% colonization of the chip; a subset of these left to grow beyond 2 weeks showed no further colonization of this zone. At 2 weeks, *A. areolatum* strains growing without competitors colonized significantly more area than did

**Table 2.** Surface area colonized by contending fungal strains at 2 weeks at 25 °C on 2.4% potato dextrose agar and on *Pinus sylvestris* wood chips ($n = 5$ for each treatment pairing and control).

| *Amylostereum areolatum* strain | Ophiostomatoid species | Ophiostomatoid strain | Space colonized by *A. areolatum* on PDA (%) | Space colonized by contender on PDA (%) | Space colonized by *A. areolatum* on wood chip (%) | Space colonized by contender on wood chip (%) |
|---|---|---|---|---|---|---|
| Aareol-1 | *Leptographium wingfieldii* | Lwin-1 | 14.8 (0.43) | 85.3 (0.43) | 12.0 (2.13) | 48.8 (7.30) |
| | | Lwin-2 | 21.8 (0.69) | 78.2 (0.69) | 6.0 (0.63) | 93.4 (2.25) |
| | | Lwin-3 | 24.8 (1.98) | 75.2 (1.98) | 15.9 (2.20) | 65.9 (2.55) |
| | | Control | 46.4 (1.44) | — | 82.7 (2.40) | — |
| ANOVA results* | | | $F_{3,16} = 112.5$, $P < 0.001$ | — | $F_{3,16} = 54.5$, $P < 0.001$ | — |
| Aareol-2 | | Lwin-1 | 13.2 (0.70) | 86.8 (0.70) | 13.9 (2.13) | 73.7 (5.66) |
| | | Lwin-2 | 9.8 (0.56) | 90.2 (0.56) | 9.0 (1.47) | 70.0 (7.70) |
| | | Lwin-3 | 24.0 (0.83) | 76.0 (0.83) | 15.2 (3.87) | 69.8 (4.63) |
| | | Control | 39.2 (1.71) | — | 76.6 (6.06) | — |
| ANOVA results* | | | $F_{3,16} = 62.0$, $P < 0.001$ | — | $F_{3,16} = 101.9$, $P < 0.001$ | — |
| Aareol-1 | *Ophiostoma minus* | Omin-1 | 20.9 (0.56) | 79.1 (0.56) | 20.7 (1.17) | 79.3 (1.17) |
| | | Omin-2 | 18.9 (0.98) | 81.1 (0.98) | 18.5 (0.90) | 80.2 (1.98) |
| | | Omin-3 | 19.7 (2.53) | 80.3 (2.53) | 15.3 (1.80) | 86.0 (2.20) |
| | | Control | 46.4 (1.44) | — | 82.7 (2.40) | — |
| ANOVA results* | | | $F_{3,16} = 189.6$, $P < 0.001$ | — | $F_{3,16} = 51.1$, $P < 0.001$ | — |
| Aareol-2 | | Omin-1 | 16.4 (0.93) | 83.6 (0.93) | 17.6 (1.54) | 82.4 (1.54) |
| | | Omin-2 | 16.5 (0.29) | 83.5 (0.29) | 18.1 (3.00) | 80.9 (2.43) |
| | | Omin-3 | 13.4 (0.50) | 86.6 (0.50) | 11.0 (1.28) | 87.8 (0.58) |
| | | Control | 39.2 (1.71) | — | 76.6 (6.006) | — |
| ANOVA results* | | | $F_{3,16} = 169.0$, $P < 0.001$ | — | $F_{3,16} = 58.19$, $P < 0.001$ | — |

**Note:** Values are given as the mean with SE in parentheses.

*Difference in the amount of substrate colonized on control substrate *versus* that with each contending species; $P < 0.001$ for Tukey's HSD of control *versus* each contending species–strain.

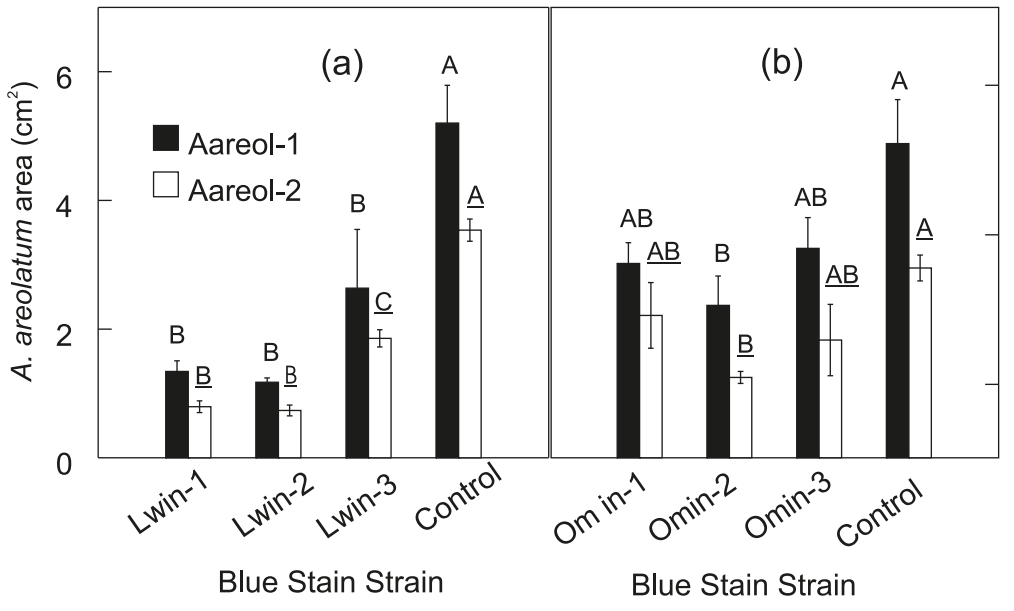
**Table 3.** *T* test values and significance for $\log_n$-transformed amount of surface area of *Pinus sylvestris* wood chip colonized at 2 weeks by each contending fungal strain at temperatures between 15 and 30 °C ($n = 5$ for all treatment pairings and control).

| *Amylostereum areolatum* strain | Ophiostomatoid species | Ophiostomatoid strain | 15 °C | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|---|---|
| Aareol-1 | *Leptographium wingfieldii* | Lwin-1 | −3.88** | −12.63*** | −4.84** | — |
| | | Lwin-2 | −17.91*** | −13.65*** | −37.08*** | — |
| | | Lwin-3 | −7.67*** | −3.68** | −14.87*** | — |
| Aareol-2 | | Lwin-1 | −3.08** | −11.24*** | −9.88*** | — |
| | | Lwin-2 | −2.36* | −1.68 | −7.79*** | — |
| | | Lwin-3 | −9.78*** | −5.90*** | −10.11*** | — |
| Aareol-1 | *Ophiostoma minus* | Omin-1 | −6.37*** | −6.48*** | −38.52*** | −19.16*** |
| | | Omin-2 | −5.25*** | −14.92*** | −28.36*** | −43.18*** |
| | | Omin-3 | −5.50*** | −9.10*** | −24.93*** | −43.29*** |
| Aareol-2 | | Omin-1 | −19.17*** | −12.13*** | −29.76*** | −85.87*** |
| | | Omin-2 | −12.28*** | −5.62*** | −16.27*** | −44.44*** |
| | | Omin-3 | −4.25** | −4.19** | — | −26.12*** |

**Note:** Ophiostomatoid species colonized more surface area in all cases.
*$p \leq 0.05$.
**$p \leq 0.01$.
***$p \leq 0.001$.



**Fig. 2.** Surface area (mean + SE) of *Pinus sylvestris* woodchip colonized by two *Amylostereum areolatum* strains in the presence of (*a*) *Leptographium wingfieldii* and (*b*) *Ophiostoma minus* compared with solo-growing control ($n = 5$ for each treatment pairing and control). Growth is presented as *A. areolatum* colony area (mean ± SE) 7 days from the start of the experiment. Results of Tukey's HSD test are indicated in regular capitals for *A. areolatum* strain Aareol-1 and in underlined capitals for strain Aareol-2.



the same strains growing with *L. wingfieldii* or *O. minus* (Table 2).

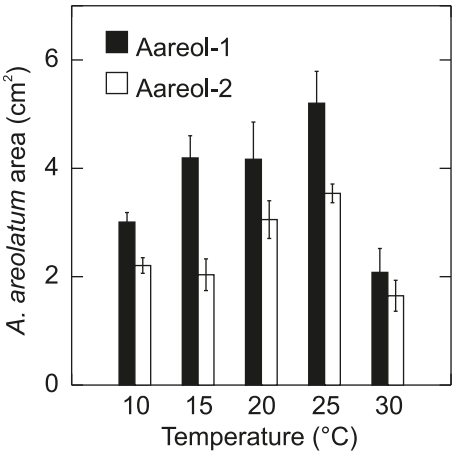
Neither *L. wingfieldii* nor *A. areolatum* was isolated from the territory of the other in subcultures taken at 4 weeks. *Ophiostoma minus* was isolated from *A. areolatum* space but *A. areolatum* was not found in space colonized by *O. minus*.

### Effect of temperature on interactions on wood substrate

At 1 week, *A. areolatum* strains colonized the greatest amount of substrate when growing at 25 °C, and Aareol-1 typically colonized more substrate than did Aareol-2 at each temperature (Fig. 3). All three fungal species grew slowly at 10 °C, the chips incubated at this temperature were never fully colonized, and the contenders did not compete for substrate. Allowing a further week of growth did not result in competition for substrate. Two *L. wingfieldii* strains (Lwin-2 and Lwin-3) grew very poorly at 30 °C and many replicates of these strains failed to grow. Several replicates of the *A. areolatum* strain Aareol-2 failed to grow in the presence of *O. minus* strain Omin-3 at 15 °C. These poorly growing treatment combinations were excluded from analyses.

With the exception of one strain combination (*A. areolatum* Aareol-2 and *L. wingfieldii* Lwin-2), in each of the tested and analyzed strain combinations the ophiostomatoid strain colonized significantly more of the chip than the *A. areolatum* strain did at temperatures between 15 and 25 °C (Table 3). There was a significant effect of temperature, *L. wingfieldii* strain, and the interaction of the two on the amount of substrate colonized by *A. areolatum* strain Aareol-1 at temperatures between 15 and 25 °C (temperature: $F_{2,36} = 3.4$, $P = 0.04$; strain: $F_{2,36} = 11.83$, $P < 0.001$; interaction: $F_{4,36} = 3.48$, $P = 0.02$). *Amylostereum areolatum* was able to colonize more substrate at 20 °C when competing with *L. wingfieldii* strain Lwin-3 than it could with the other temperature and strain combinations (Fig. 4*a*). There was a significant effect of *L. wingfieldii* strain (but not of temperature or interaction) on *A. areolatum* strain Aareol-2 (temperature: $F_{2,36} = 1.61$, $P = 0.23$; strain $F_{2,36} = 13.77$, $P < 0.001$; interaction: $F_{4,36} = 2.09$, $P = 0.10$)

**Fig. 3.** Surface area of *Pinus sylvestris* woodchip colonized by two *Amylostereum areolatum* strains grown at 10–30 °C, at 7 days from the start of the experiment ($n = 5$ for each treatment pairing and control).

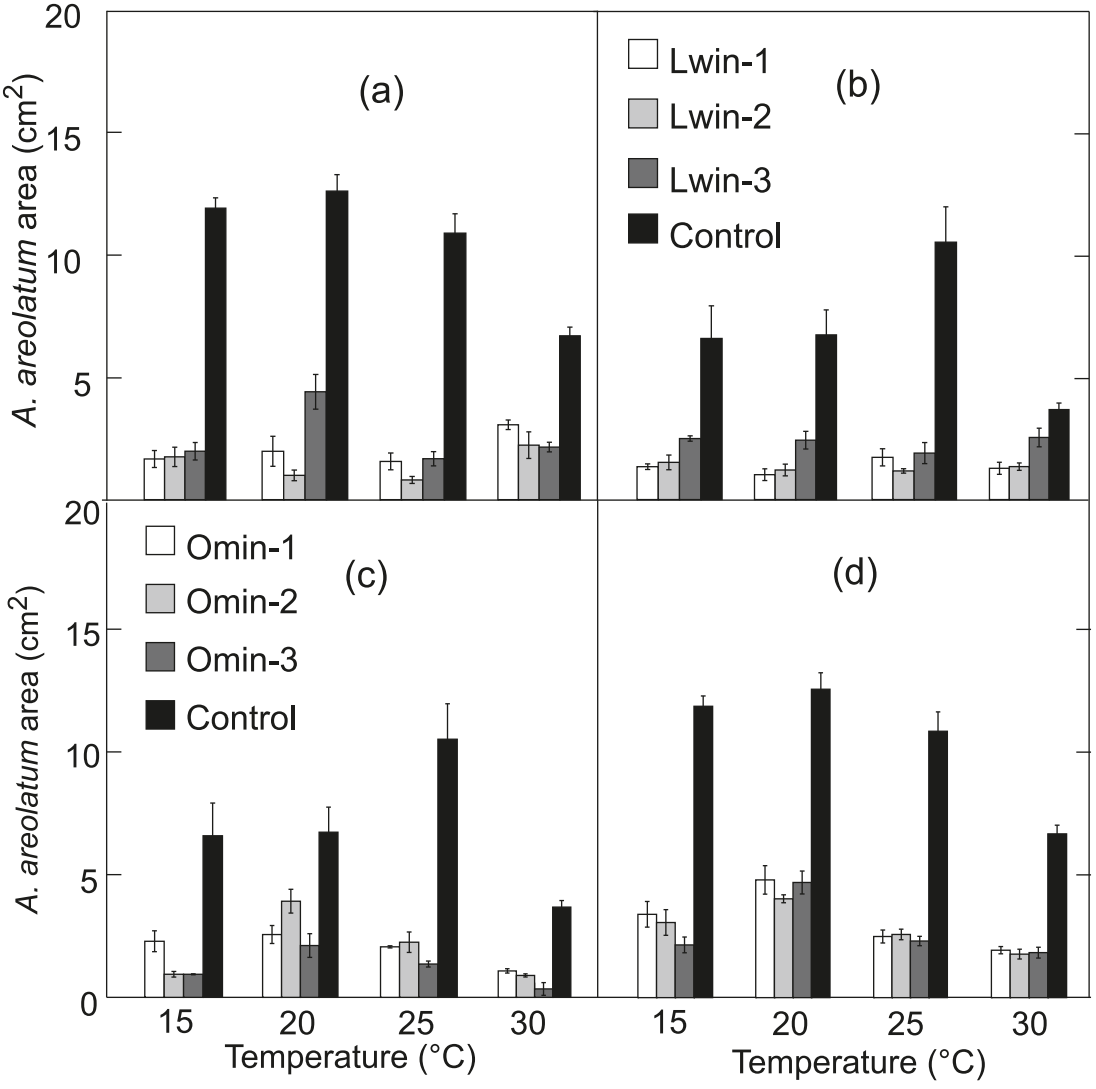



(Fig. 4*b*). At temperatures between 15 and 30 °C, there was a significant effect of temperature (but not of *O. minus* strain or the interaction of the two) on the amount of substrate colonized by *A. areolatum* strain Aareol-1 (temperature: $F_{3,48} = 40.19$, $P < 0.001$; strain $F_{2,48} = 2.19$, $P = 0.12$; interaction: $F_{6,48} = 1.50$, $P = 0.21$). This strain was able to capture more substrate at 20 °C than at other temperatures (Fig. 4*c*). There was a significant effect of temperature, *O. minus* strain, and the interaction of the two on area colonized by *A. areolatum* Aareol-2 at temperatures between 20 and 30 °C (temperature: $F_{2,36} = 54.09$, $P < 0.001$; strain $F_{2,36} = 7.59$, $P = 0.002$; interaction: $F_{4,36} = 2.97$, $P = 0.03$) and results were similar for the 15–30 °C temperature range when the incomplete data set was included in the analysis (Fig. 4*d*).

### Growth on precolonized substrate

Neither *A. areolatum* strain grew on any of the live (nonautoclaved) strains of either *L. wingfieldii* or *O. minus*, but both strains grew on all of the autoclaved ophiostomatoid colonized substrate. One strain of *L. wingfieldii* (Lwin-1) did not grow on either strain of live *A. areolatum* or on one of the autoclaved *A. areolatum* strains (Aareol-1). Two strains of

**Fig. 4.** Area of an *Amylostereum areolatum* colony in the presence *Leptographium wingfieldii* (*a*, strain Aareol-1; *b*, strain Aareol-2) and *Ophiostoma minus* (*c*, strain Aareol-1; *d*, strain Aareol-2) compared with solo-growing control ($n = 5$ for each treatment pairing and control). Growth is presented as *A. areolatum* colony area (mean ± SE) at temperatures of 15–30 °C at 2 weeks from the start of the experiment.






*O. minus* (Omin-3 and Omin-2) did not grow on live *A. areolatum* strain Aareol-1.

## Discussion

The outcomes of experimental interactions on artificial and wood substrates and at all temperatures were generally similar: *L. wingfieldii* and *O. minus* each colonized more substrate than did *A. areolatum* and both prevented *A. areolatum* from colonizing this space. Assuming that these laboratory results can be extrapolated to natural environments, this suggests that where *T. piniperda*, *S. noctilio*, and their associated fungal symbionts are found in close proximity, the growth of nutritional resources for larvae of the woodwasp may be limited. The limitation of *A. areolatum* growth through these fungal interactions could reduce the size and fecundity of adult

*S. noctilio* (Madden 1974). This could be especially deleterious at early instars, when woodwasp larvae are believed to depend on *A. areolatum* as their sole food source (*e.g.*, Madden and Coutts 1979). Therefore, fungal interactions have the potential to exert a degree of biological control in this system.

Although the ophiostomatoid competitors typically occupied more substrate than did *A. areolatum* at temperatures between 15 and 25–30 °C, the relative amount of substrate that *A. areolatum* colonized was influenced by temperature in interactions with some ophiostomatoid strains. Under natural conditions, sapwood temperatures of around 20 °C (Fig. 4) may provide relatively more favorable interaction conditions for the woodwasp fungal symbiont. Sapwood temperatures can range up to 30 °C (Stockfors 2000); at this temperature *A. areolatum* strains would likely have difficulty competing with *O. minus* strains (Figs. 4*c*, 4*d*). Trees infested by *S. noctilio* appear to have higher sapwood temperatures than uninfested trees (Jamieson 1957), and this may add competition pressure on the woodwasp symbiont after its initial establishment in a tree.

*Amylostereum areolatum* could not establish on substrate already occupied by active colonies of either of the ophiostomatoid species. However, it could establish on autoclaved colonies of the same competitors, suggesting that this pattern was a result of antibiosis rather than competition for nutrients (exploitation). Given this outcome, we expect that oviposition by *S. noctilio* would not result in successful brood development in sapwood already colonized by one of these ophiostomatoid fungi. The observed interstrain variability in the ability of each ophiostomatoid species to establish on *A. areolatum*-colonized substrate suggests that only some strains would have the potential to capture substrate already colonized by *A. areolatum* and influence woodwasp larval development. That half of the ophiostomatoid strains were unable to establish in one of the two *A. areolatum* strains is surprising in light of King's (1966) comment that the woodwasp symbiont was always overtaken by competitors. This illustrates the importance of examining interstrain differences when evaluating fungal interactions.

The degree to which these ophiostomatoid species may affect development of *S. noctilio* larvae will depend on factors such as the timing and frequency of entry of woodwasps and bark beetles (and therefore the fungal associates) into trees, and the role that *A. areolatum* plays in nutrition during later larval instars of *S. noctilio*. Adult *T. piniperda* appear most likely to enter a tree in late winter or early spring, after initial colonization by, and early development of, larval *S. noctilio* (personal observation). Oviposition by *S. noctilio* in the late summer may result in greater effects of fungal competition on larval development than oviposition in the early summer. However, phloem- and wood-boring insects other than *T. piniperda* may enter the tree shortly after oviposition by *S. noctilio* (personal observation), and could introduce ophiostomatoid fungi at early stages in woodwasp development. Not all beetles carry fungi, so the frequency with which fungal competitive interactions could occur is unknown (Solheim and Långström 1991; Jacobs *et al.* 2004). The role of *A. areolatum* in nutrition of later larval instars of *S. noctilio* is not well described, so the entry of ophiostomatoid fungi during those stages may have a limited effect on woodwasp development.

The interactions we observed followed general patterns; however, there were differences between strains. In particular, *A. areolatum* strain Aareol-1 tended to colonize more space than the other strain (Table 2, Fig. 3), showed different responses to temperature and contending strain (Fig. 4), and was more resistant to secondary-resource capture. Therefore, woodwasp larvae developing in the presence of Aareol-1 may benefit from the greater ability of that symbiont to procure and maintain nutritional resources.

Other factors may affect the outcome of interactions between *A. areolatum* and other fungi. The phytotoxic mucus deposited by *S. noctilio* during oviposition is thought to stimulate fungal growth (Boros 1968; Titze and Turnbull 1970) and may influence the outcomes of interactions between competing fungi under natural conditions. The effect of wood moisture may be another key factor. *Amylostereum areolatum* grows best in wood having less than 70% moisture content (Coutts

and Dolezal 1965), whereas *L. wingfieldii* grows more quickly in wood between 120% and 150% moisture (Hironori *et al.* 2003). At lower wood moisture content, the *S. noctilio* symbiont may fare better in competition against ophiostomatoid fungi.

Our work is the first to show quantitative evidence of the outcomes of interactions between the *S. noctilio* symbiont *A. areolatum* and species of beetle-vectored fungi. *Amylostereum areolatum* was clearly a poor competitor against *L. wingfieldii* and *O. minus* and, although it fared better at certain temperatures, it was consistently outcompeted by those fungi and excluded from substrate already colonized by them, regardless of substrate or temperature conditions. This will have reproductive consequences for *S. noctilio* if its larvae are vying for space in trees with phloem- and wood-boring beetles and could ultimately affect its population dynamics as it establishes in North American forests.



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