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# *Ips pini* (Curculionidae: Scolytinae) Is a Vector of the Fungal Pathogen, *Sphaeropsis sapinea* (Coelomycetes), to Austrian Pines, *Pinus nigra* (Pinaceae)

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**ABSTRACT** *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton, is among the most common and widely distributed pathogens of conifers worldwide. *S. sapinea* is disseminated over short distances by rain splash and moist wind, but significant knowledge gaps regarding long-range dispersal remain. Our objective was to determine whether or not the pine engraver beetle, *Ips pini* Say, is a vector of the pathogen onto Austrian pines (*Pinus nigra* Arnold). In 2004 and 2005, individuals of *I. pini* were collected with pheromone traps at two locations in central Ohio (197 and 1,017 individuals for 2004 and 2005, respectively) and screened for the presence of *S. sapinea*. In the field, fresh logs of Austrian pine were baited with pheromone lures, mechanically wounded, or left undisturbed. After 2 mo, logs were evaluated for insect feeding and the presence of *S. sapinea* along beetle galleries. Fresh logs were also inoculated in the greenhouse with adult *I. pini* that were either artificially infested or uninfested with *S. sapinea* spores to determine vectoring potential. Phoresy rates for individual collections ranged from 0 to 4.1%; average rates were 1.5 and 2.0% for 2004 and 2005, respectively. Isolation frequencies of *S. sapinea* from baited ( $15 \pm 5\%$ ) and unbaited logs ( $3 \pm 1\%$ ) differed significantly ( $P = 0.009$ ). *I. pini* was also capable of transmitting the pathogen under controlled conditions. Based on phoresy rates, association, and artificial inoculation studies, we conclude that *I. pini* is able to transmit *S. sapinea* to Austrian pine stems.

**KEY WORDS** short- and long-range dispersal, phoresy rates, pine engraver beetle, *Diplodia* tip blight, canker

*Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton [syn. *Diplodia pinea* (Desmaz.) J. Kickx; Sutton 1980], the causal agent of *Sphaeropsis* shoot blight and stem canker diseases, is among the most common and widely distributed pathogens of conifers worldwide and one of the most important limiting factors in the growth of Austrian pine (*Pinus nigra* Arnold) in the landscape (Gibson 1979, Morelet and Chandelier 1993), particularly in the midwestern United States. *S. sapinea* can result in extensive economic losses of exotic and native coniferous trees growing in managed plantations (Swart and Wingfield 1991, Zwolinski et al. 1995, Stanosz and Carlson 1996). Symptoms associated with *S. sapinea* infection include shoot blight, branch dieback, blue-stain of cut timber, top-kill, and main stem cankers (Punithalingham and Waterson 1970, Gibson 1979, Farr et al. 1989).

*Sphaeropsis sapinea* is a coelomycete that produces conidia inside a pycnidium that develops at the base

of needles, on leaf sheaths, and on cone scales (Waterman 1943, Peterson and Wysong 1968). Infection by *S. sapinea* occurs through stomata of elongating needles, expanding shoots, and injured tissue in which symptom development proceeds rapidly (Brookhouser and Peterson 1971, Chou 1978). Cankers form after the pathogen has been introduced into injured pine tissue. *S. sapinea* has been shown to be an aggressive canker-pathogen on Austrian pine after inoculation of wounded sites (Blodgett and Bonello 2003). Abiotic factors such as water deficits, hail, and heavy snow predispose trees to infection and are associated with severe damage caused by canker formation (Bachi and Peterson 1985, Nicholls and Ostry 1990, Blodgett et al. 1997).

*Sphaeropsis sapinea* is disseminated as conidial inoculum with most dissemination occurring from April to June when second- and third-year seedlings are most susceptible (Brookhouser and Peterson 1971, Palmer et al. 1988). Short-distance dispersal of the pathogen is generally attributed to rain splash and moist wind, whereas the means and nature of long-range dispersal of the pathogen are relatively unknown (Brookhouser and Peterson 1971, Palmer et al. 1988, Tainter and Baker 1996). The only animal vector of the pathogen that has been recognized in North

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America is a spittle bug, *Aphrophora parallela* Say (Haddow and Newman 1942). In Italy, an association between the pathogen and the cone bug *Gastrodes grossipes* has been found in cones of *Pinus nigra* (Feci et al. 2002).

Bark beetles (Curculionidae: Scolytinae) of the genus *Ips* attack coniferous trees of the genus *Pinus*. Most species breed in slash, broken, fallen, dying, or large limbs of trees, but some are capable of making successful primary attacks on healthy trees when conditions are favorable (Wood 1982). The pine engraver beetle, *Ips pini* Say, is found across North America (Lanier 1972). *I. pini* usually infests logging slash, cull logs, and wind thrown trees, but when the pine engraver is found in high densities or associated with other bark beetles, it does have the capacity to colonize dense, young stands or the tops of older trees (Livingston 1979, Wood 1982, Poland and Borden 1994).

Colonization of host material is initiated by males that, after constructing an entrance tunnel and releasing aggregation pheromones attracting both sexes, mate with up to three females (Wood 1982, Schenk and Benjamin 1969, Robins and Reid 1997). Females construct Y-shaped longitudinal galleries (5–25 cm in length) from the male nuptial chamber, along which eggs are deposited. Male galleries range in densities from 1.5 to 3.0 entrance tunnels/dm<sup>2</sup> (Wood 1982, Poland and Borden 1994). Numerous field observations have shown that most, if not all, Scolytinae are associated with fungi (Wood 1982). *I. pini* transports several species of fungi, of which *Ophiostoma ips* (Rumbold) Nannfeldt (Klepzig et al. 1991) was found to be transmitted to Ponderosa pine, *Pinus ponderosa* Lawson, on its elytrae (Furniss et al. 1995). *O. ips* has been found to be consistently present in logs of red pine (*Pinus resinosa* Aiton) infested with *I. pini* and missing when host material is protected from beetle attack (Leach et al. 1934).

The objective of this study was to determine whether the pine engraver beetle, *I. pini*, vectors the pathogen *S. sapinea* onto Austrian pine, *P. nigra*. To conclude that *I. pini* vectors the pathogen, it is necessary to show that (1) *I. pini* infests nondiseased Austrian pine trees under conditions suitable for transmission of the pathogen; (2) populations of *I. pini* carry inoculum of the pathogen (phoresy) in the field when in proximity to stands of Austrian pine; (3) *I. pini* is associated with diseased host material; and (4) *I. pini* can successfully transmit the pathogen to nondiseased host material under controlled experimental conditions (Leach 1940). Individuals of *I. pini* are known to associate with nondiseased Austrian pine trees when conditions are favorable (e.g., under water stress) (Furniss and Carolin 1977, Wood 1982, unpublished data), therefore satisfying the first criterion. To fulfill the second criterion, we trapped natural populations of *I. pini* to estimate phoresy rates in the field. An association between the potential vector, host, and pathogen was determined by field observation and an experiment to satisfy the third criterion. To fulfill the fourth and final criterion, a study under controlled

conditions tested the ability of *I. pini* to transmit the pathogen to nondiseased Austrian pine material.

## Materials and Methods

**Phoresy Study.** *Ips pini* were collected throughout their flight season from June to September 2004 and 2005 from Sharon Woods Metro Park (40°7' N, 82°57' W) and Inniswood Metro Park (40°6' N, 82°54' W) in Westerville, OH. *I. pini* were trapped using eight-funnel Lindgren traps baited with ipsdienol and lanierone bark-beetle lures (Phero Tech, Delta, British Columbia, Canada). Three separate Lindgren traps were hung at each site within a stand of 10–15 mature Austrian pine trees with approximate diameters at breast height ranging from 15 to 20 cm. These stands were surrounded by other pines covering an area of ~0.25 ha. Samples were collected every 3–4 d depending on the weather. In 2004 and 2005, we collected a total of 557 males and 657 females. Individuals of *I. pini* were stored at –18°C in petri plates until specimens were sorted by date, trap number, location, and sex. Differences in phoresy rates of *S. sapinea* between male and female *I. pini*, totaled for all collection times and locations, were analyzed with a G test of independence using COSTAT (CoHort Software, Monterey, CA).

Individual beetles were plated on the semiselective medium tannic acid agar (TAA), which enhances the growth of *S. sapinea* (Blodgett et al. 2003). Plates were incubated at room temperature for 6–7 d in the dark. At the end of the incubation period, plates were examined microscopically at ×70, and fungi that exhibited the growth characteristics of *S. sapinea* (Cheng-Guo et al. 1985, Blodgett et al. 2003) were isolated and subcultured on acidified potato dextrose agar (APDA).

**Confirmation of *S. sapinea*.** Putative *S. sapinea* cultures were transferred to pine needle agar (PNA) (Blodgett et al. 2003) to induce the formation of fruiting bodies and sporulation. Fruiting bodies were removed and broken onto a microscope slide. The length and width of 50 randomly selected spores were measured to confirm culture identity (Cheng-Guo et al. 1985, Hildebrand 2005). Samples were also grown in potato dextrose broth (PDB) at room temperature for 6–7 d in the dark to obtain mycelia for confirmation of the pathogen based on DNA sequence. Cultures were harvested by collecting mycelia onto filter paper in a Buchner funnel and washing with sterilized water to remove nutrient broth from the mycelial sample. Samples were stored at –80°C in 1.5-ml microcentrifuge tubes until they could be processed for DNA extraction and amplification.

DNA was extracted with a Nucleospin plant DNA extraction kit (Macherey-Nagel, Easton, PA). The fungal ITS region was amplified with two universal primers: (1) ITS1 F (5'-cttggcttagaggagtaa-3') and (2) ITS4 (5'-tctcctcgcttattgatgc-3') (Gardes and Bruns 1993). A Roche Applied Science LightCycler (Indianapolis, IN) was used to amplify the ITS region by real-time polymerase chain reaction (PCR). The

total volume of each sample totaled 20  $\mu\text{l}$  (3 mM  $\text{MgCl}_2$ , 0.3 mM each primer, 2  $\mu\text{l}$  Sybr Green Master Mix [Roche Applied Science], and 50 ng of DNA template). PCR was run using one cycle of preincubation at 95°C for 10 min, followed by 35 cycles of denaturing at 95°C for 10 s, annealing at 55°C for 5 s, and extension at 72°C for 30 s for amplification. Samples were cooled at 40°C for 30 s. PCR products were purified using the Nucleospin PCR clean-up kit (Macherey-Nagel). Purified PCR products were sequenced using the ITS1F primer at the Ohio State University Plant and Microbe Genomics Facility (<http://www.biosci.ohio-state.edu/~pmgf/>). Sequences were blasted against the NCBI database, allowing for the identification to species. Blast scores and e-values were recorded.

**Association Study.** Two healthy Austrian pine trees were cut at Sharon Woods Metropark on 1 August 2005. The trees were cut into 12 sections (length =  $88 \pm 3.02$  cm; diameter = 12.7–27.9 cm) that were randomly divided into three treatments of four logs each and immediately placed, standing upright on the ground, in an open field with close proximity to several stands of diseased Austrian pine trees. The three treatments used were (1) unbaited logs, (2) baited logs, and (3) wounded logs. The three treatment groups were spaced roughly 15 m apart in the field. Separate logs within each treatment were spaced 60 cm apart from the next replication. Unbaited logs were not treated in any way. Baited logs had pheromone lures with ipsdienol and lanierone stapled directly to the outer bark of the log. Wounded logs had four to six evenly distributed columns of six puncture wounds within each column created using a hammer and Phillips screw driver that were sterilized using 70% EtOH in the laboratory before being taken out to the field. Reisolation attempts from control wounds yielded no *S. sapinea*, indicating that no cross-contamination occurred in the wounding of host material. Wounds extended from the outer surface of the bark to the cambium. Puncture wounds in each column were 8 cm apart from each other. Logs were left in the field for 6 wk and collected and brought indoors. Logs were processed by counting the number of individual *I. pini* attack sites throughout the whole log. The bark was removed, and the presence/absence of *I. pini* galleries in the phloem was observed. A total of 20 random phloem samples, 2.5 by 2.5 cm in size, were taken from each log at the gallery sites. Samples were surface sterilized with 95% EtOH for 10 s and 1.05% NaOCl with Tween 20 (1–2 drops/liter) for 3–4 min. Samples were rinsed in distilled water for 5 min. After surface sterilization, samples were placed on TAA and processed for identification of fungal cultures as described above. The experiment was analyzed as a completely random design with three treatments (baited, unbaited, and wounded), four replications, and 20 subsamples. Differences among treatments were analyzed for log diameter, number of *I. pini* attack sites, and percentage of *S. sapinea* isolates recovered. Treatment means were separated based on Student-New-

man-Keuls mean separation tests ( $\alpha = 0.05$ ) using COSTAT.

**Inoculation Study.** In 2005, a healthy Austrian pine tree was cut at Sharon Woods Metropark (height = 13 m, circumference at breast height = 64 cm). The tree was cut into six roughly equally sized logs (length = 53–82.5 cm, diameter = 12.7–19.7 cm) and moved to the greenhouse, where the tops and bottoms of the logs were sprayed with 95% EtOH and allowed to dry. Holes, 4 mm in diameter, were drilled through the outer bark to the xylem-phloem interface at an approximate angle of 90° to the longitudinal axis of each log. A total of six columns were spaced 11 cm apart around the circumference of each log. Each column represented an individual treatment and contained six holes longitudinally spaced 8 cm apart, with each hole representing a subsample within each column. Each hole represented an individual inoculation site for a total of 36 inoculation sites on each log. The treatments used were (1) positive control (spore solution of *S. sapinea*); (2) negative control (sterile deionized/distilled water [SDW]); (3) uninfested female (UF) *I. pini*; (4) uninfested male (UM) *I. pini*; (5) infested female (IF) *I. pini*, infested with a spore solution of *S. sapinea*; and (6) infested male (IM) *I. pini*, infested with *S. sapinea* in the same way as the females. Treatments were randomized on each log/repetition.

Living *I. pini* were used to inoculate healthy Austrian pine logs. *I. pini* were collected from 1 to 31 July, 2005 from Inniswood Metro Park in Westerville, OH. *I. pini* were trapped using eight-funnel Lindgren traps baited with ipsdienol and lanierone. Three separate Lindgren traps were hung at each site, and samples were collected every 3–4 d depending on the weather. Live beetles were taken from the field, placed into petri dishes, and stored at 5°C. Beetles were removed from 5°C storage and sexed under  $\times 70$  magnification. A total of 72 male and 72 female *I. pini* were used.

For uninfested treatments, beetles were taken from the field and directly inserted into the 4-mm-diameter holes and confined to the tree by stapling an appropriately sized piece of 40- $\mu\text{m}$  wire mesh over each hole. A total of six uninfested males and females were used on each log for each of six logs. For infested treatments, 2  $\mu\text{l}$  of a *S. sapinea* spore suspension was pipetted onto the back of each of six beetles, for a total of  $\sim 1,000$  spores/beetle (of which  $\sim 33\%$  were viable). There is no literature on the minimum spore load required to obtain an infection, and as a result, spore loads were chosen arbitrarily. Spores for the spore suspension were obtained from surface sterilized Austrian pine cones bearing pycnidia. Cones were incubated in plastic containers for 1 wk and rinsed with sterile water. Spores were collected by centrifugation, and spore concentrations were calculated using a hemacytometer. The spore suspension was adjusted to  $5 \times 10^5$  spores/ml and used immediately to infest beetles in the inoculation studies. The spore suspension was allowed to sit on the back of each beetle until surface tension broke the droplet, dispersing the pathogen over the beetle's body. Infested male and

**Table 1.** *Sphaeropsis sapinea* phoresy data (frequencies) for populations of *I. pini* collected during the flight seasons 2004 and 2005 from two locations in Westerville, OH

Location	No. males	No. infested males	No. females	No. infested females	Total no. individuals	Total no. infested individuals	Phoresy rate (%)
2004							
Sharon Woods							
1-15 Jul	63	1	67	1	130	2	1.5
27 July to 19 Aug	5	0	6	0	11	0	0.0
19 Aug to 6 Oct	10	1	18	0	28	1	3.6
Inniswood							
30 June to 19 Aug	17	0	11	0	28	0	0.0
Total	95	2	102	1	197	3	1.5
2005							
Sharon Woods							
1-15 June	36	1	37	2	73	3	4.1
15-30 June	38	1	39	0	77	1	1.3
1-31 July	100	2	122	3	222	5	2.3
1-15 Aug	38	1	52	1	90	2	2.2
15-31 Aug	49	1	52	0	101	1	0.9
1-15 Sept	47	1	47	1	94	2	2.1
Inniswood							
1-30 June	57	1	98	1	155	2	1.3
1-15 Aug	64	2	72	1	136	3	2.2
1-15 Sept	33	1	36	0	69	1	1.4
Total	462	11	555	9	1,017	20	1.97

female *I. pini* were placed into 4-mm-diameter holes on the logs in their respective treatment within each repetition and were confined to their sites as described above.

For the positive control, 2  $\mu$ l of the *S. sapinea* spore solution (corresponding to 1,000 spores) was directly pipetted into each hole for its respective treatment column on each log. Each inoculation site was covered with a piece of 40- $\mu$ m wire mesh and stapled in place. The negative control consisted of 2  $\mu$ l of SDW.

After 1 mo, logs were harvested by removing the 40- $\mu$ m wire mesh screens and removing the phloem at each site. Symptoms, necrotic lesion width and length (when present), and gallery length in beetle-inoculated sites were recorded. An  $\sim$ 6.5-cm<sup>2</sup> piece of tissue was removed from each site and placed in a 1.5-ml microcentrifuge tube. Samples were stored at 5°C and processed by surface sterilization in 95% EtOH for 10 s, 4 min in a 1.05% NaOCl solution with Tween 20, and rinsed in SDW. Plates were incubated at 24°C for 6-7 d in a dark environment. At the end of the incubation period, plates were examined microscopically, and fungi that exhibited *S. sapinea* characteristics were isolated and subcultured on APDA. The cultures were transferred to PNA to induce the formation of fruiting bodies and sporulation. The length and width of 50 randomly selected spores were measured to confirm culture identity. Confirmed samples were also sequenced as described above for further confirmation of the pathogen. The experiment was analyzed as a randomized complete block design with four treatments (infested individuals, uninfested individuals, positive controls, and negative controls), six replications, and either 6 or 12 subsamples. Differences among treatments were analyzed for lesion width, lesion length, gallery length, and percentage of positive *S. sapinea* reisolations. Treatment means were separated based on Student-Newman-Keuls mean separation tests ( $\alpha = 0.05$ ) using COSTAT.

## Results

**Phoresy Studies.** The rate of isolation of *S. sapinea* was independent of whether *I. pini* was male or female ( $G = 1.00$ ;  $df = 1$ ;  $P = 0.32$ ). Adult *I. pini* from both sites carried *S. sapinea* in at least one of the years throughout the duration of our sampling (Table 1). Phoresy rates ranged from 0.0 to 3.6 (2004) and 0.9 to 4.1 (2005). Overall rates were 1.5 and 1.97% for 2004 and 2005, respectively.

**Confirmation of *S. sapinea*.** Spore measurements of putative *S. sapinea* isolates ranged from 33.8 to 38.6 and 12.5 to 15.0  $\mu$ m for lengths and widths, respectively. A subsample of eight putative isolates had a minimum sequence identity blast score of 444 bits and a maximum e-value of  $2.0 \times 10^{-123}$ .

**Association Studies.** The number of *I. pini* attack sites differed significantly ( $F_{2,9} = 23.9$ ;  $P = 0.003$ ) among baited, unbaited, and wounded logs of similar size. Austrian pine logs baited with ipsdienol and lanierone had  $670 \pm 117.2$  *I. pini* attack sites, whereas attack sites for unbaited ( $83 \pm 49.6$ ) and wounded logs ( $16 \pm 7.2$ ) were significantly less (Table 2). In addition, the isolation frequency of *S. sapinea* from baited

**Table 2.** Association between *S. sapinea* and *I. pini* on baited, unbaited, and wounded logs of *P. nigra*

Treatment <sup>a</sup>	Log diameter (cm) <sup>b</sup>	No. <i>I. pini</i> attack sites <sup>b</sup>	Percentage of <i>S. sapinea</i> isolates recovered <sup>b,c</sup>
Baited	19.7 $\pm$ 2.7a	670 $\pm$ 117.2a	15 $\pm$ 5a
Unbaited	21.9 $\pm$ 3.2a	83 $\pm$ 49.6b	3 $\pm$ 1b
Wounded	18.9 $\pm$ 2.0a	16 $\pm$ 7.2b	0 $\pm$ 0b

<sup>a</sup> Each treatment was replicated over four logs.

<sup>b</sup> Values are the means  $\pm$  SE of four replications (logs). Means within columns with a letter in common do not differ significantly at  $\alpha = 0.05$  based on Student-Newman-Keuls mean separation tests.

<sup>c</sup> Values are the means  $\pm$  SE of 20 subsamples per log.

**Table 3.** Length and width of lesion, gallery length of *I. pini* feeding, and percentage of *S. sapinea* recovered from inoculation sites on logs of *P. nigra*

Treatment <sup>a</sup>	Lesion width (cm) <sup>b</sup>	Lesion length (cm) <sup>b</sup>	Gallery length (cm) <sup>bc</sup>	Percent of <i>S. sapinea</i> isolates reisolated <sup>b</sup>
Infested individuals	3.1 ± 0.13a	9.8 ± 0.50a	3.1 ± 0.57a	31.9 ± 5.21a
Uninfested individuals	2.6 ± 0.26a	8.7 ± 0.25b	2.6 ± 0.45a	13.9 ± 4.51b
Positive control	1.6 ± 0.38b	7.3 ± 0.54c	—	2.7 ± 2.78b
Negative control	0.0 ± 0c	0.0 ± 0d	—	0.0 ± 0b

<sup>a</sup> Infested and uninfested individuals included both males and females for a total of 72 inoculation sites (6 logs × 6 sites × 2 sexes) in each of these two treatments. Positive and negative controls included 36 inoculation sites (6 logs × 6 sites) each.

<sup>b</sup> Means within columns with a letter in common do not differ significantly at  $\alpha = 0.05$  based on Student-Newman-Keuls mean separation tests.

<sup>c</sup> Gallery length is the length of feeding site produced by *I. pini*.

logs (15 ± 5%) differed significantly ( $F_{2,9} = 8.5$ ;  $P = 0.009$ ) from unbaited (3 ± 1%) or wounded logs (0.0 ± 0%; Table 2).

**Inoculation Studies.** In inoculation studies, treatments differed significantly for lesion width, lesion length, and percentage of *S. sapinea* isolates recovered ( $P < 0.001$  for all parameters; Table 3). Infested treatments were associated with lesions that were wider than lesions associated with positive and negative control and longer than lesions associated with all other treatments (Table 3). The width and length of the negative control was significantly less than all other treatments. In addition, the percentage recovery of *S. sapinea* of the infested treatment was significantly higher than all other treatments. The negative control produced no lesions, and *S. sapinea* was not recovered from any samples (Table 3).

### Discussion

Based on fulfillment of Leach's postulates (Leach 1940), *I. pini* is a vector of the pathogen *S. sapinea* onto Austrian pines. In the literature and through our studies, we found that *I. pini* is (1) repeatedly associated with nondiseased hosts (Furniss and Carolin 1977, Wood 1982, unpublished data), (2) carries the pathogen in the field, (3) is associated with diseased host material, and (4) is capable of transmitting the pathogen under controlled experimental conditions.

With respect to point 2, our estimated phoresy rates obtained by trapping *I. pini* with Lindgren traps are consistent with results from studying the incidence of other pathogens on bark beetles, e.g., the pitch canker pathogen associated with insects found on intact and chipped Monterey pine (*Pinus radiata* D. Don) branches. Using insect rearing boxes, McNee et al. (2002) estimated that the bark beetle *Pityophthorus* spp. Eichhoff carried the canker pathogen at rates ranging from 3.6 to 27%. Similarly, using baiting systems for the bark beetle, Storer et al. (2004) estimated phoresy rates for the same insect-pathogen system as ranging from 0.0 to 17.0%. Further study of the relationship between specific *Pityophthorus* species, the canker pathogen, and Monterey pines concluded that *Pityophthorus* spp. is a vector of the canker pathogen to Monterey pines (Storer et al. 2004).

Our field test with cut logs addressed the third postulate. Sampling of galleries from logs baited with pheromone lures yielded significantly higher percentages of *S. sapinea* than galleries from unbaited logs. We speculate that this is an artifact, because baited logs had higher *I. pini* attack densities, resulting in galleries that were significantly closer to each other than in the unbaited and wounded controls. We hypothesize that this higher incidence of *S. sapinea* in baited logs was caused by lesions expanding from galleries constructed by infested beetles into galleries from beetles not carrying the pathogen. Thus, the bait-induced increase in the number of attacks by potentially infested beetles increased the probability of recovering *S. sapinea* indirectly from initially noninfected sample sites.

In the greenhouse experiment, we introduced beetles that had been artificially contaminated with *S. sapinea* spores into mechanically wounded logs. Contamination of the beetles with pathogen spores resulted in higher rates of *S. sapinea* reisolation from beetle-colonized host material, satisfying Leach's fourth postulate above. Furthermore, we found that *S. sapinea* was reisolated from logs colonized by beetles that carried a natural spore load (the nonartificially infested controls) at a higher rate than the phoresy rates estimated from the field trapping study. This suggests that log infection by *S. sapinea* might have been a combination of direct vectoring by *I. pini* and natural pathogen ingress occurring after beetle colonization through the wounds produced by the insects (Haddow and Newman 1942).

The importance of the pine engraver beetle *I. pini* in the movement of *S. sapinea* within and between stands of healthy Austrian pine, as well as its importance with other potential vectors of the pathogen, is still in question. It is plausible that even failed attacks of *I. pini* on main stems or larger limbs of healthy or temporarily water-stressed trees could be sufficient to introduce the pathogen. Because it is known that *S. sapinea* can occur as a latent pathogen or endophyte (Flowers et al. 2001, Stanosz et al. 2001), once the trees are infected through the beetles, the pathogen could spread systemically and eventually sporulate from cones and needles, thus initiating a new local infection center. Although all this is speculative at present, it is

not inconceivable that *I. pini* could contribute to long-distance dispersal of *S. sapinea* in this way. Further study is needed to establish the importance of *I. pini* as a vector, so that efforts for reducing long-range dispersal of the pathogen and introduction of disease into previously unaffected stands of pine could be implemented in a rational way.

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