J Chem Ecol (2011) 37:1374–1377
DOI 10.1007/s10886-011-0046-x

RAPID COMMUNICATION

The Scent of a Partner: Ambrosia Beetles Are Attracted to Volatiles from Their Fungal Symbionts

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Received: 24 November 2011 / Accepted: 2 December 2011 / Published online: 13 December 2011

Abstract Invasive fungus-growing ambrosia beetles are an emerging threat to forest ecosystems and fruit industries, but management tools are lacking. Here we explored the potential of beetle symbionts–ambrosia fungi–as a source of attractants. Our focus was the redbay ambrosia beetle, *Xyleborus glabratus*, and its symbiotic fungus, *Raffaelea lauricola*, which are devastating lauraceous hosts in the southeastern United States. We also tested three additional co-occurring beetle species and their symbionts. Each beetle species was consistently attracted to the odors of its symbiotic fungal species, occasionally also to symbionts of other species, but never to non-symbiotic *Trichoderma*. We further confirmed attraction to ethanol (positive control) in some species. Thus, ambrosia fungi produce volatiles attractive to their vector beetles, which may have potential as novel lures for ambrosia beetle management.

Key Words Redbay ambrosia beetle · *Xyleborus* · *Xylosandrus* · Ambrosia fungi · Symbiosis · Attractants

Jiri Hulcr and Rajinder Mann have joint first authorship

Electronic supplementary material The online version of this article (doi:10.1007/s10886-011-0046-x) contains supplementary material, which is available to authorized users.

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Introduction

Invasive fungus-farming ambrosia beetles (*Xyleborus*, *Xylosandrus*) pose a growing threat to forest ecosystems and fruit industries (Hulcr and Dunn, 2011), but management tools are lacking. Here, we explored the possibility that an entirely new source of olfactory cues–volatiles from fungal symbionts–may attract ambrosia beetles, suggesting a promising direction for lure development. Ambrosia beetles colonize tree xylem, which they inoculate with one or more species of symbiotic fungi, their sole source of food. Most xyleborini beetles do not produce long-distance pheromones, and are host-generalists, relying on general cues of host decay such as ethanol (Ranger et al., 2010). *Xyleborus glabratus* appears to be an exception that is attracted to specific host volatiles (Kendra et al., 2011). Olfactory interactions between beetles and their fungi have not been studied in depth (but see Hanula et al. (2008)).

We tested whether the invasive *Xyleborus glabratus* and three other commonly co-occurring beetle species in southeastern U.S.A. (*Xyleborus ferrugineus*, *Xylosandrus crassiusculus*, and *Xyleborinus saxesenii*) are attracted to volatiles from their respective fungal symbionts, and/or volatiles from symbionts of other ambrosia beetle species, or whether they are attracted to fungi in general, including non-symbiotic fungi.

Methods and Materials

Ambrosia beetles were reared from redbays killed by laurel wilt, and from naturally infested dead poplar and loblolly pine, collected near Myrtle Beach, South Carolina, U.S.A., and Kissimmee Lake, Florida, U.S.A., in 2010. A 1% dilution of mycangium content from several representatives of

each species was cultured on potato dextrose (PD) agar. Isolations of single-hypha strains of all ambrosia-like morphotypes (*Ambrosiella*, *Ambrosiozyma*, or *Raffaelea*) followed previously published protocols (Kolarik and Hulcr, 2008). Morphotyping to genus was confirmed by amplification of ITS1 or SSU rDNA sequences using the primers ITS1 and ITS4, or NS1 and NS4, respectively (White et al., 1990; Gardes and Bruns, 1993), followed by identification in NCBI-BLAST (see Table S1 for results).

Behavioral Assays Using a 2-choice Teflon olfactometer (Analytical Research Systems, Gainesville, FL, USA) thoroughly described in Mann et al. (2011), we measured responses of beetles to volatiles from *R. lauricola* (symbiont of *X. glabratus*), *Ambrosiella xylebori* (symbiont of *X. crassiusculus*), *Ambrosiozyma ambrosiae* (symbiont of *X. ferrugineus*), *Trichoderma* sp. (antagonist of fungi and insects common in ambrosia beetle galleries), and ethanol, a known attractant of many ambrosia beetles. Fungus odor sources consisted of individual 0.25 cm^2 pieces of actively growing mycelium on PD agar medium; sterile medium was used as blank control. In case of ethanol, 1 ml of absolute alcohol was pipetted onto 5 cm of cotton wick; an untreated cotton wick was used as a blank control. A constant airflow of 0.1 l/min was maintained through both olfactometer arms, and a 0.5 l/min suction flow vacuumed the odor mixture from the olfactometer. The olfactometer was housed within a temperature controlled room (26±1°C, 60±2% RH) inside a fiber board box for uniform light diffusion from a fluorescent 900 lx light bulb. We confirmed the lack of positional bias by exposing a batch of beetles to two clean air treatments (blank odor fields).

Approximately 20 newly emerged beetles of a single species were assayed per replicate; each species replicated at least 5 times, always with new beetles. An odor source was randomly assigned to one of the olfactometer arms at the beginning of each assay and reversed after every replication. After each run, the olfactometer and the glass tubes were washed in soapy water and rinsed with distilled water. The glass tubes then were rinsed with acetone and oven dried overnight at 93.3°C, and the olfactometer stage was cleaned with absolute alcohol and air-dried. The numbers of beetles choosing the control arm vs. the treatment arm were compared with the *Chi square* test. Beetles not moving normally (exhibiting difficulty of walking or in poor condition) were excluded from analysis.

Results

We isolated diverse communities of fungi and yeasts from beetle mycangia (for details see Table S1). *Xyleborus glabratus* consistently yielded *Raffaelea lauricola* and *R. subalba* (Ascomycota, Ophiostomatales). *Xyleborus ferrugineus* commonly yielded *Raffaelea albimanens* (Ascomycota, Ophiostomatales), *Ambrosiozyma ambrosiae* (Ascomycota, Saccharomycetales), and an unidentified *Ambrosiozyma*-like strain. We selected *A. ambrosiae* as the experimental symbiont. *Xylosandrus crassiusculus* yielded *Ambrosiella xylebori/hartigi* (ambiguous BLAST assignment; Ascomycota, Microascales). We did not isolate symbionts of *Xyleborinus saxesenii*. To test responses of beetles to a non-symbiotic antagonistic fungus, we chose *Trichoderma* sp. isolated from our samples.

Beetle Responses Each beetle species for which a symbiont was available showed statistically significant attraction to its respective symbiont (Fig. 1). The two representatives of the genus *Xyleborus* were also attracted to certain symbionts from other beetle species (Fig. 1, Table 1). The non-symbiotic *Trichoderma* never triggered attraction from any beetle species. Ethanol was attractive to *X. ferrugineus* and *X. crassiusculus*, and did not elicit significant response from *X. glabratus* and *X. saxesenii* (Fig. 1).

Discussion

The tested fungus-farming ambrosia species responded positively to volatiles from their symbiotic fungi. Specificity of the reaction varied by beetle genus. In both *Xyleborus* spp., we observed attraction to several ambrosia fungi, but *Xylosandrus crassiusculus* was attracted only to its own symbiont. *Xyleborus* spp. are often associated with more symbionts than *Xylosandrus* spp. (Six et al., 2009; Harrington et al., 2010). We suggest that symbiont specificity and odor-perception specificity may be correlated traits. Attraction of ambrosia beetles to volatile cues from their fungal symbionts may function as a mechanism to locate established fungal gardens of conspecific beetles (suitable microhabitat), but also as an orientation cue within a gallery.

Our results are not consistent with Hanula et al. (2008), who did not observe attraction of *X. glabratus* to trees infested with its symbiont as compared with non-infested trees. The difference may have been caused by the unknown vigor of the fungal colonies in the logs exposed for several months in Hanula et al., or perhaps because fungal odors do not transpire readily from the inside of logs. The beetle responses to ethanol, in general, confirmed previous reports (Hanula and Sullivan, 2008; Ranger et al., 2010), except in *Xyleborinus saxeseni*.

Responses of beetles to odors of fungal symbionts were statistically significant, but often weak (Fig. 1). Further experiments may require a modified assay (ambrosia beetles are poor walkers), varying release rates of attractants, and a

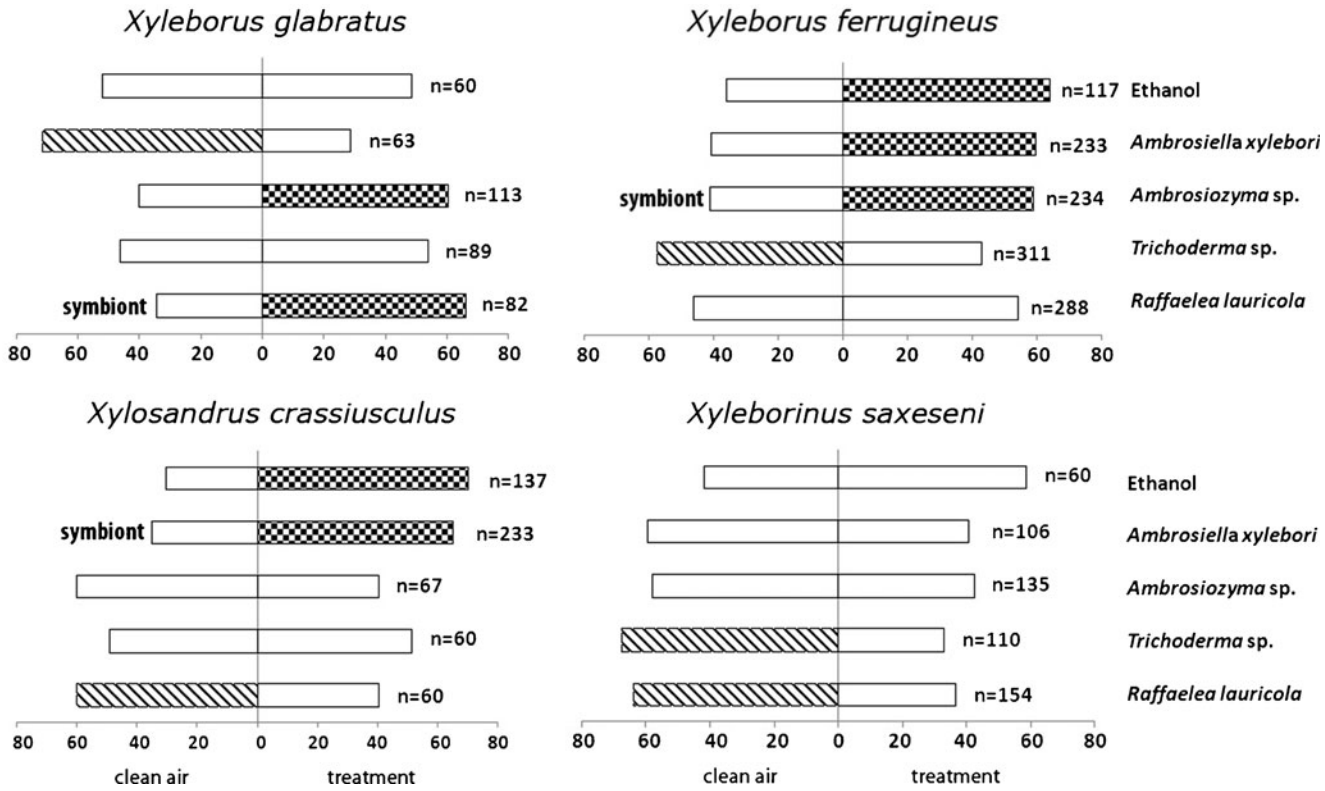

Fig. 1 Percentage of beetles attracted to fungal odors (*right bars*) vs. blanks (sterile agar media, *left bars*), and total numbers of tested individuals. Non-moving beetles were excluded. *Highlighted bars* indicate statistically significant differences (*stripes*: repulsion, *squares*: attraction). The symbiont of each beetle is highlighted (*X. saxesenii* did not have a symbiont in the study)

test of synergy with host tree volatiles. Our results indicate that fungal symbiont volatiles are a source of attractants, with a potential for development of lures for monitoring invasive ambrosia beetles.

Table 1 Responses of ambrosia beetles to volatiles from ambrosia fungi, antagonistic fungus, or ethanol. Fractions: absolute numbers of "beetles in treatment arm/beetles in control arm" summed across all trials. NA: non-active beetles

Beetle species	Response to:				
	Raffaelea lauricola	*Ambrosiozyma sp.*	*Ambrosiella xylebori*	*Trichoderma*	*ethanol*
Xyleborus glabratus	attracted SYMBIONT 54/28, $\chi2$=8.24; df = 1; *P*=0.004, NA = 36	attracted 68/45, $\chi2$=5.05; df = 1; *P*=0.02, NA = 57	repelled 18/45, $\chi2$=11.57; df = 1; *P*=0.001, NA = 19	no response 48/41, $\chi2$=0.55; df = 1; *P*=0.46, NA = 78	no response 29/31, $\chi2$=1.90; df = 1; *P*=0.27, NA = 32
Xyleborus ferrugineus	no response 156/132, $\chi2$=2; df = 1; *P*=0.16, NA = 98	attracted SYMBIONT 138/96, $\chi2$=7.54; df = 1; *P*=0.006, NA = 35	attracted 139/94, $\chi2$=8.69; df = 1; *P*=0.003, NA = 8	repelled 133/178, $\chi2$=6.51; df = 1; *P*=0.01, NA = 102	attracted 107/60, $\chi2$=13.23; df = 1; *P*<0.001, NA = 33
Xylosandrus crassiusculus	repelled 55/82, $\chi2$=5.32; df = 1; P =0.02, NA = 116	no response 27/40, $\chi2$=0.02; df = 1; *P*=0.89, NA = 12	attracted SYMBIONT 39/21, $\chi2$=5.4; df = 1; *P*=0.02, NA = 6	no response 119/114, $\chi2$=0.11; df = 1; *P*=0.74, NA = 102	attracted 42/18, $\chi2$=9.40; df = 1; *P*=0.002, NA = 5
Xyleborinus saxesenii	repelled 56/98, $\chi2$=11.45; df = 1; P =0.001, NA = 50	no response 57/78, $\chi2$=3.23; df = 1; *P*=0.07, NA = 51	no response 43/63, $\chi2$=3.77; df = 1; *P*=0.05, NA = 54	repelled 36/74, $\chi2$=13.13; df = 1; *P*<0.001, NA = 53	no response 34/26, $\chi2$=1.07; df = 1; *P*=0.3, NA = 20

Acknowledgement Nina Rountree, Jorge Peña, Angel Hoyte, Wendy Meyer and Ian W. Jackson supported the project. JH thanks Jaime Collazo and Rob Dunn for support. This project was partially funded by a USDA-SCRI grant to LLS.

References

GARDES, M. and BRUNS, T. D. 1993. ITS primers with enhanced specificity of basidiomycetes: application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2:113–118.

HANULA, J. L. and SULLIVAN, B. 2008. Manuka oil and phoebe oil are attractive baits for Xyleborus glabratus (Coleoptera Scolytinae), the Vector of Laurel Wilt. *Environ. Entomol.* 37:1403–1409.

HANULA, J. L., MAYFIELD, A. E., FRAEDRICH, S. W., and RABAGLIA, R. J. 2008. Biology and host associations of Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae), exotic vector of laurel wilt killing Redbay trees in the Southeastern United States. *J. Econ. Entomol.* 101:1276–1286.

HARRINGTON, T. C., AGHAYEVA, D. N., and FRAEDRICH, S. W. 2010. New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. *Mycotaxon* 111:337–361.

HULCR, J. and DUNN, R. R. 2011. The sudden emergence of pathogenicity in insect-fungus symbiosis threatens naïve forest ecosystems. *Philos. Trans. R. Soc. Lond. Ser. B* 278:2866–2873.

KENDRA, P. E., MONTGOMERY, W. S., NIOGRET, J., PEÑA, J. E., CAPINERA, J. L., BRAR, G., EPSKY, N. D., and HEATH, R. R. 2011. Attraction of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) to avocado, lychee, and essential oil lures. *J. Chem. Ecol.* 37:932–942.

KOLARIK, M. and HULCR, J. 2008. Mycobiota associated with the ambrosia beetle *Scolytodes unipunctatus* (Coleoptera: Curculionidae, Scolytinae). *Mycol. Res.* 113:44–60.

MANN, R. S., ROUSEFF, R. L., SMOOT, J. M., CASTLE, W. S., and STELINSKI, L. L. 2011. Sulfer volatiles from *Allium* spp. affect Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) response to citrus volatiles. *Bull. Entomol. Res.* 101:89–97.

RANGER, C., REDING, M., PERSAD, A., and HERMS, D. 2010. Ability of stress-related volatiles to attract and induce attacks by *Xylosandrus germanus* (Coleoptera: Curculionidae, Scolytinae) and other ambrosia beetles. *Agr. For. Entomol.* 12:177–185.

SIX, D. L., STONE, W. D., DE BEER, Z. W., and WOOLFOLK, S. W. 2009. *Ambrosiella beaveri*, sp. nov., associated with an exotic ambrosia beetle, *Xylosandrus mutilatus* (Coleoptera: Curculionidae, Scolytinae), in Mississippi, USA. *Antonie. Leeuwenhoek* 96:17–29.

WHITE, T. J., BRUNS, T., LEE, S., and TAYLOR, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322, *in* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), PCR protocols: a guide to methods and applications. Academic Press, Inc, New York.