Ecology and evolution of symbiotic microbial communities in fungus-farming ambrosia beetles*

Maximilian Lehenberger

Max-Planck-Institut für Chemische Ökologie

Zusammenfassung: Ökologie und Evolution von symbiotischen mikrobiellen Gemeinschaften in Pilz-züchtenden Ambrosiakäfern

All unsere heimischen Borken- und Ambrosiakäfer sind eng mit den verschiedensten Mikroben assoziiert, die ihnen unter anderem helfen, das Phloem oder Splintholz von Bäumen zu besiedeln und so das Überleben der Käfer sichern. Hier spielen vor allem filamentöse Pilze, aber auch Hefen und Bakterien eine essentielle Rolle. Obwohl diese Symbionten maßgeblich am Überleben der Käfer beteiligt sind, wissen wir nur wenig über ihre genauen Funktionen und wie sie am Erfolg der Käfer beteiligt sind. Im Rahmen meines Promotionsprojektes untersuchte ich eine Vielzahl an unterschiedlichen Thematiken und konnte dabei neue und grundlegende Erkenntnisse über die Natur dieser Symbiose erlangen. Im Einzelnen untersuchte ich (i) die Assoziation heimischer *Trypodendron*-Arten mit ihren Pilzen sowie ihr Potential zum Holzabbau, (ii) das Pilzsporenorgan „Mycetangium" einer *Trypodendron* Art mittels Mikro-Computertomographie (µCT) , (iii) die Ethanol-Toleranz von unterschiedlichen Borken- und Ambrosiakäfer-Pilzen, (iv) die Anreicherung von natürlichen Ambrosiakäfer-Nestern mit essentiellen Elementen, sowie (**v**) die Zusammensetzung von volatilen Substanzen, welche von unterschiedlichsten Pilzen abgegeben werden.

Durch meine Studien konnte ich die (i) Assoziation der europäischen *Trypodendron* Ambrosiakäfer mit mutualistischen Pilzen in der Gattung *Phialophoropsis* bestätigen und fand Hinweise auf eine neue *Phialophoropsis* Art in zwei der vier heimischen *Trypodendron* Arten. Außerdem konnte ich zeigen, dass diese mutualistischen Pilze wesentliche Polysaccharide im Holz, wie etwa Cellulose und Xylan, abbauen können. Zusätzlich untersuchte ich (ii) erstmals die Pilzsporen übertragende Struktur „Mycetangium" des Ambrosiakäfers *Trypodendron laeve* und konnte durch den Einsatz von µCT die ersten detaillierten anatomischen Aufnahmen bereitstellen. Außerdem bestätigten und ergänzten meine Studien, (iii) dass Ethanol-Toleranz unter Ambrosiakäfer-Pilzen eine wohl sehr weitverbreitete Besonderheit ist, wohingegen antagonistische Schadpilze schon durch geringe Ethanol-Konzentrationen gehemmt werden. Interessanterweise konnte ich zum ersten Mal zeigen, dass auch nahezu alle mit Borkenkäfern assoziierten Pilze stark negativ durch Ethanol beeinflusst werden. Durch die erstmalige Verwendung von Energiedispersiver Röntgenspektroskopie – Elektronenmikroskopie (EDX-REM) fand ich heraus, (iv) dass natürliche Brutsysteme von Ambrosiakäfern, im Gegensatz zum unbesiedelten Splintholz, sehr stark mit verschiedenen essentiellen Elementen (wie etwa N, P, S, K und Mg) angereichert sind. Dies ist vermutlich den Pilzsymbionten zu verdanken, welche Elemente aus unmittelbar umliegendem Splintholz abziehen und diese in den Brutsystemen der Käfer anreichern. Schließlich fand ich heraus, (v) dass nahezu alle untersuchten Borken- und Ambrosiakäfer-Pilze eine Vielzahl an flüchtigen Inhaltsstoffen produzieren, welche sich hier vor allem in die Gruppe der aliphatischen und aromatischen Alkohole und Ester eingliedern. Nicht-mutualistische Pilze sowie freilebende Arten produzierten im Vergleich eine geringere Anzahl an unterschiedlichen flüchtigen Inhaltsstoffen und emittierten zumeist geringere Mengen.

* Ph.D. Projekt, betreut von Prof. Dr. Peter Biedermann,
Lehrstuhl für Forstentomologie und Waldschutz, Albert-Ludwigs-Universität Freiburg, Germany

Keywords: ambrosia beetle, bark beetle, fungi, microbes, volatile organic compounds, ethanol, nutrition, antimicrobial, mycetangia, wood-degrading enzymes, polysaccharides

Maximilian Lehenberger, Max-Planck-Institut für Chemische Ökologie, Department für Biochemie, Hans-Knöll-Straße 8, 07745 Jena, Deutschland,
E-Mail: mlehenberger@ice.mpg.de

Introduction

Bark beetles (Curculionidae: Scolytinae) are a highly diverse group of insects encompassing more than 6000 described species worldwide, with the highest species diversity in tropical and subtropical areas (Blackman 1941, Raffa & al. 2015 and references therein). They are all known to colonize healthy and/or dead or weakened woody tissues from the major groups of terrestrial plants (Kirkendall & al. 2015). This wood-boring trait of weevils is quite old and originated multiple times at least around 90 – 120 million years ago (McKenna & al. 2009, Jordal & al. 2011, Haran & al. 2013, Gillett & al. 2014). However, the term "bark beetle" is jointly used to combine multiple species with two completely different life strategies: (i) the colonization of outer bark and phloem by bark beetles (*sensu lato*) and (ii) the colonization of sapwood (xylem) of woody plants by their descendants, the fungus-farming ambrosia beetles (*sensu stricto*) (Biedermann & Vega 2020). Phloem tissue has been described as the ancestral habitat for bark beetles in general, while the colonization of the xylem was only possible (and likely needed because of highly competitive pressure within phloem) through the evolution of mutualism with fungi (Kirkendall & al. 2015, Biedermann & Vega 2020).

Conclusively, bark and ambrosia beetles are closely related and are thus sharing multiple traits. They mostly forage in the dark, have to deal with several ubiquitous fungal antagonists, are highly adapted to their specific niche, and are associated with various microorganisms like bacteria, yeasts, and filamentous fungi (see Kirkendall & al. 2015). In most systems, filamentous fungi are playing an important role as they can fulfill essential functions such as nutritional or tree-defenses-detoxifying mutualists and tree-defenses-stimulating fungi (e.g. Six 2003, Kandasamy & al. 2019, Biedermann & Vega 2020). Here, bark beetles are generally described to be associated with certain facultative mutualistic fungi, while ambrosia beetles have long been known for their species-specific, obligate nutritional mutualism with filamentous fungi (Kirkendall & al. 2015, Biedermann & Vega 2020). Typically, the transportation of these mutualistic fungi by the beetles is maintained over generations by vertical transmission. Fungi are vectored in gland-rich pouches, the so-called mycetangia or mycangia, which are highly specialized and possibly selective structures (Francke-Grosmann 1956, Batra 1963, Six 2003). To date, scientists are aware of at least 13 evolutionary independent origins of fungus farming within the polyphyletic ambrosia beetles (~ 3,400 species worldwide) (Hulcr & Stelinski 2017, Biedermann & Vega 2020).

Besides ambrosia beetles, only two other groups of insects are known for their advanced fungus-farming (including active cooperative care of fungal cultures): the famous attine ants and the fungus-farming macro termites (Farrell & al. 2001, Müller & al. 2002). In contrast to the latter ones, ambrosia beetles colonize the sapwood of woody plants, which are typically recently dead or in an unhealthy state (Kirkendall & al. 2015). However, the infestation of woody tissue such as sapwood is a very challenging task as it is generally known to be chemically well-defended in living and in weakened trees. Further, it is an extremely nutrient-poor substrate, which is additionally biased toward certain elements, particularly carbon (C), hydrogen (H), and oxygen (O) (Filipiak & Weiner 2014, Pretzsch & al. 2014). To survive in such an extreme environment, ambrosia beetles are necessarily depending on species-specific mutualistic fungi, which they actively farm in often social family groups within their excavated galleries (Francke-Grosmann 1967, Kirkendall & al. 2015).

Besides this, the detailed role of the majority of associated fungi remains unclear within most beetle systems. Some are well-known mutualists, others may have beneficial functions while the role of some frequently isolated species is still not clear. In ambrosia beetles, only about 5 % of fungal associates are described so far (Hulcr & Stelinski 2017) and the role of several other associated fungi, yeasts, and

bacteria is even less studied (Haanstad & Norris 1985). This indicates the importance of research on fungal associates of ambrosia beetles, but also of bark beetle-associated fungi, where even fewer species were examined in detail (such as *Ips typographus* and *Dendroctonus* spp.). Within my Ph.D. project, I aimed to tackle long-standing questions on the physiological mechanisms of beetle-associated fungi that may maintain the symbiosis with their beetle hosts. Therefore, I included a broad range of ambrosia and bark beetle fungi as well as several free-living, non-mutualistic fungi in my projects to understand, compare, and highlight evolutionary mechanisms within fungus-associated scolytinae.

New aspects within the Trypodendron – Phialophoropsis mutualism

Until recently, the mutualistic association of the European ambrosia beetle genus *Trypodendron*, with the fungal genus *Phialophoropsis* (Microascales) was not safely established when I started my work. *Trypodendron* is known to cause economic damage in harvested and stored conifer and broadleaf trees (e.g., Dyer & Chapman 1965, Parini & Petercord 2006) and the most common European ambrosia beetles from this genus are *T. lineatum*, *T. signatum*, as well as *T. domesticum*. Indeed, a fourth species exists (*T. leave*) which is widespread, but relatively rare and challenging to find (Bussler & Schmidt 2008). Especially *T. lineatum* is well studied and was known to be associated with the fungus *P. ferruginea* quite some time ago (Mathiesen-Käärik 1953, Francke-Grosmann 1956, Mayers & al. 2015). Although similar fungi were isolated out of *T. domesticum* and *T. signatum* in past studies (Nakashima & al. 1992, Mayers & al. 2015), an association could not be safely confirmed due to the low number of isolations and examined beetles. Additionally, mycetangial structures are well known for three of these species (Francke-Grosmann 1956), but were not investigated for *T. leave* yet.

Here, I confirmed the association of the European ambrosia beetle genus *Trypodendron* with the fungal genus *Phialophoropsis* and additionally found species-specific differences between the fungal isolates of *T. lineatum* and *T. domesticum*/*T. signatum* (Lehenberger & al. 2018) using multiple genetic markers. *T. lineatum* was found to be associated with *P. ferruginea*, while *T. signatum,* as well as *T. domesticum,* are associated with a possible new species in the genus *Phialophoropsis*. Indeed, a recent study confirmed my results that both *T. domesticum* and *T. signatum* are sharing the same fungal symbiont, which was annotated as a new *Phialophoropsis* species (Mayers & al. 2020). In aim to transmit their *Phialophoropsis* symbionts to freshly established nests, the beetles rely on specific mycetangial structures, which were examined in detail by Francke-Grosmann (1956) for three of the four European *Trypodendron* species. Through my work, I provided the first µCT images of the mycetangium of *T. leave*, which was previously unknown (Lehenberger & al. 2019). These analyses revealed a paired, tubular, pleural-prothoracic mycetangium, which is typical for *Trypodendron* ambrosia beetles (see Francke-Grosmann 1956).

Nutritional benefits of the ambrosia beetle symbiosis

Fungus-farming ants and termites have to provide huge amounts of plant biomass for the cultivation of their fungi, which they actively collect and transfer to their colonies. In contrast, ambrosia beetles are not leaving their nests for almost their entire life cycle (Kirkendall & al. 2015). They highly depend on microbes to help them to decompose the major carbon sources in woody tissue such as cellulose, hemicelluloses, pectin, and lignin as these compounds are indigestible by insects (Douglas 2009). Filamentous fungi are of crucial importance as they can forage deeper into the wood. However, ambrosia beetle fungi are generally believed to be rather poor wood decomposers, unable to grow far into the xylem (< 2 – 3 mm) (Francke-Grosmann 1966). Their ability to colonize and decompose wood has only been poorly investigated and several questions remain open.

Here, I first examined ambrosia beetle fungi for their wood-degrading potential and showed that *Phialophoropsis* fungi can express robust amounts of endo-β-1,4-xylanase and endo-β-1,4-glucanase (Lehenberger & al. 2018). This indicates their ability to degrade and metabolize particularly cellulose and xylan as a carbon source. However, the species in the genus *Phialophoropsis* are among the very few ambrosia beetle fungi (e.g., *Fusarium euwallaceae*) which are thought or known to forage deeper within the xylem (Francke-Grosmann 1966, Eskalen & al. 2013). To date, the importance of wood-degrading enzymes excreted by bark and ambrosia beetle fungi remains open as representative studies

including other fungal mutualists are missing. Possibly, several other mutualistic fungi are receiving only low amounts (or none) of carbon out of cellulose and hemicelluloses, and instead are focusing on other woody polysaccharides such as pectin as already suggested by Francke-Grosmann (1966).

Besides carbon, other elements such as nitrogen, sulfur, phosphorous, calcium, and potassium are also highly needed by fungi and beetles but are only available in low proportions in woody tissue (Fengel & Wegener 1989, Filipiak & al. 2016). My studies showed that natural galleries of ambrosia beetles are highly enriched with several elements in contrast to the immediate surrounding xylem and completely uncolonized xylem (Lehenberger & al. 2021a). This is especially interesting as only nitrogen might be provided by N-fixing bacteria, while none of the other elements is fixated out of the atmosphere, and galleries are isolated from the external environment (e.g., Peklo & Satava 1949, Bridges 1981). This indicates that the woody tissue itself must function as a source of these elements. Here, mutualistic ambrosia beetle fungi would be good candidates for translocating and concentrating elements from the immediate surrounding xylem (< 2 mm) within the beetle's galleries as recently shown within a *Dendroctonus* bark beetle system (Six & Elser 2019, 2020). In contrast to other symbiotic microbes, such as unicellular yeasts and bacteria, filamentous fungi can forage deeper inside the wood. This enables them to translocate certain elements out of the immediate surrounding xylem, which is fed by the elemental pipeline of the colonized tree between roots and leaves (and *vice versa*) (see Matyssek & al. 2010).

Beetle-associated fungi emit a huge variety of volatile organic compounds (VOC)

In bark and ambrosia beetle systems, it has been a long-standing question, how and if they can distinguish among certain fungi within their galleries since this would be a highly beneficial adaption to locate their mutualistic fungi and separate between antagonistic microbes. The ambrosia beetle *X. saxesenii* was observed to actively remove fungal antagonists and thus, to prevent these competitive fungi from spreading (Biedermann & al. 2013, Nuotclà & al. 2019), but how the beetles are informed about the presence of such fungi remains mostly unknown. Nowadays, it is well known for some beetle-fungus symbioses, that olfaction plays a role. As shown within past studies (e.g., Hulcr & al. 2011, Kandasamy & al. 2016, 2019), volatile organic compounds can have attractive or repellent effects on beetles. In bark beetles, the study by Kandasamy & al. (2019) highlights the crucial functions of fungal volatiles in maintaining the symbiosis of *Ips typographus* with its fungal symbionts. However, our knowledge about volatiles emitted by other bark, and especially ambrosia beetle fungi is highly limited, and only a few species were examined so far.

In my studies, I screened a huge variety of different fungal bark and ambrosia beetle associates as well as antagonistic fungi for their volatile profile. I showed that most mutualistic symbionts of bark and ambrosia beetles mainly emit aliphatic and aromatic alcohols and esters similar to those described by Kandasamy & al. 2016, 2019 for bark beetle fungi (Lehenberger & al., in preparation). In contrast, antagonistic fungi emitted a lower number as well as lower amounts of volatiles. This finding is fascinating as it indicates a crucial role of fungal volatiles within several widespread bark and ambrosia beetle systems and has the potential to be one of the main drivers of the symbiosis. Additionally, the study on fungal volatile organic compounds associated with bark and ambrosia beetles can serve as the base for developing olfaction-based management strategies for several beetle species.

Novel insights into the suppression of antagonistic fungi in ambrosia beetle systems

If aggressive and fast-growing antagonistic fungi such as *Chaetomium globosum* (in case of ambrosia beetles) or *Trichoderma harzianum* (in case of bark beetles) are becoming more dominant within beetle's galleries, the risk that beetles and larvae are either dying or are at least performing poorly is quite high (e.g., Shakeri & Foster 2007, Nuotclà & al. 2019). However, it mostly remains unclear for the majority of bark and ambrosia beetles, how (and if in the case of several bark beetles) they protect their fungal cultivars against antagonistic "weedy" fungi. Past studies on the primitively eusocial *X. saxesenii* revealed that especially larvae are cleaning colony members as well as the walls in their galleries, which minimizes the abundance of certain antagonistic fungi (Biedermann & al. 2013). Further, it has been shown that as soon as ambrosia beetles are leaving the natal nest, antagonistic fungi are overgrowing the ambrosia beetle cultivars (Hubbard 1897, Francke-Grosmann 1956). Apparently,

the beetles themselves have a great influence on the composition of their fungal gardens. However, a recent study showed that the beetles are not solely suppressing antagonistic fungi (Ranger & al. 2018). Ethanol, which is naturally emitted from host trees, plays a major function in a process described as environmental screening. Ethanol-tolerant species are benefitting, while species sensitive to ethanol are inhibited. For ambrosia beetles, ethanol is also serving as an indicator for susceptible breeding substrate (e.g., Kelsey & al. 2014, Ranger & al. 2015).

Within my studies, I confirmed the ethanol tolerance in ambrosia beetle fungi for a broader range of former unstudied fungal species (Lehenberger & al. 2021b), suggesting that ethanol tolerance might be a widespread trait within several ambrosia beetle systems. Further, examined fungal antagonists were highly inhibited by ethanol, which indicates the mechanism of environmental screening (ambrosia beetle fungi benefit, while antagonists are suppressed). Interestingly, the majority of tested bark beetle fungi were highly inhibited through even low concentrations of ethanol. This is especially surprising as ethanol also naturally occurs within the phloem of trees and is additionally also produced by both bark and ambrosia beetle fungi (Lehenberger & al. in preparation). Ethanol is a general metabolic product in fungi, deriving from fermentation processes (degradation of sugars as carbon source) under oxygen-limited conditions and serves as the base for the production of ethyl acetate (e.g., Wang & al. 2016, Holt & al. 2019). Ethanol (as well as acetate) can further be used as the base for the production of higher carbohydrates (see Strijbis & Distel 2010). Therefore, it might be possible that the studied bark beetle fungi are more efficient in sugar degradation and may additionally utilize more ethanol for other processes compared to ambrosia beetle fungi.

Besides ethanol, also volatiles emitted from fungi might function as suppressors of antagonistic microbes (see also Caballero-Ortiz & al. 2018 for yeast volatiles). Several volatiles, which I identified in my studies, are known to have antifungal capabilities (Lehenberger & al. in preparation) and inhibitory effects of certain volatiles towards competitive fungi were already observed within other fungus–fungus interactions (see Amin & al. 2010, Nishino & al. 2013). However, in bark and ambrosia beetle systems, similar mechanisms mostly remain unknown to date.

Acknowledgments

I especially would like to thank Prof. Peter Biedermann for bringing ambrosia beetles and their fungi into my world, for supervising my Ph.D. thesis, and for all the great discussions in the past. Further, I am thankful to the Zoology III department of the University of Würzburg and all further colleagues of the Insect-Fungus lab / Chair of Forest Protection and Entomology of the University of Freiburg. I want to thank Dineshkumar Kandasamy and Prof. Jonathan Gershenzon for their great support during my time at the Max Planck Institute for Chemical Ecology in Jena as well as Nina Foh for her help with the EDX-SEM measurements at the FAU in Nürnberg, and of course also all other co-authors for their great work and support. Finally, I would like to thank my family and my wife Pam for their help and support during the past three years.

Reference List

Amin, F., Razdan, V., Mohiddin, F., Bhat, K. & Sheikh, P. (2010): Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens in-vitro. – Journal of Phytology, **2**(10).

Batra, L. R. (1963): Ecology of ambrosia fungi and their dissemination by beetles. – Transactions of the Kansas Academy of Science (1903-), **66**(2): 213-236.

Biedermann, P.H.W., Klepzig, K.D., Taborsky, M. & Six, D.L. (2013): Abundance and dynamics of filamentous fungi in the complex ambrosia gardens of the primitively eusocial beetle *Xyleborinus saxesenii* Ratzeburg (Coleoptera: Curculionidae, Scolytinae). – FEMS Microbiology Ecology, **83**(3): 711-723.

Biedermann, P.H. & Vega, F.E. (2020): Ecology and evolution of insect-fungus mutualisms. – Annual review of entomology, **65**: 431-455.

Blackman, M.W. (1941): Bark beetles of the genus *Hylastes* Erichson in North America (Vol. 415). US Department of Agriculture.

Bridges, J.R. (1981): Nitrogen-fixing bacteria associated with bark beetles. – Microbial Ecology, **7**(2): 131-137.

Bussler, H. & Schmidt, O. (2008): *Trypodendron leave* Eggers, 1939 - ein wenig bekannter Nutzholzborkenkäfer. – Forstschutz aktuell, **45**:11-13.

Caballero-Ortiz, S., Trienens, M., Pfohl, K., Karlovsky, P., Holighaus, G. & Rohlfs, M. (2018): Phenotypic responses to microbial volatiles render a mold fungus more susceptible to insect damage. – Ecology and Evolution, **8**(8): 4328-4339.

Douglas, A.E. (2009): The microbial dimension in insect nutritional ecology. – Functional Ecology, **23**(1): 38-47.

Dyer, E. & Chapman, J. (1965): Flight and attack of the ambrosia beetle, *Trypodendron lineatum* (Oliv.) in relation to felling date of logs. 1. – The Canadian Entomologist, **97**(1): 42-57.

Eskalen, A., Stouthamer, R., Lynch, S. C., Rugman-Jones, P. F., Twizeyimana, M., Gonzalez, A. & Thibault, T. (2013): Host range of *Fusarium* dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. – Plant Disease, **97**(7): 938-951.

Farrell, B.D., Sequeira, A.S., O'Meara, B.C., Normark, B.B., Chung, J.H. & Jordal, B.H. (2001): The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). – Evolution, **55**(10): 2011-2027.

Fengel, D. & Wegener, G. (1989): Wood: chemistry, ultrastructure, reactions. Walter de Gruyter.

Filipiak, M. & Weiner, J. (2014): How to make a beetle out of wood: multi-elemental stoichiometry of wood decay, xylophagy and fungivory. – PLoS One, **9**(12):115104.

Filipiak, M., Sobczyk, Ł. & Weiner, J. (2016): Fungal transformation of tree stumps into a suitable resource for xylophagous beetles via changes in elemental ratios. – Insects, **7**(2): 13.

Francke-Grosmann, H. (1956): Hautdrüsen als Träger der Pilzsymbiose bei Ambrosiakäfern. – Zeitschrift für Morphologie und Ökologie der Tiere, **45**: 275-308.

Francke-Grosmann, H. (1966): Über Symbiosen von xylo-mycetophagen und phloeophagen Scolitoidea mit holzbewohnenden Pilzen. – Mater. Org. **1**: 503-522.

Francke-Grosmann, H. (1967): Ectosymbiosis in wood-inhabiting insects. – Symbiosis, Academic Press: 141-205.

Gillett, C. P., Crampton-Platt, A., Timmermans, M. J., Jordal, B. H., Emerson, B. C., & Vogler, A. P. (2014): Bulk de novo mitogenome assembly from pooled total DNA elucidates the phylogeny of weevils (Coleoptera: Curculionoidea). – Molecular biology and evolution, **31**(8): 2223-2237.

Haanstad, J.O. & Norris, D.M. (1985): Microbial symbiotes of the ambrosia beetle *Xyletorinus politus*. – Microb Ecol, **11**(3): 267-276.

Haran, J., Timmermans, M.J. & Vogler, A.P. (2013): Mitogenome sequences stabilize the phylogenetics of weevils (Curculionoidea) and establish the monophyly of larval ectophagy. – Molecular Phylogenetics and Evolution, **67**(1): 156-166.

Holt, S., Miks, M.H., de Carvalho, B.T., Foulquié-Moreno, M.R. & Thevelein, J.M. (2019): The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. – FEMS microbiology reviews. **43**(3): 193-222.

Hubbard, H. V. (1897): The ambrosia beetles of the United States. – U.S. Dep. Agric. Div. Ent. Bull, **7**: 9-13.

Hulcr, J., Mann, R. & Stelinski, L.L. (2011): The scent of a partner: Ambrosia beetles are attracted to volatiles from their fungal symbionts. – Journal of Chemical Ecology, **37**(12): 1374-1377.

Hulcr, J. & Stelinski, L.L. (2017): The ambrosia symbiosis: from evolutionary ecology to practical management. – Annual review of entomology, **62**: 285-303.

Jordal, B.H., Sequeira, A.S. & Cognato, A.I. (2011): The age and phylogeny of wood boring weevils and the origin of subsociality. – Molecular Phylogenetics and Evolution, **59**(3): 708-724.

Kandasamy, D., Gershenzon, J. & Hammerbacher, A. (2016): Volatile organic compounds emitted by fungal associates of conifer bark beetles and their potential in bark beetle control. – Journal of chemical ecology, **42**(9): 952-969.

Kandasamy, D., Gershenzon, J., Andersson, M.N., & Hammerbacher, A. (2019): Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (*Ips typographus*) with its fungal symbionts. – The ISME Journal, **13**(7): 1788-1800.

Kelsey, R.G., Gallego, D., Sánchez-Garcá, F. & Pajares, J. (2014): Ethanol accumulation during severe drought may signal tree vulnerability to detection and attack by bark beetles. – Canadian journal of forest research, **44**(6): 554-561.

Kirkendall, L.R., Biedermann, P.H., & Jordal, B.H. (2015): Evolution and diversity of bark and ambrosia beetles. – Bark beetles, Academic Press: 85-156.

Lehenberger, M., Biedermann, P.H., & Benz, J.P. (2018): Molecular identification and enzymatic profiling of *Trypodendron* (Curculionidae: Xyloterini) ambrosia beetle-associated fungi of the genus *Phialophoropsis* (Microascales: Ceratocystidaceae). – Fungal Ecology, **38**: 89-97.

Lehenberger, M., Thalhammer, M., Benz, J.P. & Biedermann, P. (2019): Take good care of my fungi – Fungus-spore carrying organs in *Tryopodendron* ambrosia beetles. – Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, **22**: 195-198.

Lehenberger, M., Foh, N., Göttlein, A., Six, D. & Biedermann, P.H. (2021a): Nutrient-poor breeding substrates of ambrosia beetles are enriched with biologically important elements. – Frontiers in Microbiology, **12**: 664542.

Lehenberger, M., Benkert, M. & Biedermann, P.H. (2021b): Ethanol-enriched substrate facilitates ambrosia beetle fungi, but inhibits their pathogens and fungal symbionts of bark beetles. – Frontiers in microbiology, **11**: 3487.

Lehenberger, M., Kandasamy, D., Edelmann, P., Hammerbacher, A.D., Gershenzon, J. & Biedermann, P.H. (in preparation): Working title: Volatiles and amino acids in bark and ambrosia beetle fungi

Mathiesen-Käärik, A. (1953): Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. – Meddelanden från Statens Skogsforskningsinstitut, **43**(4):1-74.

Matyssek, R., Fromm, J., Rennenberg, H. & Roloff A. (2010): Biologie der Bäume: von der Zelle zur globalen Ebene. UTB 8450.

Mayers, C., Mcnew, D., Harrington, T., Roeper, R.W., Fraedrich, S., Biedermann, P., Castrillo, L. & Reed, S. (2015): Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. – Fungal Biology, **119**(11): 1075-1092.

Mayers, C.G., Harrington, T.C., Mcnew, D.L., Roeper, R.A., Biedermann, P.H., Masuya, H. & Bateman, C.C. (2020): Four mycangium types and four genera of ambrosia fungi suggest a complex history of fungus farming in the ambrosia beetle tribe Xyloterini. – Mycologia, **112**(6): 1104-1137.

McKenna, D.D. (2011): Towards a temporal framework for "Inordinate Fondness": reconstructing the macroevolutionary history of beetles (Coleoptera). – Entomologica Americana, **117**(1): 28-36.

Müller, Gerardo, N.M., Aanen, D.K., Six, D.L. & Schultz, T.R. (2002): The evolution of agriculture in insects. – Annual Review of Ecology Evolution and Systematics, **36**(1): 563-595.

Nakashima, T., Otomo, T., Owada, Y. & Lizuka, T. (1992): Sem observations on growing conditions of the fungi in the galleries of several ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). – Journal of the Faculty of Agriculture, Hokkaido University, **65**(3): 239-273.

Nishino, S., Parada, R.Y., Ichiyanagi, T., Maekawa, N., Shimomura, N. & Otani, H. (2013): 1-phenyl-3-pentanone, a volatile compound from the edible mushroom *Mycoleptodonoides aitchisonii* active against some phytopathogenic fungi. – Journal of Phytopathology, **161**(7-8): 515-521.

Nuotclà, J.A., Biedermann, P.H. & Taborsky, M. (2019): Pathogen defence is a potential driver of social evolution in ambrosia beetles. – Proceedings of the Royal Society B; **286**(1917): 20192332.

Parini, C. & Petercord, R. (2006): Der Laubnutzholzborkenkäfer *Trypodendron domesticum* als Schädling der Rotbuche. – Mitteilungen aus der Forschungsanstalt für Waldökologie und Forstwirtschaft Rheinland-Pfalz, **59**(06): 63-77.

Peklo, J. & Satava, J. (1949): Fixation of free nitrogen by bark beetles. – Nature, **163**: 336-337.

Pretzsch, H., Block, J., Dieler, J., Gauer, J., Göttlein, A, Moshammer, R., Schuck. J., Wendelin, W. & Wunn, U. (2014): Nährstoffentzüge durch die Holz- und Biomassenutzung in Wäldern. Teil 1: Schätzfunktionen für Biomasse und Nährelemente und ihre Anwendung in Szenariorechnungen. – Allgemeine Forst-u. Jagdzeitung, **185**(11/12): 261-285.

Raffa, K.F., Gregoire, J.C., & Lindgren, B.S. (2015): Natural history and ecology of bark beetles. – In: Bark beetles, Academic Press: 1-40.

Ranger, C.M., Schultz, P.B., Frank, S.D., Chong, J.H. & Reding, M.E. (2015): Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. – PLoS One, **10**(7): e0131496.

Ranger, C.M., Biedermann, P.H., Phuntumart, V., Beligala, G., Ghosh, S., Palmquist, D.E., Mueller, R., Barnett, J., Schultz, P.B., Reding, M.E. & Benzi, J.P. (2018): Symbiont selection via alcohol benefits fungus farming by ambrosia beetles. – Proceedings of the National Academy of Sciences, **115**(17): 4447-4452.

Shakeri, J. & Foster, H.A. (2007): Proteolytic activity and antibiotic production by *Trichoderma harzianum* in relation to pathogenicity to insects. – Enzyme and Microbial Technology, **40**(4): 961-968.

Six, D.L. & Elser, J.J. (2019): Extreme ecological stoichiometry of a bark beetle – fungus mutualism. – Ecological Entomology, **44**(4): 543-551.

Six, D.L. (2003): Bark beetle-fungus symbioses. – Insect symbiosis, **1:** 97-114.

Six, D.L. & Elser, J.J. (2020): Mutualism is not restricted to tree-killing bark beetles and fungi: the ecological stoichiometry of secondary bark beetles, fungi, and a scavenger. – Ecological Entomology, **45**(5): 1134-1145.

Strijbis, K. & Distel, B. (2010): Intracellular acetyl unit transport in fungal carbon metabolism. – Eukaryotic cell, **9**(12):1809-1815.

Wang, J., Suzuki, T., Dohra, H., Takigami, S., Kako, H., Soga, A., Kamei, I., Mori, T., Kawagishi, H. & Hirai, H. (2016): Analysis of ethanol fermentation mechanism of ethanol producing white-rot fungus *Phlebia* sp. mg-60 by RNA-seq. – BMC genomics, **17**(1):1-11.