Perception of host odours by forest pests: comparison of a wood breeding beetle (*Monochamus galloprovincialis*) and a bark breeding beetle (*Phaenops cyanea*)

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Abstract: Wahrnehmung von Wirtsdüften durch Forstschädlinge: Vergleich zwischen einem Holzbrüter und einem Rindenbrüter.

Die Forstschädlinge *Phaenops cyanea* (Coleoptera: Buprestidae) und *Monochamus galloprovincialis* (Coleoptera: Cerambycidae) befallen beide Kiefern (*Pinus sylvestris*), die geschwächt sind, z.B. durch Wasser-Stress. Die Larven von *P. cyanea* entwickeln sich in oder unter der Rinde, während Larven von *M. galloprovincialis* nach einem Fraß im Kambium des befallenen Baumes auch tief in das Holz eindringen. *P. cyanea* befällt bereits Bäume, die nur einem geringen Stress ausgesetzt waren, während *M. galloprovincialis* Bäume bevorzugt, die erheblich geschwächt oder bereits tot sind. Die vorliegende Studie soll anhand elektrophysiologischer Experimente untersuchen, welchen olfaktorischen Hinweisen die beiden Insekten bei ihrer Suche nach einem geeigneten Eiablageplatz folgen. Duftstoffe von *Pinus sylvestris* wurden auf Aktivkohle gesammelt und mit Gaschromatographie, Massenspektroskopie und Elektroantennographie untersucht (GC-MS/ EAD). Die stärksten Signale von *P. cyanea* traten im Retentionsindex-Bereich von 936 (α-Pinen) bis 1200 auf, wobei insbesondere bizyklische Terpene und Terpenoide wie α-Pinen, β-Pinen, 1,8-Cineol und trans-Verbenol detektiert wurden. *M. galloprovincialis* reagierte auf einen weiteren Bereich von Stoffen, ebenfalls beginnend mit α-Pinen, bis hin zu Stoffen mit einem Retentionsindex von ca. 1300. Es scheint eine spezifische Empfindlichkeit für monozyklische und azyklische Terpene und Terpenoide vorzuliegen, insbesondere für γ-Terpinen, Terpinolen, β-Myrcen und p-Cymen.

Key words: *Monochamus galloprovincialis,* Cerambycidae, *Phaenops cyanea*, Buprestidae, Electroantennography, GC-MS, GC-EAD

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The blue pine wood borer (*Phaenops cyanea*) and the black pine sawyer beetle (*Monochamus galloprovincialis*) (Fig. 1) both are pests of the white pine (*Pinus silvestris*) and other *Pinus* species.

Both insects have nearly the same demands regarding their breeding site. Larval development requires a fresh, unwilted inner bark. An infestation occurs on freshly cut trees or on trees suffering from stress (e.g. after dry seasons, loss of needles caused by feeding caterpillars or damage by forest fires).

Phaenops cyanea detects susceptible pines by their volatile emissions (SCHÜTZ et al. 2004) and is able to infest the trees already at a low stress level. During feeding the larvae avoid the resin ducts of the tree and thus evade the oleoresin defence. The beetle is endemic in Europe and – under favourable climatic conditions – can cause substantial damage to pine forests. It is the most significant bark-breeding beetle of white pine in the lowlands of north-eastern Germany.

Monochamus galloprovincialis is found in Europe and northern Africa. The larvae tend to a more copious feeding which makes them more susceptible to the oleoresin defence of the tree. Thus, *M. galloprovincialis* prefers trees that are weakened by a higher degree of stress. The economic damage caused by feeding of the beetle is low. However, the beetle has gained a special attention of forest scientists because of its association with the nematode *Bursaphelenchus xylophilus* which is causing the pine wilt disease (PWD) in *Pinus*. The only outbreak of the PWD within Europe is limited to an area of 258.000 ha in Portugal. (MOTA et al. 1999).

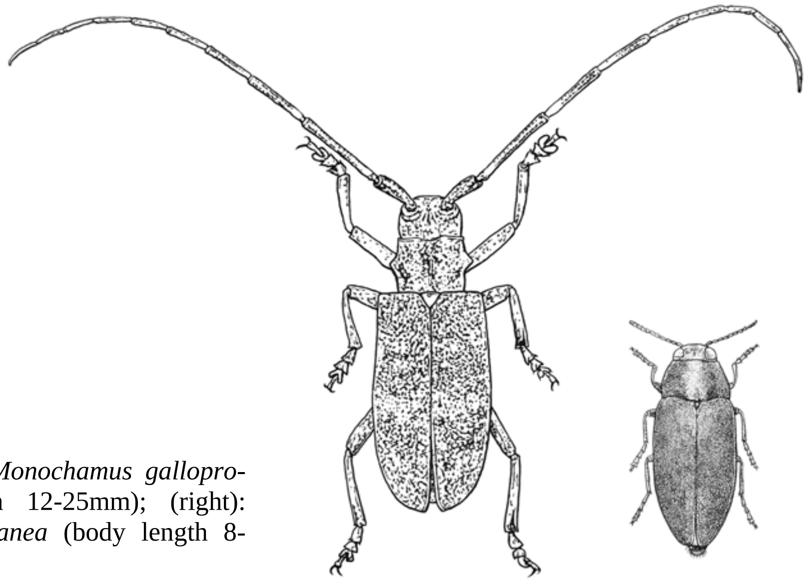

Fig. 1 (left): Female adult of *Monochamus galloprovincialis* (body length 12-25mm); (right): Adult of *Phaenops cyanea* (body length 8-11mm

Materials and Methods

Specimen of *M. galloprovincialis* were obtained from naturally infested pine wood under outdoor conditions. The beetles were used for GC-MS/EAD experiments approximately 4 weeks after hatching.

Adults of *P. cyanea* were collected from pine stems where they gathered for mating or oviposition after their maturation feeding.

Odour samples were collected from naturally grown pine trees at an age of 9 years and a height of ca. 2 m. The trees were enclosed by tents of PTFE foil. Within these tents the air was circulated through a charcoal filter with a flow of 1 l/min for a sampling time of 2 hours. Volatiles were eluted from the charcoal with a mixture of methylene chloride and methanol.

Odour samples were analysed with a gas chromatograph with combined mass spectroscopic and electroantennographic detection (WEISSBECKER et al. 2004). The GC (model 6890N, Agilent, Palo Alto, USA) employs the following temperature program: start: 50°C, hold for 1.5 min, ramp 6°C/min to 200°C, hold for 5 min. It is equipped with a split/splitless-injector operated at 250°C in the pulsed-splitless-mode and a HP-5MS column (length 30m, ID 0.25 mm, film thickness 0.25 µm, Agilent). Helium is used as carrier gas at a constant flow of 1 ml/min. The effluent of the column is splitted between a quadrupole mass spectrometer (model 5973N, Agilent) and a modified "olfactory detector port" (ODP-2, Gerstel, Mülheim, Germany).

The ODP is used to mix the effluent from the GC column with humidified air. The air flow carrying the odours is directed to the insect antenna which is fixed in a special antenna holder (FÄRBERT et al. 1997) within the sensor containment. Signals from the antenna are amplified by a factor of 100 and recorded using an A/D-converter and the HP chemstation software.

Results

Recordings with GC-MS (Fig. 2) demonstrate that pine trees emit a considerable number of different volatiles. About 50 volatiles were detected in the retention index range from 900 to 1500. Within this range reproducible EAD responses were observed both from antenna of *P. cyanea* and *M. galloprovincialis.* The high number of volatiles makes it difficult to achieve a complete separation of all compounds during the selected GC program. The temperature program was chosen as a compromise between an optimum separation and the limited lifetime of the employed antennae.

The results from GC-MS/EAD recordings show that the reproducibility of the EAD signals is very high within an insect species. However, the comparison between recordings from *M. galloprovincialis* and *P. cyanea* shows striking differences.

Substances that were preferably detected by *M. galloprovincialis* are p-cymene, γ-terpinene, and myrcene. *P. cyanea* showed a preference for β-pinene, 1,8-cineol and cis-dihydromultifidene. The identification of the last mentioned volatile is not fully assured because the pure compound is not commercially available. Both beetles show responses to p-cymenene, α-pinene, and 3-carene. A comparison of the structures of the mentioned volatiles reveals that *M. galloprovincialis* seems to prefer monocyclic or acyclic volatiles, whereas *P. cyanea* shows a certain preference for bicyclic terpenes. However, this seems to be not a strict rule since there is some overlap between the two groups.

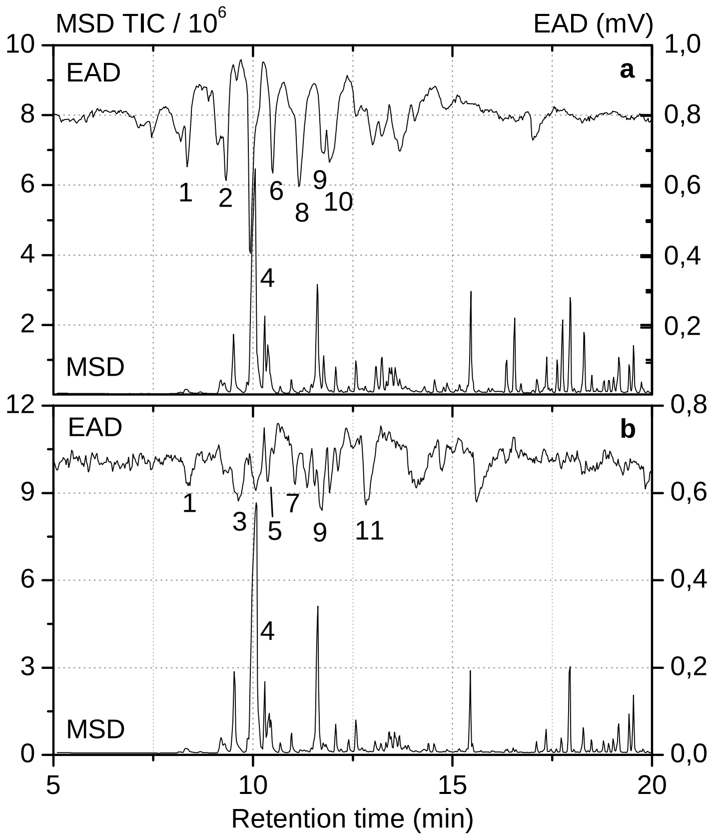

Fig. 2: GC-MS/EAD recording of odour samples from pine trees with antennae of *Phaenops cyanea* (a) and *Monochamus galloprovincialis* (b). Identification of volatiles:
1: α-pinene – 2: β-pinene – 3: myrcene – 4: 3-carene – 5: p-cymene – 6: 1,8-cineol – 7: γ-terpinene – 8: dihydromultifidene (?) – 9: p-cymenene – 10: 6-camphenone (?) – 11: myrtenol / camphor

Discussion

The presented data demonstrate that two different insect species that both infest the same host tree might have a completely different olfactory impression of their environment. It can be assumed that the insects have a specific sensitivity for compounds that are of a special significance for the suitability for infestation of a host tree. Especially terpenoids which are produced by the trees as part of the oleoresin defense are used by the insects for host recognition. The composition of resin may change in time and gives information on the defense status of a tree. Different terpenes may have completely different functions, e.g. it was suggested that the bicyclic pinenes are more notable for their solvent properties in order to mobilize the resin acids, whereas other terpenes e.g. limonene are notable for their toxicity (TRAPP & CROTEAU 2001). However, it is not clear to which extent the beetles rely on key compounds (attractants) for a targeted selection of susceptible trees or on repellents for a specific avoidance of healthy trees. No evidence was found yet, how the differences in the olfactory perception are linked to the different feeding behaviour (wood / bark) of the beetle's larvae.

It was shown before, that *P. cyanea* is able to locate pine trees that are predisposed for an infestation, e.g. trees that suffer from water-stress (SCHÜTZ et al. 2004). The compounds that were named as possible attractants for *P. cyanea* comprise bicyclic volatiles (bornylene, decahydro-methanoazulene) as well as monocyclic and acyclic volatiles (menthatriene, dimethyl-undecadienon).

The results presented here suggest that both insects, *P. cyanea* and *M. galloprovincialis*, use a complex pattern of volatiles for the detection of suitable host trees. Already healthy trees emit a huge variety of different volatiles and it is a remarkable task to detect changes in this odour bouquet.

M. galloprovincialis was observed to infest pine trees that were damaged by forest fires (MARKALAS 1997). This indicates that this beetle also responds to changes in the volatile pattern of its host tree. *M. galloprovincoalis* often occurs as a secondary pest of pine trees and may also use bark beetle semiochemicals for its orientation. It was shown that *M. galloprovincialis* is attracted to pheromones of *Ips spp.* especially if they are combined with typical volatiles of pine trees like α-pinene (PAJARES et al. 2004). A similar behaviour was also reported for other *Monochamus* species (ALLISON et al. 2001; DE GROOT & NOTT 2004). The attractive response by *M. galloprovincialis* to blends of the bicyclic α-pinene with other semiochemicals emphasizes the possibility that bicyclic terpenes play a crucial role for the host location of the beetle.

The results presented here may give some new impetus for behavioural tests in order to find out more about the olfactory orientation of *P. cyanea* and *M. galloprovincialis*.

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