Odours of wood decay as semiochemicals for *Trypodendron domesticum* L. (Col., Scolytidae)

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Abstract: Duftstoffe aus Holzalterungsprozessen als Infochemikalien für *Trypodendron domesticum* L. (Col., Scolytidae) und dessen Bedeutung innerhalb der Buchenkomplexkrankheit.

Beginnend im Jahr 1999 konnte eine unbekannte Variante der Buchenkomplexkrankheit in Beständen der Rotbuche (*Fagus sylvatica*) in Mittelgebirgsregionen beobachtet werden. Gesunde, hiebsreife Buchen wurden von dem bis dahin als klassisches Totholzinsekt zu bezeichnenden Buchennutzholzborkenkäfer (*Trypodendron domesticum*) befallen und starben vielfach ab. Oft konnten keinerlei Vorschädigungen beobachtet werden. Neben dem Interesse an dieser Art als Lagerholzschädling, gab dies Anlass zu einer chemoökologischen Studie des Wirtswahlverhaltens. Die Duftbouquets verschiedener Holzalterungsstadien unterschiedlicher Attraktivität an gefällten Buchen wurden gaschromatographisch analysiert und charakterisiert. Gleichzeitig wurde ihre Wahrnehmbarkeit über die Insektenantenne untersucht. Mit einem gekoppelten gaschromatographischen und elektroantennographischen Aufbau (GC-MS/EAD) fanden sich Hinweise auf 13 elektrophysiologisch aktive Substanzen, von denen 9 identifiziert werden konnten. Vergleichend wird die syntope Art *Hylecoetus dermestoides* L. (Col., Lymexylidae) betrachtet. Die identifizierten Duftstoffe sind verzweigte Alkohole, Aldehyde und Phenole. In Analysen zur Buchenkomplexkrankheit an stehenden, befallenen Bäumen fand sich nur eine der Substanzen wieder. Die Ähnlichkeiten und Unterschiede im Duftmuster stehender sowie gefällter Buchen werden diskutiert.

Key words: *Trypodendron domesticum*, *Xyloterus domesticus*, *Fagus sylvatica*, European beech bark disease, ambrosia beetle, wood decay, deadwood, GC-EAD

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Beginning in Belgium 1999, low mountain ranges of middle Europe were afflicted with the “European beech bark disease” (EBBD). It was first described by Hartig in 1878 as a complex disease where infestation of beech scale (*Cryptococcus fagisuga*; Hemiptera, Eriococcidae) is followed by fungal affection with *Nectria coccinea* and several white rot fungi. This often causes die back of mature beech trees, they tumble down and are colonised by woodbreeding beetles. Beside Belgium with 1 million cubic meter solid of beech wood (*Fagus sylvatica*); Luxembourg, France, and Southern Germany were affected in the last 6 years. In addition to known symptoms of EBBD, in all regions beech trees of an healthy appearance were surprisingly infested by the wood-breeding beetle *Trypodendron domesticum*. To understand mechanisms of this disease a chemo-ecological study was carried out, comparing the new phenomenon with the classical situation. A number of investigations of the involved beetles of the family of Scolytidae and Lymexylidae (BYERS 1992; KERCK 1976; KLIMETZEK 1984) suggest that the mechanisms of host-selection consist in the chemosensory differentiation of states of wood decay. The presence at the “border” between living and dying trees, *T. domesticum* turns out to be an interesting research object on xylobiont insects and physiological dying- and decaying-processes in trees.

The underlying hypotheses of this work are: 1) Volatile organic compounds change successively during aging and decay of wood and characterise the most susceptible phase and breeding site for *T. domesticum*. 2) Volatiles released by trees afflicted by the new disease phenomenon are similar to volatiles of felled, susceptible deadwood.

Material and Methods

To examine hypothesis 1), the volatiles of decaying beech logs were sampled and analysed. The perception of these compounds was scrutinised using a GC-MS/EAD setup (WEISSBECKER et al. 2004) and additionally tested with pure compounds in an EAG experiment. Volatiles were sampled with a specialised trunk sampling jacket (SCHÜTZ et al. 2004) to trap emanated volatiles from dead and living trees in a non-invasive manner. The sampling jacket surrounds the trunk and generates an enclosure of air above a bark surface of $0.5m^2$ in size. To trap volatile compounds emitted by this surface, the enclosed air was circulated with a miniature pump through a charcoal filter with a flow of 1 l/min for a sampling time of 1 hour. Enriched volatiles were eluted from the charcoal with a mixture of methylene chloride and methanol. Odour samples were repeatedly taken in march and April during the early flight period of the beetle and separated into non-infested, highly attractive, beetle-infested and fungus-infested samples respectively. The attractive sites selected by females for creating the entrance holes of breeding galleries correspond to small bark patches which can be sub-cortically differentiated by visual cues as colouration, or smell with notes of e.g. ethanol, smoke or fungus (KERCK 1976). For more detailed information, bark and wood chips of 1cm diameter were punched out, classified visually according to decay states (Fig. 1), and examined for emanating volatiles under lab conditions.

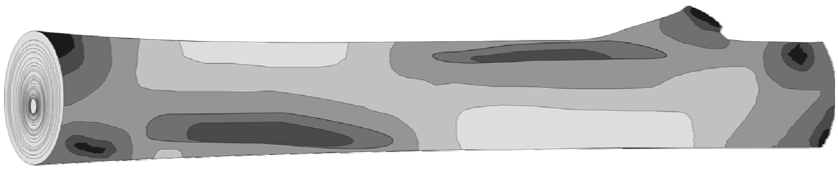

Fig.1: Distribution of physiological decay states on beech trunk (grey to black parts: fresh to seriously decayed)

The investigations on living beech trees concerning hypothesis 2) were done at a monitoring site of the Forest Research Institute Rheinland-Pfalz (FAWF), placed in the “Hunsrück” low mountain range in the forest district “Saar-Hochwald”, southern on a mountain top of 800m altitude. During the flight season between march and may, infested and non-infested living trees, all of a healthy appearance, were sampled with 7 replications each, using the sampling jacket as described.

Odour samples were analysed by combined gas chromatography-mass spectrometry and electroantennographic detection GC-MS/EAD as described by WEISSBECKER et al. (2004). For the analyses by GC (model 6890N, Agilent, Palo Alto, USA) we used the following temperature program: start: 50°C, hold for 1.5 min, ramp 6°C/min to 200°C, hold for 5 min. It was 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 was used as carrier gas at a constant flow of 1 ml/min. The effluent of the column was splitted, leading to a quadrupole mass spectrometer, and carried by a humidified air flow to a sensor containment including the insect antenna. Signals from the antenna were amplified by a factor of 100 and recorded using an A/D-converter and the HP- Chemstation software. For peak identification the National Institute of Standards and Technology mass spectral library (NIST, Gaithersburg, USA) was used.

Results

Analysis with GC-MS showed that felled beech trunks release over all more than 140 volatile compounds in noticeable amounts during the first phase of decay (0-2 years after felling), up to 70 contemporary in one bark sample. Differences between the small bark samples showed a high spatial variability of chemical processes and related volatiles within one trunk. Drawing an overview of the physiological aging processes starting with felling, the number of volatiles and emission rates increased beginning with aldehydes (exemplary GC-runs with named main compounds shown in Fig. 2 following decay progress from (a) to (d)). Several single and branched alcohols occured with beginning fermentation processes in the headspace of bark tissue (b). Beside terpenes, phenolic compounds, as 2-methoxy-phenole, 4-methoxy-phenole and 1.2-dimethoxy-benzene emanated during the most attractive phase for oviposition of *T. domesticum* (b) & (c). They vanished fast and the branched alcohols changed to longer carbon-chains. With the entrance of white rotting fungi (c) up to 30 sesquiterpenes were additionally detected in the bark samples. After degradation of lignin and cell structures, only these were found (d).

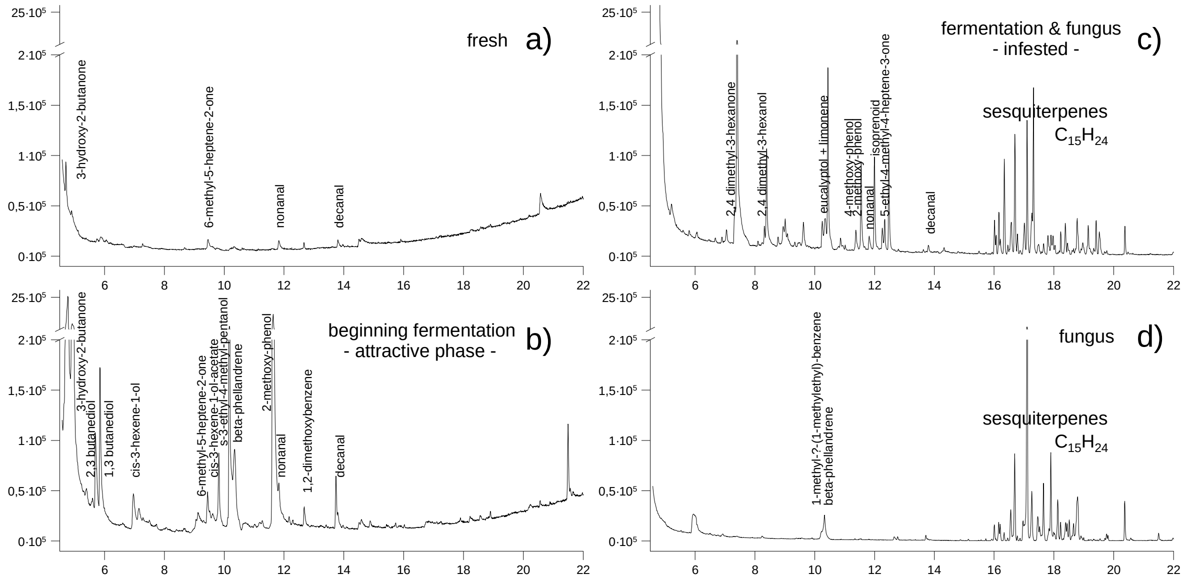

Fig. 2: Total Ion Chromatogram of physiological decay states from fresh to seriously decayed (a-d). Attractive tunnelling sites (see Fig 1): b) and c). Bark patches in state a) not yet attractive. d) unsuitable.

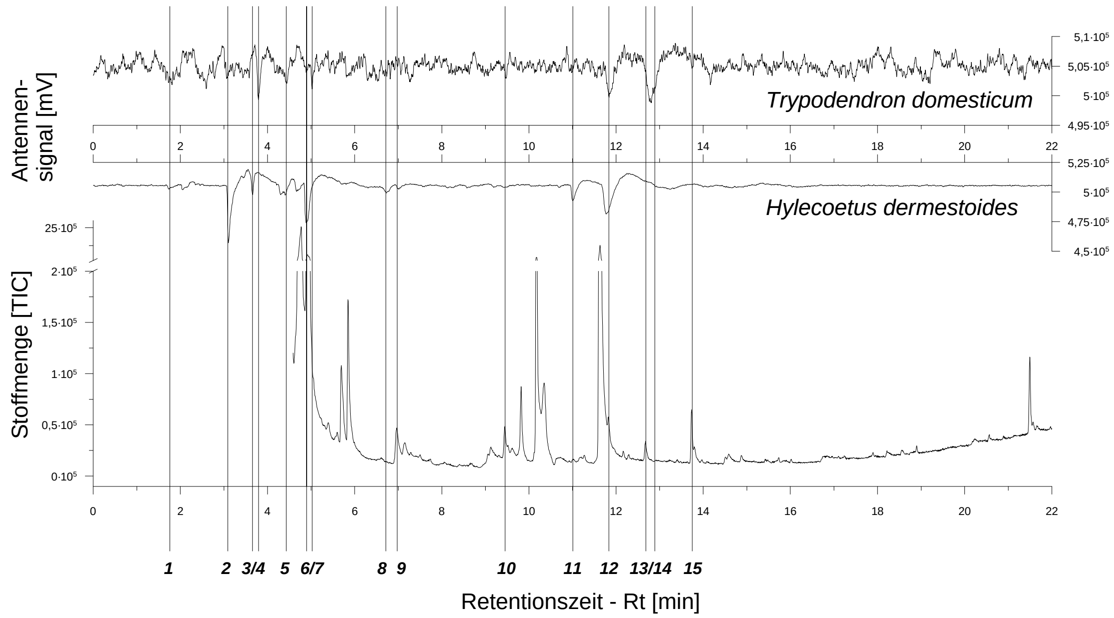

Fig. 3: Comparison of two GC-MS/EAD recordings of the same odour sample of highly attractive decaying beech bark with antenna of *T. domesticum* (upper, top) and antenna of *H. dermestoides* (upper, down). Corresponding ion-chromatogram (lower line).
Identifcation of volatiles 1: solvent (MeOH/CH_2Cl_2); – 2-5, 8, 11, 14: not identified; – 6: 3-methyl-1-butanol; – 7: 2-methyl-1-butanol; – 9: cis-3-hexene-1-ol; – 10: 6-methyl-5-heptene-2-one; – 12: 2-methoxy-phenol + nonanal; – 13: 1,2-dimethoxybenzene; – 15: decanal

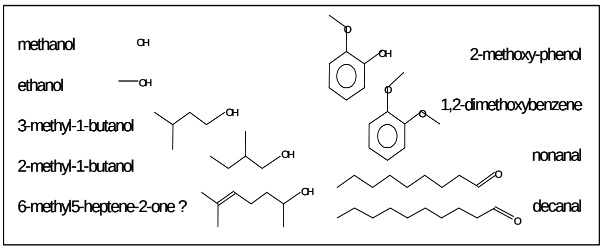

Fig. 4: Perceived substances discovered in GC-MS/EAD experiments. Ethanol, 2-methoxy-phenol, nonanal and additionally 4-methoxy-phenol were tested as pure compounds down to concentrations of 0.1 ppb in air.

GC-EAD: Recordings from antennae of female *T. domesticum* with GC-MS/EAD showed reproducible EAD signals to thirteen compounds. Nine of them were also perceived by *Hylecoetus dermestoides* L. (Coleoptera: Lymexylidae), a syntopic beetle, often found at the same host-trunks. (Fig. 3).The sensitivity of the two species is similar but differs for single compounds (e.g. nonanal & 1,2-dimethoxybenzene).

Discovered compounds which may play a role as semiochemicals in *T. domesticum* are shown in Fig 4. Four were tested as pure compounds and are perceived highly sensitive. Identification of both butanols and 6-methyl-5-heptene-2-one is not fully assured and has to be proven with synthetic compounds.

The volatile-analysis of living trees in the context of EBBD showed that proposed semiochemicals (Fig. 5) could not be found in any living trees, except of nonanal. Trees infested with *T. domesticum* did not differ significantly from uninfested trees of the same stand. Therefore, control trees were measured again one year later, and they differed clearly from the others (Fig. 5).

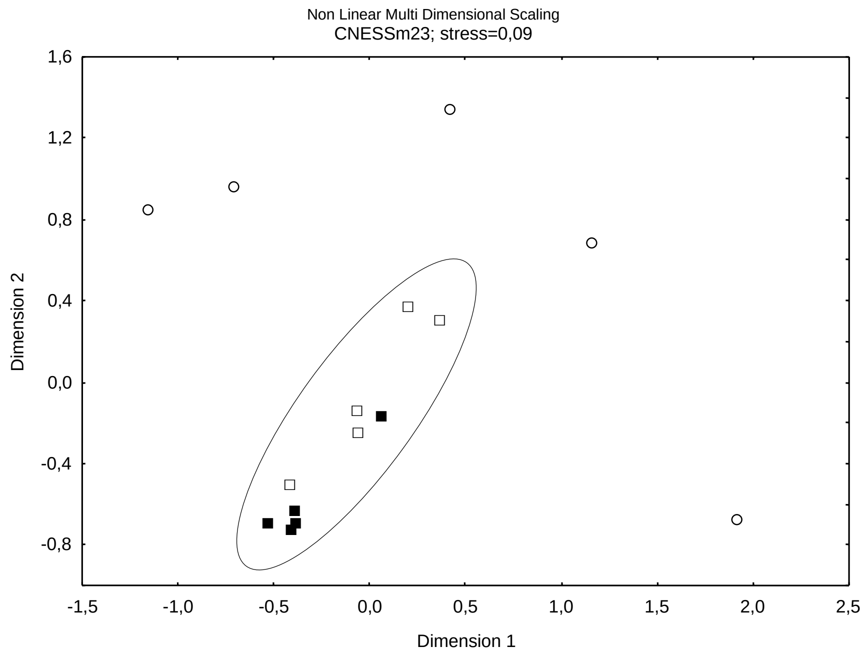

Fig. 5: Non linear multidimensional scaling: Volatile composition of living trees. Infested trees (■), non-infested (□), controls one year later (○).

Discussion

The variability of decay processes is very high. Decay processes at a trunk occur discontinuously in time-course and patch-wise in space. Depending on abiotic factors, chemical processes, and involved organisms decay related volatiles vary in presence and quantity. They are used by deadwood colonising insects in host finding. The suitability of wood for deadwood-insects is determined by its state of decay. *T. domesticum* is highly selective in choosing breeding sites at spots of a specific decay state. Our investigations showed several candidates as marking semiochemicals eliciting behavioural response. Catches of *T. domesticum* in a trap experiment showed that beside ethanol, 2-methoxy-phenol and methyl-butanol isomers may act as attractants to *T. domesticum*. Further studies should complete the list of compounds that are perceived. Specific micro-organisms and fungi are known to be distributed by *T. domesticum*. Their impact on volatile production and wood decay chemistry may reveal trophic interactions and mechanisms of host finding. The investigations on infested living trees with EBBD symptoms showed huge differences to infested deadwood. Unknown plant defense signals, which might have caused the beetle attack, or an already expired infection can be an explanation for the lack of expected semiochemicals. The homogeneity between infested living trees and uninfested controls may refer back to a stress situation in 2003 similar to all trees. The uninfested control trees measured in 2004 differ clearly in volatile composition as shown in Fig. 5. An effect of a recovery within this climatically intermediate year might be the reason for this result.

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