Volatile organic compounds emitted from fungal-rotting beech (*Fagus sylvatica*)

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Abstract: Flüchtige organische Stoffe von pilzbefallenem Buchenholz (*Fagus sylvatica*).

Holzzerstörende Insekten nutzen oft flüchtige Chemikalien, um geeignete Orte zum Fraß oder zur Eiablage zu finden. Diese Wechselbeziehung kann von volatilen Stoffen (volatile organic compounds, VOCs) beeinflusst werden, die nicht vom Holz selbst, sondern von holzbewohnenden Pilzen produziert werden.
Die VOCs von Buchenholz (*Fagus sylvatica*), das mit einem der Pilze *Trametes versicolor*, *Poria placenta* oder *Gloephyllum trabeum* inokuliert wurde, konnten mittels SPME (85 µm Carboxen™ / PDMS StableFlex™) gesammelt und mit GC-MS analysiert werden. Hauptsächlich wurden Stoffe aus drei Gruppen von chemischen Verbindungen nachgewiesen: Aliphatische Verbindungen (C_5-C_8), Monoterpene und Sesquiterpene. In Proben von *G. trabeum* waren Sesquiterpene (insbesondere Protoillud-6-en) die am häufigsten emittierte Stoffgruppe. In geringen Mengen wurde 1-Octen-3-ol und 3-Octanon nachgewiesen. Die typischen Sesquiterpene für *T. versicolor* waren α- und β-Barbaten. Die C8-Verbindungen 1-Octen-3-ol, 3-Octanon und 3-Octanol wurden in hohen Mengen gefunden. Zudem konnte 3-methyl-1-butanol nachgewiesen werden. In Proben von *P. placanta* wurden vergleichsweise geringe Mengen von VOCs gefunden, wobei eine spezifische Emission des Sesquiterpens Daucen festgestellt wurde. Zudem wurden hohe Mengen von 1-Octen-3-ol, 3-Octanon und 3-Octanol nachgewiesen. Die C_8-Verbindungen 1-Octen-3-ol, 3-Octanon und 3-Octanol, die insbesondere von *T. versicolor* und *P. placenta* emittiert werden, sind typische Pilz-Volatile. Sie können nicht nur von spezialisierten fungivoren Insekten wahrgenommen werden, sondern auch von einer Vielzahl anderer Insekten, z.B. Kartoffelkäfer und Borkenkäfer. Wahrscheinlich nutzen viele Insekten diese Stoffe, um die Anwesenheit von Pilzen zu detektieren.

Kew words: beech, VOCs, SPME, fungus, insect

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Chemo-communication is an important mode of interaction within ecosystem. The living organism in the ecosystem can deliver signals to conspecifics, to co-organisms, and unintentionally to their enemies, by emitting the volatile organic compounds (VOCs) to the atmosphere.

There are some insect-fungi-associations displaying interesting relationships. For example, some bark beetle species (PAINE et al. 1997) introduce fungi into the conifers during the attack process. Fungi take advantage by associating with the insect in transport to new trees and passage through the bark. Insects may get advantage by feeding on fungi and overcoming the tree defence. Questions arise how fungus vectoring insects can recognize the weakness of defense mechanism in the case to introduce the fungus to the host tree and successfully overcome the remaining defence mechanisms. The interspecific communication among insects, fungi and host trees are not completely clarified, but there was the evidence that a number of forest scolytids including species in the genera *Scolytus*, *Dendroctonus*, *Hylurgops*, *Trypodendron* and *Tomicus* can be attracted to the host volatile compounds (BYERS 1995).

Materials and Method

Sample preparation

Approximate 100 – year old beech (*Fagus sylvatica*) from Ebergoetzen, North - East direction from Goettingen, Germany, was used in this experiment. Parts of the sapwood were cut to samples of approximate cubic shape with about 1 x 1 x 1 cm in size. Twelve g of beech cubes were placed in 80-ml vials with septum caps and soaked with 6-ml deminerilized water. Then they were sterilized at 121 °C for 20 min. Afterwards they were inoculated either with 3 different species of wood–infecting fungi, namely *Trametes versicolor* (White rot), *Poria placenta* (Brown rot, BR) *or Gloeophyllum trabeum* (BR). Uninoculated wood samples were kept as control. Fungal cultures were incubated under dark at 22 °C and 70% relative humidity.

VOCs Sampling and analyzing

Solid phase microextraction (SPME) technique is a suitable tool for this investigation (ZHANG et al. 1994). SPME fiber, 85 μm Carboxen™/Polydimethylsiloxane (PDMS) StableFlex™ type supplied by Supelco, was used as the sampling device. The SPME syringe was firstly inserted through septum to the head space of the vials and the fiber was pushed to adsorp the VOCs. After the 30 - min sampling time was elapsed the fiber was retracted to the housing. The SPME fiber was directly subjected to the injection port of the GC at a temperature of 230 °C desorbing the VOCs. GC (Agilent Technologies, model 6890N) was performed as follows. Column: HP – 5MS, 0.25 mm x 30 m x 0.25 μm; temperature program: initial temperature 35 °C and held for 1.5 min, heated up to 200 °C with a heating rate of 6 °C/min and held at this temperature for 5 min; carrier gas was helium. The mass spectrometry (Agilent Technologies, MS model 5973N) was performed with the mass scan range of 15 – 300 amu, 230 °C source temperature and EI mode at 70 eV. The data were analyzed with Massfinder version 3, Enhance chemstation version D00.00.38 and NIST Mass Spectral Search program version 2.0a.

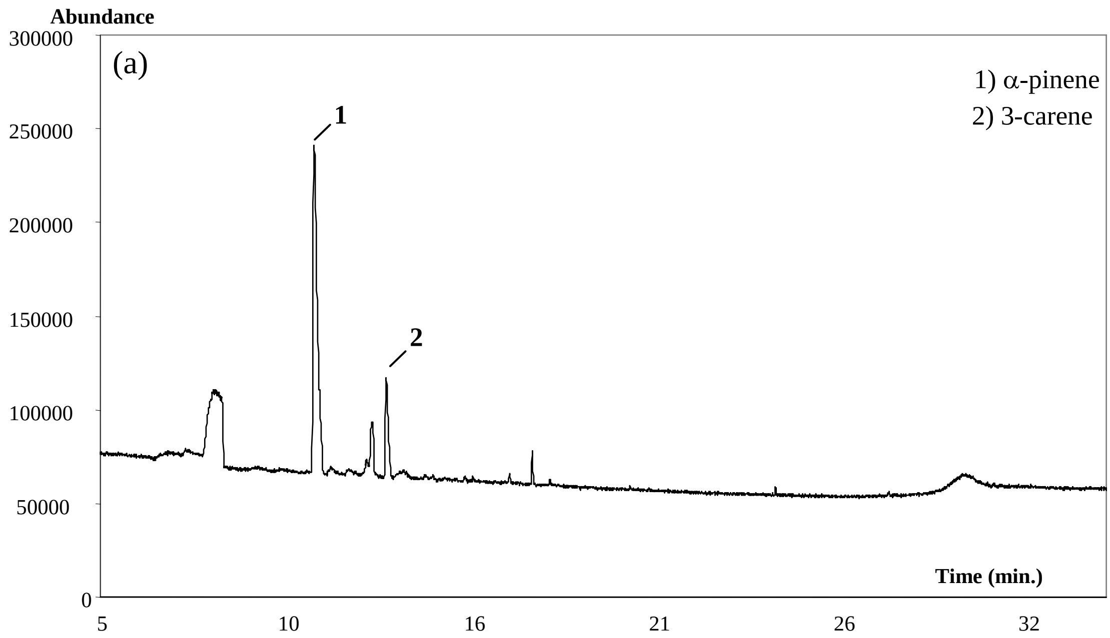

Fig. 1a – d: Chromatograms of VOCs released from (a) control beech, and beech infected with (b) *T. versicolor* (c) *G. trabeum,* and (d) *P. placenta*

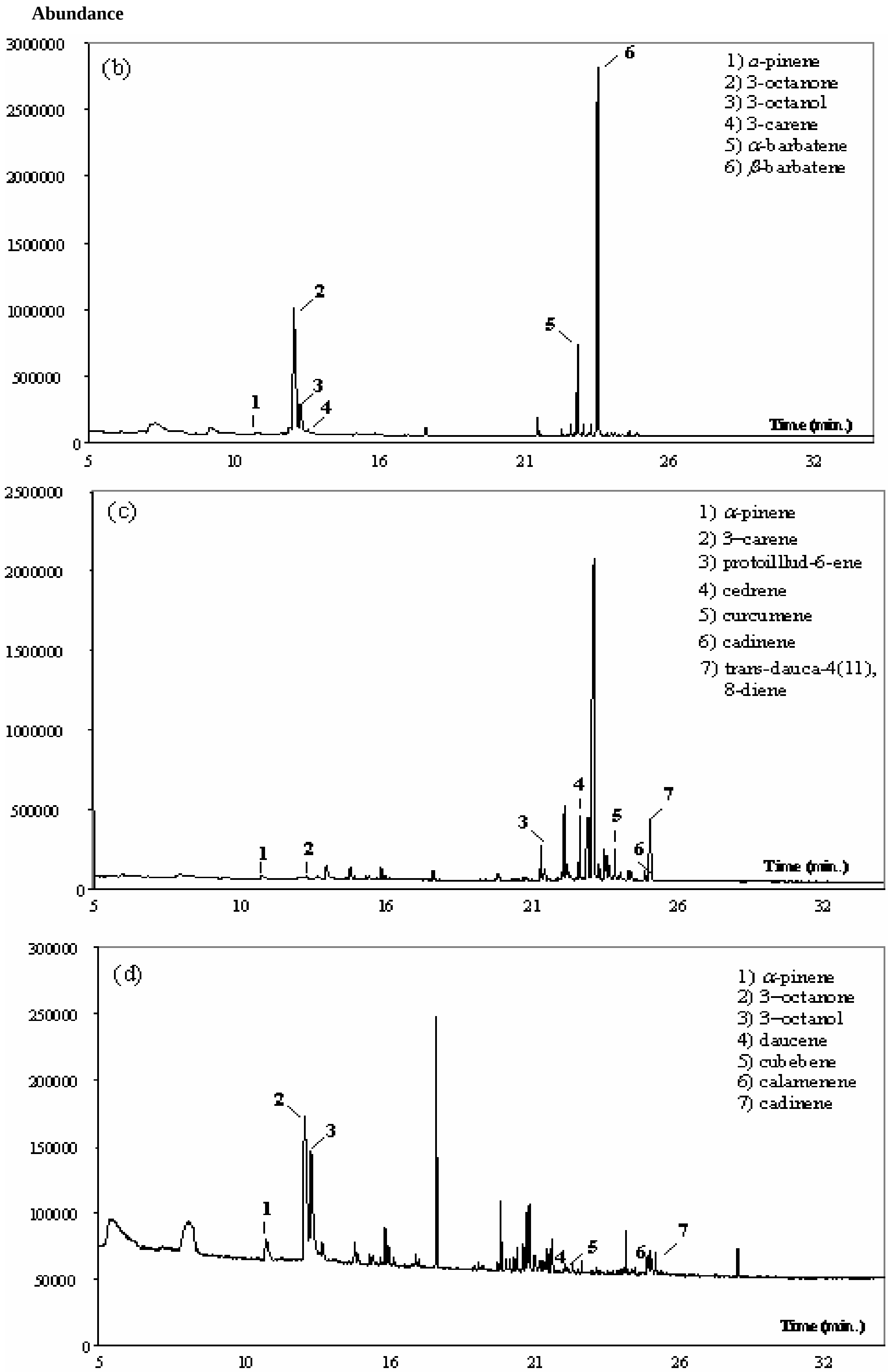

Fig. 1a – d: Chromatograms of VOCs released from (a) control beech, and beech infected with (b) *T. versicolor*, (c) *G. trabeum*, and (d) *P. placenta* (continued)

Results and Discussion

After 1-week inoculation of *T. versicolor*, *G. trabeum*, and *P. placenta* on beech, the VOCs in the head space of vials were sampled, using 85 μm Carboxen™/Polydimethylsiloxane (PDMS) fiber. Using SPME, the sampling procedure becomes convenient, less time consuming, solvent-free, and sterile. The GC-MS analysis of VOCs allows the indentification and quantification of volatile compound mixtures released by fungi. It can be seen from Fig. 1 that beech rotted with different types of fungi provided different patterns of VOCs.

Fig. 1 (a) shows the chromatogram of a control sample, where the only 2 VOCs detected were α - pinene and 3-carene. Comparing with the inoculated wood samples, the number and amounts of VOCs released from beech - *T. versicolor*, - *G. trabeum*, and - *P. placenta* were comparatively higher. Those VOCs can be broadly devided into 3 groups; $C_5 – C_8$ compounds, monoterpenes and sesquiterpenes.

3-carene, α–pinene and the C_8 compounds of 1-octen-3-ol, 3-octanone, and 3-octanol (except in *G. trabeum* sample) were generally found in these infested samples. The number of VOCs produced in infected wood samples decreased in the order *G. trabeum*, *T. versicolor* and *P. placenta* samples. The most distinguished compound was protoillud-6-ene in *G. trabeum* samples (Fig. 1 c). There were also sesquiterpenes found in these samples like cadinene, curcumene, sativene, cedrene, and *trans*-dauca-4(11),8-diene. Additionally, many non identified sesquiterpenes were detected in *G. trabeum* samples. For *T. versicolor* samples, there were 2 remarkable compounds, α– and β– barbatene. β– barbatene was already known as emission of *Fomitopsis pinicola* (FÄLDT et al. 1999). It was stated that the two beetles *Cis glabratus* and *C. quadridens* might use this compound to discriminate the host odors of chopped *F. pinicola* and *Fomes fomentarius*. For *P. placenta* sample, there were not many different VOCs released from this system compared to the other fungi. However, daucene seems to be a specific sesquiterpene emission of this fungus, because it was not found neither in *G. trabeum* nor *T. versicolor* samples. Moreover other sesquiterpenes for instance, calamenene, cubebene and cadinene, were detected.

In these experiments we demonstrated that different fungi growing on beech wood produce individually different chemical profiles. The insect ability of selective odor perception might enable the insects to find special fungus species as host or indicators of reduced tree defence. Altogether, this information promise that the measurement of VOCs released from wood might be used as a method to evaluate the infection of wood by fungi.

As a next step experiment will be carried out with insect antennae using Gas – Chromatograph and Electroantennograph detection (GC - EAD) method to find out whether they can perceive those distinguished compounds.

Acknowledgement

This work was performed with the financial support from DAAD, the Royal Thai Government and Faculty of Agro – Industry, Chiangmai University, Thailand and with kind support of Institute of Wood Biology and Technology.

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