Innovative bark beetle control – Identification of semiochemicals including attractants for *Thanasimus* spp.

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ABSTRACT: Durch den Klimawandel vermehrt auftretende Kalamitäten durch Gradationen von Borkenkäfern einhergehend mit restriktiveren Regelungen zum Einsatz von Pflanzenschutzmitteln sind Innovationen im Bereich des Polter- und Bestandesschutzes erforderlich. Flüchtige organische Verbindungen (engl.: volatile organic compounds -– „VOC“) spielen als Semiochemikalien eine zentrale Rolle bei der Partner- und Nahrungssuche von Insekten. Als synthetische Lock- und Ablenkstoffe kann mittels solcher Semiochemikalien eine artspezifische Lenkung der Insekten erfolgen.

In dieser Studie im Tharandter Wald wurde das VOC-Muster von Holzpoltern fünf verschiedener Baumarten (*Fagus sylvatica*, *Quercus robur*, *Fraxinus excelsior*, *Pinus sylvestris, Picea abies)* ohne und mit Befall artspezifischer primärer und sekundärer Borkenkäfer charakterisiert. Zehn ausgewählte VOCs wurden in Verhaltensversuchen auf ihre Eigenschaften als Semiochemikalien für den Ameisenbuntkäfer (*Thanasimus* spp.), einem der wichtigsten Borkenkäferantagonisten, untersucht. Die VOCs der Holzpolter wurden mit einer Stammabsaugkammer und Tenax®-Adsorbentien gesammelt und mittels GC-MS identifiziert. Die Verhaltensversuche erfolgten in einem Zwei-Arm-Olfaktometer.

Im VOC-Muster der Holzpolter wurden 39 VOCs identifiziert, die als Nicht-Wirtsbaum-Volatile potentiell dazu geeignet sein könnten, andere Baumarten zu maskieren und so den Borkenkäferanflug zu verringern. In den Verhaltensversuchen wurden schwach attraktive und repellente Einzelsubstanzen gefunden, die nur im Vergleich gegeneinander, nicht aber zur Kontrolle signifikante Effekte zeigen. Die Ameisenbuntkäfer hielten sich im Vergleich zu 6 weiteren VOCs signifikant länger in den Methylsalicylat-Duftfeldern auf. Der stärkste Effekt wurde im Kontrast mit dem bekannterweise repellent wirkenden Acetophenon beobachtet. Methylsalicylat ist als volatiles Produkt des Salizylsäure-Stoffwechsels als regulatorisches Signal in die Reaktion von Pflanzen auf abiotischen und biotischen Stress eingebunden. Aufgrund seiner besonderen Präsenz in Angiospermen könnte es zur Anwendung als allochtones Kairomon für die Ameisenbuntkäfer zur Reduktion von Borkenkäferbefall im Nadelholzkontext dienen.

Keywords: Biological pest control, IPM, VOC, wood stack protection, *Thanasimus*, gas chromatography, olfactometer

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Climate change favor calamities such as bark beetle mass outbreaks and the increasingly restrictive regulations for the use of insecticides in forest protection urge the forestry sector to develop novel forest protection strategies. Especially with regard to *Ips typographus*, the most destructive bark beetle in Europe, innovative control methods are needed. In managed forests, aggregation pheromones are used so far [GITAU & al., 2012], however also other volatile organic compounds (VOC) such as non-host-volatiles (NHV) can be integrated in pest management, since they impair successful host location of bark beetles [ZHANG & SCHLYTER, 2004]. Bark beetle pheromones, as well as tree VOCs as their kairomones, are also used by the main bark beetle predators such as *Thanasimus* spp. to locate the bark beetles as their prey [BAKKE & KVAMME, 1981]. In some circumstances *Thanasimus* spp. can even prevent the onset of bark beetle mass outbreaks [ALTENKIRCH & al., 2002]. *Thanasimus* spp. are known to use aggregation pheromones of bark beetle species of coniferous as well as deciduous trees at the same time. Therefore, to identify semiochemicals that e.g. might attract *Thanasimus* spp. without attracting the bark beetles themselves, we examined the VOC-pattern of five tree species from forest areas near Tharandt (Germany) and examined the effect of ten selected VOCs as semiochemicals on behaviour of *Thanasimus* spp. in order to identify new VOCs for novel pest control strategies. Such components have been found for conifer protection [Wehnert & Müller, 2012], but there is a lack of knowledge for such usage in deciduous trees. For the identification of VOC patterns, VOCs were sampled with stem enclosure chambers on felled trees of beech - *Fagus sylvatica*, oak - *Quercus robur*, ash - *Fraxinus excelsior*, pine - *Pinus sylvestris* and spruce - *Picea abies*. The sampling of VOCs emitted by trunks was done before attack of species specific bark beetles, during the attack phase and after successful colonization of trunks. VOCs were analyzed and identified with GC-MS. Ten candidate VOCs were examined in behavioral assays with *Thanasimus* spp. in a ten channel two-arm-olfactometer to unravel their behavioural activity in a walking situation.

Research question

Can we identify VOCs of deciduous trees for the protection of coniferous trees and vice versa that positively affect *Thanasimus* abundance while not attracting the respective bark beetles since those are NHVs for the latter?

Material & Methods:

Volatile analysis: The sampled wood stacks were located in forest areas near Tharandt, Dresden and consisted of trunk sections > 1 m originating from 2 – 3 trees. VOCs were sampled between March and September 2017 with Tenax® adsorbents and stem enclosure chambers according to the method of Rachow & al. (2012). The sampling was done before, during and after bark beetle colonization. Bark beetle species found on trunks were *Trypodendron domesticum* on beech, *Scolytus intricatus* and *Xylosandrus germanus* on oak, *Hylesinus fraxini* on ash, *Ips sexdentatus* and *Tomicus piniperda* on pine and *Ips typographus* as well as *Pityogenes chalcographus* on spruce. As control samples, ambient air was sampled simultaneously. The VOC patterns were analyzed with a gas chromatograph type 6890 coupled to a mass spectrometer type 5973N (both Agilent Technology, Santa Clara, USA) and identified by mass spectral comparison to the NIST Library (Version 2.0 f, NIST Gaithersburg, USA) using "Enhanced ChemStation Data Analysis" software of the device manufacturer (Version D 02.00.275). Linear retention indices were calculated and compared to literature values for further validation. Identified VOCs of replicate measures (n = 10; *Quercus robur* n = 6) were grouped into frequency tables.

Behavioural assays: In 2017 during the vegetation period, the behavioral responses of 70 individual *Thanasimus* beetles (field catches) were tested by using a two-arm-olfactometer with 10 arenas (Fig. 1). VOC's tested were 3-methyl-2-pentanol, limonene, 2-methyl-3-butyn-2-ol, methyl salicylate, pentanol, camphor, α-pinene, myrcene, 4-methyl-3-heptanone and acetophenone. Each VOC was assigned to one of the ten arenas for the whole experiment to avoid memory effects. All beetles were randomly assigned to the arenas and tested only once with each VOC. Before testing again with the next VOC, beetles were given a rest for a minimum of 60 minutes. Control experiments were conducted first, testing empty pipette tips against paraffin oil on filter paper. A humidified, constant stream of synthetic air flows through the pipette tips carrying the VOCs on filter paper, directed into the arenas leaking through openings at the middle of every arena. For VOC testing, each VOC dissolved in paraffin at a concentration of 10^{-3} (w/w) was loaded on filterpaper and located on the "odor-side" while paraffin oil on filter paper was located at the "control-side". The beetles were tracked for three minutes with the live-tracking-video-system "EthoVision XT 8.0" (Noldus Information Technology, Wageningen, NL). For the tracking analysis a mask was created dividing each arm of the arena in two zones, separating a choice zone near the entrance of the airstream (Fig. 2). Non-decisive beetles that did not move away from the release point were not considered for further analysis. To analyse independence of the olfactometer, control assays were analyzed with a chi-square-test. Differences in staying durations in different zones was analyzed with an analysis of variance followed by a Bonferroni post-hoc test for examine differences among the odors tested.

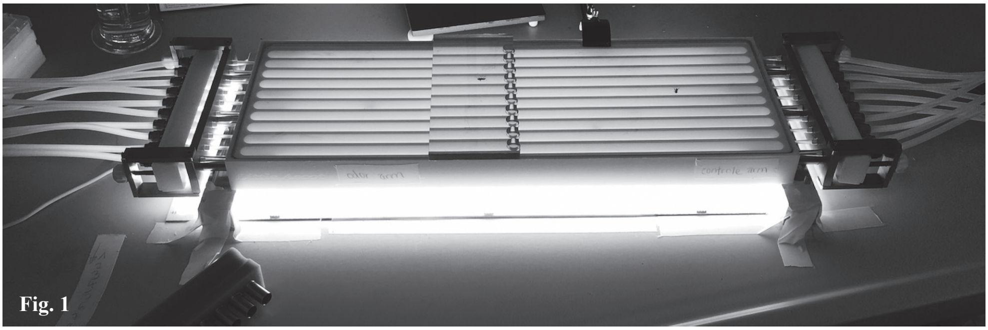

Fig. 1

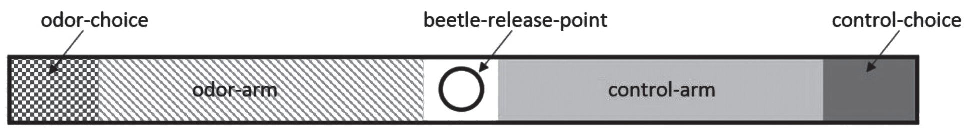

Fig. 2

Results

Volatile analysis: Table 1 summarizes VOCs found in deciduous and coniferous trees before, during and after bark beetle attack. Overall, deciduous tree species emitted most VOCs before attack with a decrease during the colonization, while number of VOCs released from coniferous trees were equal before and during bark beetle attack. VOCs shared by both groups were only 14 before attack with an increase to 26 during and after attack (Table 1).

Table 1: Number of VOCs of all tree species from stem enclosure chamber sampling.

	Before outbreak	During outbreak	After outbreak	Total
VOC total	128	114	98	337
Emitted by deciduous tree species	78	54	42	174
Emitted by coniferous tree species	36	34	31	101
Emitted by both	14	26	25	65

VOCs are of potential interest for masking wood stacks when occurring in at least 40% of the replicates at one time and only emitted by deciduous or coniferous trees (Table 2). By applying these criteria, altogether 39 VOCs could be identified, which could possibly be able to mask other tree species as non-hosts and thereby reduce the bark beetle infestation. These VOCs could only be reliably identified at least at two deciduous tree species and of both conifer species. 28 VOCs from deciduous tree species belong mainly to the chemical groups of aromatic compounds (5), alkenes (5) and alkanes (5). In contrast, 11 VOCs from coniferous tree species belong mainly to the chemical groups of terpenes (8).

Behavioural assays: Exclusion of non-decisive beetles resulted in different replicates for each VOC. The control experiment confirmed that the beetles did not prefer a particular side of the olfactometer. The analysis of variance showed a significant difference ($p=0.02$) in the odor choice-zone in terms of staying durations. Table 3 shows all VOC comparisons of the staying durations of the odor choice-zone, which showed a trend ($p \leq 1.00$) or a significant difference. It turned out that methyl salicylate occurred in all VOC-comparisons of the odor choice-zone durations (Table 3). The comparison of the staying durations between methyl salicylate and acetophenone is significant with a p-value of 0.01. Furthermore, the staying durations of methyl salicylate and 3-methyl-2-pentanol as well as methyl salicylate and 4-methyl-3-heptanone are slightly significant ($\alpha=0.1$). The other VOC-comparisons show only a slight tendency to be significant.

Table 2: Potential VOCs for masking wood stacks.
Bold VOCs: Found at every time (before, during and after bark beetles infestation).

Deciduous tree VOC			Coniferous tree VOC		
VOCs	CAS	Chemical group	VOCs	CAS	Chemical group
undecane	000124-11-8	alkene	α-terpinolene	000586-62-9	monoterpene
nonane	000111-84-2	alkane	**pinocarvone**	019890-00-7	ketone, monoterpene
heptanal	000111-71-7	aldehyde	**α-longipinene**	005989-08-2	sesquiterpene
anisole	000100-66-3	aromatic compounds	(+)-epi- bicyclosesqui-phellandrene	054324-03-7	sesquiterpene
phenol	000108-95-2	phenol	**1-methyl-4-(1-methylethenyl)-benzene**	001195-32-0	alkylbenzene, monoterpene
1-decene	000872-05-9	alkene	1,3,8-p-menthatriene	021195-59-5	aromatic compounds
hexanoic acid, 2-ethyl-methyl ester	000816-19-3	ester	**(+)-sativene**	003650-28-0	sesquiterpene
benzeneacetaldehyde	000122-78-1	aldehyde	bornylene	000464-17-5	monoterpene
α-cumyl-alcohol	000617-94-7	alcohol	tricyclene	000508-32-7	monoterpene
1-undecene	000821-95-4	alkene	d-fenchyl alcohol	001632-73-1	alcohol
undecane	001120-21-4	alkene	myrtenal	000564-94-3	aldehyde
2,6-dimethyl-cyclohexanol	005337-72-4	alcohol			
1,3-dimethoxy-benzene	000151-10-0	aromatic compounds			
benzoic acid	000065-85-0	organic acid			
naphthalene	000091-20-3	aromatic compounds			
dodecane	000112-40-3	alkane			
decanal	000112-31-2	aldehyde			
β-cyclocitral	000432-25-7	aldehyde, monoterpene			
1-tridecene	002437-56-1	alkene			
tridecane	000629-50-5	alkane			
tetradecane	000629-59-4	alkane			
(1-butylheptyl)-benzene	004537-15-9	aromatic compounds			
6(E), 8(E)-heptadecadiene	999281-78-6	alkene			
3-octanone	000106-68-3	ketone			
octanal	000124-13-0	alcohol			
thujopsene	000470-40-6	sesquiterpene			
ethylbenzene	000100-41-4	aromatic compounds			
1-octen-3-ol	003391-86-4	alcohol			

Table 3: Differences of staying durations of the odor choice-zone (Bonferroni post-hoc test). [*** significance level 95% (α=0.05), ** significance level 90% (α=0.1), * = trend]

Compared staying duration (Odor-Zone)	p-value	level of significance
methyl salicylate & 2-methyl-3-butin-2-ol	0.36	*
methyl salicylate & 3-methyl-2-pentanol	0.06	**
methyl salicylate & 4-methyl-3-heptanone	0.06	**
methyl salicylate & acetophenone	0.01	***
methyl salicylate & camphor	0.96	*
methyl salicylate & myrcene	0.33	*

Discussion

Volatile analysis: This study showed that VOC-occurrence differs between tree species and infestation phases. 28 VOCs that might serve for the protection of coniferous trees and 11 VOCs that might protect deciduous trees were detected in the different VOC-patterns. The majority of VOCs that occurred at deciduous trees could not be detected at coniferous trees and vice versa. VOCs for a potential protection of coniferous trees mainly belong to the chemistry group of aromats and alkenes, whereas the VOCs of deciduous trees mainly belong to the chemistry group of mono- and sesquiterpenes. The extent of occurring VOCs is triggered by environmental factors such as temperature [Zhang & al., 1999]. In general, it is already known that NHVs can cause an anti-aggregative effect in phytophagous insects [Gitau & al., 2012]. α-longipinene was found in higher concentrations in the VOC-patterns of *P. abies*, which were not colonized by *I. typographus* and survived the infestation healthy. This might declare α-longipinene as a potential VOC for the protection of coniferous wood stacks. Benzoic acid and dodecane have been proposed as a conifer protection, but were in literature referred to be possible attractants for insects [Hanover, 1975; Vrkočová & al., 2000]. With all VOCs listed in Table 2, EAG-studies with bark beetles and *Thanasimus* spp. should be conducted to determine perceptibility. Afterwards, behavioral tests could clarify whether the VOCs are suitable for the protection of wood stacks or the attraction of *Thanasimus* spp..

Behavioural assays: *Thanasimus* spp. showed different behavioral reactions under the influence of VOCs in the olfactometer experiments. The differences in the staying durations of the odor choice-zone, especially for methyl salicylate, gave interesting results. According to Zhang & Schlyter (2004) methyl salicylate is a VOC emitted by angiosperms and a NHV to conifer inhabiting bark beetles. Only bark beetles that are non-native in Europe responded with antennal reactions to methyl salicylate [Zhang & Schlyter, 2004]. *Thanasimus* spp. stayed significantly longer in the odor choice-zone of methyl salicylate as in the odor choice-zone of acetophenone, which is known as a repellent-acting VOC for *Thanasimus* spp. [Wehnert, 2013]. Thus, it can be assumed that methyl salicylate, which is not known as being attractive for European bark beetles, has an attractive effect on *Thanasimus* spp..

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