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ORIGINAL ARTICLE

Identification of male produced compounds in the bark beetle *Polygraphus subopacus* and establishment of (*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol as an aggregation pheromone component

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Abstract
Bark beetles of the genus *Polygraphus* have recently been involved in large bark beetle outbreaks in central Sweden, together with the European spruce bark beetle *Ips typographus*. Three species of *Polygraphus* can be found in this region; *Polygraphus poligraphus*, *Polygraphus punctifrons* and *Polygraphus subopacus*. Efficient pheromone traps would facilitate further investigations of these species and their role in bark beetle outbreaks. Pheromone compounds have previously been identified in *P. poligraphus* and *P. punctifrons*, but not in *P. subopacus*. Thus, we allowed males and females of *P. subopacus* to bore in the bark of stem sections of Norway spruce (*Picea abies*) in the laboratory. Volatile organic compounds from boring insects were sampled with SPME and analysed with GC–MS and several male-specific compounds were observed. The male specific compounds were 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 3-methyl-2-butenal, grandisol, fragranol, (*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol, (*E*)-2-(3,3-dimethylcyclohexylidene)-ethanol, (*Z*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde, (*E*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde, geranial and γ-isogeraniol. (*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol, [(*Z*)-DMCHE], was identified from GC–MS analysis to be the major male-specific compound while the (*E*)-isomer, [(*E*)-DMCHE], was found as a minor compound. These two compounds gave positive responses in EAG analyses with antennae from males and females of *P. subopacus*. Thus, (*Z*)- and (*E*)-DMCHE were used in a field experiment in central Sweden but only (*Z*)-DMCHE was found to be attractive to males and females of *P. subopacus*. Consequently, (*Z*)-DMCHE was established to be a component of *P. subopacus* aggregation pheromone.

Keywords *Polygraphus subopacus* · (Z)–2-(3,3-dimethylcyclohexylidene)-ethanol · Bark beetle · SPME · GC–MS · EAG

Introduction

Some bark beetle species of the genus *Polygraphus* can cause considerable damage to their host trees. *Polygraphus rufipennis* is a pest on black spruce in North America (Bowers et al. 1991) and *Polygraphus proximus* is a pest on Siberian fir in Russia (Kerchev 2014). In Sweden, there are three species of *Polygraphus* beetles; *P. poligraphus*, *P. punctifrons* and *P. subopacus. P. poligraphus* has recently been involved in large bark beetle outbreaks in central Sweden, together with the European spruce bark beetle *Ips typographus* (Wulff 2015). However, *P. punctifrons* and *P. subopacus* were also, to some extent, present in the killed trees (Schroeder, personal communication). The role of the *Polygraphus* species in these bark beetle outbreaks is still not clear and needs to be examined further.

Polygraphus use aggregation pheromones to coordinate mass attacks on trees, and male-produced pheromone compounds have been identified for *P. rufipennis* (3-methyl-3-buten-1-ol, Bowers et al. 1991), *P. poligraphus* [(−)-(*R*)-terpinen-4-ol, Schurig et al. 1985, (−)-(*R*)- and (+)-(*S*)-terpinen-4-ol, Rahmani et al. 2015] and for *P. punctifrons* [(+)-(*1R,2S*)-grandisol and (−)-(*R*)-terpinen-4-ol,

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Rahmani et al. 2019]. No pheromone has yet been identified for *P. subopacus* and little has been published about the species. However, males of *P. subopacus* have been shown to produce acoustic signals when exposed to others males of the same species. These signals were found to be species-specific when compared to acoustic signals from *Polygraphus proximus* males and *Polygraphus nigrielytris* males (Kerchev 2020). *P. subopacus* breeds on Norway spruce (*Picea abies*) and it has been reported to attack standing trees which are weakened or have recently died (Ehnström and Axelsson 2002). However, findings of *P. subopacus* in bark samples from snapped and uprooted trees show that they can also attack lying tree trunks (Jakus 1998). *P. subopacus* has also been found in medium-sized branches (3 cm diameter) which have been left on clear cut areas as cutting residues (Gedminas et al. 2007). *P. subopacus* is thought to occur only locally in the northern parts of Sweden (Ehnström and Axelsson 2002).

In this work, our aim was to investigate the chemical communication of *P. subopacus* and to identify the pheromone of the species to be used as bait in traps for monitoring of population levels and early detection in areas outside its distribution area.

Materials and methods

Collection of insects and host spruce tree material

In June 2016, stem sections were cut from 10 small (mean diameter 1.3 m = 10 cm; mean height 7 m) dying Norway spruce trees, assessed to be colonized by *P. subopacus*, in the province of Jämtland. The stem sections were placed in emergence boxes in a climate room (20 °C, 20 h day length) which were emptied on a daily basis except for during weekends. Beetles were stored in 5 °C for 2–8 days until they were used in the experiments.

Stem sections from weakened Norway spruce trees were cut 2 to 12 days before they were used in the experiments (diameter 10–15 cm, length of each stem section 50 cm). They were stored outdoors until used, to minimize their risk of drying out. The stem sections were stored in net bags to avoid colonization of other insects.

Sampling of volatiles from boring beetles

Males and females of *P. subopacus* were placed on stem sections of Norway spruce. Microcentrifuge tubes, 1.5 ml (Brand®, Wertheim, Germany), were nailed onto the stem sections and the bottoms and lids were cut off. One beetle was introduced into each microcentrifuge tube and the open end was sealed with aluminium foil. Boring of beetles into the bark was demonstrated by presence of frass in the tube. Volatile organic compounds (VOCs) were sampled by introducing an SPME fiber into the microcentrifuge tube, exposing the fiber for 30 min. Before sampling, the fiber was conditioned in the GC inlet at 250 °C for 5 min or until clean. Background emissions were measured in the same way but without introducing an insect into the microcentrifuge tube. Instead, a hole of 2.5 mm in diameter was drilled manually in the bark. All experiments were conducted between August 15, 2016 and November 7, 2016 and at room temperature (20–22 °C). For all samples, an SPME fiber with a 65-µm polydimethylsiloxane/divinylbenzene coating (PDMS/DVB, Pink 57,326-U, Supelco, Bellefonte, PA, USA) was used.

On August 15, 2016, two males were placed on a stem section of Norway spruce. Both males started boring into the bark and one of them was sampled 5, 6, 7, 9, 12, 13, 14, 15, 16, 19 and 22 days after it started boring. The other male was sampled only on day 6 and 7, as it did not seem to produce any VOCs.

On August 25, 2016, two additional males were placed on a stem section and both started boring into the bark. They were sampled on day 3, 4, 5, 6, 7, 10, 11 and 13.

On October 12, 2016, seven males and seven females were placed separately on a stem section 5 males and 5 females started boring into the bark. Of these insects, 4 males were found to produce volatiles when sampled with SPME–GC–MS. One was sampled on days 5, 7, 8, 9, 10, 11, 12, 13, 14, 18 and 25 and another one on days 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18 and 25. Two males were sampled on days 6, 7 and 8. The one which did not produce VOCs was sampled on day 5 and 7. In the 5 females, no volatiles were observed other than those from the spruce background. Two females were sampled on day 6, one on day 6 and 12, one on day 7 and one on day 7 and 12.

Background emissions were sampled from each stem section. After a hole was drilled manually, volatiles were sampled on the same day in all cases (day 1) and also on day 2, 3 and 8 in one case, day 2, 3, 5, 8 and 9 in one case and on day 2 in one case.

Analysis of collected volatiles

SPME samples were analysed with a Hewlett-Packard 6890 N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP 5973 mass spectrometer (MS). The MS was operating in electron impact (EI 70 eV) ionisation mode using helium as mobile phase (flow rate 1 mL/min) with the inlet set to 250 °C, splitless injection. Transfer line was set to 250 °C.

Two types of columns were used, a mid-polar HP5-ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J&W Scientific, Folsom, CA, USA) and a polar VF23-ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J&W Scientific, Folsom, CA, USA). The

temperature program used for both columns was started at 50 °C, held for 2 min, then the temperature was increased by 10 °C per minute up to 230 °C, where it was held for 5 min. The HP5-ms column was used for all insects and background samples, whereas the VF23-ms column was used for three of the males and their corresponding spruce backgrounds. For both columns, a slower program was also tried, where the temperature was increased by 5 °C per minute. This did not increase separation significantly, at least not for the compounds considered relevant. Desorption time for the sampled volatiles on the SPME fiber was in all cases 5 min at 250 °C.

Raw MS data was analyzed using the Workstation 7.0.0 (Agilent) software. Compounds were identified by comparing their mass spectra with the NIST 14 library, then comparing the mass spectra and the retention times with those of synthetic references.

Electroantennography (EAG)

EAG responses were recorded with glass electrodes filled with Beadle-Ephrussi Ringer Solution (Ephrussi and Beadle 1936). The probe, micro manipulators, IDAC4 signal amplifier, CS-55 stimulus controller and GcEad 2014 v1.2.5 software were all from Ockenfels SYNTECH GmbH, Buchenbach, Germany. The reference electrode was connected to the decapitated head of the insect and the recording electrode to the tip of one of the antennae. A constant flow of humified air (50 ml/min) was applied 1 cm above the antenna. The compounds to be tested were diluted in *n*-hexane to concentrations of 100 ng/µl. 10 µl of each test solution was applied to a 00A grade filter paper (1 cm^2) that was folded and inserted to a Pasteur pipette. The filter paper was dried for at least 5 min before the experiment started. The pipette was inserted to the stream of humified air from the stimulus controller and a short air puff through the pipette delivered the substance to the antenna. *n*-Hexane was used as a blank and (*Z*)-DMCHE as a control of the response of the antenna. Each compound was tested on 4 male and 4 female insects but *n*-nonane was only tested on two males and three females due to a lack of insects.

Field study

Based on GC peak areas and EAG responses, two male-produced compounds were used in a field study, together and in combination, to investigate their attractiveness to males and females of *P. subopacus*. The field study was conducted in Norway spruce forests in the province of Medelpad, county of Västernorrland, in central Sweden. Black Ecotraps (Fytofarm Ltd.) were used with collection jars for dry catches. Dispensers were hung just above the collection jars, around 80–90 cm above the ground. The traps were spaced 30 m apart and they were placed at least 20 m into the forest. Four treatments were randomly assigned to positions at 10 different locations and each location was considered a replication. A randomized block design was used for the field study and treatments were not rotated. The treatments were (1) (*Z*)-DMCHE, (2) (*E*)-DMCHE, (3) (*Z*)-DMCHE together with (*E*)-DMCHE and (4) unbaited control traps (containing only *n*-nonane). Traps were emptied once per week (with the exception of two locations which were not emptied the first week, and a third location which was not emptied during the second week). Catches were stored in a freezer (18 °C) until the insects could be counted and identified. Species identification and sex determination (Lekander 1959) was done using a stereomicroscope with 14-90X magnification. The experiment was conducted between June 26 and July 29, 2017, and was combined with a larger study aimed at examining interactions between three *Polygraphus* species. The daily maximum temperature at the weather station closest to the locations used in the field study varied between 12.4 ºC and 24.1 ºC, whereas the total rainfall during this period was 76.1 mm (SMHI 2020).

Chemicals and dispensers

3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol and 3-methyl-2-butenal were purchased from Sigma-Aldrich (Schnelldorf, Germany). Both isomers of DMCHE (trade names Grandlure II and (*E*)-isomer of Grandlure II) were purchased from Bedoukian Research (Danbury, CT, USA), as was racemic grandisol, (Grandlure I) and a 1:1 mixture of (*Z*)- and (*E*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde (Grandlure III and IV). Racemic fragranol was synthesized previously at our laboratory (Rahmani et al. 2019). Citral was bought from Acros Organics (Geel, Belgium). Benzaldehyde and benzyl alcohol were bought from Sigma Aldrich. 7-Methyl-3-methylene-6-octen-1-ol (γ-isogeraniol) was synthesized according to the method described by Yong et al. (2001), the method was slightly modified and is described in detail in the Supplementary Information. *n*-Nonane 99% was bought from Alfa Aesar (Heysham, Lancashire, UK).

The dispensers used for the field experiments were"wick baits" (Birgersson et al. 2012). For each dispenser, the compound(s) were dissolved in 4 mL of *n*-nonane and contained in a 4 mL glass vial. The dissolved compound(s) were allowed to evaporate through a teflon tube, 6 cm × 1,5 mm i.d. which was lined with cotton yarn and inserted through a drilled hole in the lid of the vial. For (*Z*)-DMCHE, 12.5 mg was used for each dispenser and for (*E*)-DMCHE, 1.25 mg was used. Based on previous evaporation studies with similar compounds, the average release rate in a fume hood at the laboratory (22–25 °C and with an air flow of 0.5–0.6 m/s) was expected to be 0.46 mg/day of (*Z*)-grandlure II and 0.046 mg/day of (*E*)-grandlure II (Viklund et al. 2019). All

lures lasted for the duration of the field study and did not need to be replaced.

Statistical analyses

The number of *P. subopacus* and *P. poligraphus* caught in each treatment was compared using a binomial sign test with a significance level of $\alpha = 0.05$. The summed catches from each trap over four weeks was used in the analysis, and each location was considered a replication. Statistical tests were performed in Microsoft Excel, using built-in functions.

Results and discussion

Analysis of collected volatiles

In total, 9 males and 5 females started boring into the spruce bark and were sampled with SPME and analysed with GC–MS using a HP5-ms column. Several compounds were found in the VOCs from males (Fig. 1), but not in females or in the spruce background. The identities of these male-specific compounds were determined based on retention times and fragmentation patterns which were compared to

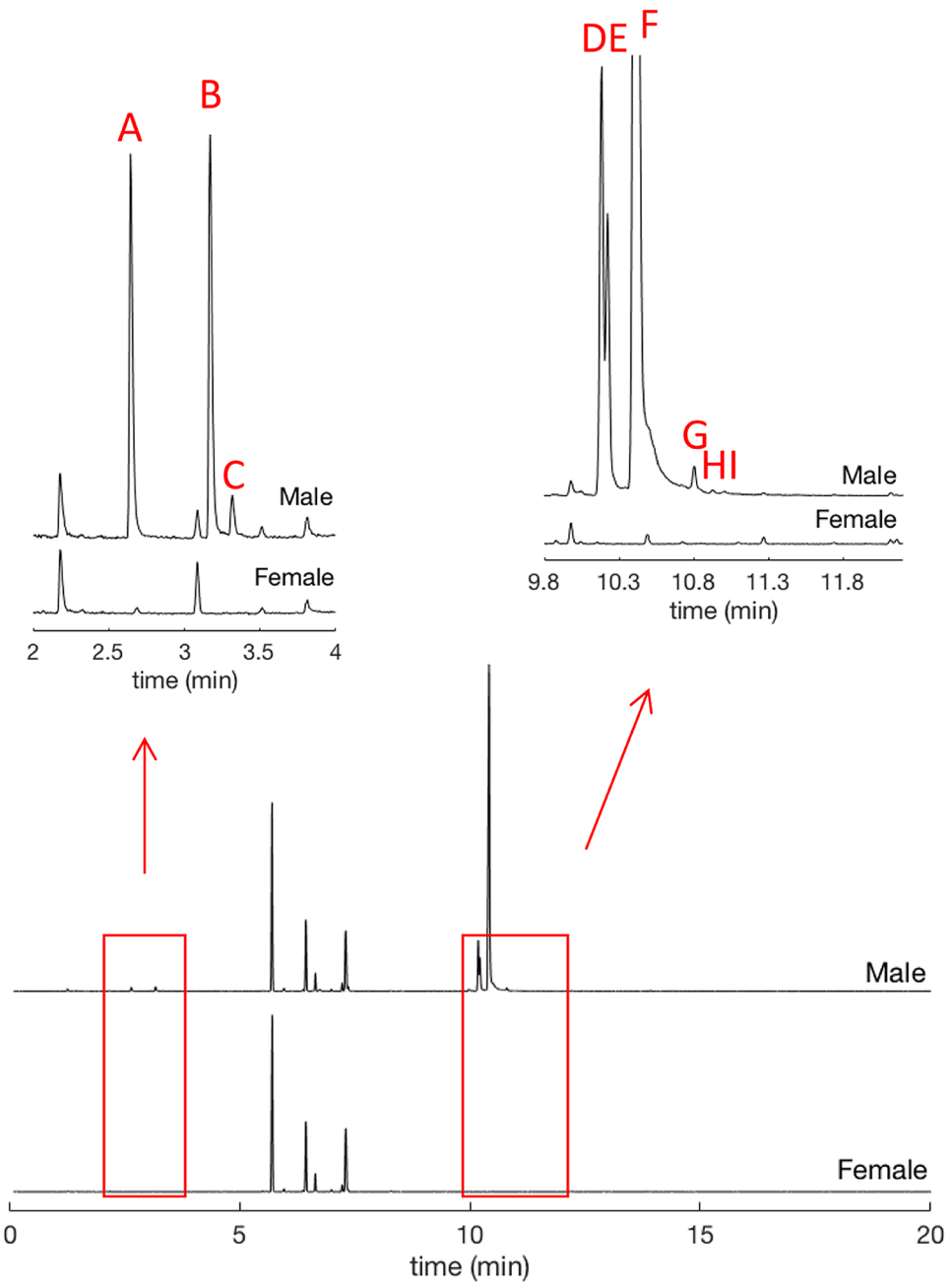

Fig. 1 Chromatograms showing male-specific peaks from SPME–GC–MS analyses of a representative *P. subopacus* male and female. The insects were sampled 7 days after they started boring into the bark. A HP5-ms column was used for the analyses with a temperature program that started at 50 °C for 2 min and then the temperature was increased by 10 °C per minute up to 230 °C where it was held for 5 min

the NIST 14 library and to synthetic references (Supplementary Information, Figures S1-S9).

The MS library suggested that Compound **A** (Fig. 1, rt: 2.64 min, m/z(%): 86(40), 68(99), 67(65), 56(100), 41(82)) was 3-methyl-3-buten-1-ol (F match 928, R match 935). When a synthetic reference of 3-methyl-3-buten-1-ol was injected, both retention time and mass spectrum matched the insect produced compound and its identity could be confirmed. 3-Methyl-3-buten-1-ol is the pheromone of *Polygraphus rufipennis*, a closely related species (Bowers et al. 1991) and it is also part of the pheromone of the bark beetle *Ips cembrae* (Zhang et al. 2000).

Compound **B** (Fig. 1, rt: 3.17 min) had a fragmentation pattern (m/z(%): 86(23), 71(100), 53(29), 43(26), 41(37)) which was similar to 3-methyl-2-buten-1-ol (F match 938, R match 944), according to the MS library. Comparison with both retention time and mass spectrum of a synthetic reference showed that the emitted compound was indeed 3-methyl-2-buten-1-ol.

Compound **C** (Fig. 1, rt: 3.32 min) seemed to be the aldehyde of B, 3-methyl-2-butenal (F match 922, R match 951, m/z(%): 84(100), 83(50), 55(44), 53(9), 41(24)) and once again, after an injection of a synthetic reference both retention time and mass spectrum confirmed that the male produced compound was 3-methyl-2-butenal.

Compounds **A**, **B** and **C**, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol and 3-methyl-2-butenal, are all produced by males of the walnut twig beetle, *Pityophthorus juglandis.* In this species, 3-methyl-2-buten-1-ol is the primary aggregation pheromone component (Seybold et al. 2015).

Compounds **D** and **E** eluted nearly at the same time (Fig. 1, rt: 10.18 min and 10.22 min) and their mass spectra were almost identical (m/z(%): 154(0.4), 109(38), 68(100), 67(68), 69(21) for compound **D** and m/z(%): 154(0.4), 109(38), 68(100), 67(67), 69(18) for compound **E**). The suggestion from the MS library for both compounds was grandisol (F match 948, R match 948 for compound **D**; F match 939, R match 939 for compound **E**), the main pheromone component of *Polygraphus punctifrons* (Rahmani et al. 2019). The fact that two GC peaks were present on a non-chiral column led us to believe that one was grandisol and the other one the *trans*-isomer, fragranol. Injection of synthesized references confirmed this hypothesis. The retention time of compound **D** matched the grandisol reference and the retention time of compound **E** matched the fragranol reference. The stereochemistry of these compounds was not further examined.

The largest GC peak representing the male-specific compound **F** showed a retention time of 10.42 min (Fig. 1, m/z(%): 154(13), 136(84), 121(86), 93(87), 69(100)). The suggestion from the MS library was that the identity of this compound was (*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol (F match 956, R match 956). When references of the (*Z*)- and (*E*)-isomer were run, retention times and mass spectra of both compounds were very close to the male specific compound (Fig. 2). A slower temperature programme where the temperature was increased by 5 °C/min did not increase the separation significantly. But, when a VF23-ms column was used, the (*E*)- and (*Z*)-isomers separated and it was clear that both compounds were present in the VOCs from males (Fig. 3). The major male specific compound **F1** was (*Z*)-2-(3,3-dimethylcyclohexylidene)-ethanol, (*Z*)-DMCHE (m/z(%): 154(13), 136(84), 121(86), 93(87), 69(100)), but the (E)-isomer, (*E*)-DMCHE was also present as a minor compound, **F2.** The retention times were 11.85 min and 11.95 min respectively on the VF23-ms column. Both compounds **F1** and **F2** are known pheromone compounds in other beetles, among others the soybean stalk weevil *Sternechus subsignatus* (Ambrogi et al. 2012), the pepper weevil *Anthonomus eugenii* (Eller et al. 1994) and the bark beetle *Pityogenes quadridens* (Byers et al. 2013).

Compounds **G** (Fig. 1, rt:10.80 min) and **H** (Fig. 1, rt: 10.92 min) had somewhat similar fragmentation patterns. The suggestions from the MS library was that these compounds were aldehydes of **F1** and **F2**. Compound **G** had a fragmentation (m/z(%): 152(30), 137(100), 109(51), 81(36),

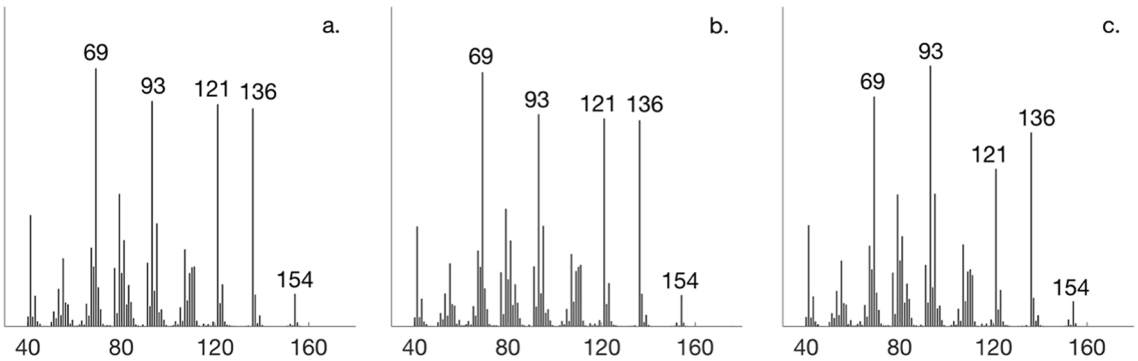

Fig. 2 MS-spectra showing (**a**) the major male specific compound F, (**b**) (*Z*)-DMCHE and (**c**) (*E*)-DMCHE

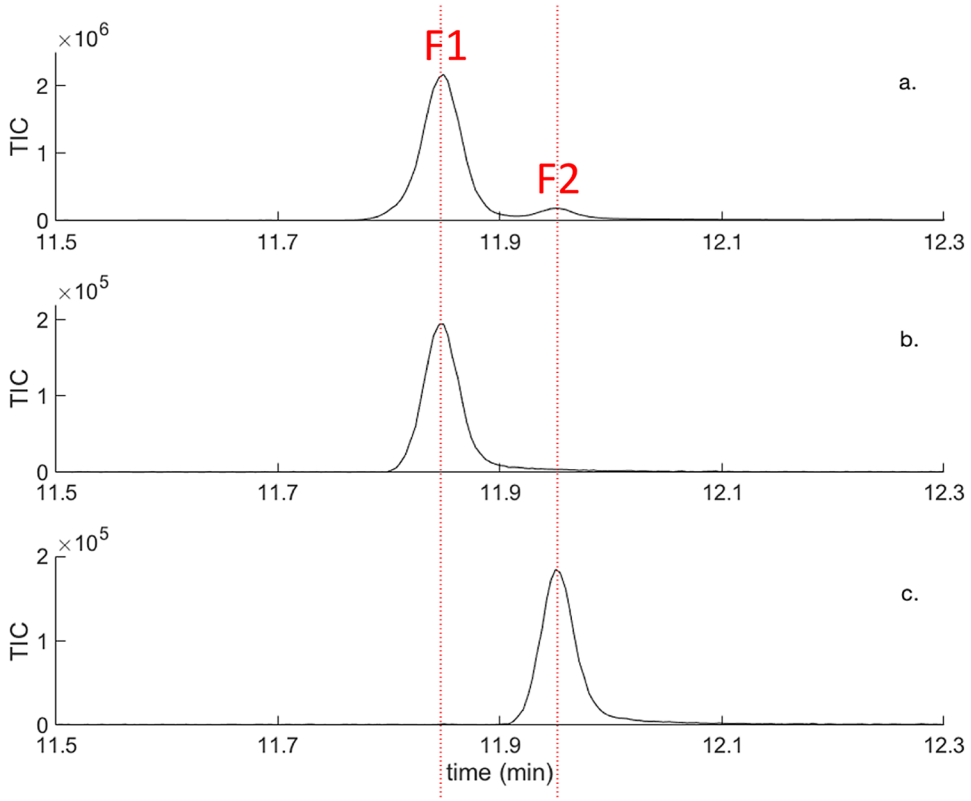

Fig. 3 GC-chromatograms from SPME–GC–MS analysis of (**a**) *P. subopacus* male, and synthetic references of (**b**) (*Z*)-DMCHE and (**c**) (*E*)-DMCHE. A VF23-ms column was used for the analyses with a temperature program that started at 50 °C for 2 min and then the temperature was increased by 10 °C per minute up to 230 °C where it was held for 5 min

69(30)) similar to (*Z*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde, (*Z*)-DMCHA (F match 902, R match 903) and compound **H** gave a fragmentation (m/z(%): 152(79), 137(61), 109(100), 81(61), 69(54)) similar to (*E*)-2-(3,3-dimethylcyclohexylidene)-acetaldehyde, (*E*)-DMCHA (F match 853, R match 859). A synthetic reference of these two compounds in a 1:1 ratio (Grandlure III and IV) confirmed that these male produced aldehydes were indeed compounds **G** and **H**. These two aldehydes are part of the pheromone in several beetles, such as the pepper weevil *Anthonomus eugenii* (Eller et al. 1994), the cotton boll weevil *Anthonomus grandis* (Tumlinson et al. 1969), the cranberry weevil *Anthonomus musculus* (Szendrei et al. 2011), the pecan weevil *Curculio caryae* (Hedin et al. 1997) and the bark beetle *Pityogenes quadridens* (Byers et al. 2013).

Compound **I** (Fig. 1, rt: 11.01 min) presented as a very small CG peak with a fragmentation pattern (m/z(%): 152(5), 109(16), 84(28), 69(100), 41(40)) similar to geranial (F match 744, R match 838). F and R match values were rather low since the peak height was only three times the noise level. However, a synthetic reference of citral confirmed that the insect specific GC peak matched with the second eluting peak. Citral consists of two isomers, neral and geranial, and the mass spectra of these two compounds are quite similar. However, retention times are different on an HP5-ms column and geranial elutes after neral (Shimizu et al. 2004). We concluded that compound **I** was geranial which is also a pheromone component in a wide variety of species, among others the Ambrosia beetle *Platypus koryoensis* (Kim et al. 2009).

When the VF23ms column was used, another very small GC peak, compound **J**, was seen emitted from the males and its mass spectrum matched γ-isogeraniol (R match 803, F match 803, m/z(%): 41(47), 69(100), 93(16), 111(25), 121(2)). The retention time on the VF23-ms column was 11.32 min, which was the same as for the synthethic reference. The presence of γ-isogeraniol in some of the males could also be confirmed, with expected characteristic mass fragments, at retention time 10.25 min on the HP5-ms column.

In addition to these eleven male-specific compounds, some other GC peaks were noted in *P. subopacus* males (Supplementary Information, Figures S7-S10). Benzaldehyde (rt: 6.15 min on the HP5-ms column) was present in all males, but only in trace amounts in one of the females. Benzyl alcohol (rt: 12.45 min on the VF23-ms column) was also present in the males, but as it coeluted with GC peaks from the background on both columns, it was not possible to determine whether it was also present in the spruce background or in the females. When the HP5-ms column was used, two additional, small GC peaks which may be male specific were noted (rt: 6.72 and 6.74 min). However, their mass spectra did not generate good matches in the MS library as they coeluted with GC peaks from the background,

and they were not seen when the VF23-ms column was used and thus their identities were not further investigated.

To summarize, eleven male-specific compounds were identified (Fig. 4). These compounds were found in SPME samples from the males but not in samples from the females or from the spruce background. The major compound in all males was (*Z*)-DMCHE (compound F1, trade name Grandlure II). The males seemed to produce a maximal amount of compounds during the first week after the insects started boring into the bark. However, the main compound could still be detected in two males after 25 days. No female-specific compounds were found in our study, since all VOCs seen in the females were also seen in the males and/or in the spruce background.

Electroantennography

The identified male-specific compounds were tested for activity on *P. subopacus* antennae (Table 1, Fig. 5). Fragranol could not be tested due to a lack of compound of high enough chemical purity. Antennae of males and females of *P. subopacus* could detect (*Z*)-DMCHE, (*E*)-DMCHE, grandisol and to some extent (*Z*)-DMCHA and (*E*)-DMCHA. The beetles' antennae could also detect *n*-nonane, which was used as a solvent in the dispensers in the field study. They could not detect 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 3-methyl-2-butenal, citral or γ-isogeraniol. The two compounds which seemed to occur in larger amounts in males (benzaldehyde and benzyl alcohol) were also tested, although these compounds did not generate a strong response from the antennae. A weak EAG signal (denoted X in Table 1 and Fig. 5) was defined as a response (peak height) which was less than half of the response generated by (*Z*)-DMCHE, but still significantly larger than the response generated by *n*-hexane. Any response which was stronger than a weak EAG signal was considered a strong signal (denoted XX in Table 1, Fig. 5).

Table 1 EAG response of *P. subopacus* to different synthetic substances

Compound	Response
3-Methyl-3-buten-1-ol	0
3-Methyl-2-buten-1-ol	0
3-Methyl-2-butenal	0
Grandisol	XX
(*Z*)-DMCHE	XX
(*E*)-DMCHE	X/XX
(*Z*)-DMCHA	0/X
(*E*)-DMCHA	X
Citral	0
γ-isogeraniol	0
Benzaldehyde	0
Benzyl alcohol	0/X
Nonane	X/XX

XX = strong, X = weak, 0 = none (≤ Hexane), 0/X, X/XX = response varies

Field study

(*Z*)-DMCHE and (*E*)-DMCHE were tested in the field, as they appeared to be the major male-produced compounds and as our results from the EAG study showed that *P. subopacus* antennae reacted strongly to these compounds. They were also tested in combination (Table 2). The beetles' antennae could also clearly detect grandisol in our EAG study, but grandisol was not tested in the field since the stereochemistry of the compound emitted from the beetles had not been determined. The *trans*-isomer fragranol,

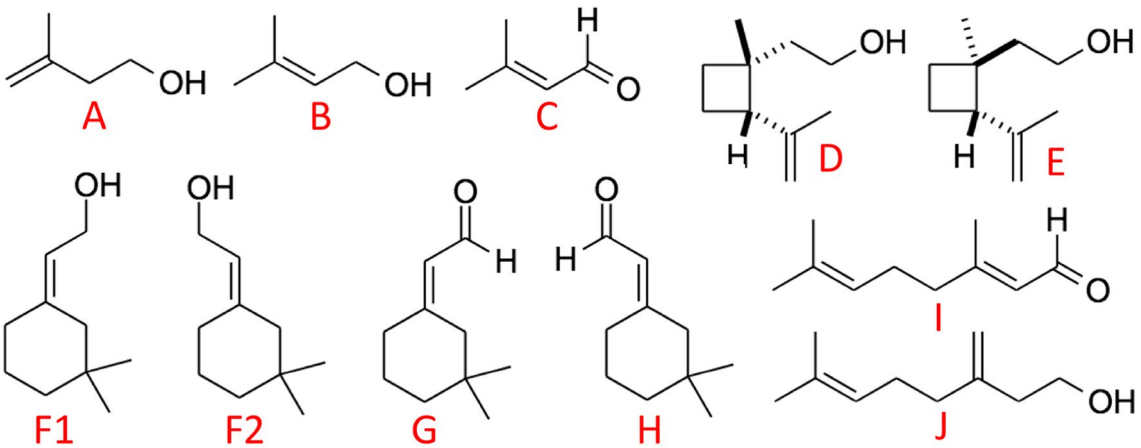

Fig. 4 Structures of male specific compounds found when sampling with SPME when *Polygraphus subopacus* were boring into spruce logs

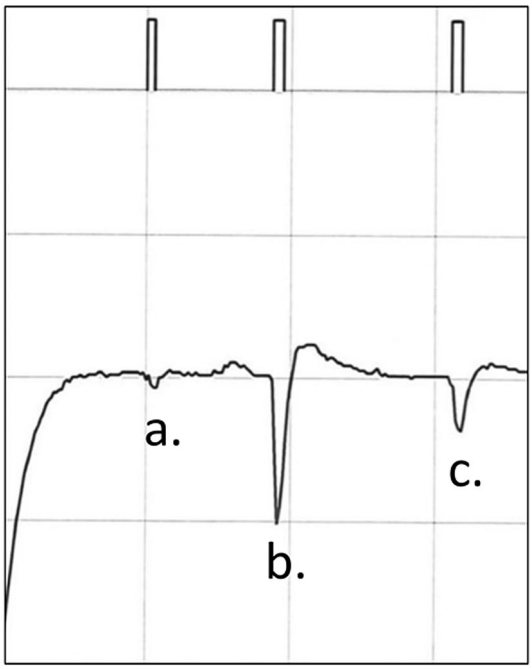

Fig. 5 Example of EAG responses of *P. subopacus* antennae. **a** *n*-Hexane (0), (**b**) (*Z*)-DMCHE (XX) and (**c**) (*E*)-DMCHA (X). XX = strong, X = weak and 0 = none (≤ *n*-hexane)

which also occured in the males of *P. subopacus*, was not at that time available for field test.

Traps baited with (*Z*)-DMCHE caught significantly more males and females of *P. subopacus* compared to the control traps ($P < 0.001$ for both sexes). The combination of (*Z*)- and (*E*)-DMCHE did not significantly change the catches as compared to only (*Z*)-DMCHE, and (*E*)-DMCHE on its own did not attract more *P. subopacus* than the control traps. Of all *P. subopacus* caught in traps baited with (*Z*)-DMCHE 38% were males.

Side catches consisted mostly of *P. poligraphus* and (*Z*)-DMCHE was more attractive to this species than the control traps ($P < 0.001$ for males and $P = 0.01$ for females). (*E*)-DMCHE did not attract *P. poligraphus* on its own and did not significantly increase the catches when combined with (*Z*)-DMCHE.

n-Nonane on its own caught very few individuals of both species. In total, only 7 *P. subopacus* and 4 *P. poligraphus* were caught in the 10 control traps baited with *n*-nonane during the entire field study. For comparison, 1855 *P. subopacus* and 323 *P. poligraphus* were caught in total in the 10 traps baited with (*Z*)-DMCHE.

Both *P. poligraphus* and *P. subopacus* were active for the duration of the field study, and both species were caught at all locations.

Conclusions

Several male specific compounds were produced by *P. subopacus* when beetles were boring in the bark; 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 3-methyl-2-butenal, grandisol, fragranol, (*Z*)-DMCHE, (*E*)-DMCHE, (*Z*)-DMCHA, (*E*)-DMCHA, geranial and γ-isogeraniol.

The major male specific compound was found to be (*Z*)-DMCHE and we could show that it was attractive to *P. subopacus* in field tests. To some extent, this compound was also attractive to *P. poligraphus* and the reason for that remains to be discovered since (*Z*)-DMCHE does not seem to be produced by male *P. poligraphus* (Rahmani et al. 2015).

(*E*)-DMCHE was also produced by *P. subopacus* males but was not attractive on its own, and in combination with (*Z*)-DMCHE it did not increase or decrease the attractiveness for either species in the field. It is likely that some of the other male produced compounds which we identified

Table 2 Trap catches of *P. subopacus* and *P. poligraphus* between June 26 and July 29, 2017. Catches are shown as a mean per trap from the entire study period with a 95% confidence interval (95% CI). The proportion of males caught by each treatment is also shown. There were 10 replications in the experiment ($N = 10$)

Treatment	Number of *P. subopacus*		Number of *P. poligraphus*	
	Mean per trap, (95% CI), $N = 10$	Proportion of males (%)	Mean per trap, (95% CI), $N = 10$	Proportion of males (%)
(*Z*)-DMCHE	186 (±93)*	38	32 (±25)*	69
(*E*)-DMCHE	0 (±0)	100[a]	1 (±2)	70
(*Z*)-DMCHE and (*E*)-DMCHE	116 (±49)*	35	50 (±50)*	70
Control (*n*-nonane)	1 (±1)	71	0 (±1)	50[b]

[a]Only one *P. subopacus* was caught in total in the traps baited with (*E*)-DMCHE

[b]Four *P. poligraphus* were caught in total in the 10 control traps

*Denotes statistically significant differences compared to the control traps

are also part of the pheromone of *P. subopacus* and may be required in order to make a species-specific bait. Grandisol and fragranol are good candidates for future field studies as they generated rather large GC peaks in our analysis of the male emitted compounds and as the insects responded well to racemic grandisol in our EAG study. However, adding racemic grandisol to (*Z*)-DMCHE is not likely to make the bait species-specific as we know from previous studies that *rac*-grandisol does not repel *P. poligrahus* in the field (Rahmani et al. 2019).

Furthermore, *Polygraphus subopacus* was caught at all ten locations used in our field experiment, most of them being production forests. This finding suggests that *P. subopacus* is a more common species than previously thought, at least in the spruce forests of Medelpad.

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Authors' contributions LV, EH and MS designed the study. MS provided beetles for laboratory analyses. LV conducted the SPME–GC–MS study and the field study. JB conducted the EAG analyses. LV wrote the first draft of the manuscript and all authors contributed to the final version of the manuscript.

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Data availability Data is presented in the Supplementary Information. Additional data can be provided by the authors upon request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors do not have any conflicts of interest to declare.

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