Are different volatile pattern influencing host plant choice of belowground living insects?

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Abstract: Beeinflussen verschiedene Volatilenmuster die Wirtspflanzenwahl unterirdisch lebender Insekten?

Zum besseren Verständnis der Orientierung und Fraßpräferenz von Maikäferlarven *Melolontha hippocastani* FABR. (Coleoptera: Scarabaeidae) im Boden wurden Wahltests durchgeführt. Den Larven von *M. hippocastani* wurden Karotten (*Daucus carota* ssp. *sativus*) und Eichenwurzeln (*Quercus* sp.) zur Auswahl angeboten. Die Duftstoffe von Karotte und Eichenwurzel wurden auf Aktivkohle gesammelt und mit Gaschromatographie – Massenspektroskopie untersucht (GC-MS). Unverletzte Karotten sowie Eichenwurzeln unterschieden sich in ihren Volatilenmustern deutlich voneinander. Darüber hinaus konnten Unterschiede im Volatilenmuster unverletzter, mechanisch verletzter sowie angefressener Wurzeln nachgewiesen werden.

Key words: choice test, GC-MS, *Melolontha hippocastani*, root volatiles, Scarabaeidae

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Cockchafers of the genus *Melolontha* (Coleoptera: Scarabaeidae) can be severe pests in forestry, agriculture and horticulture. Gradation of the two most important species, the forest cockchafer *M. hippocastani* FABR. and the European cockchafer *M. melolontha* L., occurs currently in several parts of central Europe.

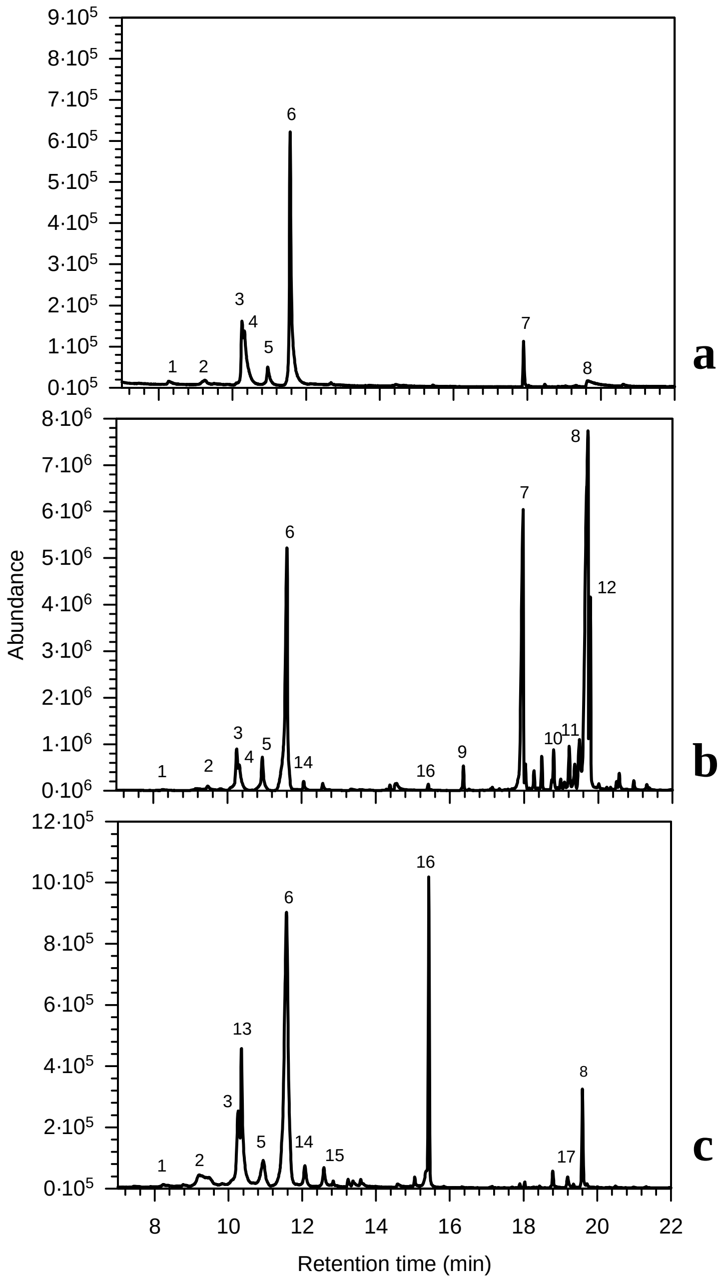
Orientation behaviour of the adult beetles has been the focus of recent studies (REINECKE et al. 2002 a, b, 2005). However, especially the larvae are dreaded because their belowground damage is not visible directly after feeding. There are a lot of speculations about belowground living insects and their way of living, but until now there were not that many experimental investigations. A rather unknown topic is the orientation behaviour of soil living organisms, which is also subject of some publications (HORBER 1954, HAUSS & SCHÜTTE 1976, HASLER 1986, HIBBARD et al. 1994, JEWETT & BJOSTAD 1996, BERNKLAU & BJOSTAD 1998A, BERNKLAU & BJOSTAD 1998B, BERNKLAU et al. 2005).

Material and methods

The plants investigated were organically cultivated carrots (*Daucus carota* ssp. *sativus*) and about 5 year old oak trees (*Quercus* sp.) from a forest near Göttingen. The larvae (L3) of *M. hippocastani* were collected in a forest near Darmstadt.

Prior to measurements the roots were washed carefully to remove the soil. During this process the capillary roots and the external bark were only slightly mechanically damaged (undamaged roots). Moreover massive mechanical damage was inflicted by cutting the roots into pieces by a knife (mechanical damage) and roots were damaged by feeding of the larvae (insect damage). Volatiles released by these differently damaged roots were collected.

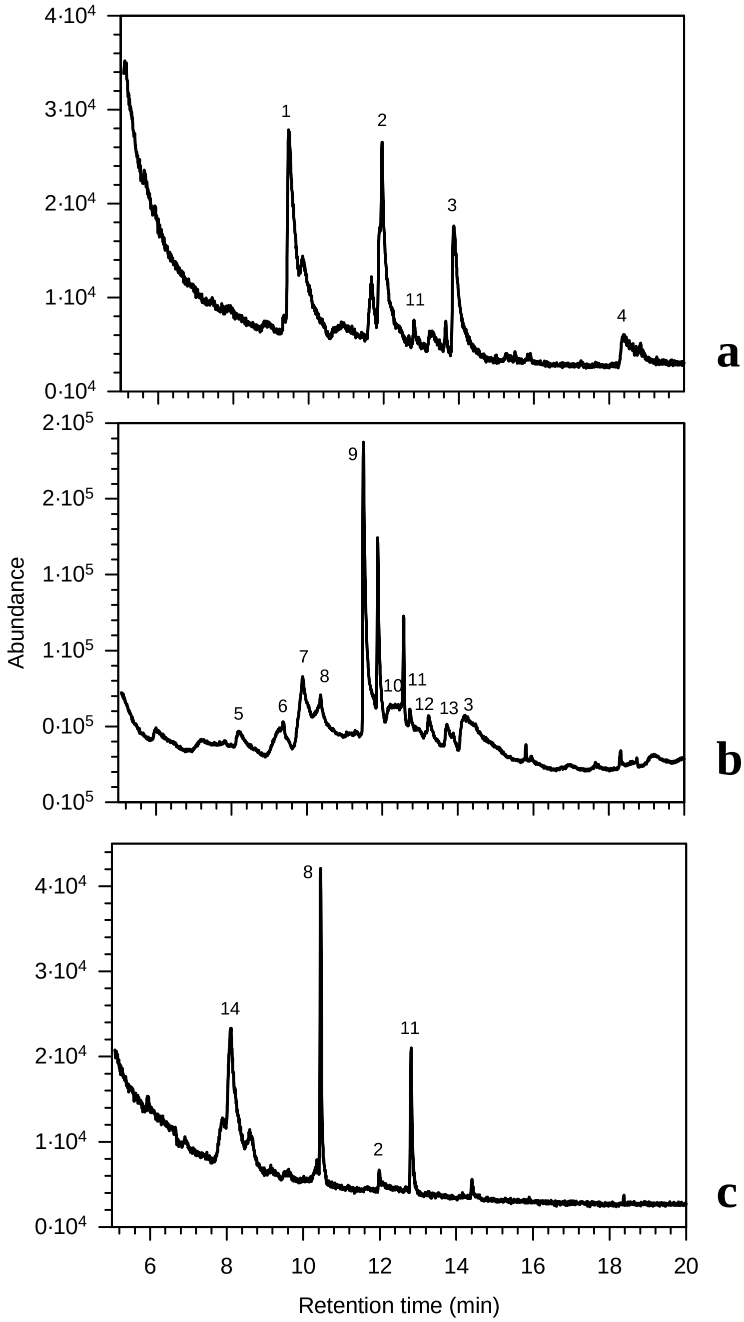
For collecting root volatiles (N=5-10) were enclosed into bags of PTFE foil. At the beginning and at the end of each behavioural experiment, root volatiles were collected from the bare roots using the closed-loop-stripping-analysis (CLSA) method (BOLAND et al. 1984). Within these bags the air was circulated through a charcoal filter with a flow of 1 l/min for a sampling time of 3 hours for oak roots. Carrots were sampled only for 1 hour in order to avoid overloading charcoal trap and GC column. Volatiles were eluted from the charcoal with a 2+1 mixture of methylene chloride and methanol. Odour samples were analysed by coupled gas chromatography-mass spectrometry (WEISSBECKER et al. 2004). The GC (model 6890N, Agilent, Palo Alto, USA) employed the 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 two GC-columns were employed for identification: HP-5MS column (length 30m, ID 0.25 mm, film thickness 0.25 µm, Agilent) and HP-Innovax 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 odour compounds were identified by comparison of retention times and MS-data of reference compounds. Compounds not available as reference standards were identified tentatively by the comparison of retention time and mass spectra with the NIST library and the MASS FINDER library (marked by *).



1→ α-pinene
2→ β-pinene
3→ o-cymene
4→ limonene
5→ 1,4-p-menthadiene *
6→ terpinolene *
7→ caryophyllene
8→ unknown sesquiterpene
9→ δ-elemene *
10→ α-curcumene *
11→ β-bisabolene *
12→ α-caryophyllene
13→ 2-ethyl-hexan-1-ol
14→ 1,3,8-p-menthatriene *
15→ 1-ethyl-3,5-dimethyl benzene
16→ bornyl acetate
17→ 2,4-bis(1,1-dimethylethyl)-phenol *

* → tentatively identified

Fig. 1: Total Ionchromatograms of root volatiles from *Daucus carota* ssp. *sativus*
a) undamaged root
b) mechanically damaged root
a) root damaged by feeding



1→ methylheptenone
2→ 3-methyl-3-cyclohexen-1-one *
3→ decanal
4→ geranylacetone
5→ α-pinene
6→ ethyltoluene
7→ 3-carene
8→ eucalyptol
9→ linalooloxide
10→ nonanal
11→ camphor
12→ epoxylinalool *
13→ dodecane
14→ methoxybenzene

* → tentatively identified

Fig. 2: Total Ionchromatograms of root volatiles from *Quercus* sp.
a) undamaged root
b) mechanically damaged root
c) root damaged by feeding

During the dual choice tests the larvae of *M. hippocastani* (N=20) were kept individually in 20 cm high black 10 l-plastic buckets with a diameter of 28 cm together with carrots and oak trees. The distance between the two plants was about 15 cm. The larva was placed halfway from carrot and oak and about 10 cm below soil surface. Humous, sandy and clayey soil was used as a substrate. The experiment was performed in June 2004 in the glasshouse under controlled conditions (photoperiod 16 hours, 10 kLux, 19-25°C, 40-50% relative humidity). Position of the larvae in relation to the roots and feeding traces of larvae at the roots were assessed in order to evaluate the decision of the larvae.

After one week the roots were visually inspected for signs of feeding damage. The experiment was prolonged for those larvae that did not show any clear decision for one of the plants.

Results

Undamaged oak roots predominantly release fatty acid derivatives whereas damaged oak roots release phenols and monoterpenoids (Fig. 2). The quantities of volatile compounds released by oak roots were about a factor 100 lower compared to carrot roots of the same fresh weight. Both undamaged and mechanically damage roots caused volatile patterns distinctive from the volatile pattern caused by larval feeding (Fig. 1, Fig. 2)

In dual choice tests feeding preferences of larvae of *M. hippocastani* were observed: carrots were clearly favoured if the larvae had the opportunity to decide between carrots and oak roots. After 3 weeks, 4 larvae had died during the experiment. Four of the remaining 16 larvae (=25%) caused feeding traces on both of the roots (by considering only the main roots of the oak trees and carrots), 1 larvae (=6%) showed no decision for one of the plants. 11 larvae (=69%) fed on the roots of carrots exclusively.

According to the null hypothesis the roots of carrots and oaks would be accepted to the same extent. The validity of the null hypothesis was checked by the sign test with correction for continuity. The test statistic u was 2,581989 and thus lead to the rejection of the null hypothesis for $\alpha = 0.05$. Therefore the acceptance of the carrots by the larvae was significantly higher than the acceptance of the oak roots.

Volatile pattern released by carrot roots and oak roots differ both qualitatively and quantitatively. Undamaged carrot roots release predominantly monoterpenoids whereas damaged carrot roots release sesquiterpenoids (Fig. 1).

Diskussion

The common hypothesis about the behaviour of soil living organisms suggests that orientation towards host plant roots of *Melolontha*-larvae is principally guided by a CO_2-gradient (HORBER 1954, HAUSS & SCHÜTTE 1976, HASLER 1986) which is caused by plant root respiration. This means that CO_2 for soil inhabiting polyphagous larvae could function as a non specific lure to find their potential host plants. In addition, volatile secondary plant substances released by the roots might be utilized by the larvae as an important additional cue for orientation and choice of host plants. However, it is important to consider that the composition of the root volatiles is not only influenced by the species but also by the physiological status of the plants (mechanical damage, feeding damage, colonisation by microorganisms). Moreover, the rhizosphere is inhabited by numerous microorganisms modifying plant root volatiles. Additionally plant volatiles might be transformed by these microorganisms, which, in turn, release their own volatile metabolites. Thus, it is a demanding task for the larvae to find the proper food source belowground.

ENE (1942) mentioned, that the orientation of *M. melolontha* larvae is depending more on the quality of the root than on the plant species. THIEM observed some years later (1949), that not only the root tissue quality is important for the behaviour, because in his experiments the larvae preferred clearly carrots over potatoes (*Solanum tuberosum*). However, HOFFMEISTER (1957) performed experiments with larvae of *M. melolontha* and found out, that the level of lignification is an important factor of the choice by the larvae. It is unlikely that these discriminations can be performed on the basis of CO_2-gradient only. Thus, differentiation of plant species by *Melolontha*-larvae needs a contribution of secondary plant metabolites. Moreover it was shown that volatiles released by damaged roots have an impact on orientation behaviour of belowground invertebrates (RASMANN et al. 2005). Insect pathogenic nematodes are attracted by damage induced root volatiles. This suggests that similar mechanisms of volatile guided orientation might be used by invertebrates belowground in a similar way to mechanisms known from insects aboveground. Larvae of *M. melolontha* might serve as a first potential example.

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