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Soil substrates affect responses of root feeding larvae to their hosts at multiple levels: Orientation, locomotion and feeding

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Abstract

The role of soil characteristics for cue-directed behaviour of rhizophagous insects remains largely unexplored to date. Here, we studied behavioural responses of polyphagous cockchafer larvae (*Melolontha melolontha*; Scarabaeidae) to roots of one of its preferred host plants, dandelion (*Taraxacum* sectio *ruderalia*; Asteraceae). Plants were grown in substrates with different adsorptive capacities, i.e. in vermiculite or sand. A behavioural assay was developed which allowed both monitoring the release of attractive CO_2 from roots and observation of larval behaviour at the same time. In sand, larvae got closer to the roots than in vermiculite where they eventually stopped orienting towards the roots: However, their total locomotory activity and the number of turning events/stationary points were higher in vermiculite. Larval behaviour was not correlated with CO_2 concentrations in both vermiculite and sand. On average, larvae kept a greater distance to vermiculite-grown roots. However, a feeding bioassay revealed that the larvae consumed more of vermiculite-grown roots than of sand-grown ones. This result showed that the weaker orientation of larvae towards vermiculite-grown roots was not due to the lower palatability of these roots. Vermiculite might adsorb foraging-relevant cues of dandelion roots, alter composition of root exudates, and/or physically impair oriented movements of soil-dwelling insects. Our study shows that the type of substrate affects responses of rhizophagous larvae to host roots at multiple levels independently of root-derived CO_2 gradients.

Zusammenfassung

Die Rolle von Bodeneigenschaften für Signalstoff-orientiertes Verhalten von wurzelfressenden Insekten ist bisher weitgehend unerforscht. Wir untersuchen die Orientierung von polyphagen Maikäferlarven (*Melolontha melolontha*; Scarabaeidae) zu einer ihrer bevorzugten Wirtspflanzen, Löwenzahn (*Taraxacum* sectio *ruderalia*; Asteraceae). Pflanzen wurden in Substraten mit unterschiedlichem Adsorptionsvermögen, Vermikulit oder Sand, angezogen. Ein Verhaltenstest wurde entwickelt, welcher eine

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gleichzeitige Messung von wurzelbürtigem CO_2 und die Beobachtung von Larvalverhalten ermöglichte. In Sand kamen die Larven den Wurzeln näher als in Vermikulit, wo sie schließlich aufhörten, sich zu den Wurzeln zu orientieren. Die Summe ihrer Bewegungsaktivitäten und die Anzahl der Drehungen/Wendepunkte waren in Vermikulit dennoch höher. Das Larvalverhalten war weder in Vermikulit noch in Sand mit der Konzentration an CO_2 korreliert. Obwohl die Larven sich in Vermikulit durchschnittlich in größerem Abstand zu den Wirtswurzeln aufhielten, zeigte ein Fraßtest, dass die Larven mehr von den in Vermikulit als von den in Sand gewachsenen Wurzeln konsumierten. Demnach ist die beobachtete schwächere Orientierung zu den in Vermikulit gewachsenen Wurzeln nicht mit einer geringeren Schmackhaftigkeit dieser Wurzeln verbunden. Wir nehmen an, dass Vermikulit für die Larven relevante chemische Signalstoffe adsorbiert, die Zusammensetzung von Wurzelexsudaten beeinflusst und/oder Vermikulit gezielte Bewegungen von Insekten im Boden physikalisch beeinträchtigt. Unsere Studie zeigt, dass die Art des Substrats das Verhalten Futter suchender wurzelfressender Insektenlarven auf unterschiedlichen Ebenen unabhängig von wurzelbürtigen CO_2-Gradienten beeinflusst.

Keywords: Belowground; Cockchafer larvae; *Melolontha melolontha*; Scarabaeidae; Soil; Sand; Vermiculite; *Taraxacum* sectio *ruderalia*; Chemical cues; Biotest

Introduction

Numerous herbivorous insect species spend at least part of their lives belowground foraging for plant roots. They may locate their resource randomly or by orienting along gradients of chemicals released by plant roots. Several rhizophagous insect species are known to be attracted by CO_2 released by respiring roots (Johnson & Gregory 2006). Furthermore, a wide range of other root-derived compounds is used by insects when foraging for roots, e.g. sugars, amino acids and lipids (Wensler & Dudzinski 1972), isoflavonoids (Johnson, Gregory, Greenham, Zhang, & Murray 2005), phenolic compounds (Rogers & Evans 2014), hydroxamic acid (Rogers & Evans 2013), or volatiles, such as low molecular weight alcohols, esters, and aldehydes (Johnson & Gregory 2006; Johnson & Nielsen 2012). Despite an increasing number of studies on belowground chemo-ecological interactions between insects and plant roots (e.g. Rasmann et al. 2005; Robert et al. 2012; D'Alessandro et al. 2013; Erb & Lu 2013), we are only beginning to understand how rhizophagous insects locate their host plant roots.

The impact of the type of soil on host foraging behaviour of rhizophagous insects by chemical cues has to our knowledge not been considered so far, although the soil matrix type affects the adsorbance of chemicals (Eilers et al. 2015), and thus, also translocation of root-derived compounds and establishment of chemical gradients in the rhizosphere (Kuzyakov & Domanski 2000; Kuzyakov, Raskatov, & Kaupenjohann 2003). Furthermore, the type of soil may significantly affect the soil microbiota, and thus, the compounds that are detectable in a rhizosphere (Berg & Smalla 2009). The biotic and abiotic factors that plant roots are exposed to in various types of soils may determine the chemicals that are available in a rhizosphere and thereby affect the chemically mediated foraging behaviour of rhizophagous herbivores (Rovira 1969; Bertin, Yang, & Weston 2003; Erb & Lu 2013).

For example, the clay mineral vermiculite (Sutherland & Hillier 1974; Reinecke, Müller, & Hilker 2008; Vaughan, Tholl, & Tokuhisa 2011) or similar substrates (Wolfe, Husband, & Klironomos 2005), which have been used in studies on root – herbivore interactions, are typical examples for highly sorptive substrates. Other studies on root location by herbivores have been conducted by growing plants in white sand (Rasmann et al. 2005; Robert et al. 2012), a substrate with lower adsorption capacities than regular soil. In several studies on host location by rhizophagous herbivores the roots were offered to the herbivores on moist filter paper with no substrate at all (Rogers & Evans 2013, 2014). In other settings cut root pieces (Sutherland & Hillier 1974; Weissteiner 2010) or washed roots with all substrate removed were used as stimulus source.

In the present study we investigated the impact of the type of soil substrate on orientation of a rhizophagous insect to root chemicals. We used the root-feeding European cockchafer (*Melolontha melolontha*) and its preferred host plant (Hauss 1975; Hauss & Schütte 1976), the ubiquitous ruderal plant dandelion (*Taraxacum* sectio *ruderalia*), as a model system. We compared orientation behaviour of cockchafer larvae to dandelion roots grown either in pure white sand or in vermiculite. These two substrates represent extremes along a continuum in terms of specific surface area and adsorptive capacities. Vermiculite is a three layer clay mineral with a remarkable adsorption potential for compounds with divergent chemical properties (Abate & Masini 2007; Abollino, Giacomino, Malandrino, & Mentasti 2007; Froehner, Furukawa, Maceno, & da Luz 2009), whereas sand is an inert silicate material with marginal adsorption potential. Due to differences in particle size and parent rock material, the substrates show differences in pH (Oades 1988), specific surface area, density, aeration, and soil-water retention characteristics (Kettler, Doran, & Gilbert 2001).

CO_2 has for a long time been postulated as the only, or at least the major attractant for cockchafer larvae (Klingler 1957; Hasler 1986). We also monitored the CO_2 contents in