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Identification of olfactory receptor neurons in *Uraba lugens* (Lepidoptera: Nolidae) and its implications for host range

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ABSTRACT

Phytophagous insects detect volatile compounds produced by host and non-host plants, using species-specific sets of olfactory receptor neurons (ORNs). To investigate the relationship between the range of host plants and the profile of ORNs, single sensillum recordings were carried out to identify ORNs and corresponding active compounds in female *Uraba lugens* (Lepidoptera: Nolidae), an oligophagous eucalypt feeder. Based on the response profiles to 39 plant volatile compounds, 13 classes of sensilla containing 40 classes of ORNs were identified in female *U. lugens*. More than 95% (163 out of 171) of these sensilla contained 16 classes of ORNs with narrow response spectra, and 62.6% (107 out of 171) 18 classes of ORNs with broad response spectra. Among the specialized ORNs, seven classes of ORNs exhibited high specificity to 1,8-cineole, (±)-citronellal, myrcene, (±)-linalool and (*E*)-β-caryophyllene, major volatiles produced by eucalypts, while nine other classes of ORNs showed highly specialized responses to green leaf volatiles, germacrene D, (*E*)-β-farnesene and geranyl acetate that are not produced by most eucalypts. We hypothesize that female *U. lugens* can recognize their host plants by detecting key host volatile compounds, using a set of ORNs tuned to host volatiles, and discriminate them from non-host plants using another set of ORNs specialized for non-host volatiles. The ORNs with broad response spectra may enhance the discrimination between host and non-host plants by adding moderately selective sensitivity. Based on our finding, it is suggested that phytophagous insects use the combinational input from both host-specific and non-host specific ORNs for locating their host plants, and the electrophysiological characterization of ORN profiles would be useful in predicting the range of host plants in phytophagous insects.

1. Introduction

The intricate interaction between insects and plants is receiving increasing attention from biologists, and 'How does an insect locate and recognize its host plant?' is still a key question (Schoonhoven et al., 2005). Phytophagous insects have species-specific range of host plants for oviposition and feeding, and the composition of phytochemicals is directly related to the host specificity (Dyer et al., 2007). Plants synthesize species-specific sets of primary and secondary metabolites, and the volatile compounds released from the plants travel through the air as an odor plume containing a number of incompletely mixed filamentous odor strands (Baker et al., 1998). Thus, the usual habitats of phytophagous insects are full of highly complex dynamic mixtures of volatile compounds with a continuously changing temporal and spatial structure. Locating suitable host plants in such a complex odor environment is a daunting task for phytophagous insects.

Chemoreception is the major sensory modality for host location in phytophagous insects (Schoonhoven et al., 2005; Andersson, 2007) although other sensory modalities may also be involved. In a number of species, host location is guided by the trail of volatile compounds produced by their host plants, and the composition of the volatile compounds appears to be important in this process (Bengtsson et al., 2006; Webster et al., 2010b; Bruce and Pickett, 2011; Späthe et al., 2013), playing a critical role in both host recognition and non-host avoidance in phytophagous insects (Bruce and Pickett, 2011). Studies indicate that phytophagous insects such as Lepidoptera can recognize

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the host-specific blends of volatile compounds (Bengtsson et al., 2006; Tasin et al., 2010), where the ratio of constituents in the blend is important in distinguishing between host and non-host plants (Webster et al., 2010b; Bruce et al., 2005). The attractiveness of a blend of volatile compounds to phytophagous insects can be lost or decreased by changing the blend ratio or adding non-host volatile compounds (Webster et al., 2010b). For the fine-tuned detection and discrimination of plant volatiles insects have highly specialized olfactory sensory system.

The underlying sensory mechanism for the perception of blend ratios of plant volatiles is not yet well understood although the recognition of blend ratios has been relatively well described in multi-component pheromone system in some insects (Baker, 2002; Baker et al., 2004; Heinbockel et al., 2004; Domingue et al., 2007). In moths, specialized ORNs appear to accommodate the recognition of specific blend ratios, which is enhanced by the co-compartmentalization of ORNs (Baker et al., 1998; Quero et al., 2001; Heinbockel et al., 2004; Clifford and Riffell, 2013). Phytophagous insects detect volatile compounds using a set of differentially tuned olfactory receptor neurons (ORNs) mostly present in the antennae. Studies show that phytophagous insects usually possess various classes of highly specialized ORNs for plant volatiles, often with other classes of ORNs with broad response spectra (Shields and Hildebrand, 2001; Yuvaraj et al., 2013; Park et al., 2013). Studies also show that phytophagous insects have a set of ORNs specialized for non-host plant volatiles (Andersson et al., 2009), which indicates that the sensory inputs from both host specific and non-host specific ORNs are used for host-location. Our aim was to identify ORNs and their corresponding active volatile compounds to inform us which host and non-host volatile compounds are used as critical cues for host location and discrimination. Our interest arises from the desire to be able to find new methods for predicting the host range of invasive photophagous insects. We might be able to make accurate predictions of the host range by comparing the composition of plant volatile emanation with the profile of ORNs and their corresponding active compounds in an invasive insect, even in the absence of any reliable host-plant records from the country of origin of the invading insect.

The gum leaf skeletonizer, *Uraba lugens* Walker (Lepidoptera: Nolidae), is oligophagous, feeding mostly on many *Eucalyptus* and closely related Australian species in the Myrtaceae (*Corymbia calophylla*, *Corymbia ciriodora*, *Corymbia ficifolia*, *Corymbia intermedia*, *Lophostemon confertus* and *Lophostemon sauveolens*) (Cobbinah, 1978; Farr, 2002; Potter and Stephens, 2005; Berndt and Allen, 2010). This species, native to Australia, invaded into New Zealand around 1995 and is now well established in the northern area of New Zealand (Suckling et al., 2005). Some other non-eucalypt tree species including the unrelated *Betula pendula* Roth (Betulaceae) and *Liquidambar* have also been identified as 'novel hosts' in New Zealand although being utilized only occasionally (Berndt unpublished data). No native hosts have been recorded in New Zealand as anything other than extremely rare oviposition hosts (Withers et al., 2011). Here we investigated the profile of ORNs in female *U. lugens* and compared the profile with the compositions of their host and non-host plant volatiles. Antennal ORNs and their corresponding active stimulus compounds were electrophysiologically identified by using the single sensillum recording (SSR) technique, and their response profiles were characterized by measuring the sensitivity and selectivity of individual ORNs to a range of plant volatile compounds. The ORNs were classified into distinct classes depending on their response characteristics, and the relationship of the ORN profile to the host specificity in *U. lugens* was evaluated. The implication of the results to the potential usefulness of ORN profiling for predicting the host plant range of invasive insects is discussed.

2. Materials and methods

2.1. Insects

U. lugens were collected as eggs or larvae from *Eucalyptus* and *Lophostemon* spp. from the field population in Auckland, New Zealand. Larvae were reared on adult *Eucalyptus nitens* freshly cut foliage within plastic ventilated containers in the laboratory. Pupae were shipped to Lincoln, Canterbury, New Zealand and kept in a physical containment 2 (PC2) quarantine room at 25 °C and 14:10 L:D until emergence. Each pupa was individually kept in a plastic container with a cotton dental roll soaked with 10% sucrose solution. Adult moths were sexed according to their antennal morphology. Two- to five-day-old unmated female moths were used for our electrophysiology experiments.

2.2. Test compounds and odor presentation

Thirty-nine host or non-host plant volatile compounds were used as test stimuli in our study (Table 1). At least 13 of the plant volatile compounds investigated for SSR measurements are present in eucalypt plants (Li et al., 1994; Betts, 2000; Yassaa et al., 2000; Zini et al., 2001, 2002; Tsiri et al., 2003; Viturro et al., 2003; Ogunwande et al., 2005), the host plant of *U. lugens*. The source, purity and presence of the test compounds in eucalypts are reported in Table 1. Each compound was dissolved in hexane as a 500 ng/μl solution, except the green leaf volatile compounds (Mix-GLV, Table 1) that were prepared in mineral oil at the same concentration. The test compounds were divided into seven groups (Table 1), and the mixture solution of each group was also prepared in hexane (or in mineral oil for group Mix-GLV) at a concentration of 500 ng/μl for each compound in the group. Hexane and mineral oil were used as the solvent control stimuli. Serial dilutions of some compounds were also prepared in hexane or mineral oil for measuring dose–responses of ORNs.

Presentation of test chemicals to the insect antennae was similar to previous studies (Park and Baker, 2002; Park and Hardie, 2004; Park et al., 2013). A 20-μl aliquot of each test solution was applied onto a piece (5 × 30 mm) of filter paper (Whatman No 1, USA), and the filter paper strip was inserted into a glass Pasteur pipette (146 mm, Fisher Scientific, USA) after being evaporated for 10 s in air. The tip of the pipette was inserted into a small hole (2 mm diameter, 10 cm from the outlet to the antennae) in a glass main airflow tube with a continuous, charcoal-filtered and humidified airflow (600 ml/min) over the antennal preparation. A 0.1-s pulse of charcoal-filtered airflow (10 ml/s) was injected through the wide end of the Pasteur pipette odor cartridge for stimulation, using an electronic airflow controller (CS-55, Syntech, Hilversum, The Netherlands). The wide end of the Pasteur pipette was covered with a piece of aluminum foil when not in use, to reduce evaporation. Each odor stimulus cartridge was used less than 10 times.

2.3. Single sensillum recording

A female moth was mounted on a Plasticine® block with U-shaped thin copper wire restraints, and each antenna was further fixed using fine copper wires. The preparation was then positioned in the middle of the charcoal-filtered and humidified main airstream. A fine tip (tip diameter < 10 μm) glass electrode (0.86 mm ID, A–M Systems Inc., USA) filled with 0.1 M KCl was inserted into a compound eye to serve as the reference electrode. An electrochemically sharpened tungsten electrode (tip diameter < 0.1 μm) was used as a recording electrode and the position