Journal of Asia-Pacific Entomology 18 (2015) 165–168

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Electrophysiological activity of the *Sirex noctilio* ovipositor: You know the drill?

R.A. Hayes [a,c,*], M.W. Griffiths [a,c], H.F. Nahrung [b,c]

[a] Agri-Science Queensland, Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, Dutton Park, Queensland, Australia
[b] Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, Queensland, Australia
[c] Forest Industries Research Centre, University of the Sunshine Coast, Maroochydore DC, Queensland, Australia

ARTICLE INFO

Article history:
Received 12 November 2014
Revised 9 January 2015
Accepted 15 January 2015
Available online 24 January 2015

Keywords:
Electrophysiology
Ovipositor
Host selection
Forestry
Woodwasp

ABSTRACT

Sirex woodwasp (*Sirex noctilio* F: Hymenoptera: Siricidae) is a major worldwide pest of pine (*Pinus*) species. The female woodwasp undertakes exploratory drills with the ovipositor prior to egg-laying to assess tree suitability. Previous work has shown that this behaviour is associated with assessing the osmotic pressure of the tree. Here we show that, in addition, the ovipositor is electrophysiologically active and capable of detecting ethanol and chemical components of solvent extracts of pine needles and bark. Scanning electron micrographs of the ovipositor show the presence of structures which may have a chemoreceptive function. Our research expands our knowledge of the role that the *Sirex* ovipositor plays in egg-laying site selection.

Introduction

The Sirex woodwasp (*Sirex noctilio* F: Hymenoptera: Siricidae) is a serious pest of conifers (especially *Pinus* spp.) outside its native Eurasian distribution. The woodwasp preferentially attacks stressed trees, independent of whether this stress is environmental or occurs through silvicultural practices (Hurley et al., 2007). As a result of its economic importance and a desire for improved management practices, the species has been the subject of a large volume of work investigating its ecology and control (see for example Rawlings and Wilson, 1949; Madden, 1971, 1974, 1977; Spradbery and Kirk, 1978; Madden and Coutts, 1979; Tribe and Cillie, 2004; Hurley et al., 2007; Slippers et al., 2012).

The woodwasp has an obligate mutualistic relationship with a basidiomycete fungus, *Amylostereum areolatum*. The fungus is introduced into the tree during oviposition and is essential for the initiation of insect development. Larvae feed on the fungus and tunnel through the wood (Talbot, 1964; Coutts and Dolezal, 1965, 1969; Madden and Coutts, 1979; Carnegie et al., 2005). Also introduced into the tree during oviposition is a phytotoxic mucus which acts to dry out the tree (Kile and Turnbull, 1974; Ryan and Hurley, 2012), creating an ideal environment for the fungus to grow. Trees drilled by *S. noctilio* die from the combination of mucus and fungus (Madden and Coutts, 1979). With each of these different elements in play, oviposition behaviours and outcomes are both complex and critical for the woodwasp (Madden, 1974; Spradbery, 1977; Spradbery and Kirk, 1978; Madden and Coutts, 1979; Madden, 1988; Slippers et al., 2012; Wermelinger and Thomsen, 2012).

On locating a potential host, female woodwasps initially assess tree suitability through antennal tapping on the bark surface. In a search for an understanding of this behaviour, early work showed solvent extracts of pine phloem to be attractive to female woodwasps (Madden, 1968), and the electrophysiological response of the woodwasp antennae to volatiles from *Pinus radiata* was examined. Various volatile compounds were determined to be important in the attractiveness of a tree, especially α- and β-pinene and 3-carene, as well as a number of other monoterpene hydrocarbons with much lower concentrations (Simpson, 1976; Simpson and McQuilkin, 1976). The two pinene isomers form the basis of the commercially-produced Sirex Lure, now used in detection programmes worldwide (Morgan and Stewart, 1972; Simpson, 1976; Hurley et al., 2007; Bashford and Madden, 2012).

On trees deemed suitable, the female wasp then probes with the ovipositor, making either single or multiple drill holes approximately 12 mm into the sapwood through a single entry hole in the bark. Single drills are generally made in trees with osmotic pressure $>18 \times 10^5$ Pa (Madden and Coutts, 1979) and are injected with the phytotoxic mucus and fungus arthrospores but no eggs. In multiple drills, initial drill sites are inoculated with a single egg each, and the final drill contains the mucus and fungus (Coutts and Dolezal, 1965). These behaviours also enable the wasp to further assess tree suitability, allowing assessment of the oleoresin pressure and moisture content of the

* Corresponding author at: Agri-Science Queensland, Department of Agriculture, Fisheries and Forestry, GPO Box 267, Brisbane QLD 4001, Australia. Tel.: +61 7 3255 4408.
E-mail address: andrew.hayes@daff.qld.gov.au (R.A. Hayes).

http://dx.doi.org/10.1016/j.aspen.2015.01.003
1226-8615/

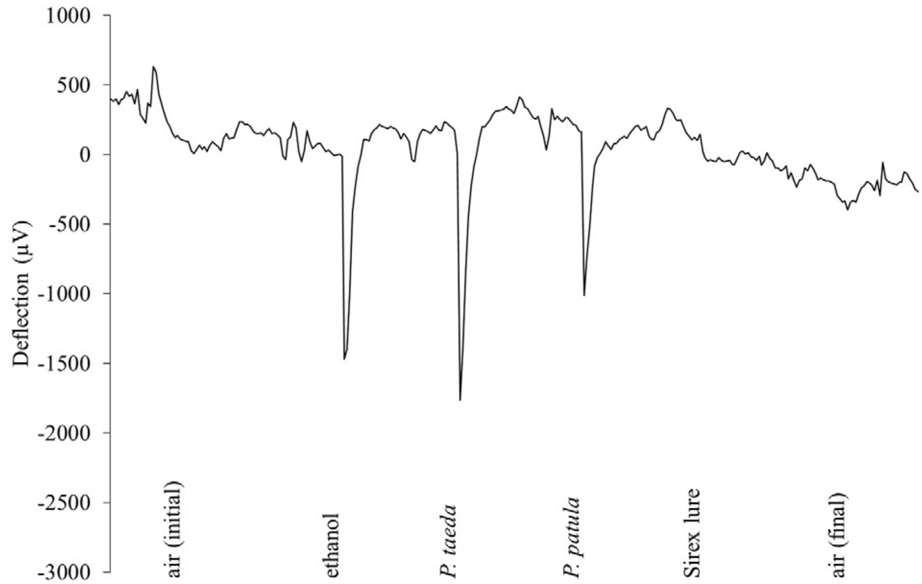

Fig. 1. Representative electrophysiological responses of the ovipositor from a *Sirex noctilio* woodwasp female to extracts from pine bark. Response peaks are labelled with the treatment. Sirex lure is a commercial lure.

sapwood which are critical for larval survival (Coutts and Dolezal, 1969; Madden, 1968, 1971; Morgan and Stewart, 1972; Neumann and Minko, 1981; Neumann et al., 1987). If the tree is too dry then larvae don't survive, however if the tree is too wet, fungi (such as blue stain fungus, e.g. *Ophiostoma*, *Diplodia*) can develop which are believed to kill the larvae (Coutts and Dolezal, 1965).

The role of the ovipositor in assessing host suitability has been further demonstrated by qualitatively examining ovipositors on isolated abdomens for their response to solutions of various NaCl concentrations, and comparing the amount and type of movement as an indicator of response. Flexing and reciprocal movements comparable to those observed in an ovipositing female were elicited predominantly by stimulation of the ovipositor with 0.1 M and 0.2 M NaCl (Madden, 1974). Madden (1974) concluded that female *S. noctilio*'s ability to assess potential host trees is directly related to the osmotic quality of the phloem, and that the ovipositor itself is stimulated by phloem fluids.

Despite the hypothesised role of the *Sirex* ovipositor in determining suitability of a tree for oviposition, to our knowledge the chemosensory role of the *Sirex* ovipositor has been thus far unexplored. Contact chemoreceptors are present on the ovipositors of a variety of insect species (Rice, 1976; Hood Henderson, 1982; Waladde, 1983; Crnjar et al., 1989; Maher and Thiéry, 2004; Maher et al., 2006; Faucheux, 2012), including Hymenoptera (Nacro and Nénon, 2009). The presence of these sensilla is likely to be associated with oviposition in fresh plants: indeed, the uniporous sensilla are known to respond to chemicals such as salts, water, and amino acids (Rice, 1976; Chadha and Roome, 1980). Evidence that the sensilla respond to host odours from *Pinus* spp. would support the hypothesis that in addition to the previously reported assessment of osmotic pressure, the role of ovipositor drilling behaviour observed in *Sirex* is to assess chemical characteristics of the wood and/or bark.

Methods

Solvent extracts were prepared from bark of two pine taxa, *Pinus taeda*, and *P. patula*. Both taxa are grown in southern Queensland, where *S. noctilio* has recently established (Carnegie and Bashford, 2012), and are known hosts of *S. noctilio* (Ryan and Hurley, 2012). Bark (498.5 ± 4.5 mg) from three trees per taxon (approximately half inner and half outer bark by mass) was ground to a fine powder with a coffee grinder (Braun), and extracted with ethanol (Sigma) (3 ml) for 24 h in darkness at 4 °C. The extract was filtered (Whatman filter paper) and stored in the freezer (−20 °C) until use (methods modified from Cadahia et al., 1997; Eyles et al., 2003).

To avoid any interactions due to the solvents, bark alone was also tested as an odour source. Ground bark (~200 mg) from two pine taxa (*P. taeda* and the hybrid of *P. caribaea* var. *hondurensis* × *P. elliottii* var. *elliottii*) was packed into a glass Pasteur pipette between two wads of glass wool. The control odour in this instance was a glass pipette containing only the glass wool.

Sirex noctilio females were collected upon emergence from caged pine logs obtained from Passchendaele State Forest, southern Queensland (28.5°S, 151.8°E). The ovipositor and sheath from each female were cut from the body under a phosphate buffered saline solution (pH 7.4, Sigma). The ovipositor was separated from the protective sheath and both ends were covered in electroconductive gel (Medtel, Australia) and mounted across two electrodes connected to an EAG Combiprobe manipulator (Syntech) in a constant stream of humidified air (flow rate = 19.9 ml/s) at ambient temperature (~22 °C). The bark extracts as well as a commercial lure (Sirex Wood Wasp, AlphaScents), and air and solvent blanks were puffed (14.8 ml of air over 0.5 s) over

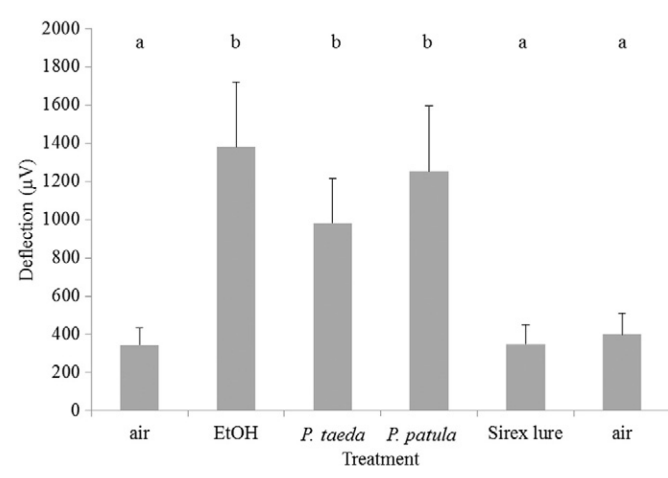

Fig. 2. Mean (± SEM) response of sixteen *Sirex noctilio* woodwasp female ovipositors to ethanol extracts from pine bark. Columns labelled with the same letter are not significantly different from each other.