

ORIGINAL CONTRIBUTION

Host selection by *Ibalia leucospoides* based on temporal variations of volatiles from the hosts' fungal symbiontN. Jofré¹, M. B. Pildain^{2,3}, A. M. Cirigliano⁴, G. M. Cabrera⁴, J. C. Corley^{1,3} & A. S. Martínez^{1,3}¹ Grupo de Ecología de Poblaciones de Insectos, Instituto de Tecnología Agropecuaria. Estación Experimental Bariloche, San Carlos de Bariloche, Río Negro, Argentina² Área de Protección Forestal, Centro de Investigación y Extensión Forestal Andino Patagónico, Esquel, Chubut, Argentina³ CONICET, Argentina⁴ Departamento de Química Orgánica y UMYMFOR (CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina**Keywords***Amylostereum areolatum*, biocontrol, foraging, headspace analysis, semiochemical, *Sirex noctilio***Correspondence**

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Abstract

Insect parasitoids locate hosts via reliable and predictable cues such as volatile emissions from hosts and/or host plants. For insects that depend on mutualistic organisms, such as many wood-boring insects, symbiont-derived semiochemicals may represent a source of such cues to be exploited by natural enemies. Ultimately, exploitation of these signals may increase fitness by optimizing foraging efficiency. Female parasitoids of *Ibalia leucospoides* use volatiles from the fungal symbiont *Amylostereum areolatum* of their host *Sirex noctilio* to find concealed host eggs and young larvae within the xylem. We hypothesize that the temporal pattern of fungal emissions may indicate not only the presence of host larvae but also be used as a cue that indicates host suitability and age. Such information would allow female parasitoids to discern more efficiently between hosts within ovipositor reach from those already buried too deep into the xylem and out of reach. In this context, we assessed the behaviour of *I. leucospoides* females to volatiles of *A. areolatum* in a 'Y'-tube olfactometer at regular intervals over 30 days. We concurrently examined the fungal volatiles by headspace sampling through solid-phase microextraction (SPME) followed by gas chromatography mass spectrometry (GC-MS). We observed that females were attracted to volatiles produced by two-week-old fungal cultures, a period that matches when older larvae are still within ovipositor reach. Four chemical compounds were detected: ethanol, acetone, acetaldehyde and the sesquiterpene 2,2,8-trimethyltricyclo[6.2.2.0^{1,6}]dodec-5-ene, with each compounds' relative abundance changing over time. Results are discussed in the context of parasitoids fitness. Future studies involving electrophysiology, different collection techniques and further behavioural assays will help in identifying the compounds that convey temporal information to female parasitoids and have the potential for being used in integrated pest management programmes.

Introduction

Fitness of insect parasitoids – insects whose larval stages rely on a second organism to develop – is tightly

linked to successful host location for oviposition. Female parasitoids must locate their hosts within a complex environment and thus resort to the use of information, not only from their hosts, but also from

the host's microhabitat or other indirect sources associated with its presence or activity. During this search, parasitoids that forage for herbivorous hosts face what has been termed the reliability–detectability problem (Vet and Dicke 1992), while stimuli from the host itself are the most reliable indicators of its presence, their detectability is selected to be low. Conversely, plants on which herbivores feed may provide abundant information which is readily detectable but is a less reliable predictor of herbivore presence. One way in which parasitoids deal with this problem is by exploiting stimuli produced by specific interactions of the host with its food (Turlings et al. 1990; Vet and Dicke 1992; De Moraes et al. 1998; Meiners et al. 2000; Hilker et al. 2002; Blassoli Moraes et al. 2005).

Ibalia leucospoides Hochenwarth (Hymenoptera, Ibalidae) is a solitary, koinobiont parasitoid that uses the woodwasp *Sirex noctilio* Boidin (Hymenoptera, Siricidae) as host. This parasitoid, of Palearctic origin, is used in biocontrol programmes of its host, a global invasive pine pest (Fischbein and Corley 2015), and is nowadays established throughout the invasion range of its host (Zondag 1959; Taylor 1967; Neumann and Morey 1984; Tribe and Cillie 2004; Carnegie et al. 2005). Female parasitoids oviposit inside *S. noctilio* eggs or young larvae burrowed inside the xylem of pines. *Sirex noctilio* females drill ca. 1.5 cm deep holes into tree stems and introduce, together with the eggs, a phytotoxic venom and spores of the symbiotic fungus *Amylostereum areolatum* Boidin (Basidiomycota, Amylostereaceae) (Spradbery 1977). Fungal growth reduces the moisture content of the wood, presumably favouring host egg hatching and facilitating boring activity while providing nutrients to host larvae (Coutts and Dolezal 1965; Gilmour 1965; Neumann and Minko 1981; Thompson et al. 2013, 2014). *Ibalia leucospoides* females must therefore find concealed *S. noctilio* eggs and larvae within the xylem to be able to oviposit.

Past studies have shown that secondary metabolites released by *A. areolatum* are used by *I. leucospoides* as semiochemicals to locate the host concealed within the tree (Madden 1968; Spradbery 1974; Martínez et al. 2006; Bryant 2010). Furthermore, a recent study reports that also the host, *S. noctilio*, uses fungal volatiles to locate trees that have been previously infected by conspecifics (Fernández Ajó et al. 2015). It has been shown that fungal growth may help foraging *I. leucospoides* females to optimize patch-choice decisions by detecting concentration differences of fungal volatiles, used as a proxy to assess relative host densities available for parasitization (Martínez et al.

2006; Corley et al. 2010; Fischbein et al. 2012). Yet, studies on the nature of *A. areolatum* secondary metabolites that could influence *I. leucospoides* behaviour are scarce. Madden (1968) suggested acetaldehyde to be involved in the recognition of host eggs and larvae of *I. leucospoides*, Titze (1970) demonstrated that *A. areolatum* also produces pyruvic acid and a polysaccharide–protein complex in anaerobiosis, and more recently Bryant (2010) identified linalool, p-anisaldehyde, (3-ethylphenyl) ethanone and 1-(4-ethylphenyl) ethanone as fungal volatile emissions that could be used as semiochemicals by *I. leucospoides*. From an applied point of view, elucidating the role and identity of fungal volatiles used by *I. leucospoides* to locate hosts could facilitate management of *S. noctilio* by controlling the spatial distribution of biocontrol agents (e.g. as the attractive compound in push–pull strategies) (Cook et al. 2007).

We investigated the host searching behaviour of *I. leucospoides* in relation to *A. areolatum* volatile changes through time. In particular, we studied the change in composition of the volatiles derived from *A. areolatum* and how *I. leucospoides* behaviour is affected by these volatiles through time. We hypothesized that fungal volatiles could facilitate the location of hosts concealed within the xylem and furthermore provide additional information on host age and reachability.

Materials and Methods

Insect rearing

Both parasitoids and hosts were obtained from pines (*Pinus contorta* var. *murrayana*) collected in plantations located in NW Patagonia, during 2008–2011. Pine trees showing typical signs of woodwasp attack (chlorotic foliage and resin droplets on main stem) were felled, cut into 1-m-long billets and kept at ambient conditions, in locker-type cages. During the flight season, insects were collected daily from cages. Female parasitoids used for bioassays were 24–48 h old.

Fungal culture

Amylostereum areolatum mycelia was extracted from female woodwasp mycangia following the protocol described in Thomsen and Harding (2011) and transferred to a Petri dish with artificial culture medium based on agar–agar, yeast, malt and pine extract. Mycelia from one female were sufficient to cultivate

two Petri dishes. Additional Petri dishes containing sterile culture medium were kept under the same conditions to be used as olfactory and humidity controls (Martínez and Hardie 2009). Cultures were maintained in the dark and at ambient temperature conditions ($22 \pm 2^\circ\text{C}$) for the duration of each treatment (5, 10, 14, 17, 21, 24 and 30 days). The mycelium and culture medium used in the bioassays were excised from the cultures and controls, respectively, by removing a 170 mm^2 disc with a puncher (this amount elicits a behavioural response in female *I. leucospoides*, Martínez et al. 2006).

Behavioural assays

Ibalia leucospoides females were exposed to *A. areolatum* volatiles in a glass Y-tube olfactometer (base 13 cm, arms 7 cm, diameter 2 cm) between 10 am and 3 pm. The base of the device was connected to an air pump creating an even air flow of 1 l/min. The air was filtered through 100 g of activated charcoal before entering the system, after which it was rehumidified by passing through damp cotton wool. Stimuli (test and control) were placed in two individual Perspex dark containers ($7 \times 7 \times 7\text{ cm}$) which were attached to the end of the olfactometer arms. To avoid visual asymmetries, the Y-tube was placed inside a white Perspex box ($50 \times 50 \times 25\text{ cm}$), covered by a red, see-through detachable cover (Martínez et al. 2006).

In each replicate (i.e. one female wasp), a single female wasp was introduced into the base of the olfactometer and given a short period to adapt (1 min) and a maximum of 15 min to choose between the labyrinth arms. A choice was considered to be made when the animal passed a line arbitrarily set at 5 cm into each arm. After each replicate, the fungal disc was discarded and the olfactometer washed, dried and ventilated to avoid pseudoreplication (Ramírez et al. 2000). After each replicate, the olfactometer was rotated 180° along the longest axis so that the left arm's new position was at the right side of the large Perspex box and vice versa for the second arm inside the Perspex box. Additionally, the position of the odour sources was interchanged between arms according to a pre-defined randomization protocol to avoid biases due to unwanted asymmetries in the set-up. The experiments were performed at room temperature ($23 \pm 1^\circ\text{C}$) and humidity ($47 \pm 7\%$) in natural lighting conditions. The response to volatiles produced by *A. areolatum* of 5, 10, 14, 17, 21, 24 and 30 days old was evaluated; between 14 and 28

individuals were used for each age treatment. Binomial tests were performed with responding wasps of each fungal age to assess biases in the response towards fungal volatiles vs. clean air.

Headspace collection and analysis

Plugs of actively growing mycelia were inoculated in 100-ml flasks containing 2 ml malt yeast broth (MY) containing 2% (v/v) malt extract (Difco) and 0.2% (v/v) of yeast extract (Britania) and incubated at 24°C in darkness for 30 days. Volatile collections were performed directly from the flasks at days 5, 10, 14, 17, 21, 24 and 30 after being inoculated, also in the dark. Collections were carried out by solid-phase microextraction (SPME), a method successfully used with fungal volatile compounds (Evans et al. 2008), using a fibre coated with carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, Poole, Dorset, UK). The fibre was exposed to the headspace of the flask during 20 min at $20^\circ\text{C} \pm 2$ in the dark. Three collections (i.e. replicates) were made per time window. The same number of collections was made with uninoculated media for each age.

Analysis was undertaken on a Shimadzu GC-17A Gas Chromatograph (GC) coupled to a Shimadzu QP-5000 Mass Spectrometer (MS) (Shimadzu, Kyoto, Japan). The GC was fitted with a $30\text{ m} \times 0.25\text{ mm}$ I.D. TR-5MS fused silica capillary column with a $0.25\text{-}\mu\text{m}$ film thickness (Thermo Fisher Corporation, Waltham, MA). Oven temperature was maintained at 45°C for 1 min and raised by $20^\circ\text{C}/\text{min}$ to 300°C . The injector temperature was held constant at 250°C and operated in splitless mode. Ionization for mass analysis was by electron ionization at 70 eV and an interface at 280°C . The carrier gas was helium.

Following sampling, the fibres were immediately introduced to the gas chromatograph's injector. To achieve sharp SPME injection bands, the inlet liner was replaced with a narrow bore (0.75 mm I.D.) liner. Volatile compounds were identified on the basis of their retention times and mass spectra, in comparison with those of reference compounds under the same GC-MS conditions and with mass spectra from National Institute of Standard and Technology (NIST) and Wiley libraries spectra. For comparative purposes, normalization of individual compounds was performed relative to the minimum amount detected (represented as 1 when normalized) between replicates and treatments for the same compound. Compounds appearing in control runs were not considered in the analysis.

Results

Ibalia leucospoides females showed a significant response towards the 14-day-old fungus culture: 80% of responding females were positively biased towards the fungal volatiles ($n = 28$; binomial test; $P < 0.01$, fig. 1). Five-, 10-, 17-, 21-, 24- and 30-day-old fungus did not result in a differential response by the parasitoids ($n = 23, 14, 20, 19, 19$ and 20 , respectively; binomial test; $P > 0.5$).

Four volatile organic compounds (VOCs) were identified throughout the 30 days sampled on the basis of their mass spectrum and comparison with library data: acetaldehyde, ethanol, acetone and 2,2,8-trimethyltricyclo [6.2.2.0^{1,6}]dodec-5-ene (TMTD) (fig. 2). The absolute identification of the latter compound would require preparation of the compound as a reference which is beyond the scope of this work. Acetaldehyde was recorded at similar levels in days 5 and 10. Acetone and ethanol were found at days 5, 10 and in less concentration at days 14 and 17, after which time it was no longer detectable. TMDT presented a different emission profile, being measurable at low amounts at days 5, 10 and 14 and at larger concentrations thereafter (fig. 3).

Discussion

This is the first report of the behavioural responses of female *I. leucospoides*, a parasitoid of the woodwasp *S. noctilio*, to compounds elicited by *A. areolatum* cultures of different ages. Results indicate that

I. leucospoides females are attracted to volatiles released by 14-day-old fungal cultures and that fungal volatile emissions change with time. Fungal semiochemicals, detected near the *S. noctilio* drilling site (i.e. small spatial scale), in addition to conferring information about the spatial location of a possible host, could additionally indicate the developmental stage of the concealed *S. noctilio* egg/larvae, which could optimize foraging time for female parasitoids and have important fitness consequences.

Sirex noctilio is an invasive woodwasp whose larvae develop inside the wood of attacked trees. Adults have a short (3–4 months) flight season, while immature stages spend most of the life cycle (generally 1 year but can extend up to 3) inside the xylem of pines. In the field, a single pine tree can be exposed to *S. noctilio* attacks during the summer months and therefore can host *S. noctilio* in different developmental stages. Moreover, it is known that attacks by *S. noctilio* are highly aggregated, even when populations are low; therefore, a single tree is likely to receive successive attacks by several female hosts. As a result in a given time, only a proportion of woodwasp larvae are available hosts for *I. leucospoides* females: because of their relatively short ovipositor (23 ± 0.2 mm, personal observation), hosts that are burrowed deep inside the wood may not be accessible for oviposition (i.e. *I. leucospoides* has a limited window for parasitism and not all hosts within a tree fall within this window). Female *I. leucospoides* were only attracted to volatiles from two-week-old fungi, coinciding with the time in which woodwasp larvae start burrowing deeper into

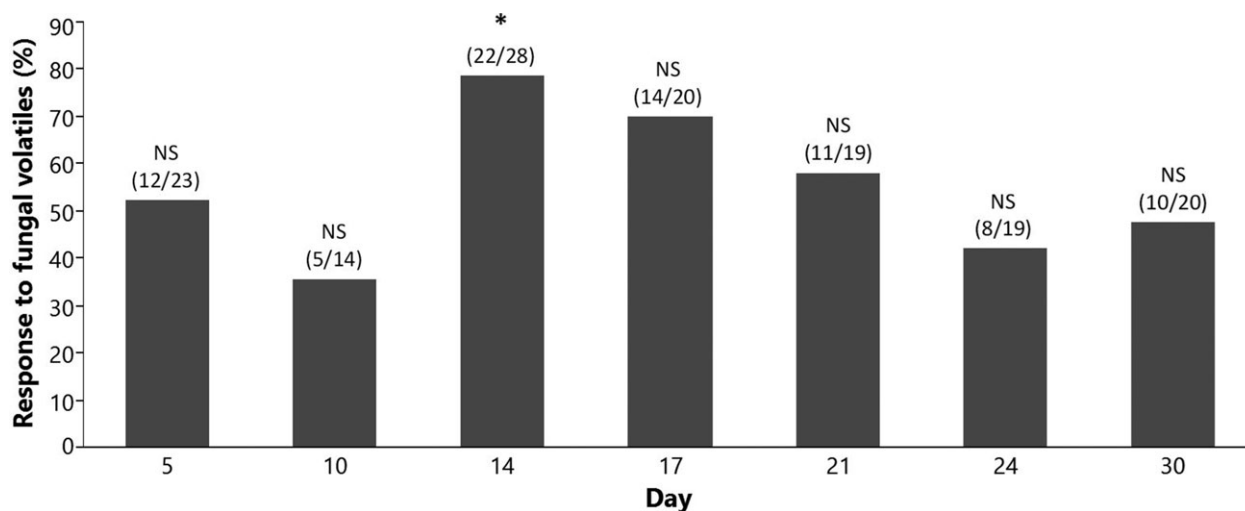


Fig. 1 Percentage of *Ibalia leucospoides* females that responded to *Amylostereum areolatum* volatiles of different ages vs. a sterile culture medium (control). ns = Non-significant ($P > 0.05$), * = $P < 0.01$, binomial test. Numbers between brackets above bars indicate insects that responded towards *A. areolatum* volatiles and the total insects tested.

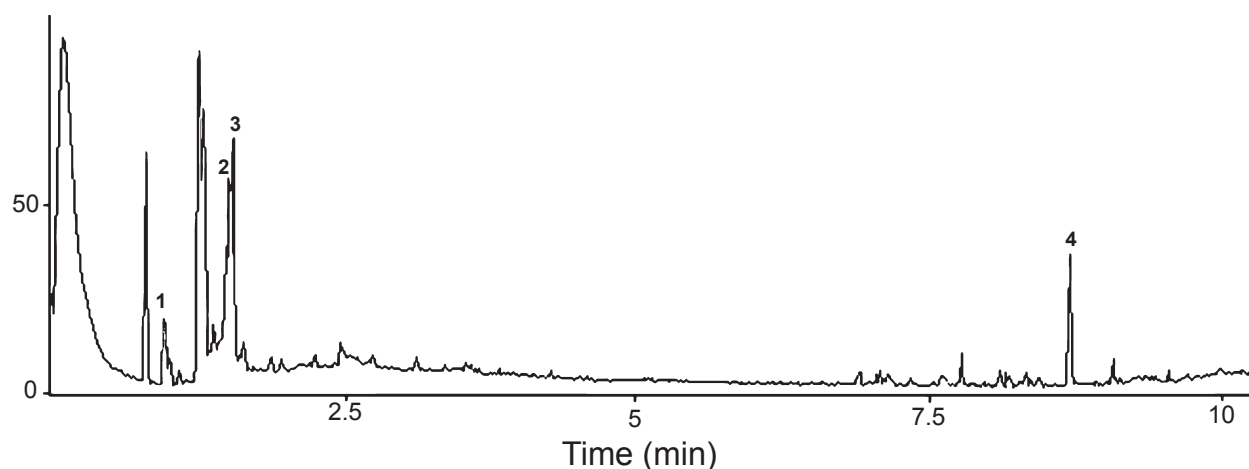


Fig. 2 Representative gas chromatogram of the volatile fraction emitted by *Amylostereum areolatum* detected by GC-MS using SPME extraction at 10 days of fungal growth. 1: Acetaldehyde; 2: ethanol; 3: acetone; 4: 2,2,8-trimethyltricyclo [6.2.2.0]dodec-5-ene. Non-identified peaks correspond to those emitted by the control (i.e. uninoculated culture media).

the wood (Madden 1968) and making them not reachable. For foraging *Ibalia leucospoides* females, being attracted to (and parasitizing) the older stages still within ovipositor reach could be advantageous as mortality rates for *Sirex noctlio* eggs and neonate stages can reach up to 60% (Haavik et al. 2015).

Results also indicate that fungal volatile composition changes with age. GC-MS analyses of headspace collections of *A. areolatum* resulted in four compounds being identified: acetone, acetaldehyde, ethanol and TMTD. Throughout the sampled period, the relative amounts of individual compounds varied. Three of the detected compounds: acetone, ethanol and acetaldehyde, have been shown to act as semiochemicals in different organisms. Acetone and ethanol are emitted by several fungi and plant species. For instance, acetone is known to affect the flight behaviour in the weevil *Pissodes strobi* (Sahota et al. 1998) and generate attraction to the cerambycid *Monochamus alternatus* (Ikeda et al. 1981) while ethanol is a known compound emitted by fungal species (Kenealy and Dietrich 2004; Okamoto et al. 2010) and known to be attractive to various species of bark beetles (Joseph et al. 2001; Allison et al. 2004). Acetaldehyde is known to induce an antennal response in *I. leucospoides* (Madden 1968) and to act as an attractant in *Drosophila* species and some fruit fly parasitoids such as *Biosteres longicaudatus* and *Leptopilina heterotoma* (Greany et al. 1977; Dicke et al. 1984; Hoffmann and Parsons 1984). TMTD, a sesquiterpene hydrocarbon not well known as a natural constituent, has yet no known semiochemical function. Previous studies have identified this compound in *Haplopappus berterii*

flowers, normally visited by pollinating insects, but no conclusive results in relation to its function as an infochemical were obtained (Urzúa et al. 2007).

Parasitoids only oriented preferentially to the volatiles of 2-week-old cultures of *A. areolatum* from which three compounds, acetone, ethanol and TMTD, were detected. In younger cultures (less than 14 days) which did not elicit a bias in response, acetone, ethanol and acetaldehyde were produced at higher levels but TMTD concentrations were similar throughout. Conversely, in older cultures, emissions of acetaldehyde, acetone and ethanol were not detected while TMTD increased. Madden (1968) suggested that acetaldehyde is an attractant for *I. leucospoides* females, but in our assay, we did not detect this compound in the volatile profile of the attractive 14-day-old cultures. Additionally, although Bryant (2010) reported that linalool, p-anisaldehyde, (3-ethylphenyl) ethanone and 1-(4-ethylphenyl) ethanone were emitted by 21-day-old *A. areolatum* cultures, we did not detect these compounds. The fact that these compounds were not detected by our sampling method could be due to differences in volatile capture methods; Bryant (2010) used Porapak Q while this study used SPME. In addition to host kairomones, it is likely that a blend, rather than one single fungal compound, aids in host location/selection. Several studies have shown that blends elicit stronger behavioural responses than individual components; moreover, in some species individual components do not induce any behavioural response (see Bruce and Pickett 2011 for review). Furthermore, volatiles used by *I. leucospoides* may be produced as a result of interactions between the fungus

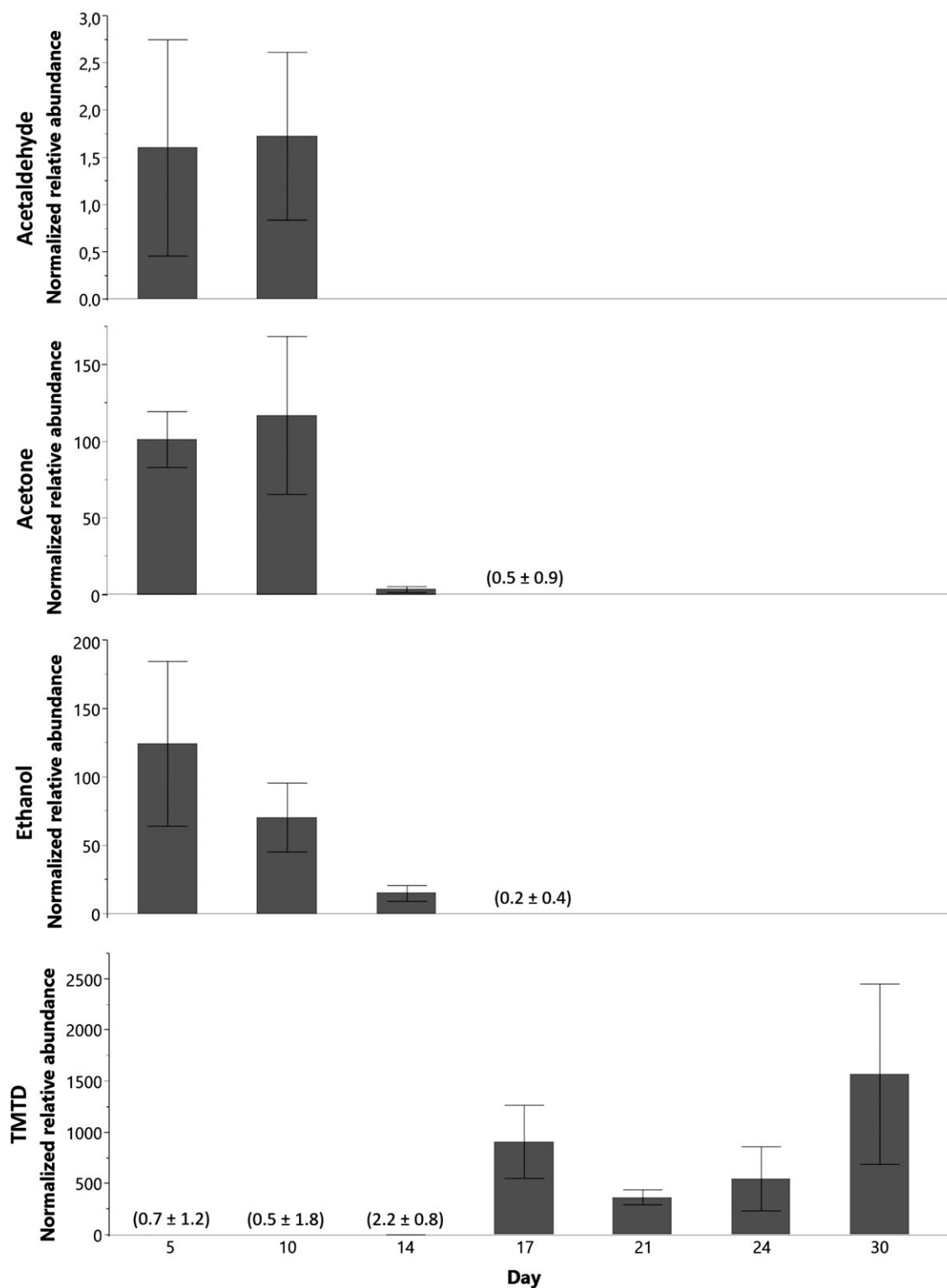


Fig. 3 *Amylostereum areolatum* volatile organic compounds collected via SPME and analysed with GC-MS at various times over a 30-day-long period. For comparative purposes, normalization of a compound was performed relative to the minimum amount detected of the same compound. Three replicates were performed for each time window. Bars indicate mean value, and error bars indicate the standard error. Numbers in brackets above days with no bars indicate amounts (mean \pm SE) detected that were too small to be represented graphically.

and the pine trees and facilitate determination of host suitability for foraging parasitoid wasps.

Identification of the compounds involved in host selection process has the potential to facilitate the design and implementation of effective and sustainable pest control programs. Such compounds may be used in integrated pest management schemes to enhance the effectiveness of natural enemies by controlling their abundance and distribution (Cook et al. 2007), and in recent years, special attention has been given to the development of devices to attract natural enemies to target areas (Mallinger et al. 2011; Heuskin et al. 2012; Rodriguez-Saona et al. 2012). Our study tested only VOC emissions through SPME, but it seems likely that other fungal compounds, detected by other techniques, may aid in maximizing foraging efficiency. In this sense, we consider that future studies involving different volatile collection techniques, electrophysiology and further behavioural assays should help in determining which of these compounds, and any others, are relevant from a behavioural point of view and their impact in host selection.

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References

- Allison JD, Borden JH, Seybold SJ, 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). *Chemoecology*, 150, 123–150.
- Moraes MCB, Laumann R, Sujii ER, Pires C, Borges M, 2005. Induced volatiles in soybean and pigeon pea plants artificially infested with the neotropical brown stink bug, *Euschistus heros*, and their effect on the egg parasitoid, *Telenomus podisi*. *Entomol Exp Appl*, 115, 227–237.
- Bruce TJ, Pickett J, 2011. Perception of plant volatile blends by herbivorous insects-finding the right mix. *Phytochemistry*, 72, 1605–1611.
- Bryant PW, 2010. Kairomonal attraction of the parasitoid *Ibalia leucospoides* (Hymenoptera: ibaliidae) to volatiles of the fungus *Amylostereum areolatum*, an obligate symbiont of the European woodwasp. State University of New York College of Environmental Science and Forestry, *Sirex noctilio*.
- Carnegie AJ, Eldridge RH, Waterson DG, 2005. History and management of *Sirex wood* wasp in pine plantations in New South Wales, Australia. *N Z J For Sci*, 35, 3–24.
- Cook SM, Khan ZR, Pickett JA, 2007. The use of push-pull strategies in integrated pest management. *Annu Rev Entomol*, 52, 375–400.
- Corley JC, Villacide JM, van Nouhuys S, 2010. Patch time allocation by a parasitoid: the influence of con-specifics, host abundance and distance to the patch. *J Insect Behav*, 23, 431–440.
- Coutts MP, Dolezal JE, 1965. *Sirex noctilio*, its associated fungus, and some aspects of wood moisture content. *Aust Forest Res*, 1, 3–13.
- De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH, 1998. Herbivore-infested plants selectively attract parasitoids. *Nature*, 393, 570–573.
- Dicke M, Van Lenteren JC, Boskamp GJF, van Dongen-van LE, 1984. Chemical stimuli in host-habitat location by *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae), a parasite of *Drosophila*. *J Chem Ecol*, 10, 695–712.
- Evans JA, Eyre CA, Rogers HJ, Boddy L, Müller CT, 2008. Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. *Fungal Ecol*, 1, 57–68.
- Fernández Ajó AA, Martínez AS, Villacide JM, Corley JC, 2015. Behavioural response of the woodwasp *Sirex noctilio* to volatile emissions of its fungal symbiont. *J Appl Entomol*, 139, 654–659.
- Fischbein D, Corley JC, 2015. Classical biological control of an invasive forest pest: a world perspective of the management of *Sirex noctilio* using the parasitoid *Ibalia leucospoides* (Hymenoptera: Ibalidae). *Bull Entomol Res*, 105, 1–12.
- Fischbein D, Bettinelli J, Bernstein C, Corley JC, 2012. Patch choice from a distance and use of habitat information during foraging by the parasitoid *Ibalia leucospoides*. *Ecol Entomol*, 37, 161–168.
- Gilmour JW, 1965. The life cycle of the fungal symbiont of *Sirex noctilio*. *N Z J For*, 10, 80–89.
- Greany PD, Tumlinson JH, Chambers DL, Boush GM, 1977. Chemically mediated host finding by *Biosteres (Opus) longicaudatus*, a parasitoid of tephritid fruit fly larvae. *J Chem Ecol*, 3, 189–195.
- Haavik LJ, Dodds KJ, Allison JD, 2015. Do native insects and associated fungi limit non-native woodwasp, *Sirex noctilio*, survival in a newly invaded environment? *PLoS One*, 10, e0138516. doi:10.1371/journal.pone.0138516.
- Heuskin S, Lorge S, Godin B, Leroy P, Frère I, Verheggen FJ, Haubruge E, Wathélet JP, Mestdagh M, Hance T, Lognay G, 2012. Optimisation of a semiochemical slow-release alginate formulation attractive towards *Aphidius ervi* Haliday parasitoids. *Pest Manag Sci*, 68, 127–136.
- Hilker M, Kobs C, Varama M, Schrank K, 2002. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. *J Exp Biol*, 205, 455–561.

- Hoffmann AA, Parsons PA, 1984. Olfactory response and resource utilization in *Drosophila*: interspecific comparisons. *Biol J Linn Soc*, 22, 43–53.
- Ikeda T, Yamane A, Enda N, Matsuura K, Oda K, 1981. Attractiveness of chemical-treated pine trees for *Mono-chamus alternatus* hope (Coleoptera: Cerambycidae). *J Jap For Soc*, 63, 201–207.
- Joseph G, Kelsey RG, Peck RW, Niwa CG, Service UF, Way J, 2001. Response of some Scolytids and their predators to ethanol and 4-allylanisole in pine forests of central Oregon. *J Chem Ecol*, 27, 697–715.
- Kenealy WR, Dietrich DM, 2004. Growth and fermentation responses of *Phanerochaete chrysosporium* to O₂ limitation. *Enzyme Microb Technol*, 34, 490–498.
- Madden JL, 1968. Behavioral responses of parasites to the symbiotic fungus associated with *Sirex noctilio*. *Nature*, 218, 189.
- Mallinger RE, Hogg DB, Gratton C, 2011. Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *J Econ Entomol*, 104, 115–124.
- Martínez AS, Hardie J, 2009. Hygroreception in olfactometer studies. *Physiol Entomol*, 34, 211–216.
- Martínez AS, Fernández-Arhex V, Corley JC, 2006. Chemical information from the fungus *Amylostereum areolatum* and host-foraging behaviour in the parasitoid *Ibalia leucospoides*. *Physiol Entomol*, 31, 336–340.
- Meiners T, Westerhaus C, Hilker M, 2000. Specificity of chemical cues used by a specialist egg parasitoid during host location. *Entomol Exp Appl*, 95, 151–159.
- Neumann FG, Minko G, 1981. The sirex wood wasp in Australian radiata pine plantations. *Aust For*, 44, 46–63.
- Neumann FG, Morey JL, 1984. Influence of natural enemies on the sirex wood wasp in herbicide-treated trap trees of radiata pine in north-eastern Victoria. *Aust For*, 47, 218–224.
- Okamoto K, Imashiro K, Akizawa Y, Onimura A, Yoneda M, Nitta Y, Maekawa N, Yanase H, 2010. Production of ethanol by the white-rot basidiomycetes *Peniophora cinerea* and *Trametes suaveolens*. *Biotechnol Lett*, 32, 909–913.
- Ramírez CC, Fuentes-Contreras E, Rodríguez LC, Niemeyer HM, 2000. Pseudoreplication and its frequency in olfactometric laboratory studies. *J Chem Ecol*, 26, 1423–1431.
- Rodríguez-Saona C, Blaauw BR, Isaacs R, 2012. Manipulation of natural enemies in agroecosystems: habitat and semiochemicals for sustainable insect pest control. In: Integrated pest management and pest control - current and future tactics. Ed. by Larramendy ML, Soloneski S, InTech, Rijeka, Croatia, 89–126.
- Sahota TS, Leal I, White E, Manville JF, Ibaraki A, Hollmann J, 1998. Acetone affects flight behaviour and expression of the vitellogenin gene of *Pissodes strobi*. *Can Entomol*, 130, 383–384.
- Spradbery JP, 1974. The responses of *Ibalia* species (Hymenoptera: Ibalidae) to the fungal symbionts of siricid woodwasp hosts. *J Entomol*, 48, 217–222.
- Spradbery JP, 1977. The oviposition biology of siricid woodwasps in Europe. *Ecol Entomol*, 2, 225–230.
- Taylor KL, 1967. The introduction, culture, liberation, and recovery of parasites of *Sirex noctilio* in Tasmania. Melbourne, Australia, 1962–1967.
- Thompson BM, Grebenok RJ, Behmer ST, Gruner DS, 2013. Microbial symbionts shape the sterol profile of the xylem-feeding woodwasp, *Sirex noctilio*. *J Chem Eco*, 39, 129–139.
- Thompson BM, Bodart J, McEwen C, Gruner DS, 2014. Adaptations for symbiont-mediated external digestion in *Sirex noctilio* (Hymenoptera: Siricidae). *Ann Entomol Soc Am*, 107, 453–460.
- Thomsen IM, Harding S, 2011. Fungal symbionts of siricid woodwasps: isolation techniques and identification. *For Pathol*, 41, 325–333.
- Titze JF, 1970. Some aspects of the metabolism of *Amylostereum areolatum*, the fungal symbiont of *Sirex noctilio*, in cultures on the wood of *Pinus radiata*. *Aust Forest Res*, 4, 19–22.
- Tribe GD, Cillie JJ, 2004. The spread of *Sirex noctilio* (Hymenoptera: Siricidae) in South African pine plantations and the introduction and establishment of its biological control agents. *Afr Entomol*, 12, 9–17.
- Turlings TCJ, Tumlinson JH, Lewis WJ, 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250, 1251–1253.
- Urzúa A, Santander R, Echeverría J, Rezende MC, 2007. Secondary metabolites in the flower heads of *Haplopappus berterii* (Asteraceae) and its relation with insect-attracting mechanisms. *J Chil Chem Soc*, 52, 1142–1144.
- Vet LEM, Dicke M, 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu Rev Entomol*, 37, 141–172.
- Zondag R, 1959. Progress report on the establishment in New Zealand of *Ibalia leucospoides*, a parasite of *Sirex noctilio*. *New Zeal For Res Notes*, 20, 1–10.