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Behavioural responses of *Vespula germanica* (Hymenoptera: Vespidae) wasps exposed to essential oils

Micaela Buteler,[1]* Mariana Lozada,[1] Paola D'Adamo,[1] Romina Ayelen Luz Melo[1] and Teodoro Stadler[2]

[1]*Instituto de Investigaciones en Biodiversidad y Medio Ambiente (INIBIOMA) CONICET, Universidad Nacional del Comahue, Quintral 1250, Bariloche, 8400, Rio Negro, Argentina.*
[2]*Instituto de Medicina y Biologia Experimental (IMBECU), CCT Mendoza, CONICET, Parque Gral. San Martin, 5500, Mendoza, Argentina.*

Abstract Secondary plant metabolites such as those present in essential oils can be toxic to herbivorous insects and also repel attack, given they play a role in selection and acceptance of host plants. However, few studies have dealt with the use of plant-based repellents to manage invasive wasps. The objective was to assess essential oils with potential as wasp repellents, and to gain further insight as to how they affect the learning capacities of these insects. Five essential oils were tested on *Vespula germanica* (Fabricius) wasps in field choice and no-choice tests. When given a choice, foragers avoided the treated baits almost completely. Wind tunnel bioassays demonstrated that wasps recognise the repellent essential oils through olfactory cues, leading to a dose-dependent decreased response to the food stimulus. The effect of the repellents on the cognitive ability of *V. germanica* workers was also studied in relation to food search efficiency. Naive workers landed on the treated baits, although it took them longer than to land on control baits. When workers were allowed to forage on a food bait twice before a repellent was added, an effect on relocation behaviour was observed. The returning workers arriving to a feeding site with essential oil were reticent to land on it, suggesting that there is indeed a repellent effect and not just masking of the food source. A push–pull system combining attractive baits with the use of plant-based repellents is a promising management strategy in urban settings for this insect pest.

Key words German yellowjacket, insect pest management, invasive species, repellent.

INTRODUCTION

Species of the genus Vespula spp. are social insects belonging to the Vespidae family native to the Palaearctic Region. In the last century, *Vespula germanica* (Fabricius) has invaded forested and urban areas in New Zealand, Australia, South Africa, North America, Canada, Chile and Argentina. This invasive species may adversely affect natural ecosystems (Thomas *et al*. 1990; Sackmann *et al*. 2001) and numerous economic activities, such as livestock, beekeeping (Mayer *et al*. 1987; Bacandritsos *et al*. 2006), tourism and other human activities (Akre & MacDonald 1986; Rust & Su 2012).

Current management practices available include the use of insecticides directly on the nest or workers, and the use of toxic baits or baited traps (Beggs *et al*. 2011). In Argentina, there are no commercial traps available. Homemade traps containing meat vary in efficacy and are not practical given that they need to be replaced every 2–3 days. Field studies testing toxic baits containing fipronil suggest these can be useful tools to manage vespids (Harris & Etheridge 2001; Sackmann *et al*. 2001; Hanna *et al*. 2012). The few tools available to control this pest coupled with the health concerns associated with the use of synthetic insecticides such as fipronil (Tingle *et al*. 2003) require industry and the scientific community to place greater emphasis on research into new insecticidal products based on less toxic substances. Recent studies suggest that the use of repellents to ward off vespid wasps could lead to the development of new control techniques for these insects (Zhang *et al*. 2013; Boevé *et al*. 2014).

V. germanica interferes with human activities in urban outdoor spaces and residential areas because they are attracted by food and garbage (Akre & MacDonald 1986). Thus, the use of repellents could prove useful to deter them from homes and urban recreational areas. The field of plant-based insect repellents is moving forward as consumers demand means of protection from arthropod bites and stings that employ chemicals that have low mammalian toxicity, are pleasant to use, and have minimal environmentally impact (Regnault-Roger 1997; Isman 2006; Maia & Moore 2011). Particularly, essential oils contain various secondary metabolites that are related to a plant's defence system against herbivorous insects and can also have behavioural action against predatory insects (Nerio *et al*. 2010).

The use of commercialised essential oils in insect pest control may provide an opportunity to overcome the disadvantages of synthetic pesticides (Isman *et al*. 2007), and with a

*butelermica@gmail.com

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few exceptions essential oils or their products have largely low toxicity or are non-toxic to vertebrates (Stroh *et al*. 1998; Regnault-Roger *et al*. 2012). However, essential oils may have some toxic effects and have the potential to cause a dermal reaction, inhalatory effects or oral toxicity, and therefore their safety of application must be considered on a product-by-product basis (Rubel *et al*. 1998; Hammer *et al*. 2006; Rutherford *et al*. 2007; Regnault-Roger *et al*. 2012).

Most of the research on insect repellents has been conducted on products to repel mosquitoes (Tawatsin *et al*. 2001; Fradin & Day 2002; Patel *et al*. 2012), but little research effort has been devoted towards using repellents to directly ward off wasps (Zhang *et al*. 2013; Boevé *et al*. 2014). Such repellents could be used to deter wasps from houses or recreational areas and in combination with baited traps, in a 'push–pull' scenario (Cook *et al*. 2007). For this, a better understanding of the bioactivity of different plant-based repellents, and how they affect the foraging behaviour of *V. germanica*, is needed.

V. germanica may forage on large, stationary food sources (e.g. carrion and nectar sources) in addition to hunting for prey (Greene 1991). As scavengers, wasps cannot deplete this stationary source in one visit; therefore, they learn to associate contextual cues with food location so as to repeatedly relocate a foraging site (D'Adamo & Lozada 2003, 2007, 2009, 2011, 2014; Lozada & D'Adamo 2006, 2009, 2011, 2014). For example, visual cues, provided by local landmarks learned during an orientation flight, may be used to relocate a feeding area (Takagi *et al*. 1980; Raveret Richter & Jeanne 1985; Lehrer & Collett 1994). Thus, on considering the use of repellents to manage vespid wasps, it is necessary to assess how foragers react to the addition of a repellent to a known food source, as well as to determine whether they exploit resources in proximity to a repellent odour.

The present study tested the repellent effect of five different plant essential oils on *V. germanica*. These were chosen either because the oils or some of their components had been tested against wasps (Zhang *et al*. 2013; Boevé *et al*. 2014), because their terpenoid components are known insect repellents and insecticides (Regnault-Roger *et al*. 2012), and they are readily available and/or their fragrance would make them attractive to potential end users. The objective was to identify potential repellents based on plant essential oils, to assess their efficacy, and to gain further insight as to whether these compounds are repellent or if they only mask food odours and how the learning capacities of the wasps are affected by a repellent. For this purpose, we evaluated the effect of the repellents on the ability of wasps to locate a food source or to select from a treated versus an untreated food source, as well as the effect of a repellent on the relocation of a known food source.

MATERIALS AND METHODS

Pure essential oils used were tea tree oil (essential oil from *Melaleuca alternifolia*: CAS Number: 68647-73-4), black pepper oil (essential oil from *Piper nigrum*: CAS Number: 8006-82-4), lemongrass oil (*Cymbopogon citratus* oil: CAS Number: 8007-02-1), incense oil (*Myrocarpus frondosus*: CAS Number 8016-36-2) and sweet orange oil (*Citrus sinensis*: CAS No: 8008-57-9). All essentials oils were purchased from Casa Gaia® (Buenos Aires, Argentina).

Bioassays were conducted in the Moreno Lake Area in Bariloche (41°S, 71°W), Rio Negro, Argentina, according to procedures outlined by D'Adamo and Lozada (2007). All experiments were carried out under natural conditions during the period of major activity of *V. germanica* wasps (February–April) in 2014. Four different kinds of experiments were conducted in suburban areas, in similar weather conditions (sunny and still), where temperature ranged from 22 to 27°C.

Choice tests

Choice tests were conducted to compare the preference of foraging wasps for food baits treated with pure essential oils or untreated. All experiments involved observing the behaviour of foraging wasps on or near the bait, which consisted on a white plastic dish (diameter = 7 cm) with another smaller container (2.5 cm diameter) holding 15 g of ground fresh bovine meat placed on top. Treated baits consisted of the application of 100 μL of each essential oil onto a filter paper (Whatman N°1) placed on the white dish of the bait. The volume applied was sufficient to cover the area of the filter paper not covered by the meat container and the meat did not touch the essential oil. The treated and untreated baits were placed 50 cm away from each other. The experiment commenced when the first wasp was observed overflying the bait, and then the total number of wasps hovering and landing on the bait were recorded over 10 min. The wasps that landed were removed from the experiment with a laboratory wash bottle modified as a wasp vacuum to prevent an effect of local enhancement by which wasps attract each other to food resources (D'Adamo *et al*. 2000). The experiment was replicated 10 times. Statistical analyses of preference tests were performed using a chi-square test (Statistica 6.0 2001). Analysis of variance (ANOVA proc mixed procedure) (SAS 2012) was used to determine whether there was a difference among the different essential oil treatments in the repellent effect. The number of wasps landing on the baits was the response variable, and each experiment was considered a random factor. LSMEANS comparisons were conducted with the Tukey option in SAS.

No-choice tests

No-choice tests were conducted by assessing the time it took wasps to arrive to control or treated baits. The test was conducted on a transect established along the shore of the Moreno Lake, where the wasps normally come to forage and drink water. The field tests were conducted from 09:00 h to 11:00 h. The observer walked along the transect and stopped every 50 m, where a treated or untreated bait was placed on the ground in random order. Treated and control baits were prepared in the same way as those described above for choice tests, but in this case control and treated baits were not available at the same time. After placing a bait, the time it took the first wasp to arrive to the bait was recorded and the bait was removed from the ground. Then the observer walked another 50 m and placed a different bait. This distance between sampling points ensured using different wasps at each observation point according to Lozada and D'Adamo (2006), who observed wasps remain on average around 20 min searching for food at a location even after the food has been removed. The experiment was replicated 25 times with treated and 25 times with untreated baits for each essential oil. The average time it took for the treated and untreated baits was compared with a Wilcoxon matched pairs test (Statistica 6.0 2001).

Olfactory dose–response tests

A wind tunnel was used to assess the response of *V. germanica* workers to odours from different doses of lemongrass and tea tree oil applied on a food bait, respectively. The wind tunnel was made of plexiglass tubes, with dimensions of 25 cm × 25 cm × 120 cm. Laminar flow was achieved by passing the air through several layers of cheesecloth mounted at the upwind screen, as described by Baker and Linn (1984). On one end there was a fan to ensure a low wind speed of 0.4 m/s and create a laminar airflow according to Reed and Landolt (1990). The point source of the volatiles was positioned at the upwind end of the tunnel and the wasps were introduced into the wind tunnel on the other end. The wind tunnel was placed on the ground in an area infested by wasps. The wasps were attracted to the entrance of the tunnel by a ground meat food bait prepared in the same way as the baits used in choice tests. Once they entered, the door was closed and the bait was removed. The experiment consisted of allowing one individual wasp to enter the wind tunnel and observing its behaviour during 5 min or until it landed on the stimulus. After 5 min, the wasps were aspirated up and removed from the experiment. The experiment was replicated with 25 different wasps for each treatment.

Food baits used as stimulus were prepared in the same way as the baits used for choice tests with varying amounts of essential oils. The doses of essential oil were 100 μL, 500 μL and 1 mL. The plastic dish containing meat and essential oils was covered with a dark plastic cup (6 cm diameter) that had ten 2 mm holes in it. The holes allowed for the odour to be released without a visual stimulus. Untreated ground meat served as control. A pheromonal stimulus consisting of 100 μL of methanolic *V. germanica* head extract (D'Adamo *et al*. 2001) was also used as positive control. Statistical analyses of wind tunnel results were performed using ANOVA (PROC GLIMMIX procedure) (SAS 2012). Treatments consisted of the different amounts of each essential oil on food bait. Response variables used were hovering over food bait before landing, landing on food bait or cutting, analysed with a binomial distribution. Each subject wasp was considered a random factor.

Relocation behaviour as affected by a repellent

This experiment was modified from the experiments conducted by D'Adamo and Lozada (2011). An individual forager was allowed to feed from a bait prepared in the same way as the baits described above for the choice tests, placed on the ground. An observer sat close to this array at 0.5 m distance. When a forager arrived at the dish and was collecting food, it was marked according to D'Adamo and Lozada (2011) with a dot of washable paint on the abdomen for identification. This marking procedure caused minimal disturbance to wasps. Any other wasp visiting the dish was removed with a handheld insect aspirator to work with only one individual per experiment. At each trial, the studied wasp collected food from the dish, then departed for the nest, and returned a few minutes later. We considered the wasp departure to have occurred when it flew away from the dish with a piece of meat within its mandibles. An individual wasp was used for only one experiment and one treatment.

The wasp was allowed to collect food a second time, and before it arrived back to the bait a third time the bait was replaced by one of six treatments: fresh meat bait (positive control), fresh bait with 100 μL of tea tree or lemongrass essential oil placed underneath the plastic dish on a filter paper, untreated filter paper (negative control), and filter paper treated with 100 μL of tea tree or lemongrass. The behaviour of the returning wasps was observed for 10 min, and it was recorded whether it landed on the bait and if it cut a piece of meat before leaving the site. In the case of the filter paper treatments containing no meat, it was recorded whether the wasp landed or hovered over it, and if so how many times. Each treatment was repeated 20 times with different individuals. The type of observed behaviour in the learning tests was compared among the treatments, evaluating whether the wasps hovered over the bait before landing, if they landed on it and if they removed a piece to take with them to the nest. Statistical analyses were performed using ANOVA (PROC GLIMMIX procedure) (SAS 2012). Response variables used were hovering over food bait before landing, landing on food bait or cutting, analysed with a binomial distribution. Each subject wasp was considered a random factor. Differences in the number of times a type of behaviour occurred between wasps returning to a control bait ($n = 20$) and those returning to a bait treated with essential oil ($n = 20$) were analysed using a Student t-test.

RESULTS

Choice tests

There was a clear preference for the untreated baits in choice tests. Significantly fewer wasps landed on treated baits when given a choice between meat and meat that was placed over a filter paper treated with lemongrass ($\chi^2 = 57.04$; df = 1; $P < 0.0001$), black pepper ($\chi^2 = 23.04$; df = 1; $P < 0.0001$), tea tree ($\chi^2 = 45.76$; df = 1; $P < 0.0001$), sweet orange ($\chi^2 = 36.48$; df = 1; $P < 0.0001$) or incense ($\chi^2 = 50.45$; df = 1; $P < 0.0001$) essential oils (Fig. 1). There were no differences in the number of foragers landing in control baits across experiments (F = 0.85; df = 4, 45; $P = 0.5$) showing that the density of wasps was comparable in all of them, with an overall mean of 6.5 ± 0.35. The overall number of foragers landing on treated baits was 1.3 ± 0.2, although it differed by treatment (F = 3.08; df = 4, 45; $P = 0.02$). There were more landings in baits treated with black pepper than on the other essential oils tested (Tukey contrasts, $P < 0.05$) (Fig. 1).

No-choice tests

There was a significant difference between the time it took the first wasp to arrive to a control and a treated bait for all the essential oils (Fig. 2). Wasps found and landed on control baits faster than on baits treated with lemongrass (Z = 3.614, $n = 25$, $P < 0.0001$), tea tree (Z = 3.17; $n = 25$; $P = 0.002$), sweet orange (Z = 2.18; $n = 25$; $P = 0.028$), incense (Z = 3.108, $n = 25$, $P = 0.001$) and black pepper (Z = 3.871, $n = 25$, $P < 0.0001$).

Under the conditions of the study, proportionally it took on average 2.05 (±0.34), 2.98 (±0.3), 2.5 (±0.5), 3.3 (±0.5) or 3.1 (±0.9) times longer to reach the treated than the control bait when it was treated with incense, lemongrass, sweet orange, black pepper and tea tree, respectively.

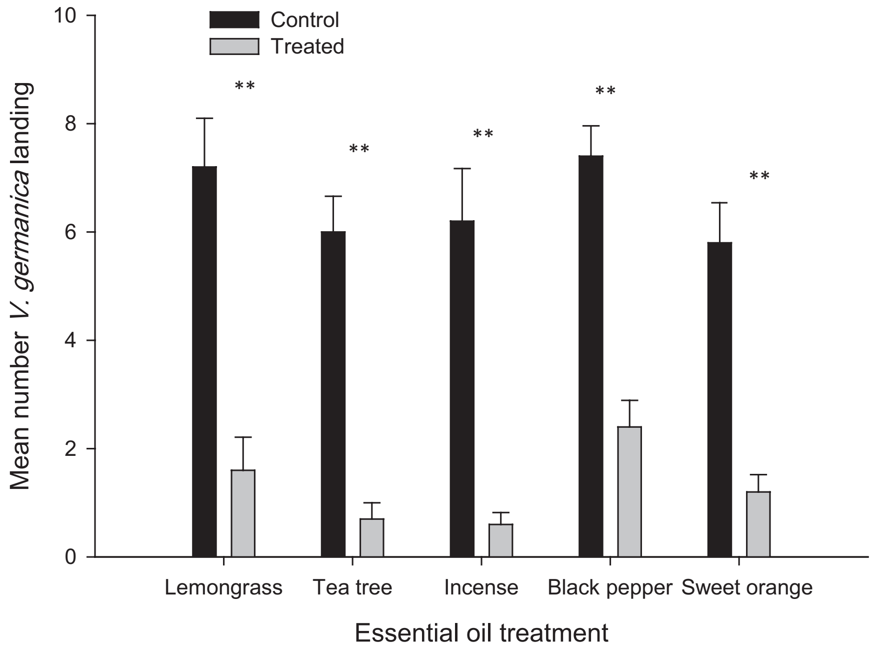

Fig. 1. Mean number of *Vespula germanica* wasps landing on control food baits and baits treated with different essential oil treatments in choice tests. **Indicates significant differences α = 0.001. *n* = 10.

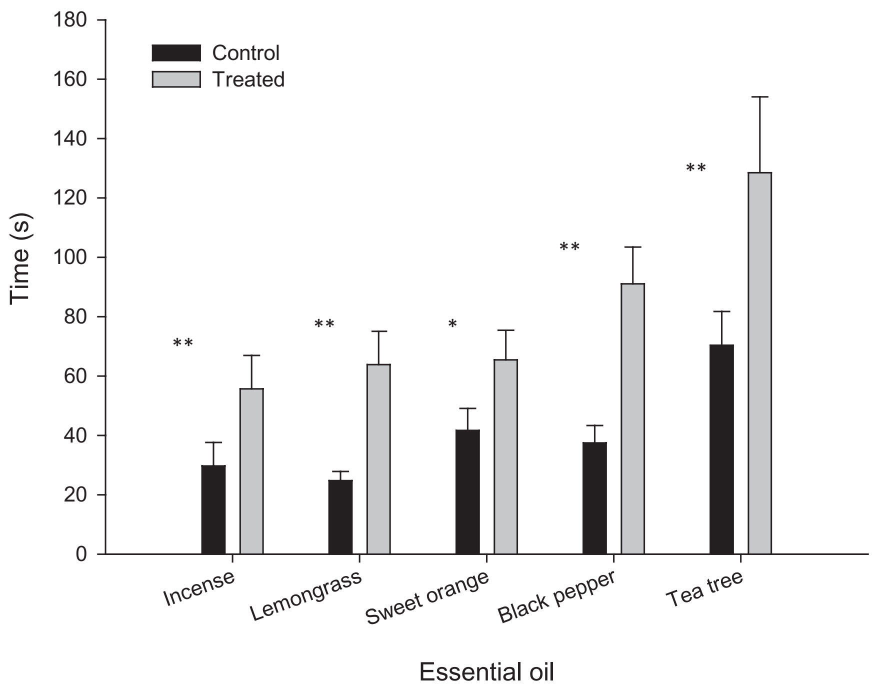

Fig. 2. Time taken for the first *Vespula germanica* worker (mean and SE) to arrive to control food baits, or to baits treated with 100 μL of each essential oil in field tests. Wilcoxon matched pairs test comparisons were made for each essential oil. **Indicates significant differences α = 0.001; *n* = 25.

Table 1 Behavioural response of foraging *Vespula germanica* wasps to different stimuli and the addition of varying amounts of repellent essential oils (tea tee or lemongrass) added to an attractive food bait in a wind tunnel ($n = 25$)

Stimulus	Addition of repellent	Amount of repellent (μL)	Number wasps hovering	Number wasps landing	Number not responding to stimulus	Proportion of wasps landing
Blank	No	None	1	0	24	0
Meat bait	No	None	0	24	1	0.96
Head extract	No	None	5	16	4	0.64
Meat bait	Lemongrass	100	5	10	10	0.4
Meat bait	Lemongrass	500	7	3	15	0.12
Meat bait	Lemongrass	1000	4	1	20	0.04
Meat bait	Tea tree	100	7	7	11	0.25
Meat bait	Tea tree	500	4	5	16	0.20
Meat bait	Tea tree	1000	6	2	17	0.08

Two types of behaviours were observed in responding wasps: hovering around the stimulus or landing on it.

Table 2 Behavioural response of *Vespula germanica* wasps returning to food baits

Bait on two first visits	Bait on third visit	Amount of repellent (μL)	Behaviour on third visit to feeding site		
			Wasps hovering over bait (%)	Wasps landing on bait (%)	Wasps cutting meat (%)
Meat	Meat	None	4a	100a	100b
Meat	Meat + lemongrass	100	85b	80a	25a
Meat	Meat + tea tree	100	90b	80a	25a

Wasps were allowed to collect food twice before placing the repellent on the bait. Wasps returning a third time to the feeding sites encountered either a control bait (ground meat) or one treated with a repellent essential oil (ground meat plus lemongrass or tea tree), $n = 20$. The percentage of wasps presenting each behaviour is shown, and those with the same letter are not statistically different at $\alpha = 0.05$.

Olfactory dose–response tests

Typically the wasps remained hovering in the area of the wind tunnel entrance for a few seconds and then either remained on the ground or started flying. An insect was considered to respond to the stimulus if it flew towards the source and did a close-range casting in front of the source (=hovering), or landed on it. In wasps that responded to the stimulus, the flight was almost straight with very small zigzagging towards the end of the tunnel where the stimulus was (chemo-anemotaxis). In non-responders, the flight was erratic, going up and down, and it was considered random. Wasps flew upwind when exposed to food volatiles, and exhibited close-range hovering over the source and landing on the container 96% of the time (Table 1). Wasps also exhibited chemo-anemotaxis, hovering and landing, when exposed to head extracts of adult wasps of the same species. Addition of different amounts of essential oils resulted in a dose-dependent decreased response to the food stimulus ($F = 6.70$; $df = 2, 144$, $P = 0.002$). When 1 mL of tea tree or lemongrass oil was added to the source, only 4% and 8%, respectively, landed on the food bait, with no significant differences between oils ($F = 0.22$; $df = 1, 144$, $P = 0.64$).

Relocation behaviour as affected by a repellent

There were significant differences between the behaviour of the trained wasps returning to the control bait and the wasps returning to a treated bait with respect to hovering ($F = 8.29$; $df = 2,38$; $P = 0.001$) and cutting ($F = 4.82$; $df = 2,38$; $P = 0.01$) (Table 2). Wasps returning to a control bait typically landed on it as soon as they arrived and cut a piece of meat before leaving the feeding site. Wasps returning to a bait treated with either lemongrass or tea tree oil usually did not land on the bait right away as opposed to those arriving to control baits. Wasps landed on treated baits after hovering over it for a few minutes, and when they did they only remained on it for a few seconds. Those wasps that did land on a treated bait usually left the feeding site without a piece of meat. When wasps returned to the feeding site to find an untreated filter paper, they hovered over it and landed on it several times during the observation period (Table 3). This behaviour was expected based on the work done by Lozada and D'Adamo (2006), who found that *V. germanica* wasps remember feeding sites and keep coming to them up to at least an hour after their first visit. Interestingly, wasps returning to the feeding site to

Table 3 Behavioural response of *Vespula germanica* wasps returning to a feeding site

Bait on 2 first visits	Bait on third visit	Amount of repellent (μL)	Mean number of times landing on third visit to feeding site
Meat	Nothing	None	4.9 ± 0.52a
Meat	Lemongrass	100	0.8 ± 0.25b

Wasps were allowed to take food with them twice before removing the bait. Wasps returning a third time to the feeding sites encountered either a blank (untreated filter paper) or a repellent essential oil (100 μL of lemongrass applied to filter paper), $n = 20$. Means ± SE are presented, and those means with the same letter are not statistically different at $\alpha = 0.05$.

find a filter paper treated with essential oils had a significantly different behaviour ($t = 6.53$; df = 39; $P < 0.0001$) and avoided landing on it (Table 3).

DISCUSSION

In the present work we report the comparative repellent effect of lemongrass, tea tree, black pepper, incense and sweet orange essential oils on *V. germanica* wasps. All the essential oils tested had a marked repellent effect, although black pepper was slightly less effective than the others in choice tests. Moreover, the wind tunnel assessed the individual response of *V. germanica* workers to olfactory cues, suggesting it is a good device to study the behaviour of this species and warrants more studies to assess the response of foraging wasps to different baits. This is particularly interesting considering that most of the literature on Vespula spp. management options are related to the use of attractive baits, and most studies are conducted in the field with traps baited with different lures (Davis *et al*. 1972; Landolt 1998; Day & Jeanne 2001; El-Sayed *et al*. 2009). It could be more cost-effective in such cases where the aim is to identify attractive compounds to use a wind tunnel or laboratory bioassay (Boevé *et al*. 2014) to screen for potential active compounds before conducting a field study.

Zhang *et al*. (2013) tested the repellency of essential oils including lemongrass on several species of wasps of which *V. germanica* was less than 10%. The current study allowed for the study of the behaviour of *V. germanica* alone in a wind tunnel as well as in a field setting, and corroborated their results on lemongrass oil. On the other hand, Boevé *et al*. (2014) tested repellency of essential oils and pure chemicals in a laboratory bioassay that used several individuals from different species simultaneously and for extended periods of time, which could have led to odour as well as other types of interactions that may have confounded the results. While Boevé *et al*. (2014) found consistent repellency of (−)-terpinen-4-ol on wasps, the essential oil of tea tree was not among the most repellent tested by these authors.

V. germanica wasps have good learning skills (D'Adamo & Lozada 2003, 2007, 2009, 2011, 2014; Lozada & D'Adamo 2006, 2009, 2011, 2014; Moreyra *et al*. 2006, 2014), and foragers are efficient at relocating a known food resource many times until its depletion. Moreover, it is known that the handling time decreases after the workers have visited a feeding site more than once (Moreyra *et al*. 2012). Interestingly, our results showed that foragers returning to a bait treated with essential oils avoided landing on it or did not take any part of it back to the nest. This behaviour suggests that the effect is not due to masking, given that foragers knew there was a food source at that location. Rather, they preferred not to exploit it when they perceived the odour of the essential oils. Thus, the results of the wind tunnel coupled with the learning tests show that at least part of the repellent effect observed is due to an olfactory cue.

Tea tree is known to contain terpinen-4-ol as one of the major terpenoids present, and is particularly interesting due to its popularity in cosmetic products and its known antimicrobial properties (Huynh *et al*. 2012). Limonene, terpinen-4-ol, b-caryophyllene, spathulenol and globulol have been found present throughout the year in incense essential oil (Cabrera *et al*. 2015). In the case of lemongrass, geranial (citral-a), neral (citral-b) and myrcene are the main components (Hanaa *et al*. 2012). Orange essential oil is typically composed mainly of limonene, followed by myrcene and other minor terpenoid components (Tao *et al*. 2009; Kamal *et al*. 2011). B-caryophyllene can be one of the main components of black pepper (Menon *et al*. 2011). Further research could focus on testing these main components individually in behavioural bioassays and conducting gas chromatography coupled with electroantennographic detection (GC-EAD) studies on the essential oil volatiles to determine which ones are responsible for the repellency, given the only GC-EAD studies have been done with other species of wasps, and most of these chemicals have not yet been studied at all (Zhang *et al*. 2013).

Usually essential oils have a better efficacy as repellents when used in mixtures, probably due to the detoxification mechanism of insects (Rossi *et al*. 2012; Rossi & Palacios 2013). Thus, future studies could focus on the formulation of a combination of individual terpenoids specifically targeting Vespula spp. to improve efficacy and standardisation of the product.

The present work shows new insights on how the utilisation of essential oil repellents in outdoor settings can be highly beneficial, given the negative impact of *V. germanica*. The results obtained have implications for management of these insects. The differences in the response towards the repellents observed between choice and no-choice tests suggest that if the wasps have a choice, they will avoid a bait in proximity to a repellent. If the only resource in sight is next to a repellent odour, the wasps may still utilise that food source, and the response will vary if the wasps have had experience with the food source before addition of the repellent or not. These results agree with those reported by Zhang *et al*. (2013) and Boevé *et al*. (2014), and suggest that a push–pull system (Cook *et al*. 2007) that combines attractive baits together with repellents has potential as a practical tool to diminish potential injuries caused by these harmful insects, particularly in urban settings.

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