



Development of *Melaleuca* oils as effective natural-based personal insect repellents

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Abstract

For many decades effective insect repellents have relied on synthetic actives such as *N,N*-diethyl-*meta*-toluamide. Increasingly, consumers are seeking natural-based alternatives to many everyday products including insect repellents. While many studies have been published detailing the potential of essential oils to act as insect repellents, few oils have been identified as viable alternatives to synthetic actives. This study details the process involved in the selection of Australian essential oils effective as repellents and the subsequent testing of natural-based insect repellents using the selected oils. Using a combination of laboratory-based and field-based testing, oil from *Melaleuca ericifolia* was identified as being an effective insect repellent. When formulated into three different bases: an alcohol-based spray, an emulsion and a gel, these *Melaleuca*-based repellents were shown to be as effective at repelling mosquitoes *Aedes vigilax* (Skuse) (Diptera: Culicidae) and *Verrallina carmentis* (Edwards) (Diptera: Culicidae), the bush fly *Musca vetustissima* (Walker) (Diptera: Muscidae), and biting midges *Culicoides ornatus* (Taylor) (Diptera: Ceratopogonidae) and *Culicoides immaculatus* (Lee & Reye) (Diptera: Ceratopogonidae) as a synthetic-based commercial repellent. This study has shown that effective insect repellents based on natural active ingredients can deliver repellency on par with synthetic actives in the field. Three *Melaleuca*-based formulations have been registered as repellents and are now commercially available.

Key words botanical repellent, deet, essential oil, *Melaleuca*, mosquito.

INTRODUCTION

In Australia, insects such as the bush fly, *Musca vetustissima* (Walker), biting midges and mosquitoes can impact human health and quality of life. The bush fly is a non-biting insect, which is found throughout Australia and can be an extreme nuisance to humans and livestock. The bush fly is also known to transmit eye infections and enteric diseases (Hughes 1970). Biting midges, also known as sandflies, are found predominantly in northern Australia and are blood-sucking insects responsible for acute discomfort, irritation and severe local reactions that can be immediate or delayed although in Australia they are not known to transmit disease (Whelan 2003). Mosquitoes in Australia are known to transmit a number of disease-causing pathogens including dengue virus (DV), Ross River virus (RRV), Barmah Forest virus (BFV), Murray Valley encephalitis virus (MVEV) and Kunjin virus (KUNV) (Russell & Kay 2004). DV is found only in Queensland, MVEV and KUNV are found in Western Australia, the Northern Territory and Queensland but are extending into the more temperate regions of New South Wales and Victoria, whereas

RRV and BFV are generally common and widespread throughout Australia (Tong & Hu 2001; Russell & Kay 2004; Horwood & Bi 2005). Mosquito bites can also cause a range of responses such as localised discomfort, irritation and swelling to anaphylaxis (Fradin 1998).

In Australia, insect bites are mostly a nuisance. However, insect bites can have serious health implications in other parts of the world. Protection from annoying insects and their bites is best achieved by avoiding infested habitats, wearing protective clothing and using insect repellents (Fradin & Day 2002). In many circumstances, applying insect repellent to the skin may be the only feasible way to protect against insect bites and transmission of disease (Fradin & Day 2002). Commercially available insect repellents can be divided into two categories: synthetic chemicals and plant-derived essential oils. The most efficacious and broadly used synthetic insect repellent with a strong safety record for the last six decades is *N,N*-diethyl-*meta*-toluamide, or deet (Osimitz & Grothaus 1995; Sudakin & Trevathan 2003; Katz *et al.* 2008).

In 1998, the United States Environmental Protection Agency (US EPA) completed a re-registration eligibility for deet (US EPA 1998). Based on animal studies designed to assess acute and chronic toxicity, mutagenicity, oncogenicity and developmental, reproductive and neurological toxicity,

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deet was classified to be of low to very low toxicity (US EPA 1998; Sudakin & Trevathan 2003). The US EPA concluded that insect repellents containing deet would generally not cause unreasonable risks to humans or the environment, but required additional label warnings and restrictions for those formulations intended to be protective for children and other sensitive individuals (US EPA 1998; Sudakin & Trevathan 2003). In addition, deet has been found to damage spandex, rayon, acetate and leather (Brown & Hebert 1997; Katz *et al.* 2008). It can dissolve plastics, vinyl and labels, is greasy and has an unpleasant odour (Brown & Hebert 1997; Fradin 1998; Katz *et al.* 2008). In spite of these drawbacks, deet is a highly effective insect repellent and remains the standard of currently available insect repellents (Fradin 1998).

Although reports of adverse effects in humans associated with the dermal application of deet are rare (Sudakin & Trevathan 2003; Katz *et al.* 2008), many consumers are reluctant to apply deet to their skin due to concerns about safety and comfort, and they deliberately seek out other alternatives based on naturally derived materials such as botanical essential oils (Fradin & Day 2002).

Thousands of plant-derived essential oils have been tested as potential sources of insect repellents. None of those tested to date demonstrate the broad effectiveness and duration of deet, but a few show repellent activity (Fradin 1998; Fradin & Day 2002). Plants whose essential oils have been reported to have repellent activity include citronella, cedar, eucalyptus, lemongrass, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint (Fradin 1998; Fradin & Day 2002; Katz *et al.* 2008). Unlike synthetic insect repellents, plant-derived repellents have been poorly studied. When tested, most of these essential oils tend to give short-lasting protection, usually less than 2 h (Fradin 1998).

Currently, the US EPA's registered plant-derived essential oils approved for application to the skin include oil of citronella and oil of lemon eucalyptus (*p*-menthane-3,8-diol; PMD) (Katz *et al.* 2008). Limited data are available from studies that directly compare the efficacy of citronella-based products with that of deet-based products. In one study, a 1000-fold higher concentration of citronellol (one of the active chemicals in citronella oil) compared with that for deet was required to prevent 90% of mosquitoes from landing on their targets (Wright 1975). Other studies have shown that citronella can be an effective repellent, but it provides shorter complete protection time than most deet-based products (Fradin 1998).

It is notable that in 2005, the US Centres for Disease Control endorsed PMD as an active ingredient in personal insect repellents (Carroll & Loye 2006a). Studies suggest that PMD may confer longer lasting protection than other plant-based repellents, generally at higher concentrations than that required for deet. For example, 20% PMD had a similar estimated mean protection time as 15% deet against mosquitoes (Barnard & Xue 2004). In the field, 30% PMD was found to be of superior repellency against mosquitoes compared with 15% deet in a 4 h test (Moore *et al.* 2002). In another study the percentage repellency did not differ significantly, but 20% deet

seemed to be slightly superior to 25% PMD against mosquitoes (Barnard *et al.* 2002). However, PMD is obtained from the hydrodistillation of the essential oil from the leaves of the lemon eucalyptus and thus the repellent is not the essential oil of the lemon eucalyptus, but the residue of the oil distillation process (Barnard & Xue 2004; Carroll & Loye 2006a).

There is increasing consumer desire for natural alternatives to deet and other synthetic insect repellents. However, the consumer is generally, and wisely, unwilling to compromise on efficacy simply because they chose a natural-based repellent. While it may be desirable to develop insect repellents based on natural active ingredients, very few materials have been found to be reliably effective in laboratory or field tests. The difficulty in formulating a natural-based insect repellent with the efficacy of deet is highlighted by the lack of such repellents on the market.

In Australia the sale of insect repellents is controlled by the Australian Pesticides and Veterinary Medicines Authority (APVMA). An insect repellent intended for human use must be approved by the APVMA, a process that includes an examination of its safety and efficacy. We have recently developed formulations based on natural Australian essential oils for personal protection against mosquitoes, the bush fly and biting midges that deliver insect repellency equivalent to that of commercially available synthetic repellents. Three insect repellents have now been approved by the APVMA and are marketed as MOOV Insect Repellent Gel, MOOV Insect Repellent Roll-on and MOOV Insect Repellent Spray. The development work leading to the registration and marketing of the MOOV natural-based repellents is described in this paper.

MATERIALS AND METHODS

Repellents

For initial screening tests in the laboratory, essential oils from Australian plants were prepared in a simple cream base consisting of cocoa butter, lecithin, myristyl alcohol and water. The essential oils studied were from *Backhousia citriodora*, *Backhousia anisata* and *Melaleuca ericifolia* purchased from FPI Oceania, Alstonville, NSW; *Callitris columellaris* (wood) and *Callitris columellaris* (leaf) purchased from Good Oil Plantation Group, Gordon, NSW; *Leptospermum liversidgei* and *Leptospermum petersonii* purchased from Tea Tree Traders, Cronulla, NSW; *Eremophila mitchellii*, *Melaleuca linariifolia* and *Melaleuca uncinata* purchased from GR Davis, Riverstone, NSW and *Callitris glaucophylla* purchased from Solvents Australia, Mona Vale, NSW.

Oil from *M. ericifolia* and *B. citriodora* were chosen for further laboratory testing. Oil from *M. ericifolia* at 4% w/w and oil from *B. citriodora* at 1% w/w were combined and prepared as three different natural-based insect repellent formulations (i.e. an alcohol solution, an emulsion and a gel). Oil from *M. ericifolia* was chosen for field testing. Oil from *M. ericifolia* at 5% w/w was prepared as three formulations as described above.

Off! Skintastic Personal Insect Repellent Spray (NRA Approval No. 56411/0902) purchased from SC Johnson Wax, Lane Cove, NSW, containing 69.75 g/L deet and 27.9 g/L di-*N*-propyl isocinchomeronate was used as the synthetic-based insect repellent for comparative field testing.

Laboratory testing

Australian essential oils, each formulated into a cream base, were screened in the laboratory to determine their efficacy as potential insect repellents. Framed cages (30 cm × 30 cm × 30 cm) fitted with synthetic gauze mesh and a cloth sleeve was used for the study. There were five cages and each volunteer used the same cage throughout testing. Each cage contained 50 five-to-ten-day-old *Aedes aegypti* (Linnaeus) female mosquitoes. The mosquitoes were provided with a 10% sugar solution as food. New mosquitoes were used for each new day of testing. In the event of some mosquitoes being 'knocked down' during a single day of testing, the mosquito numbers were supplemented to ensure that at least 50 were used in each replicate.

Five volunteers randomly selected from staff at the University of Technology, Sydney, NSW were tested, consisting of both males and females between the ages of 18 and 55 years. The area from the wrist to the elbow was treated with 2.0 g of repellent applied by weighing onto a piece of foil and then distributing evenly over the forearm by rubbing with two fingers protected by a surgical glove. The application of repellent to either the left or right arm was varied and the repellents were rotated between volunteers so that each volunteer tested a different repellent on each day. The hands of the volunteers were protected with white cotton gloves and there was no other skin apart from the forearm exposed to the mosquitoes thus preventing any unwanted bites. Immediately following repellent application, one forearm was placed into the cage and was exposed for a period of 1 min. The number of mosquitoes landing and probing on the exposed skin was noted and then repeated for the opposing forearm with no repellent applied. The procedure was then repeated 30, 60 and 120 min post repellent application. Testing was repeated four times to complete five replicates for each repellent over several days.

Prior to repellent application at the start of each new day, each volunteer was checked for inherent repellency to the mosquito. Both forearms of each volunteer were inserted separately into the testing cage for 1 min and the number of landings of duration longer than 1 s was recorded. Volunteers with less than 10 landings in 1 min were rejected from the study.

Based on the initial screening results for the Australian essential oils (Table 1), the efficacy of 4% w/w oil from *M. ericifolia* combined with 1% w/w oil from *B. citriodora* was formulated into an alcohol, emulsion or gel base and tested using the laboratory testing method described above.

Field testing

Based on laboratory testing (Table 2), 5% w/w oil from *M. ericifolia* was formulated into an alcohol, emulsion or gel base

Table 1 The mean number of landings over 1 min and percentage repellency of *Aedes aegypti* mosquitoes at various times post application of Australian essential oils in laboratory tests

Essential oil	<i>n</i>	Concentration (% w/w)	Mean number of landings (±SE) (min post application)			% repellency (min post application)				
			0	30	60	120	0	30	60	120
None	5	–	44.8 ± 11.2	43.6 ± 10.3	24.8 ± 5.5	41.2 ± 7.8	–	–	–	–
<i>Bac-khousia citriodora</i>	5	5	8.0 ± 3.8	23.4 ± 7.4	25.8 ± 10.0	32.0 ± 6.5	82.1	46.3	0	22.3
<i>Bac-khousia anisata</i>	5	2	12.8 ± 3.9	18.6 ± 3.7	24.2 ± 9.3	34.4 ± 9.4	71.4	57.3	2.4	16.5
<i>Callitris columellaris</i> (wood)	5	5	14.2 ± 9.6	21.4 ± 6.3	19.8 ± 11.1	47.2 ± 11.1	68.3	50.9	20.2	0
<i>Callitris columellaris</i> (leaf)	5	10	10.4 ± 3.3	20.4 ± 8.2	40.4 ± 20.4	41.4 ± 11.8	76.8	53.2	0	0
<i>Callitris glaucophylla</i>	5	5	4.2 ± 1.7	7.0 ± 2.7	10.6 ± 3.6	26.4 ± 8.0	90.6	83.9	57.3	35.9
<i>Eremophilla mitchelli</i>	5	10	7.6 ± 2.2	15.8 ± 5.6	18.8 ± 6.3	29.8 ± 5.4	83.0	63.8	24.2	27.7
<i>Leptospermum liversidgei</i>	5	5	5.2 ± 2.2	27.0 ± 11.2	34.4 ± 15.5	57.0 ± 15.0	88.4	38.1	0	0
<i>Leptospermum petersonii</i>	5	5	2.8 ± 1.5	31.2 ± 8.6	33.4 ± 17.9	49.8 ± 16.1	93.8	28.4	0	0
<i>Melaleuca linariifolia</i>	5	10	1.8 ± 0.9	9.6 ± 4.0	12.2 ± 4.5	25.0 ± 6.8	96.0	78.0	50.8	39.3
<i>Melaleuca ericifolia</i>	5	10	0.8 ± 0.5	6.2 ± 1.9	14.4 ± 4.1	22.4 ± 4.9	98.2	85.8	41.9	45.6
<i>Melaleuca uncinata</i>	5	10	4.8 ± 0.9	17.0 ± 2.0	20.4 ± 5.3	27.0 ± 8.8	89.3	61.0	17.7	34.5

Table 2 The mean number of landings over 1 min and percentage repellency of *Aedes aegypti* mosquitoes at various times after application of a formulation containing 5% w/w oil from *Melaleuca ericifolia* and 1% w/w oil from *Backhousia citriodora* in laboratory tests

Repellent	n	Mean number of landings (\pm SE) (min post application)				% repellency (min post application)				
		Pre-treatment	0	30	60	120	0	30	60	120
None	5	111.8 \pm 32.8a	105.4 \pm 33.5b	93.2 \pm 21.3b	81.6 \pm 18.7a	99.2 \pm 19.7a	—	—	—	—
4% w/w <i>Melaleuca ericifolia</i> + 1% w/w <i>Backhousia citriodora</i> in alcohol	5	136.4 \pm 37.7a	2.8 \pm 2.0a	20.8 \pm 7.3a	45.6 \pm 15.3a	57.6 \pm 17.0a	97.3a	77.7a	44.1	41.9
4% w/w <i>Melaleuca ericifolia</i> + 1% w/w <i>Backhousia citriodora</i> in emulsion	5	110.04 \pm 34.5a	2.4 \pm 0.9a	32.0 \pm 14.1a	48.8 \pm 16.4a	55.0 \pm 16.8a	97.7a	65.7a	40.2	44.6
4% w/w <i>Melaleuca ericifolia</i> + 1% w/w <i>Backhousia citriodora</i> in gel	5	112.4 \pm 42.2a	4.2 \pm 3.5a	16.8 \pm 4.8a	38.8 \pm 12.1a	34.4 \pm 8.3a	96.0a	82.0a	52.5	65.3

Means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.005$).

and their ability to provide repellency against mosquitoes, the bush fly and biting midges in the field was compared with a synthetic insect repellent containing 69.75 g/L deet and 27.9 g/L di-*N*-propyl isocinchomeronate. Before field testing commenced the Australian regulatory authorities withdrew approval for the use of *Backhousia* oils for topical application in insect repellents and so only *Melaleuca* oil was tested.

Field testing was based on a standard protocol (Carroll & Loye 2006b). At the start of each new day of testing before repellent application, a check was made that there was sufficient insect abundance by confirming that insects landed on both untreated legs of all volunteers. This also checked the inherent repellency of the volunteers to the insects. Volunteers with an inadequate number of landings were rejected from the study.

Each of the volunteers' legs was marked just below the knee and just above the ankle to define the area of repellent application. The repellents were applied at the requisite rate per lower leg. To prevent cross-contamination of repellents, the applicators wore surgical gloves. One repellent was applied to one leg, and a different repellent to the other. The repellents were allocated approximately evenly to either the left or right leg and the repellents were rotated between volunteers so that each volunteer tested a different repellent on each day. No other repellents were applied to any volunteers on the assessment day, and volunteers washed the treated area thoroughly between assessments, volunteers were free to walk, stand or sit. The residual activity of the repellents was assessed 30, 60, 120 and 180 min post application. The number of insects landing over a 10 min period (3 min period for biting midges) for greater than 1 s at the various assessment times was counted by the study volunteers. Both limbs were counted simultaneously after volunteers were trained in doing so. Landings were defined as resting on the limb not merely touching the limb. After the insects had settled they were disturbed by moving the limb. Testing was repeated four times to complete five replicates for each repellent.

Mosquitoes

The testing was conducted over five consecutive days in a mangrove area in Cairns, Queensland, from 18 to 22 April 2005, between 15:00 and 19:00 h. The mosquito fauna of this area is dominated by *Aedes vigilax* (Skuse) and *Verrallina carmentis* (Edwards). *V. carmentis* is a known human biter and is a vector of RRV (Webb *et al.* 2008). *Ae. vigilax* is also a human biter and is a vector of RRV and BFV (Harley *et al.* 2000). Five volunteers randomly selected from staff at the University of Technology, Sydney, NSW were tested, consisting of both males and females between the ages of 18 and 60 years. Volunteers wore special suits to protect themselves from mosquito bites. The suits (The Original Bugshirt – Elite Edition) consisted of a two piece suit, dense material trousers and jacket fitted with a fully enclosed headnet. One of the trouser legs was rolled up and taped to expose the treated leg. The other trouser leg (control), with no repellent applied, was rolled up

only during an assessment. The test subjects also wore thick cotton gloves to protect their hands from bites. The repellents were applied at a rate of 3.6 g per lower leg.

Bush fly

The testing was conducted over five consecutive days in a farmland area in Quinalow, Queensland, from 10 to 14 December 2001, between 06:45 and 11:00 h. Five volunteers randomly selected from staff at the University of Technology, Sydney, NSW were tested, consisting of both males and females between the ages of 18 and 65 years. Volunteers wore shorts to expose the lower leg below the knee. The repellents were applied at a rate of 3.6 g per lower leg except for the natural-based gel insect repellent which was applied at a rate of 1.8 g per lower leg.

Biting midges

The testing was conducted over 7 days in a mangrove area in Cairns, Queensland, from 27 April to 4 May 2002, between 08:00 and 11:15 h. This area is populated with *Culicoides ornatus* (Taylor) and *Culicoides immaculatus* (Lee & Reye). Five volunteers randomly selected from staff at the University of Technology, Sydney, NSW were tested, consisting of both males and females between the ages of 18 and 55 years. Volunteers wore shorts to expose the lower leg below the knee. The repellents were applied at a rate of 3.6 g per lower leg except for the natural-based gel insect repellent which was applied at a rate of 1.8 g per lower leg.

Statistical analysis

Data were analysed by one-way ANOVA using SPSS 12.0 software. Post hoc comparisons were made among the various groups using Dunnett's T-test. Data are expressed as mean \pm standard error where n represents the number of replicates. $P < 0.05$ was considered to be statistically significant.

The percentage repellency values reported are pooled across all replicates and the mean repellency for all volunteers was calculated using the following formula: Percentage repellency = (number of landings on untreated limb – number of landings on treated limb)/number of landings on untreated limb \times 100.

RESULTS

Laboratory testing

Initial screening of 11 Australian essential oils in the laboratory indicated that *B. citriodora*, *B. anisata*, *C. glaucophylla*, *E. mitchelli*, *M. linariifolia*, *M. ericifolia* and *M. uncinata* had the most potential to be effective and persistent insect repellents based on the mean number of landings and percentage repellency of mosquitoes (Table 1).

There were no significant differences in the mean number of mosquitoes landing on forearms assigned to be treated with the

repellent formulations compared with forearms assigned to be the untreated controls before the repellents were applied (Table 2; $P = 0.95$, d.f. = 3). All three repellent formulations tested significantly reduced the mean number of mosquitoes landing on treated compared with untreated forearms (Table 2; $P < 0.005$, d.f. = 3) after 0 and 30 min. Although not significant (Table 2; $P = 0.069$, d.f. = 3), the three repellent formulations continued to reduce the mean number of mosquito landings 2 h post application. However, laboratory studies can only be regarded as indicative, not predictive, of final field performance.

Field testing

Mosquitoes

There were no significant differences in the mean number of mosquitoes landing on lower legs treated with the repellents compared with untreated lower legs at the pre-treatment assessment (Table 3; $P = 0.62$, d.f. = 4). All four repellents tested were highly effective against mosquitoes and significantly reduced the mean number of mosquitoes landing on treated compared with untreated lower legs (Table 3; $P < 0.001$, d.f. = 4 for all time periods) to almost zero at each assessment time over the 3 h study period (Table 3). The three natural-based repellents were of comparable efficacy and not significantly different to Off! Skintastic in repelling mosquitoes over the 3 h study period, and continued to provide repellency of 97.4% or better (Table 3).

Bush fly

There were no significant differences in the mean number of bush flies landing on lower legs treated with the repellents compared with untreated lower legs at the pre-treatment assessment (Table 4; $P = 0.89$, d.f. = 4). All four repellents tested were highly effective against the bush fly and significantly reduced the mean number of bush flies landing on treated compared with untreated lower legs (Table 4; $P < 0.001$, d.f. = 4 for all time periods) to almost zero at each assessment time over the 3 h study period (Table 4). The three natural-based repellents were of comparable efficacy and not significantly different to Off! Skintastic in repelling bush flies over the 3 h study period, and continued to provide repellency of 92.1% or better (Table 4).

Biting midges

There were no significant differences in the mean number of biting midges landing on lower legs treated with the repellents compared with untreated lower legs at the pre-treatment assessment (Table 5; $P = 0.91$, d.f. = 4). All four repellents tested were found to be highly effective against biting midges and significantly reduced the mean number of biting midges landing on treated compared with untreated lower legs (Table 5; $P < 0.001$, d.f. = 4 for all time periods) to almost zero at each assessment time over the 3 h study period. The three natural-based repellents were of comparable efficacy and not

Table 3 The mean number of landings over 10 min and percentage repellency of *Aedes vigilax* and *Verrallina carmentis* mosquitoes at various times post application of four repellent formulations to the lower leg in field trials

Repellent	n	Mean number of landings (\pm SE) (min post application)			% repellency (min post application)					
		Pre-treatment	30	60	120	180	30	60	120	180
None	5	38.4 \pm 4.3a	40.6 \pm 5.9b	50.4 \pm 7.7b	56.0 \pm 8.6b	53.8 \pm 6.7b	—	—	—	—
Off! Skintastic	5	52.2 \pm 7.8a	0a	0a	0a	0a	100.0a	100.0a	100.0a	100.0a
5% w/w <i>Melaleuca ericifolia</i> in alcohol	5	40.6 \pm 4.7a	0a	0a	1.8 \pm 1.2a	1.4 \pm 0.9a	100.0a	100.0a	96.8a	97.4a
5% w/w <i>Melaleuca ericifolia</i> in emulsion	5	36.8 \pm 5.9a	0a	0a	0a	0.4 \pm 0.2a	100.0a	100.0a	100.0a	99.3a
5% w/w <i>Melaleuca ericifolia</i> in gel	5	48.6 \pm 14.1a	0a	0.2 \pm 0.2a	0.6 \pm 0.4a	1.0 \pm 1.0a	100.0a	99.6a	98.9a	98.1a

Means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.001$).

Table 4 The mean number of landings over 10 min and percentage repellency of the bush fly at various times post application of four repellent formulations to the lower leg in field trials

Repellent	n	Mean number of landings (\pm SE) (min post application)			% repellency (min post application)					
		Pre-treatment	30	60	120	180	30	60	120	180
None	5	48.4 \pm 8.9a	36.0 \pm 6.8b	28.6 \pm 7.1b	33.6 \pm 8.4b	28.0 \pm 3.9b	—	—	—	—
Off! Skintastic	5	44.8 \pm 8.9a	2.4 \pm 1.9a	1.8 \pm 1.4a	1.8 \pm 1.0a	1.8 \pm 0.9a	93.3a	93.7a	94.6a	93.6a
5% w/w <i>Melaleuca ericifolia</i> in alcohol	5	42.8 \pm 8.6a	0a	0a	0.6 \pm 0.4a	1.8 \pm 1.0a	100.0a	100.0a	98.2a	93.6a
5% w/w <i>Melaleuca ericifolia</i> in emulsion	5	49.2 \pm 21.4a	0a	0.2 \pm 0.2a	0.8 \pm 0.4a	1.8 \pm 1.4a	100.0a	99.3a	97.6a	93.6a
5% w/w <i>Melaleuca ericifolia</i> in gel	5	33.4 \pm 6.5a	1.2 \pm 1.0a	1.2 \pm 1.0a	0.8 \pm 0.6a	2.2 \pm 1.2a	96.7a	95.8a	97.6a	92.1a

Means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.001$).

Table 5 The mean number of landings over 3 min and percentage repellency of *Culicoides ornatus* and *Culicoides immaculatus* biting midges at various times post application of four repellent formulations to the lower leg in field trials

Repellent	n	Mean number of landings (\pm SE) (min post application)				% repellency (min post application)				
		Pre-treatment	30	60	120	180	30	60	120	180
None	5	36.2 \pm 3.4a	42.6 \pm 12.7b	50.2 \pm 16.8b	42.0 \pm 5.3b	27.8 \pm 4.3b	—	—	—	—
Off! Skintastic	5	42.2 \pm 11.8a	0a	0a	0.2 \pm 0.2a	0.8 \pm 0.6a	100.0a	100.0a	99.5a	97.1a
5% w/w <i>Melaleuca ericifolia</i> in alcohol	5	43.2 \pm 10.8a	0.2 \pm 0.2a	1.4 \pm 0.7a	4.0 \pm 1.3a	5.4 \pm 2.7a	99.5a	97.2a	90.5a	80.6a
5% w/w <i>Melaleuca ericifolia</i> in emulsion	5	35.2 \pm 6.4a	0a	0.2 \pm 0.2a	0.4 \pm 0.2a	1.2 \pm 0.8a	100.0a	99.6a	99.0a	95.7a
5% w/w <i>Melaleuca ericifolia</i> in gel	5	34.6 \pm 5.6a	0.2 \pm 0.2a	0.6 \pm 0.6a	0.4 \pm 0.2a	1.4 \pm 0.7a	99.5a	98.8a	99.0a	95.0a

Means followed by the same letter are not significantly different ($P > 0.05$). Means followed by a different letter are significantly different ($P < 0.001$).

significantly different to Off! Skintastic in repelling biting midges over the 3 h study period, and continued to provide repellency of 80.6% or better (Table 5).

DISCUSSION

Laboratory testing of Australian essential oils revealed that oil from *M. ericifolia* at 4% w/w mixed with oil from *B. citriodora* at 1% w/w provided repellency against *Ae. aegypti* in three different formulations. The laboratory testing indicated that while the gel base appeared to be the most suitable base for the essential oils (Table 2; 65.3% repellency at 2 h), both the alcohol and the emulsion bases also showed persistent repellency (Table 2; 44.6% and 41.9% repellency respectively at 2 h). Formulations containing oil from *M. ericifolia* at 5% w/w were adopted for field studies designed to test their ability to provide repellency against mosquitoes, the bush fly and biting midges compared with a synthetic insect repellent.

In the mosquito field study, the natural-based repellent formulations containing 5% w/w oil from *M. ericifolia* provided similar repellency rates (Table 3; up to 99.3% at 3 h) compared with the synthetic repellent Off! Skintastic (Table 3; 100% at 3 h) against mosquitoes. No differences in repellency were observed between the *Melaleuca* oil formulations. Importantly, the repellency rate for Off! Skintastic found in this study was similar to that found by others in the field tested over 3 h (Frances *et al.* 2005).

Many other studies have been published regarding the efficacy of essential oils as potential mosquito repellents with mixed results. Fewer published studies have specifically examined Australian essential oils, including *Melaleuca* oils, as potential mosquito repellents. For example, oil from *Eucalyptus citriodora* tested in the laboratory at 23 $\mu\text{g}/\text{cm}^2$ was found to provide no repellency against *Ae. albopictus* (Skuse) after 1 h (Zhu *et al.* 2006). In another laboratory study, the protection period against *Ae. aegypti* ranged from only 1 h for oil from *Eucalyptus globulus*, 2.5 h for both *E. citriodora* and *Eucalyptus radiata*, 3.5 h for *Eucalyptus dives*, 6 h for *Melaleuca leucadendron* (cajeput) and 8 h for *Melaleuca quinquenervia* (niaouli) compared with 6 h for deet, when all tested at 20%. Interestingly, all of these oils as well as deet provided 8 h protection against *Anopheles stephensi* (Liston) and *Culex quinquefasciatus* (Say), except for oil from *E. globulus* which provided 6 h protection against *A. stephensi* (Amer & Mehlhorn 2006). The authors concluded that the *Ae. aegypti* mosquito showed a high tolerance to essential oils (Amer & Mehlhorn 2006).

Although a protection time against mosquitoes of up to 8 h was found in the study by Amer and Mehlhorn (2006), the *Melaleuca* oils used were different species and at fourfold the concentration to those tested in this study. Further, the study by Amer and Mehlhorn (2006) is a laboratory study and therefore can only be regarded as indicative, not predictive, of final field performance which is investigated in this study. However, the potential of different *Melaleuca* oils as mosquito repellents is

apparent. This is further emphasized by another laboratory study which evaluated the repellency of forty essential oils extracted from Australian plants against mosquitoes, where oil from *Melaleuca bracteata* was among the most effective, as well as oils from *Dacrydium franklini*, *Backhousia myrtifolia* and *Zieria smithii* (Penfold & Morrison 1952).

In yet another laboratory study, the behavioural responses of *Ae. aegypti* to essential oils extracted from *M. leucadendron*, *Litsea cubeba* and *Litsea salicifolia* were examined in an excito-repellency test chamber. Significantly higher escape rates were observed from contact chambers treated with 0.5–6% *M. leucadendron* and 0.5–6% *L. cubeba* compared with 0.5–6% *L. salicifolia*, regardless of test concentrations (Noosidum *et al.* 2008). The authors concluded that all three botanicals exhibited significant irritant and repellent properties against *Ae. aegypti* requiring further investigation for possible use as active ingredients in topical and indoor dispersed repellent systems (Noosidum *et al.* 2008). This again demonstrates the potential of different *Melaleuca* oils as mosquito repellents at concentrations similar to those used in this study.

For both the bush fly and biting midge field studies, the natural-based repellent formulations containing 5% w/w oil from *M. ericifolia* provided similar repellency rates (Tables 4,5; up to 92.1% and 95.7% respectively at 3 h) compared with the synthetic repellent Off! Skintastic (Tables 4,5; 93.6% and 97.1% respectively at 3 h). No differences in repellency were observed between the *Melaleuca* oil formulations. No other laboratory or field studies could be found in the literature regarding the use of essential oils as repellents against the bush fly. Further, there are few laboratory and field studies published regarding the use of essential oils as repellents against biting midges, and none using *Melaleuca* oils.

In laboratory tests, 50% PMD was found to give total protection against the biting midge *Culicoides variipennis* (Coquillett) for 5 h (Trigg & Hill 1996). In field testing, 50% PMD was reported to have excellent long-duration efficacy similar to that of 20% deet against *Culicoides impunctatus* with both repellents giving 98% protection from biting 8 h after application and 99.5% protection for PMD and 97% protection for deet up to 10 h after application (Trigg 1996). In other field testing, 20% PMD lotion and 26% PMD spray were found to have mean protection times against *Leptoconops carteri* (Hoffman) of 291 and 313 min respectively compared with 360 min for 20% deet (Carroll & Loye 2006a). Half of the PMD subjects received no bites, and the true median protection time probably exceeded the test duration (Carroll & Loye 2006a). Although PMD appears to provide superior repellency against biting midges compared with *Melaleuca* oil used in this study, albeit at 4- to 10-fold the concentration, it is not a true essential oil but the residue of the oil distillation process (Barnard & Xue 2004; Carroll & Loye 2006a).

While many studies have been published detailing the potential of essential oils to act as insect repellents, few oils have been identified as viable alternatives to synthetic actives in the field. In this novel study, on which a patent has been based, we developed three natural-based insect repellent formulations for personal protection against mosquitoes, the bush

fly and biting midges, which can deliver repellency on par with commercially available synthetic actives using Australian essential oils.

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REFERENCES

- Amer A & Mehlhorn H. 2006. Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitology Research* **99**, 478–490.
- Barnard DR & Xue RD. 2004. Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae). *Journal of Medical Entomology* **41**, 726–730.
- Barnard DR, Bernier UR, Posey KH & Xue RD. 2002. Repellency of IR3535, KBR3023, para-menthane-3,8-diol, and deet to black salt marsh mosquitoes (Diptera: Culicidae) in the Everglades National Park. *Journal of Medical Entomology* **39**, 895–899.
- Brown M & Hebert AA. 1997. Insect repellents: an overview. *The Journal of the American Academy of Dermatology* **36**, 243–249.
- Carroll SP & Loye J. 2006a. Field test of a lemon eucalyptus repellent against *Leptoconops* biting midges. *Journal of the American Mosquito Control Association* **22**, 483–485.
- Carroll SP & Loye J. 2006b. PMD, a registered botanical mosquito repellent with deet-like efficacy. *Journal of the American Mosquito Control Association* **22**, 507–514.
- Fradin MS. 1998. Mosquitoes and mosquito repellents: a clinician's guide. *Annals of Internal Medicine* **128**, 931–940.
- Fradin MS & Day JF. 2002. Comparative efficacy of insect repellents against mosquito bites. *New England Journal of Medicine* **347**, 13–18.
- Frances SP, Marlow RM, Jansen CC, Huggins RL & Cooper RD. 2005. Laboratory and field evaluation of commercial repellent formulations against mosquitoes (Diptera: Culicidae) in Queensland, Australia. *Australian Journal of Entomology* **44**, 431–436.
- Harley D, Ritchie S, Phillips D & van den Hurk A. 2000. Mosquito isolates of Ross River virus from Cairns, Queensland, Australia. *American Journal of Tropical Medicine and Hygiene* **62**, 561–565.
- Horwood CM & Bi P. 2005. The incidence of Ross River virus disease in South Australia, 1992 to 2003. *Communicable Diseases Intelligence* **29**, 291–296.
- Hughes RD. 1970. The seasonal distribution of bushfly (*Musca vetustissima* Walker) in South-East Australia. *Journal of Animal Ecology* **39**, 691–706.
- Katz TM, Miller JH & Hebert AA. 2008. Insect repellents: historical perspectives and new developments. *The Journal of the American Academy of Dermatology* **58**, 865–871.
- Moore SJ, Lenglet A & Hill N. 2002. Field evaluation of three plant-based insect repellents against malaria vectors in Vaca Diez Province, the Bolivian Amazon. *Journal of the American Mosquito Control Association* **18**, 107–110.
- Noosidum A, Prabaripai A, Chareonviriyaphap T & Chandrapatya A. 2008. Excito-repellency properties of essential oils from *Melaleuca leucadendron* L., *Litsea cubeba* (Lour.) Persoon, and *Litsea salicifolia* (Nees) on *Aedes aegypti* (L.) mosquitoes. *Journal of Vector Ecology* **33**, 305–312.
- Osimitz TG & Grothaus RH. 1995. The present safety assessment of deet. *Journal of the American Mosquito Control Association* **11**, 274–278.
- Penfold AR & Morrison FR. 1952. Some Australian essential oils in insecticides and repellents. *Soap, Perfumery and Cosmetics* **52**, 933–934.

- Russell RC & Kay BH. 2004. Medical entomology: changes in the spectrum of mosquito-borne disease in Australia and other vector threats and risks, 1972–2004. *Australian Journal of Entomology* **43**, 271–282.
- Sudakin DL & Trevathan WR. 2003. DEET: a review and update of safety and risk in the general population. *Journal of Toxicology and Clinical Toxicology* **41**, 831–839.
- Tong A & Hu W. 2001. Climate variation and incidence of Ross River virus in Cairns, Australia: a time series analysis. *Environmental Health Perspectives* **109**, 1271–1273.
- Trigg JK. 1996. Evaluation of a eucalyptus-based repellent against *Culiscoides impunctatus* (Diptera: Ceratopogonidae) in Scotland. *Journal of the American Mosquito Control Association* **12**, 329–330.
- Trigg JK & Hill N. 1996. Laboratory evaluation of a eucalyptus-based repellent against four biting arthropods. *Phytotherapy Research* **10**, 313–316.
- United States Environmental Protection Agency (US EPA). 1998. Prevention, pesticides and toxic substances (7508W). Re-registration Eligibility Decision (RED) DEET. EPA738-R-98-010.
- Webb CE, Doggett SL, Ritchie SA & Russell RC. 2008. Vector competence of three Australian mosquitoes, *Verrallina carmentis*, *Verrallina lineata*, and *Mansonia septempunctata* (Diptera: Culicidae), for Ross River virus. *Journal of Medical Entomology* **45**, 737–740.
- Whelan P. 2003. Biting midges or 'sandflies' in the NT. *The Northern Territory Disease Control Bulletin* **10**, 1–12.
- Wright RH. 1975. Why mosquito repellents repel. *Scientific America* **233**, 104–111.
- Zhu J, Zeng X, YanMa *et al.* 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *Journal of the American Mosquito Control Association* **22**, 515–522.

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