

## Efficacy of *Leptospermum petersonii* oil, on *Plutella xylostella*, and Its Parasitoid, *Trichogramma pretiosum*

PURWATININGSIH,<sup>1</sup> N. HEATHER,<sup>2</sup> AND E. HASSAN<sup>2,3</sup>

J. Econ. Entomol. 105(4): 1379–1384 (2012); DOI: <http://dx.doi.org/10.1603/EC11382>

**ABSTRACT** The efficacy of lemon-scented tea tree oil (LSO), *Leptospermum petersonii* (FM. Bailey), was evaluated against the diamondback moth, *Plutella xylostella* (L.) under laboratory conditions. Feeding activity and development of larval stages were significantly reduced on broccoli leaves that had been dipped in LSO. Oviposition deterrence was also found when an adult stage was exposed to treated leaves. Fecundity dropped by >50% at concentrations >0.5%. The LC<sub>50</sub> value for third instar larvae was estimated to be 2.93% 7 d after treatment. Experiments were also conducted under greenhouse conditions to assess the efficacy of LSO against the diamondback moth. Our results suggest that LSO has modest potential for development as a botanical insecticide. The oil was also tested at concentrations from 0.5 to 6% for oviposition deterrence of an egg parasitoid of the diamondback moth, *Trichogramma pretiosum* (Riley). LSO deterred parasitization in choice tests but not in no-choice tests. LSO did not cause mortality of *T. pretiosum* during 24 h in a contact toxicity test. We conclude that LSO had no significant effects on the parasitoid, and therefore LSO is compatible with this biocontrol agent for integrated management of the diamondback moth.

**KEY WORDS** repellent, diamondback moth, lemon-scented tea tree oil (LSO), deterrent

The diamondback moth, *Plutella xylostella* (L.), is one of the most important insect pests in cabbage and other related vegetables in the family Brassicaceae. In Australia, the presence of diamondback moth was detected over 100 yr ago (Eziah et al. 2008). Control of this pest, to date, has depended on synthetic chemical insecticides. However, the over use of these insecticides has resulted in the development of insecticide resistance. In Queensland, resistance of *P. xylostella* to synthetic insecticides was first reported in the 1980s (Wilcox 1986, Altmann 1988). The same pattern of resistance in *P. xylostella* was also reported in Indonesia, Thailand, Malaysia, Taiwan, United States, and Central America (Talekar and Shelton 1993). The long-term use of synthetic insecticides has also been reported to cause serious problems on beneficial insects, the environment, and human health (Magaro and Edelson 1990). Recently, considerable efforts have gone into screening plants that have biologically active agents against insects with the goal of developing Integrated Pest Management (IPM) programs. Many plant essential oils can potentially be integrated with alternative techniques and strategies to control insect pests, including *P. xylostella*. Plant essential oils comprise many bioactive components

that are biodegradable and selective in their activity, thereby their application would be environmentally acceptable and compatible with an IPM program (Isman 2000, Koul et al. 2008).

Lemon-scented tea tree oil (LSO), obtained from the native Australian plant *Leptospermum petersonii* (FM. Bailey), has a range of chemical components. It is rich in aldehydes ranging from citronellal to neral and geranial. There are two recognized oil chemotypes based on their hydrocarbons. The first chemotype, Penfold's 'variety A' contains mainly monoterpenes while the other chemotype contains sesquiterpenes, with either  $\beta$ -caryophyllene or globulol/viridiorol/ spathulenol as major components. The existence of a third chemotype, Penfold's 'variety B' contains geraniol acetate (21–38%) and geraniol (21–29%), as well as citral (neral and geranial) as principal components of the oil (Brophy et al. 2000). Information on the use of LSO is very limited. This oil showed good antifungal activity against *Phytophthora cactorum* (Lebert & Cohn) Schroet, *Microsporium canis* (Bodin), *Trichophyton mentagrophytes* (Charles-Philippe Robin Sabour), and *Microsporium gypseum* (Guiart & Griggorakis) (Lee et al. 2008, Park et al. 2007). The current study was designed to examine the effect of LSO oil on the insect pest, the diamondback moth, and its parasitoid, *Trichogramma pretiosum* (Riley) in the laboratory. These results are intended to facilitate research to develop new agents for insect pest control based on bioactive chemical compounds from plants as alternatives to synthetic insecticides.

<sup>1</sup> Biology department, Faculty of Math and Natural Sciences, University of Jember, Kampus Tegal Boto, Jl. Kalimantan 37 Jember, East-Java, Indonesia.

<sup>2</sup> School of Agriculture and Food Sciences, University of Queensland, Gatton Campus, Gatton-4343

<sup>3</sup> Corresponding author, e-mail: e.hassan@uq.edu.au.

## Materials and Methods

**Insect Rearing.** *P. xylostella* larvae used in this study were initially obtained from a laboratory colony reared on broccoli plants (*Brassica oleracea* variety Italica). Rearing was done in the secure Controlled Environment (CE) room, a photoperiod of 14:10 (L:D) h at  $25 \pm 2^\circ\text{C}$  and  $54 \pm 10\%$  RH, in the glass-houses of the School of Agriculture and Food Science, University of Queensland Gatton, using the rearing procedure of Talekar and Lin (1998). The parasitoids, *T. pretiosum* used in these experiments were purchased from "Bugs for Bugs" (Queensland, Australia) in the form of parasitized lepidoptera (*Ephesia kuehniella* Zeller) eggs. The parasitized *E. kuehniella* eggs were stored in a chamber at  $5^\circ\text{C}$  then kept at room temperature for 2 d before use in experiments to allow it to emerge from the pupal stage.

**Preparation of Oil.** The LSO was obtained from Southern Cross Botanicals Pty Ltd. (Knockrow, NSW, Australia). Based on the certificate analysis from Southern Cross Botanicals Pty. Ltd., the components of the LSO used here, produced by steam distillation of the leaves, are geranial (31.9%), neral (26.5%), citronellal (19.2%), citronellol (2.1%), and linalool (2.3%). The oil was applied as a freshly prepared emulsion in a solution of distilled water and 0.5 ml emulsifier (Tergitol TM XD [43%] and Tergitol TMN-10 [57%] 100 g net surfactant). Known amounts of LSO were dissolved in the control solution to obtain a 10% stock solution. Various concentrations of oil suspensions were then prepared by diluting appropriate volumes of stock solution in the control solution.

**Lethal Concentration (LC<sub>50</sub>) Estimation.** Six different concentrations defined by a logarithmic scale were tested (0.5, 1.0, 2.0, 4.0, and 6% [vol:vol]) with three replications of each. Each replicate consisted of 10 third instar *P. xylostella*; a total of 180 larvae were used. A leaf dipping method was used to evaluate the toxicity of LSO against third instars of the diamond-back moth. Leaf discs (3 cm diameter) were punched from leaves of 7-wk-old broccoli seedlings. Each leaf disc was dipped into one of six different concentrations of test oil solution for 10 s. Control leaf discs received the control solution only. After air-drying for  $\approx 30$  min, the treated leaves were placed on wet Styrofoam in a container (7 cm diameter, 3 cm height). Groups of 10 third instar larvae were transferred onto each leaf discs. After 24 h, the larvae were removed from the containers and placed into new containers with fresh leaf discs. The discs were removed and replaced with fresh discs everyday for 1 wk. Mortality, assessed at 7 d, was corrected using Abbott's (1925) formula. The median LC<sub>50</sub> was calculated using probit analysis (Finney 1971).

**Antifeedant Tests.** Feeding deterrent effects were evaluated under choice and no-choice conditions. In this assay concentrations 0.5, 1.0, 2.0, 4.0, and 6% (vol: vol) of essential oil were used. In the choice test, broccoli leaf discs of 3 cm diameter were punched from leaves of 7-wk-old seedlings using a cork borer. The leaf discs were dipped in the appropriate oil

emulsion concentrations for 10 s and air dried for 30 min in a fume hood. One treated leaf disc was placed in a plastic container (7 cm diameter, 3 cm height) as described in the previous section, together with an untreated leaf. Ten third instar *P. xylostella*, starved for 4 h, were used in each replicate. Each treatment was replicated thrice, giving a total of 30 larvae in each treatment. In the no choice tests, the treated and untreated leaf discs were placed in separate plastic containers. The number of larvae used in this method was the same as described in the choice method with 10 third instar *P. xylostella* in each replicate. After 24 h, larvae were removed and the frass brushed from the leaf discs. Then the residual leaf discs areas were placed on a piece of paper for digitizing the area using an Epson scanner (Seiko Epson Corporation, Sydney, Australia) on 300 dpi. The area of the remaining leaf disc in this image was measured using the computer program, ImageJ 1.41o (open sources program on <http://rsb.info.nih.gov/ij>). The antifeedant index (AFI) for the choice method was calculated as  $\text{AFI} = (\text{C}-\text{T}) / (\text{C}+\text{T}) * 100\%$ , and for no-choice methods as  $\text{AFI} = (\text{C}-\text{T}) / \text{C} * 100\%$ , where C and T denoted the consumed area of control leaf and treated leaf discs, respectively (Simmonds et al. 1989, Ling et al. 2008).

**Development Assay of *P. xylostella*.** Ten third instar larvae of diamondback moth were placed on leaf discs (3 cm diameter) that had been dipped in six different concentrations used for the feeding deterrent assay. Each treatment had three replicates and each replicate had 10 larvae, a total of 180 larvae were used for the experiments. Records were made daily of living and dead individuals and larval development. Larval development was expressed as the ability of larvae to grow and molt to the next instar and this can be expressed by a growth index and relative growth index values. Larval growth index expressed by the growth rate can be calculated from the distribution of living and dead larvae at each instar, and the number of larvae pupating. The larval growth was calculated using the indices introduced by Zhang et al. (1993).

**Effect of LSO on Oviposition and Egg Hatch.** The surviving female adults from the development assay experiment were used in this test. Leaves from 8 wk-old broccoli seedlings were detached, dipped into one of six different concentrations of test oil solution, as in previous experiments, and then the leaf was put in a small vial (1.5 cm diameter, 2.5 cm height) with water in it to maintain moisture. The detached leaves were put in a container (3 cm diameter, 11 cm height) together with a cotton swab moistened with a 10% of honey solution. Two female and two untreated male adults were released in the container. Each treatment was replicated three times, giving a total of 72 adults used for these experiments. The total numbers of eggs deposited on the leaves were counted after 3 d and the numbers of hatched eggs (F1) were recorded after an additional 3 d.

**Efficacy of LSO in the Greenhouse.** Experiments were conducted at the School of Agriculture and Food Sciences, University of Queensland Gatton. Broccoli seedlings, as above, were planted in plastic pots (3 cm

**Table 1.** Antifeedant effect of *Leptospermum petersonii* oil on the third instar larvae of *Plutella xylostella* in choice trials and no choice trial

Concentration (%)	AFI choice (mean $\pm$ SD) <sup>a</sup> (%) <sup>b</sup>	F value; df; P < F	AFI no-choice (mean $\pm$ SD) (%) <sup>b</sup>	F value; df; P < F
0	—	61.03; 4,14; 0.0001	0.86 $\pm$ 0.18d	42.02; 5,17; 0.0001
0.5	20.23 $\pm$ 1.66d		53.58 $\pm$ 2.24b	
1	32.44 $\pm$ 6.25c		58.4 $\pm$ 6.26b	
2	34.84 $\pm$ 9.89c		67.71 $\pm$ 10.8c	
4	41.04 $\pm$ 6.55b		67.75 $\pm$ 11.39cb	
6	63.16 $\pm$ 1.56a		87.17 $\pm$ 8.8a	

<sup>a</sup> Based on 30 third instars for each rate.

<sup>b</sup> Means followed by the same letter are not significantly different (Least Significant Difference test;  $P < 0.05$ ).

diameter, 6 cm height) in a mixture of composted bark, Osmocote Plus 8–9 mo (2 kg), Osmocote Plus 3–4 mo (1 kg), Nutricote 7 mo (2 kg), coated iron (1.3 kg), SaturAid (1.2 kg), Polimite (1.2 kg), and Osmoform (1.3 kg). Plants were watered regularly and 7 wk-old plants were used in these experiments. An emulsion of LSO was tested at concentrations of 0, 0.5, 1, and 2% (vol:vol). One percent rotenone (Rotenone Garden Dust) was used as a positive control. Treatments were arranged in a randomized block design, with replicates and 10 plants/treatments/blocks. Plants were spaced 15 cm within rows and 30 cm between rows in each treatment. Spaces between treatments and blocks were 50 and 75 cm, respectively. Each plant was infested with seven third-instar *P. xylostella* obtained from the laboratory culture. The plants were sprayed with oil emulsion concentrations by using 50 ml plastic spray bottles. Approximately 20 ml of oil solution was applied to each plant. Rotenone (dust) was applied directly to broccoli leaves. The number of surviving larvae was counted at 1 and 7 d after spraying. The larval mortality was calculated from the number of live larvae before and after treatment.

**Effect of LSO on a Parasitoid (*T. pretiosum*).** The deterrent effects of LSO on the egg parasitoid *T. pretiosum* were evaluated using choice and no-choice methods. In this assay, concentrations of 0.5, 1.0, 2.0, 4.0, and 6% (vol:vol) essential oil were used. Ten *P. xylostella* eggs ( $\leq 48$  h) were pasted onto a piece of filter paper (1  $\times$  5 cm). Each filter paper represented one replication and there were five replications at each concentration. Each filter paper was dipped in the oil emulsion for 5 s and then dried for 30 min. A filter paper with eggs dipped into emulsified water served as a control. In the no-choice assay, one mated female parasitoid (age 24 h) was introduced into a glass tube (1  $\times$  15 cm) containing a filter paper with 10 eggs. A streak of honey solution (10%) was applied to the inner glass of the tube as food for the adult parasitoid. In the choice method, two mated females were introduced into a tube containing treated eggs pasted on filter paper and control filter paper with eggs. The tubes were plugged with cotton wads. After 5 d, the number of parasitized eggs was recorded. The change of egg color to black was used to determine parasitism, as this indicates *T. pretiosum* development inside the *P. xylostella* eggs (BioResources 2012). A direct contact assay was also conducted on adult *T.*

*pretiosum*. Ten adult *T. pretiosum*,  $\leq 48$  h old, were introduced to a glass tube (1  $\times$  15 cm), where a square of filter paper (1  $\times$  5 cm) that had been dipped in different oil emulsions was previously inserted. The parasitoid mortality was recorded every 24 h for 5 d.

**Statistical Analysis.** All data were analyzed using SAS computer system for Windows 6.12. Insect mortality data were analyzed using probit analysis (Finney 1971, Crawley 1993). AFI, insect relative growth index, and insect growth index data were analyzed using analysis of variance (ANOVA) for a General Linear Model. For greenhouse experiments, percent mortalities were corrected using Abbott's transformation. ANOVA was performed on the arcsine-transformed percentage mortality. The differences between means were assessed using the Least Significant Difference (LSD) test ( $P < 0.05$ ). For parasitism experiments, data were analyzed using ANOVA in a completely randomized block design. The treatment means were compared using the LSD ( $P < 0.05$ ) (Zar 1993).

## Results and Discussion

**LC<sub>50</sub> Estimation.** The range of concentrations used in this study were evaluated at 7 d after treatment because no mortality occurred 24 h after exposure to LSO. The estimated LC<sub>50</sub> value was 2.93% (range, 95% CI: 1.93–4.76%) ( $n = 180$ ; Slope Coefficient =  $0.09 \pm 0.02$ ;  $\chi^2 = 13.2$ ;  $df = 3$ ). Concentration dependent mortality was observed during the experiments. Although there is a lack of information on the efficacy of LSO as an insecticidal agent, some studies found that the chemical components of this oil had antifungal, acaricidal, and antimicrobial effects (Wilkinson and Cavanagh 2005, Park et al. 2007, Lee et al. 2008, Loughlin et al. 2008). Studies on oils obtained from others plants of the genus *Leptospermum* (*L. scoparium* variety *leptophyllum*, *L. odoratum* (Cheel), *L. lanigerum* (Smith), *L. liversidgei* (RT Baker & HG Sm.), *L. citratum* (J. F. Bailey & C. T. White), *L. ericoides* (A. Rich)) have reported acaricidal, antifungal, antibacterial, and antimicrobial properties (Short and Johnson 1926). The volatile constituents  $\alpha$ -pinene (77%),  $\alpha$ -terpineol (2%), citral (1%), aromadendrene (10%), and phenol (2.5%) obtained from these species were considered to be the principal antimicrobial components (Briggs et al. 1938). Jeong et al. (2008) and Jeong et al. (2009) reported that *L. scoparium* (J.R. et G.

**Table 2. Relationship of *Leptospermum petersonii* oil at concentrations to growth index, relative growth index, and mortality of *Plutella xylostella***

Concentration (%)	GI (mean ± SD) <sup>a,b</sup>	RGI (mean ± SD) <sup>b</sup>	Mortality (%)
0	0.98 ± 0.03a		3.33
0.5	0.92 ± 0.10b	0.94 ± 0.08a	16.67
1	0.93 ± 0.07b	0.93 ± 0.08a	16.67
2	0.91 ± 0.09b	0.92 ± 0.10a	13.33
4	0.87 ± 0.13c	0.91 ± 0.13a	20
6	0.73 ± 0.14d	0.73 ± 0.16b	53.33

<sup>a</sup> Based on 30 third instars for each concn.

<sup>b</sup> Means followed by the same letter are not significantly different (Least Significant Difference test;  $P < 0.05$ ).

Forst.) oil has toxicity to house-dust mites and stored-food mites *Dermatophagoides farinae* (Hughnes), *D. pteronyssinus* Troussart, and *Tyrophagus putrescentiae* (Schrank), with LD<sub>50</sub> values of 0.54, 0.67, and 1.12 µg/cm<sup>2</sup>, respectively. *L. scoparium* seeds contain leptospermone and its derivatives which are triketone, natural products found primarily in *Eucalyptus* and *Leptospermum*. The presence of triketones in *Leptospermum* has been lined to high antimicrobial activity. These components are also confirmed as the active constituents responsible for acaricidal activity (Douglas et al. 2004). These or other chemical constituents in the LSO could be responsible for the insecticidal activity.

**Antifeedant Tests.** Antifeedant effects of LSO on diamondback moth larvae were indicated by leaf consumption. In both the choice and no choice test, all the rates of LSO significantly deterred feeding by third instar diamondback moth ( $P < 0.001$ ). Generally speaking, the antifeedant rates obtained in the choice feeding experiments were higher than in the no choice tests. Furthermore, LSO in both the choice and no choice methods exhibited antifeedant activity in a dose dependent manner (Table 1). However, the specific chemicals responsible for this activity were not further investigated.

There are few publications regarding the effects of LSO on insect pests. Maguranyi et al. (2009) reported that LSO oil exhibited a repellent effect against *Aedes aegypti* (L.) but they did not identify active principles. However, geranial, neral, citronellal, and linalool are reported to be responsible for antifeedant activity

**Table 4. Mortality of *Plutella xylostella* larvae sprayed with *Leptospermum petersonii* oil or dusted by Rotenone in a glasshouse trial**

Treatment	Concentration (%)	Mortality (%) + SD <sup>a</sup>	
		1 dat <sup>b</sup>	7 dat
<i>L. petersonii</i> oil	0.5	4.25 ± 0.59a	15.50 ± 1.04b
	1	3.25 ± 0.53a	15.50 ± 0.64c
	2	8.75 ± 1.09b	23.00 ± 1.09d
Rotenone	1	1.25 ± 0.33a	24.25 ± 1.17d
Control		1.00 ± 0.30a	2.75 ± 0.45a

<sup>a</sup> Means followed by the same letter are not significantly different (Least Significant Difference test;  $P < 0.05$ ).

<sup>b</sup> dat = day after treatment.

against several insects. For example linalool and terpinene-4-ol, which were also found in LSO, possess antifeedant effects on *Thrips tabaci* (Lindeman) (Koschiers et al. 2002). Similarly, neral, citronellal, citronellol, and linalool distilled from *Cymbopogon winterianus* Jowitt (Poaceae) and other aromatic plants have also been shown to have antifeedant and repellent effects on *Spodoptera frugiperda* (J.E. Smith), *A. aegypti*, and *Helicoverpa armigera* (Hubner) (Labinas and Crococo 2002, Cockcroft et al. 1998, Tripathi 2002).

**Development assay of *P. xylostella*.** Exposure of third instar larvae of the diamondback moth to several concentrations of LSO had a significant effect on the growth index ( $F = 51.46$ ;  $df = 5,17$ ;  $P = 0.0001$ ) and relative growth index ( $F = 26.42$ ;  $df = 4,14$ ;  $P = 0.0001$ ) of larvae. The oil resulted in slower development of the larvae, which was more pronounced as the concentrations increased. In addition, mortality increased with higher concentration (Table 2). Developmental disruption was observed as some larvae and pupae failed to molt to the next stage. Generally, treated pupae were smaller than untreated ones. The mode of action of the oil is not known, however, the slower growth of the larvae might be caused by the antifeedant effect of the oil alone.

Geranial, which is the major chemical component in LSO, has been shown in several studies to repel the cow tick, *Hyalomma* sp., and the pharaoh ant, *Monomorium pharaonis* L., and has oviposition deterrent effects on the longicorn beetle, *Maechotypa diphyis* (Pascoe) (Khallaayoune et al. 2009, Huang and Shu 2002, Yoo et al. 2002).

**Table 3. Effect of *Leptospermum petersonii* oil on oviposition and egg hatch by *Plutella xylostella***

Concentration (%)	(mean ± SD) (%) <sup>a,b</sup> of egg lay <sup>c</sup>	F value; df	P < F	(mean ± SD) (%) <sup>b</sup> of egg hatch <sup>d</sup>	F value; df	P < F
0	295 ± 72.11a	19.79; 5,17	0.0001	211.33 ± 23.09a (71.64%)	48.42; 5,17	0.0001
0.5	206 ± 45.13b (30.17%)			138.33 ± 26.73b (67.15%)		
1	172.33 ± 41.33bc (41.58%)			110.67 ± 10.97bc (64.21%)		
2	137 ± 45.88cd (53.56%)			94.33 ± 5.51cd (68.85%)		
4	116.33 ± 51.07de (60.57%)			66.67 ± 5.67d (57.31%)		
6	78 ± 39.59e (73.56%)			27 ± 7.81e (34.61%)		

<sup>a</sup> Based on two female treated from larval stage and two untreated male for each concn.

<sup>b</sup> Means followed by the same letter are not significantly different (Least Significant Difference test;  $P < 0.05$ ).

<sup>c</sup> Number in parentheses represent of the percentage reduced of egg laying as compared with control.

<sup>d</sup> Number in parentheses represent of the percentage of egg hatching from the previous egg production.

Table 5. Effect *Leptospermum petersonii* oil on oviposition by *Trichogramma pretiosum* in choice and no choice test

Concentration (%)	Choice			No choice	
	No. of eggs parasitized		% increase (+) or decrease (-) from untreated control <sup>a</sup>	No. eggs parasitized	% increase (+) or decrease (-) from untreated control <sup>a</sup>
	Treated	Untreated			
0.5	9.8	10	-2.0a	10	2.04a
1	9.4	9.6	-2.1a	9.8	0.00a
2	8.4	9.2	-8.7b	9.6	-2.04a
4	9.4	9.8	-4.1bc	9.8	0.00a
6	8.6	9.6	-10.4c	10	2.04a
Control				9.8	

<sup>a</sup> Means followed by the same letter are not significantly different (Least Significant Difference test;  $P < 0.05$ ).

**Effect of LSO on Oviposition and Egg Hatch.** Increasing concentrations of LSO decreased oviposition and subsequent larval eclosion in adults that developed from treated larvae. Egg laying was reduced significantly from 30 to 73% when compared with the control ( $F = 19.79$ ;  $df = 5,17$ ;  $P = 0.0001$ ) and egg hatch was slightly reduced in treatments groups except at the highest concentration ( $F = 48.42$ ;  $df = 5,17$ ;  $P = 0.0001$ ) (Table 3). The chemical constituents responsible for this activity are unknown. Neral has been reported to have an oviposition deterrent effect on the leafhopper, *Amrasca devastans* (Distant.) (Saxena and Basit 1982).

Efficacy of LSO in the greenhouse LSO had a significant effect on mortality of *P. xylostella* larvae at both 24 h ( $F = 6.44$ ;  $df = 7,199$ ;  $P = 0.0001$ ) and 7 d after application ( $F = 84.33$ ;  $df = 7,199$ ;  $P = 0.0001$ ). After 24 h, only the highest concentration resulted in significantly higher mortality over the control. However, after 7 d, all concentrations of oil resulted in significantly higher mortality than the control, and the highest concentration was comparable in efficacy to rotenone. In general, the efficacy of the oil was low (23% larval mortality at the highest concentration) but comparable to rotenone (24.25% larval mortality) (Table 4).

Increasing larval mortality was observed at 3 d after application, but this did not increase between days 3–7. Most larvae died as they failed to molt. The low efficacy of the oil is likely because of the fact that the concentrations applied were below the  $LC_{50}$  (2.93%). In this experiment, the highest concentration used was 2% because phytotoxic (i.e., seedling mortality) was observed after 24 h in the broccoli seedlings when sprayed with the oil concentration over 2%. This phytotoxic effect may be related to the characteristic thinness of broccoli seedling leaves. Tariq et al. (2010) observed phytotoxicity when they applied *Acorus calamus* (L.) extracts on cotton that has thin leaves; however, there were no phytotoxic effects observed in plants with thicker leaves such as mango and coconut.

**Effect of LSO on a Parasitoid (*T. pretiosum*).** In choice tests, oviposition by *T. pretiosum* on treated *P. xylostella* eggs decreased very slightly but significantly ( $P = 0.002$ ). In the no choice test, no significant difference from the control was seen ( $P > 0.5$ ) (Table 5). In the contact test, there was no mortality from LSO on *T. pretiosum* at any concentration tested. The para-

sitoids all survived for 5 d after exposure. The life span of adult parasitoids emerging from moth eggs is normally 7–20 d (BioResources 2012). During the rearing of parasitoids in this study, we found that no parasitoids survived beyond 7 d.

This is the first report of the effects of LSO on a parasitoid insect. The chemical constituents of the oil have been reported to repel insects. Our results differ from those of Reeves and Miller (2010) who reported that a 2% of aqueous solution of geraniol repelled *A. aegypti*. Geraniol, which is the major constituent of LSO, had no toxic effect on adults of *T. pretiosum*, but no-choice experiments supported the observation that LSO did not adversely affect *T. pretiosum*.

In conclusion, LSO had modest insecticidal action against diamondback moth larvae, but is phytotoxic at concentrations above 2% under glasshouse conditions. LSO has an antifeedant effect and also inhibits larval growth and oviposition in *P. xylostella*. Adult survivors produced fewer eggs than controls. Importantly, LSO appeared harmless to the egg parasitoid, *T. pretiosum*. LSO should be developed further along with parasitoids as components of an IPM program against *P. xylostella*. However, our results need to be validated under field conditions.

## Acknowledgments

We thank ADS (Australian Development Scholarship) for the financial support during this Ph.D. study, Alan Lisle for help with the statistical analysis, Lara Senior (DEEDI Gatton Research Center), and Dough George and Murray B. Isman (the *Journal of Economic Entomology* editor) for critically reviewing the manuscript.

## References Cited

- Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Altmann, J. A. 1988. An investigation of resistance in cabbage moth (*Plutella xylostella* L.) to pyrethroids in the Lockyer Valley. Graduate Diploma thesis, Queensland Agricultural College, Lawes, Queensland, Australia.
- BioResources. 2012. *Trichogramma pretiosum*. (<http://www.bioresources.com.au/pretiosum/PretiosumGeneral.htm>).
- Briggs, L. H., A. R. Penfold, and W. F. Short. 1938. *Leptospermone*, part 1. *J. Chem. Soc.* 7: 1193–1195.

- Brophy, J. J., R. J. Goldsack, A. Punruekvong, A. R. Bean, P. I. Forster, B. J. Lepschi, J. C. Doran, and A. C. Rozefelds. 2000. Leaf essential oils of the genus *Leptospermum* (Myrtaceae) in eastern Australia, part 7. *Leptospermum petersonii*, *L. liversidgei* and allies. *Flav. Frag. J.* 15: 342–351.
- Cockcroft, A., J. B. Cosgrove, and R. J. Wood. 1998. Comparative repellency of commercial formulation of deet, permethrin and citronellal against the mosquito *Aedes aegypti*, using collagen membrane techniques compared with human arm test. *Med. Vet. Entomol.* 12: 289–294.
- Crawley, M. J. 1993. *GLIM for ecologists*. Blackwell Science Publication, Oxford, United Kingdom.
- Douglas, M. H., J. W. van Klink, B. M. Smallfield, N. B. Perry, R. E. Anderson, P. Johnstone, and R. T. Weavers. 2004. Essential oils from New Zealand manuka: triketone and other chemotypes of *Leptospermum scoparium*. *Phytochemistry* 65: 1255–1264.
- Eziah, V. J., H. A. Rose, A. D. Clift, and S. Mansfield. 2008. Susceptibility of four field populations of the diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to six insecticides in the Sydney region New South Wales, Australia. *Aust. J. Entomol.* 47: 355–360.
- Finney, D. J. 1971. *Probit analysis*. Cambridge University Press, Cambridge, United Kingdom.
- Huang, C. Y., and T. H. Su. 2002. Repellency and toxicity of semiochemicals on pharaoh ant, *Monomorium pharaonis* (Hymenoptera : Formicidae). *Plant Prot. Bull.* 44: 125–134.
- Isman, M. B. 2000. Plant essential oils for pest and disease management. *Crop Prot.* 19: 603–608.
- Jeong, E. Y., G. M. Kim, and S. H. Lee. 2008. Acaricidal activity of triketone analogues derived from *Leptospermum scoparium* oil against house-dust and stored-food mites. *Pest Manag. Sci.* ([www.interscience.com](http://www.interscience.com)).
- Jeong, E. Y., J. H. Jeon, H. W. Kim, M. G. Kim, and H. S. Lee. 2009. Antimicrobial activity of leptospermone and its derivatives against human intestinal bacteria. *Food Chem.* 115: 1401–1404.
- Khallaayoune, K., J. M. Biron, A. Chaoui, and G. Duvallet. 2009. Efficacy of 1% geraniol (Fulltec) as a tick repellent. *Parasite* 16: 3.
- Koschier, E. H., K. A. Sedy, and J. Novak. 2002. Influence of plant volatiles on feeding damage caused by the onion thrips *Thrips tabaci*. *Crop Prot.* 21: 419–425.
- Koul, O., S. Walia, and G. S. Dhaliwal. 2008. Essential oils as green pesticides: potential and constraints. *Biopestic. Int.* 4: 63–84.
- Labinas, A. M., and W. B. Crocorno. 2002. Effect of java grass (*Cymbopogon winterianus* Jowitt) essential oil on fall armyworm *Spodoptera frugifera* (J.E. Smith) (Lepidoptera: Noctuidae). *Maringá* 24: 5.
- Lee, Y. S., J. Kim, S. C. Shin, S. G. Lee, and I. K. Park. 2008. Antifungal activity of myrtaceae essential oils and their components against three phytopathogenic fungi. *Flav. Frag. J.* 23: 23–28.
- Ling, B., G. Y. Wang, J. M. Zhang, and G. Liang. 2008. Antifeedant activity and active ingredients against *Plutella xylostella* from *Momordica charantia* leaves. *Agric. Sci. China.* 7: 1466–1473.
- Loughlin, R., B. F. Gilmore, P. A. McCarron, and M. M. Tunney. 2008. Comparison of the cidal activity of tea tree oil and terpinen-4-ol against clinical bacterial skin isolates and human fibroblast cells. *Appl. Microbiol.* 46: 428–433.
- Magaro, J. J., and J. V. Edelson. 1990. Diamondback moth (Lepidoptera: Plutellidae) in South Texas: a technique for resistance monitoring in the field. *J. Econ. Entomol.* 83: 1201–1206.
- Maguranyi, S. K., C. E. Webb, S. Mansfield, and R. C. Russel. 2009. Are commercially available essential oils from Australian native plants repellent to mosquitoes? *J. Am. Mosq. Control Assoc.* 25: 3.
- Park, M. J., K. S. Gwak, I. Yang, W. S. Choi, H. J. Jo, J. W. Chang, E. B. Jeung, and I. G. Choi. 2007. Antifungal activities of the essential oils in *Syzygium aromaticum* (L.) Merrer Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. *J. Microbiol.* 45: 460–465.
- Reeves, W. K., and M. Miller. 2010. Aqueous 2% geraniol as a mosquito repellent failed against *Aedes aegypti* on ponies. *J. Am. Mosq. Control Assoc.* 26: 340–341.
- Saxena, K. N., and A. Basit. 1982. Inhibition of oviposition by volatiles of certain plants and chemicals in the leafhopper *Amrasca devastans* (Distant), *J. Chem. Ecol.* 8: 329–338.
- Short, W. F., and D. Johnson. 1923. *Leptospermone*, part 1. *Rep. Aust. Assoc. Sci.* 16: 223.
- Simmonds, M.S.J., W. M. Blaney, S. V. Ley, G. Savona, M. Bruno, and B. Rodriguez. 1989. The antifeedant activity of clerodane diterpenoids from *Teucrium*. *Phytochemistry* 28: 1069–1071.
- Talekar, N. S., and A. S. Shelton. 1993. Biology, ecology, and management of the diamondback moth. *Annu. Rev. Entomol.* 38: 275–301.
- Talekar, N. S., and M. Y. Lin. 1998. Training of diamondback moth. AVRDC Publication No. 98-472. ([http://libntrs.avrdc.org.tw/fulltext\\_pdf/eb0050.pdf](http://libntrs.avrdc.org.tw/fulltext_pdf/eb0050.pdf)).
- Tariq, R. M., S.N.H. Naqvi, S. M. Zafar, and A. S. Burrero. 2007. Toxic effects of botanical pesticide from *Acorus calamus* (AC) and *Annona squamosa* (AS) against boll-worms at Ari-Tandojam Sindh-Pakistan. *Pak. J. Entomol. Kar.* 22: 31–36.
- Tripathi, A. K. 2002. Feeding deterrent and growth inhibitory effect of *Lippia alba* oil towards crop insect-pests. *J. Med. Arom. Plant Sci.* 24: 486–488.
- Wilcox, P. R. 1986. Resistance of cabbage moth (*Plutella xylostella*) to pyrethroids in Lockyer Valley. Graduate Diploma thesis, Queensland Agricultural College, Lawes, Queensland, Australia.
- Wilkinson, J. M., and A.M.A. Cavanagh. 2005. Antibacterial activity of essential oils from Australian native plants. *Phytother. Res.* 19: 643–646.
- Yoo, J., G. Kim, S. Lee, S. Shin, J. Park, and S. Park. 2002. Insecticidal activity and ovipositional repellency of monoterpenoids against *Moechotypa diphysis* adults (Coleoptera: Cerambycidae). *Korean J. Appl. Entomol.* 42: 285–292.
- Zar, J. H. 1993. *Biostatistical analysis*, 3rd ed. Prentice-Hall, London, United Kingdom.
- Zhang, M., S. K. Chaudhuri, and I. Kubo. 1993. Quantification of insect growth and its use in screening of naturally occurring insect control agents. *J. Chem. Ecol.* 19: 6.

Received 13 November 2011; accepted 11 June 2012.