Ovicidal, larvicidal, and behavioural effects of some plant essential oils on diamondback moth (Lepidoptera: Plutellidae)

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Abstract—Alternatives to synthetic insecticides are desirable for management of diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), an insect pest of global importance. Many essential oils derived from aromatic plants have demonstrated toxicity and behaviour altering effects on insect pests, and are considered low-risk alternatives to synthetic insecticides. We conducted laboratory experiments to determine the biological activity of several low-cost, commercially available essential oils against *P. xylostella.* Experiments testing ovicidal effects, larvicidal effects, larval feeding deterrence, and adult oviposition deterrence were done with essential oils derived from *Artemisia abrotanum* Linnaeus (Asteraceae), balsam fir (*Abies balsamea* Linnaeus (Pinaceae)), black pepper (*Piper nigrum* Linnaeus (Piperaceae)), eucalyptus (*Eucalyptus polybractea* (Baker) (Myrtaceae)), garlic (*Allium sativum* Linnaeus (Amaryllidaceae)), rosewood (a blend of different oil constituents), tansy (*Tanacetum vulgare* Linnaeus (Asteraceae)), and thyme (*Thymus zygis* Linnaeus (Lamiaceae)), using concentrations of 1, 2.5, and 5% v/v. Although all essential oils had some level of bioactivity against certain *P. xylostella* life stages, essential oils from garlic, rosewood, and thyme were most effective overall, demonstrating significant ovicidal and larvicidal activity, as well as deterrent effects on larval feeding and settling behaviour, and adult oviposition. Although variable phytotoxicity was observed with essential oils at 2.5% and 5% v/v concentrations, the results suggest that rosewood, garlic, and thyme essential oils have potential in management of *P. xylostella.*

Introduction

Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is a pest of Brassicaceae crops, causing losses estimated to exceed USD 1 billion annually. The insect is thought to have its origins in the Mediterranean region, but is now found throughout the world wherever Brassicaceae crops are found (Talekar and Shelton 1993; Sarfraz *et al.* 2006). In most cropping systems, insecticides are relied upon heavily to manage *P. xylostella*. Unfortunately, *P. xylostella* has an inordinate ability to evolve resistance, rendering many active ingredients from all major classes of insecticides ineffective against many populations of this pest (Liu *et al.* 1981; Tabashnik *et al.* 1990, 1997; Zhao *et al.* 2002, 2006; Troczka *et al.* 2012). Alternatives to synthetic insecticides are therefore desirable for *P. xylostella* management.

Plant essential oils are secondary metabolite mixtures of monoterpenes, phenols, sesquiterpenes, and other compounds from aromatic plants that can protect plants from herbivores or pathogens. When isolated and tested against insects, many essential oils have demonstrated insecticidal activity, but also a number of sublethal effects, including disruption of growth, development, reproduction, and behaviour (Isman 2006; Regnault-Roger *et al.* 2012). Though regulated application of essential oils is presently somewhat limited in North America and Europe, essential oils

Received 2 October 2016. Accepted 31 January 2017. First published online 12 May 2017.

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Subject editor: Cécile Le Lann
doi:10.4039/tce.2017.13

can be a valuable pest management tool, particularly in developing countries where synthetic insecticides may be less affordable or available (Isman 2008; Isman *et al.* 2011).

Considering its global prominence as a pest, the amount of research investigating the potential of essential oils in *P. xylostella* management has been somewhat limited. Crude plant extracts of mahogany, *Melia volkensii* Gürke (Meliaceae), deterred feeding (Akhtar and Isman 2004), and himachalene and atlantone enriched fractions of essential oils from wood chips of Himalayan cedar, *Cedrus deodara* (Roxburgh ex Don) Don (Pinaceae), were insecticidal (Chaudhary *et al.* 2011) to *P. xylostella.* Thymol and 1,8-cineole were toxic to *P. xylostella* larvae and were synergised when mixed with the essential oil pulegone (Kumrungsee *et al.* 2014), essential oil of the herb *Chenopodium ambrosioides* (Linnaeus) Mosyakin and Clemants (Amaranthaceae) exhibited both insecticidal and antifeedant activity against this insect (Wei *et al.* 2015), and *n*-hexane fractions from leaves of the citrus plant *Zanthoxylum armatum* Candolle (Rutaceae) demonstrated larvicidal activity against *P. xylostella* (Kumar *et al.* 2016). In a broad screen of 66 plant essential oils; pennyroyal (*Mentha pulegium* Linnaeus, Lamiaceae), rosemary (*Rosmarinus officinalis* Linnaeus, Lamiaceae), and sage (*Salvia officinalis* Linnaeus, Lamiaceae) essential oil showed particularly good fumigant toxicity against *P. xylostella* larvae, but all essential oils were less potent fumigant than dichlorvos (Yi *et al.* 2007).

Our goal in the present laboratory study was to test the effectiveness of several commercially available essential oils against *P. xylostella*. The essential oils we used have not, to our knowledge, previously been tested against *P. xylostella.* However, they have shown activity against other insect pests, and some of the essential oils we tested have chemical constituents with demonstrated activity against this pest. Based on previous work showing susceptibility of *P. xylostella* to thymol and 1,8-cineole (Kumrungsee *et al.* 2014), we predicted that thyme and eucalyptus essential oil would have good bioactivity in our experiments, but were uncertain how *P. xylostella* would respond when exposed to other essential oils. Acute toxicity to eggs and larvae, and behaviour-modulating effects on larvae and adults were examined.

Material and methods

Plant and insect maintenance

Cabbage (*Brassica oleracea capitata* Linnaeus (Brassicaceae), cultivar Golden Acre) plants were grown from seeds in 100-mm-diameter pots containing Pro-Mix (Rivière-du-Loup, Québec, Canada) potting soil in a greenhouse under ambient temperature. Soluble fertiliser was given at planting, and plants were watered as needed. Insect rearing and experiments used 3–4-week-old plants or foliage.

Plutella xylostella eggs, larvae, and adults were from a colony maintained at the Faculty of Agriculture, Dalhousie University, Nova Scotia, Canada. Larvae were reared in mesh cages ($33 \times 33 \times 33$ cm) (Bugdorm, Brea, California, United States of America) in a temperature controlled growth chamber (20 ± 1 °C; 16:8 light: dark hours; 65 ± 5% relative humidity). Pupae that developed were placed on foliage in another mesh cage ($48 \times 48 \times 93$ cm) containing cabbage plants. Adults that emerged were provided 10% sugar solution via saturated cotton dental wicks placed in a glass Petri plate and allowed to lay eggs on cabbage plants.

Essential oils

Eight essential oils were tested. Six of these were purchased from a commercial supplier (New Aromatics, Mississauga, Ontario, Canada) and were from the following botanical sources: balsam fir, *Abies balsamea* Linnaeus (Pinaceae); eucalyptus (Blue mallee), *Eucalyptus polybractea* (Baker) (Myrtaceae); black pepper, *Piper nigrum* Linnaeus (Piperaceae); garlic, *Allium sativum* Linnaeus (Amaryllidaceae); rosewood (a blend of different oil constituents); and thyme, *Thymus zygis* Linnaeus (Lamiaceae). The composition of these essential oils was indicated in the certificate of analysis provided by vendor (Table 1).

In addition, plant material of *Tanacetum vulgare* Linnaeus (Asteraceae) and *Artemisia abrotanum* Linnaeus (Asteraceae) was collected in Truro (Nova Scotia, Canada) in July and August 2012 from plants growing on the Dalhousie Agricultural Campus. Voucher specimens of the material (*T. vulgare* n.109–18002 and *A. abrotanum* n.109–18001) were deposited in the A.E. Roland Herbarium, Dalhousie University. *Tanacetum vulgare* flowers and *A. abrotanum* aerial parts were

https://doi.org/10.4039/tce.2017.13 Published online by Cambridge University Press

Table 1. Essential oils and their major constituents used in toxicity tests against diamondback moth, *Plutella xylostella*.

Essential oil	Source	Major components (%)
Artemesia	*Artemisia abrotanum*	Davanone (isomer) (31.1) Davanone (isomer) (14.8) Davana ether (6.4) Eucalyptol (5.1) 6-Methyl-5-octen-2-one (4.0) *cis*-Carvone oxide (3.2) Caryophyllene oxide (3.8)
Balsam fir*	*Abies balsamea*	α,β-Pinene (45.8) l-Bornyl acetate (3.6)
Eucalyptus*	*Eucalyptus polybractea*	1,8-Cineole (87.4) α-Pinene (1.7) β-Pinene (1.0)
Garlic*	*Allium sativum*	Diisopropyl disulphide (36.6) Diisopropyl trisulphide (22.3)
Pepper black*	*Piper nigrum*	β-Caryophyllene (24.2) δ-3-Carene (14.2)
Rosewood*	Blend of different oil constituents	Linalool (86.9)
Tanacetum	*Tanacetum vulgare*	β-Thujone (92.4) Artemisia Ketone (1.6) Eucalyptol (1.5) 4-Terpineol (1.2)
Thyme*	*Thymus zygis*	Thymol (50.4)

*Purchased from New Directions Aromatics (www.newdirectionsaromatics.ca). Composition as indicated in the provided certificate of analysis.

subjected to hydrodistillation for four hours using Clevenger-type apparatus (Fisher Scientific, Ottawa, Ontario, Canada). The *T. vulgare* and *A. abrotanum* extracts were dried over anhydrous sodium sulphate and then stored in sealed vials at 4 °C. Previous work from our lab has shown that essential oils from *T. vulgare* and *A. abrotanum* have insecticidal activity (Faraone *et al.* 2015).

For *T. vulgare* and *A. abrotanum* analysis of essential oils, mass spectra were obtained on an Agilent model 5975 C MSD mass spectrometer (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada), coupled directly to an Agilent 7890 A gas chromatograph fitted with a ZB-5HT Inferno (0.25 mm i.d. × 30 m, 0.25 µm film thickness, fused silica capillary column). The gas chromatography-mass spectrometry was done under the following conditions: two minutes at 70 °C, 2 °C/minute up to 210 °C, and maintained at this temperature up to 120 minutes. Compounds were identified by a combination of retention indices and mass spectra found within library and standards (Collin *et al.* 1993; Khodakov *et al.* 2009). Quantification of the constituents of essential oil (expressed in percentage) was carried out by peak area normalisation measurements in an Agilent 7890 A GC-FID under the same chromatographic condition. The identification was based on the comparison of mass spectra and retention indexes with published results (Adams 2007) and by injection of standard solutions. *Tanacetum vulgare* essential oil consisted of a high percentage of β-thujone, and *A. abrotanum* essential oil was composed mainly of davanone isomers (Table 1).

Preliminary trials found that most essential oils tested had little bioactivity at concentrations < 1.0%. Therefore, for experiments essential oils were diluted to concentration of 5.0, 2.5, and 1.0% (v/v essential oil) in distilled water with 0.1% v/v Tween-80. For all experiments, distilled water with 0.1% v/v Tween-80 was used as a control.

Ovicidal activity

Plutella xylostella eggs (24 hours old) were exposed to the treatments of essential oil or

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control solution by placing groups of 10 eggs onto 15 mm filter paper discs (Whatman number 1) moistened with 40 µL of treatment solution. After one hour, eggs were transferred to untreated 2.0 cm diameter leaf discs (10 eggs/disc) in 30 mL transparent plastic cups (TRA, Truro, Nova Scotia, Canada) and covered with a lid. Eggs were held in a growth chamber (20 ± 1 °C; 16:8 light: dark hours; 65 ± 5% relative humidity). Larval emergence was recorded after 72 hours, and the per cent eggs that hatched and had larvae emerge was used to determine ovicidal activity per cup. All treatments were run at the same time and there were three replicate cups per treatment. The entire experiment was conducted three times.

Larvicidal activity

Cabbage leaf discs (2.0 cm diameter), each containing one first instar, were placed individually in 100-mm Petri dishes and sprayed with 2 mL of treatment or control solution in a Potter spray tower (Burkard Scientific, Uxbridge, United Kingdom). Treated leaf discs and larvae were then transferred to individual 30-mL transparent plastic cups with a lid and incubated in a growth chamber under conditions described above. Mortality of larvae was determined after 72 hours based on responsiveness to probing with a camel hair brush. All treatments were run at the same time and there were 10 larvae (cups) per treatment. The entire experiment was conducted three times.

Larval deterrence bioassay

Leaf discs (2.0 cm diameter) were cut from fresh cabbage leaves and individually sprayed with 2-mL treatment or control solution using a Potter tower. Sprayed leaf discs were dried in a fume hood for ~10 minutes. For bioassay, one leaf disc treated with essential oil and one control leaf disc were each placed on a filter paper moistened with distilled water and placed on opposing sides of a 100-mm plastic Petri plate. The bottom of Petri plate was adjoined to a tube through a centre hole that fed into a 15-mL glass vial. Using a moist camel hair brush, a single third instar was released into the glass vial and the Petri plate was then covered and sealed using Parafilm® (Fisher Scientific, Ottawa, Ontario, Canada). The larva was allowed to climb the glass vial and enter the Petri plate. Petri plates were held under ambient conditions on a laboratory bench and after 24 hours we recorded whether the larva in each dish had settled and/or was feeding on the treated or control leaf disc. When feeding occurred, larvae were always found on foliage. Larvae found off leaf discs were considered to have been deterred from feeding. There were five replicates per treatment, and the entire experiment was conducted three times.

Adult deterrence bioassay

Three-week-old potted cabbage plants were sprayed to drip with treatment or control solution using a hand-held trigger pump sprayer to evenly cover the leaf surface. The spray was allowed to dry for 15 minutes. In $25 \times 25 \times 25$ mesh cages we placed groups of three treatment or three control plants on opposite sides of a cage, separated by at least 10 cm. Six male and six female 24–48-hour-old moths, were collected from rearing cages and released in a single treatment cage. The moths were provided 10% honey solution as a source of food and allowed to mate and lay eggs. After 48 hours, treatment and control plants were removed and the number of eggs on treatment versus control plants was determined. The bioassay was replicated three times per treatment. Essential oil activity was expressed as an oviposition deterrent index: $\mathrm{ODI} = [100 \times (C - T)]/(C + T)$, where C is the number of eggs laid on control plants, and T the number of eggs laid on plants treated with essential oil. Positive values indicate a deterrent effect, whereas negative values indicate an attraction to the treatment.

Statistical analysis

The effect of plant source of essential oil (eight levels) and concentration (three levels) on diamondback moth ovicidal activity, larval toxicity (number alive out of 10), larval choice (% of larvae that chose foliage treated with essential oil versus control foliage), and oviposition deterrent index was determined using a 3×8 factorial in three blocks (the three times where the whole experiment was conducted were used as blocks; but for oviposition deterrent index, the design had three replications instead of blocks) design. For ovicidal and larvae toxicity responses, because the interaction between plant source and concentration was not significant, and to compare the treatments with essential oil with the control, a second phase analysis was completed using a

https://doi.org/10.4039/tce.2017.13 Published online by Cambridge University Press

randomised block design with three blocks and nine treatments. This used the average of the three concentrations for ovicidal activity (because concentration effect was not significant) and for the 5% concentration only for larvae toxicity. This second phase analysis was not required for larval choice and oviposition deterrent index analyses because the control treatment was included in the calculation of the values of these response variables. However, within a given choice scenario, to determine if the percentage of larvae that choose foliage treated with essential oil and control-treated foliage were significantly different, and if the oviposition deterrent index for plants treated with essential oil and control-treated plants was significantly different, the same analysis was conducted by adding "choice" with two levels (essential oil and control) as a third factor of interest.

For each response, the validity of model assumptions (normal distribution and constant variance of the error terms; independence of the error terms were ensured by proper randomisation) were verified by examining the residuals as described in Montgomery (2013). All analyses were completed using SAS software (SAS 2014). For significant ($P < 0.05$) effects, multiple means comparisons were completed by comparing the least squares means of the corresponding treatments or treatment combinations. Letter groupings were generated using a 5% level of significance for the main effects and using a 1% level of significance for interaction effects to protect Type I experimental error rate from over inflation.

Results

Ovicidal activity

Emergence of larvae from eggs exposed to the different treatments with essential oil was not affected by the concentration of essential oil to which eggs were exposed ($P = 0.995$), or the interaction of plant source and treatment concentration of essential oil ($P = 0.999$). Plant source of essential oil did, however, have a significant effect on larval emergence from treated eggs ($P < 0.001$). Emergence of larvae was significantly lower from eggs treated with any essential oil than from control eggs ($P < 0.05$), but was most pronounced for treatments with garlic and rosewood, where larval emergence was 2.5–5.5% that of control eggs (Table 2). Larval emergence from eggs treated with tansy and thyme was also less than one-third that of the control.

Table 2. Mean ovicidal activity (larval emergence from treated eggs, *x*/10), larval toxicity (number larvae alive after treatment, *x*/10), and oviposition deterrence (measured as an oviposition deterrence index (ODI); an index of egg laying on treated versus untreated plants) of different essential oil (EO) treatments against diamondback moth, *P. xylostella*.

EO plant source	Larval emergence	Larvae toxicity	ODI*
Control	8.78a	8.33a	–
Art	7.48b	4.67b	19.7b
BF	7.00bc	9.33a	70.6ab*
Euc	5.74c	8.33a	59.2ab*
Gar	0.22f	3.33b	75.4ab*
Pep	7.04b	4.00b	117.4ab*
RW	0.48ef	3.67b	155.2a*
Tan	2.93d	8.00a	13.6b
Thy	1.56e	4.00b	90.8ab*
SEMdiff†	0.60	0.74	50.4

Notes: Within a column, means sharing the same letter are not significantly different.

* Significant differences between the ODI of plants treated with EO and control.

† SEMdiff = standard error of the difference between two means.

Art, artemesia (*Artemisia abrotanum*); BF, balsam fir (*Abies balsamea*); Euc, eucalyptus (*Eucalyptus polybractea*); Gar, garlic (*Allium sativum*); Pep, black pepper (*Piper nigrum*); RW, rosewood blend (various species); Tan, tansy (*Tanacetum vulgare*); Thy, thyme (*Thymus zygis*).

Larvicidal activity

Survival of larvae exposed to the different treatments of essential oil was significantly affected by concentration of essential oil ($P < 0.001$) and the plant source of essential oil ($P < 0.001$), but not by the interaction of these terms ($P = 0.276$). This suggested that differences among concentrations were consistent across plant sources, and differences among plant sources were consistent across the three concentrations. Mean survival (*x*/10) of larvae exposed to 5% concentrations of essential oil (5.7/10 survival) was significantly lower ($P < 0.05$) than mean survival with exposure to 2.5% concentrations of essential oil (7.2/10 survival),

https://doi.org/10.4039/tce.2017.13 Published online by Cambridge University Press

which in turn was significantly lower ($P < 0.05$) than survival with 1% treatments of essential oil (8.17/10 survival) (larval toxicity standard error of the difference between two means = 0.45). Exposure to balsam fir, eucalyptus, and tansy essential oil has no effect on larval survival ($P < 0.05$). All other treatments of essential oil significantly reduced larval survival ($P < 0.05$), but were at most only 60% lower than that seen in the control treatment (Table 2).

Larval deterrence bioassay

The choice of larvae to feed or settle upon untreated versus foliage treated with essential oil was not affected by concentration of essential oil ($P = 0.217$). However, the plant source of the essential oil had a significant effect on larval choice ($P = 0.003$). Most treatments of essential oil had no strong effect on larval choice, with the percentage of larvae choosing foliage treated with essential oil ranging from 33 to 65% (Table 3). However, foliage treated with 1 or 2.5% *Artemisia*, 2.5% garlic, and 5% rosewood had a significantly lower percentages ($\leqslant 15\%$) of larvae feeding and settling upon it than the control foliage alternative (Table 3). On the other hand, some treatments with essential oil, like 5% balsam fir, 1% tansy, and 1% rosewood were attractive to *P. xylostella* larvae, with significantly more larvae found on those treatments than on control foliage. The effect of plant source was not consistent across all concentrations, resulting in a significant interaction of plant source and concentration of essential oil ($P = 0.012$).

Adult deterrence bioassay

In the cage experiment where adult *P. xylostella* were given choice of ovipositing on treated versus untreated plants, oviposition was significantly affected by plant source of the essential oil ($P = 0.018$) and concentration of essential oil ($P = 0.024$), but not the interaction of those terms ($P = 0.215$). A positive oviposition deterrence index was found for all treatments, indicating some level of deterrence due to treatment with essential oil in all cases. Whereas plants treated with *A. abrotanum* and tansy did not deter oviposition by adult moths relative to control plants, all other treatments did (Table 2). Moths tended to be most deterred from laying eggs on plants treated with black pepper, rosewood, and thyme essential oil (Table 2), with intermediate levels of oviposition deterrence observed with the other treatments. Mean oviposition deterrence was greater in the 5% treatments with essential oil (oviposition deterrence index = 103.2) than in the 1% treatments with essential oil (oviposition deterrence index = 35.9) ($P < 0.05$). The mean oviposition deterrence index for the 2.5% treatments with essential oil (oviposition deterrence index = 86.6) was intermediate and not significantly different from the high and low percent

Table 3. Mean percent of diamondback moth, *Plutella xylostella*, larvae that chose to feed or settle upon cabbage foliage treated with different concentrations of essential oil (EO) versus untreated foliage.

EO plant source	Concentration (%)	% Choosing EO-treated foliage*
Art	1.0	6.7g*
	2.5	15.0efg*
	5.0	49.4abcde
BF	1.0	43.3bcdef
	2.5	65.0abcd
	5.0	75.6ab*
Euc	1.0	41.7bcdef
	2.5	50.0abcd
	5.0	41.1bcdefg
Gar	1.0	46.7abcdef
	2.5	15.0efg*
	5.0	56.1abcd
Pep	1.0	71.1abc
	2.5	51.7abcd
	5.0	40.0cdefg
RW	1.0	73.3abc*
	2.5	33.3defg
	5.0	13.3fg*
Tan	1.0	80.0a*
	2.5	55.0abcd
	5.0	60.0abcd
Thy	1.0	35.0defg
	2.5	33.3defg
	5.0	51.7abcd
SEMdiff†		17.2

Notes: Within a column, means sharing the same letter are not significantly different.

* Significant differences in larvae choice between EO-treated and control-treated foliage.

† SEMdiff = standard error of the difference between two means.

Art, artemesia (*Artemisia abrotanum*); BF, balsam fir (*Abies balsamea*); Euc, eucalyptus (*Eucalyptus polybractea*); Gar, garlic (*Allium sativum*); Pep, black pepper (*Piper nigrum*); RW, rosewood blend (various spp.); Tan, tansy (*Tanacetum vulgare*); Thy, thyme (*Thymus zygis*).

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treatments with essential oil (oviposition deterrence index standard error of the difference between two means = 24.7).

We observed that treatments with high concentrations of essential oil elicited variable degrees of phytotoxicity to cabbage plants in this experiment. This effect was most prominent with the 2.5 and 5% v/v garlic treatments with essential oil, particularly at the leaf margins where drops would accumulate following sprays. Phytotoxicity was mild with the other essential oils at these concentrations and absent in all 1% v/v treatments with essential oil.

Discussion

Several of the essential oils we tested – mainly those of garlic, rosewood, and thyme – were quite effective at suppressing *P. xylostella* egg hatch and killing larvae, although activity against larvae was generally lower. Previous studies showing toxicity of these essential oils to Lepidoptera and other insects corroborate our findings. Allicin, an organosulphur compound derived from garlic, reportedly has ovicidal, larvicidal, and adulticidal activity against *Musca domestica* Linnaeus (Diptera: Muscidae), could partially account for the ovicidal and larvicidal activity we observed against *P. xylostella*. Garlic has shown ovicidal activity against red spider mite, *Oligonychus coffeae* Nietner (Acari: Tetranychidae) (Roobakkumar *et al.* 2010), and larvicidal, fumigant, or repellant activity against a wide range of insect pests, including *P. xylostella* (Samarasinghe *et al.* 2007), and other Lepidoptera in laboratory experiments (Machial *et al.* 2010; Yang *et al.* 2012; Ribeiro *et al.* 2015), although applications of garlic extract for Lepidoptera pest suppression in the field have not always been effective (Endersby *et al.* 1992).

Rosewood essential oil has demonstrated a fairly wide range of insecticidal activity as well, previously found to be effective against greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) adults, nymphs, and eggs (Choi *et al.* 2003), and *P. xylostella* larvae (Yi *et al.* 2007). Thyme essential oil and its constituents have received considerable attention for their insecticidal properties (Isman 2006; Regnault-Roger *et al.* 2012), including Lepidoptera pests of vegetables. For example, Machial *et al.* (2010) and Jiang *et al.* (2012) reported good contact or residual toxicity of thyme essential oil against third instar cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). Similar to our findings, Kumrungsee *et al.* (2014) found that thymol, the main component of thyme essential oil, was larvicidal to *P. xylostella* larvae. The same authors also reported good activity of 1,8-cineole against *P. xylostella*, and although this compound comprised 87% of our eucalyptus essential oil, we found no larvicidal activity from eucalyptus essential oil.

The ability of essential oils, when applied to plants or crop commodities, to affect the feeding behaviour of insects has received almost as much study as their insecticidal activity (Isman 2006; Regnault-Roger *et al.* 2012), and the ability of an essential oil to both kill and repel insects would likely boost prospects for use in pest management. Overall, we found no dose-response effect of concentration of essential oil on larval choice of treat versus control foliage, and although essential oil overall did affect larval choice, most treatments with essential oil had no strong effect on larval choice. *Artemisia abrotanum* and certain garlic and rosewood treatments with essential oil had significantly lower percentages ($\leqslant 15\%$) of larvae on them than on the control foliage, meaning that in addition to having ovicidal and larvicidal activity discussed above, garlic and rosewood essential oils also deter larvae. Thyme essential oil, though toxic to *P. xylostella* eggs and larvae, did not deter *P. xylostella* larvae in our experiments. Similarly, thyme essential oil had low to moderate feeding deterrence against *T. ni* (Jiang *et al.* 2012). On the other hand, thymol has been shown to deter feeding of other Lepidoptera larvae (Hummelbrunner and Isman 2001), indicating that species specificity or thyme composition of essential oil could be important in determining repellency effects.

Certain balsam fir and tansy treatments with essential oil, which were non-toxic to *P. xylostella* larvae, were attractive to larvae. There are many examples of essential oils or their volatile constituents being attractive to pests (*e.g.*, Braverman *et al.* 1999; Koschier *et al.* 2000; Hanula *et al.* 2013) and beneficial insects (Cseke *et al.* 2007; Dev *et al.* 2010), perhaps mimicking the activity of kairomones or pheromones in many instances

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(Werker 1993; Muller and Buchbauer 2011). It is unclear why 5% balsam fir, 1% tansy, and 1% rosewood treatments were attractive to *P. xylostella* larvae, given that many of the key constituents in these essential oils were also present in other essential oils tested. Our finding that rosewood essential oil was toxic to *P. xylostella* eggs and larvae, a deterrent to larvae at a 5% v/v, but attractive to larvae at 1% v/v was surprising, but points to the potential biphasic nature of attraction and repulsion to essential oil. It would be valuable to confirm the reproducibility of this finding in order to test hypotheses of biphasic attraction and repulsion of essential oils, which when coupled with toxicity might reveal an interesting attract-and-kill phenomenon.

A significant amount of work has investigated the repellency effects of essential oils on insect behaviours such as oviposition (Isman 2006; Regnault-Roger *et al.* 2012). In choice tests with adult mated *P. xylostella*, treatment of cabbage plants with six of the eight essential oils we tested resulted in oviposition deterrence. The effects of oviposition deterrence were strongest with rosewood, black pepper, thyme, and garlic essential oil. In experiments with the moth *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), thyme and garlic essential oils resulted in 80% repellence of oviposition (Ribeiro *et al.* 2015). Similarly, egg laying by sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), was reduced almost 60% on thyme plants treated with essential oil (Yang *et al.* 2009), and black pepper essential oil inhibited oviposition and repelled pulse beetle, *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Chrysomelidae) (Upadhyay *et al.* 2007). We observed a clear effect of concentration of essential oil on oviposition deterrence, such that the oviposition deterrence index for the 1% v/v treatments was only one-third that of the 5% v/v treatment, and less than half that of the 2.5% v/v treatments. However, the 2.5% and 5% v/v treatments with essential oil were generally phytotoxic to plants along leaf margins where drops accumulated, potentially limiting their practical application as oviposition deterrents of *P. xylostella*. The effect at these concentrations was most notable with garlic essential oil, but less so with other essential oils. The potential for phytotoxic effects at high concentrations of essential oil would have to be tested on different crops of different ages in different settings in order to evaluate their practical use in pest management.

In conclusion, results of our laboratory experiments suggest that rosewood, garlic, and thyme essential oils have potential in management of *P. xylostella* through toxicity to eggs and larvae, and deterrence of larvae and adults. Although field applications of essential oils have been less impressive than laboratory results, particularly in terms of low persistence and efficacy relative to synthetic compounds, there clearly have been successful applications of essential oils for pest control in the field (Isman *et al.* 2011). Furthermore, problems of limited efficacy, phytotoxicity, and persistence could be surmounted through use of plant essential oils as synergists (Hummelbrunner and Isman 2001; Faraone *et al.* 2015), or through microencapsulation of essential oils (Yang *et al.* 2009; Rani *et al.* 2014). Further experiments are needed to determine if and how our laboratory results could translate into economical protection from *P. xylostella* in field or greenhouse crop production.

Acknowledgements

This research was supported by an Atlantic Canada Opportunities Agency, Atlantic Innovation Fund (Project number 197853), and the Agriculture and Agri-Food Canada Organic Science Cluster II, in partnership with the Organic Federation of Canada (Project number AIP-CL02).

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